REGISTRATION REPORT

# Part B

# Section 9

# Ecotoxicology

Detailed summary of the risk assessment

Product code: A23109A

Product name: **ORONDIS VIP**

Chemical active substances:

Metalaxyl-M, 174.4 g/L
Oxathiapiprolin, 30 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(New authorisation)

Applicant: Syngenta
Submission date: June 2022
Evaluation date: December 2022
MS Finalisation date: December 2023

# Version history

| When | What |
|---|---|
| July 2022 | dRR submitted by applicant to the Polish Ministry of Agriculture and Rural Development |
| September 2022 | Submission to the evaluation unit |
| December 2022 | zRMS finalized dRR evaluation |
| December 2023 | Updates following cMS comments |

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# 9 Ecotoxicology (KCP 10)

Oxathiapiprolin is owned by Corteva Agriscience International Sàrl (formally DuPont International Operations Sàrl; change effective January 4, 2021) (hereafter called "Corteva"), Syngenta has a Letter of Access in place to access data owned by Corteva relevant for this evaluation. Study summaries for new studies on oxathiapiprolin can be found in Appendix 2. For actual reports, please refer to data owner.

**Review Comments:**

This document describes the acceptable use conditions required for registration of A23109A, a dispersible concentrate containing 174.4 g/L metalaxyl-M and 30 g/L oxathiapiprolin, for use as a fungicide on field and protected vegetable crops.

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

Since this document is based on the information provided by the applicant, all review comments, additions and corrections have been made using commenting boxes or highlighted in grey.

## 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 | 11a | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Use-No. *** | **Member state(s)** | **Crop and/or situation (crop destination / purpose of crop)** | **F, Fn, Fpn G, Gn, Gpn or I **** | **Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)** | **Application** | | | | | **Application rate** | | | **PHI (days)** | **Remarks: e.g. g safener/ synergist per ha** | **Conclusion** | | | | | | |
| | | | | | **Method / Kind** | **Timing / Growth stage of crop & season** | **Max. number a) per use b) per crop/ season** | **Min. interval between applications (days)** | **L product/ha a) max. rate per appl. b) max. total rate per crop/season** | **g OXTP/ha a) max. rate per appl. b) max. total rate per crop/season** | **g MFX/ha a) max. rate per appl. b) max. total rate per crop/season** | **Water L/ha Min / max** | | | **Birds** | **Mammals** | **Aquatic organisms** | **Bees** | **Non-target arthropods** | **Soil organisms** | **Non-target plants** |
| **Zonal uses (field or outdoor uses, certain types of protected crops)** | | | | | | | | | | | | | | | | | | | | | |
| PL-3 | Poland | Broccoli | F | *Hyaloperonospora parasitica* | foliar | BBCH 12 - 49 | a) 2 b) 2 | 7-10 | a) 0.5 b) 1 | a) 15 b) 30 | a) 87.2 b) 174.4 | 200-800 | 20 | - | A | A | A | A | A | A | A |
| PL-27 | Poland | Leek | F | *Peronospora destructor* | foliar | BBCH 12 - 48 | a) 2 b) 2 | 12-14 | a) 0.5 b) 1 | a) 15 b) 30 | a) 87.2 b) 174.4 | 200-800 | 14 | - | A | A | A | A | A | A | A |
| PL-29 | Poland | Lettuce | F | *Bremia lactucae* | foliar | BBCH 12 - 49 | a) 2 b) 2 | 7 | a) 0.5 b) 1 | a) 15 b) 30 | a) 87.2 b) 174.4 | 200-800 | 10 | max 2 app per year in same field | A | A | A | A | A | A | A |
| PL-31 | Poland | Onion | F | *Phytophthora porri* | foliar | BBCH 12 - 48 | a) 2 b) 2 | 7 | a) 0.5 b) 1 | a) 15 b) 30 | a) 87.2 b) 174.4 | 200-800 | 14 | | A | A | A | A | A | A | A |

* Use number(s) in accordance with the list of all intended GAPs in Part B, Section 0 should be given in column 1. The critical use patterns are reported only for Poland as they cover all the other intended uses as listed in the GAP table in Part B, Section 0.

** 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)

The acute and long-term risks of A23109A to birds and mammals were assessed from toxicity exposure ratios between toxicity endpoints, estimated from studies with A23109A, metalaxyl-M and oxathiapiprolin, and maximum residues occurring on food items following applications according to the proposed use pattern.

Risk of secondary poisoning has also been assessed, as oxathiapiprolin and some of its metabolites have log $P_{ow}$ values of > 3.0. The risk to birds and mammals from exposure *via* drinking water has also been assessed.

The TER values, calculated for recommended scenarios, all exceed the trigger values of 10 for acute risk and 5 for long-term risk (including secondary poisoning), indicating that the risk to birds and mammals is acceptable following use of A23109A according to the proposed use pattern.

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

The PEC/RAC ratios, using worst-case Step 1 or Step 2 $PEC_{SW}$ values for metalaxyl-M, oxathiapiprolin and their metabolites are less than the trigger value of 1, for all aquatic organisms.
The PEC/RAC ratios, using worst-case $PEC_{SW}$ values for A23109A, are also less than the trigger value of 1, for all aquatic organisms.

The toxic unit analysis indicated that neither metalaxyl-M nor oxathiapiprolin was driving the toxicity of the mixture therefore a quantitative mixture toxicity risk assessment was conducted. The $RQ_{mix}$ values for all organism groups are below the trigger value of 1 when worst case Step 1 or Step 2 $PEC_{SW}$ values are considered.

Acceptable risks to aquatic organisms following the proposed uses of A23109A have therefore been demonstrated without the need for any application mitigation.

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

The risk to honeybees was assessed following SANCO/10329/2002 rev.2 and EPPO, 2010 as proposed in the list of guidance documents relevant to the implementation of Regulation 1107/2009, published in the official EU Journal 2013/C 95/01 and 95/02.

The risk of A23109A to honeybees was assessed from hazard quotients, estimated from acute oral and contact studies with metalaxyl-M, oxathiapiprolin and A23109A. The acute oral and contact hazard quotients were less than the relevant trigger of 50, indicating that the risk to honeybees is acceptable following use of A23109A according to the proposed use pattern.

In addition, the acute risk to honeybees was assessed from hazard quotients (HQ) and Exposure Toxicity Ratios (ETRs) following EFSA Bee Guidance Document (2013) using endpoints from acute oral and contact studies with metalaxyl-M and oxathiapiprolin. Acute contact HQ and oral ETR values were less than the relevant triggers at the screening step, indicating acceptable acute risk to adult honeybees.

The chronic adult and larval risk of A23109A to honeybees was assessed from ETRs following EFSA Bee Guidance Document (2013) using endpoints from chronic adult oral and larval studies with metalaxyl-M and oxathiapiprolin. At the screening step assessment the ETR values for chronic adult oral

and larval toxicity were below the relevant trigger values for oxathiapiprolin, as was the larval ETR for metalaxyl-M, thereby demonstrating acceptable risks to honeybees following the proposed uses of A23109A.

A Tier 1 chronic adult oral risk assessment for metalaxyl-M in the treated crop and for lettuce also in weeds, representing the worst case exposure scenario, was conducted. The ETR values for all proposed uses were below the relevant trigger values thereby demonstrating an acceptable chronic risk to adult honeybees following the proposed uses of A23109A.

The screening step assessment for the combined effects of metalaxyl-M and oxathiapiprolin indicated an acceptable risk to honeybees for acute oral and contact exposure and larval exposure but a Tier I risk assessment was necessary for chronic adult oral exposure. However, an assessment of the contribution of each active substance to the mixture toxicity showed that metalaxyl-M was driving the toxicity (>90%) therefore the Tier I chronic adult oral risk assessment for the mixture is covered by the Tier I risk assessment for metalaxyl-M which demonstrated acceptable risks to honeybees following the proposed uses of A23109A.

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

At Tier 1, the in-field HQ values based on the $LR_{50}$ were greater than the trigger value for the worst-case intended use scenarios. However, extended laboratory test data for four species, including *Typhlodromus pyri* and *Aphidius rhopalosiphi*, confirmed that the in-field risks following the proposed uses of A23109A were acceptable.

At Tier 1, the off-field HQ values based on the $LR_{50}$ were below the trigger value for the worst-case intended use scenarios thereby demonstrating that the off-field risks following the proposed uses of A23109A were acceptable.

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

**Soil meso- and macrofauna**

The risk of A23109A to earthworms was assessed from acute and long-term toxicity exposure ratios (TERs) between the selected toxicity endpoints for A23109A, metalaxyl-M, oxathiapiprolin and their relevant metabolites, and the maximum $PEC_{soil}$. The acute and long-term TER values derived are greater than the Regulation (EU) 546/2011 triggers of 10 and 5, respectively, indicating that the risk to earthworms is acceptable following use of A23109A according to the proposed use pattern.

The risk of A23109A to other non-target soil macro-organisms, as represented by *Folsomia* and *Hypoaspis* was assessed from long-term toxicity exposure ratios (TERs) between the selected no-effect concentrations or $EC_{10}$ values, derived from laboratory tests on A23109A, metalaxyl-M, oxathiapiprolin and their relevant metabolites, and the maximum $PEC_{soil}$. The long-term TER values are all greater than the recommended trigger value of 5, indicating that the risk to soil macro-organisms is acceptable following use of A23109A according to the proposed use pattern.

**Soil micro-organisms**

The risk of A23109A, metalaxyl-M, oxathiapiprolin and their relevant metabolites to soil micro-organisms was evaluated by comparison of the maximum concentrations with effects <25% derived from laboratory tests, with the maximum $PEC_{soil}$.

All the effect levels exceeded the relevant $PEC_{soil}$ values, indicating that the risk to soil micro-organisms is acceptable following the use of A23109A according to the proposed use pattern.

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

Screening test rates up to and including 1000 mL/ha were tested for six plant species with formulation A23109A. Less than 50% effect on seedling emergence and vegetative vigour on all six species was observed at the maximum test rate of 1000 mL A23109A/ha. This indicates that the risk to non-target terrestrial plants in off-crop areas is acceptable following use of A23109A according to the proposed use pattern.

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

Tests on other non-target species are not required.

## 9.1.2 Grouping of intended uses for risk assessment

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

**Table 9.1-2: Critical use pattern of A23109A grouped according to crops**

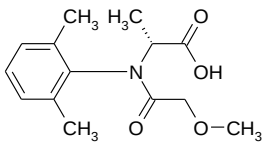
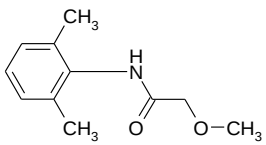
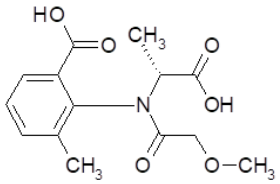
| Grouping according to criterion | | | |
|---|---|---|---|
| **Group** | **Intended uses** | **relevant use parameters for grouping** | **relevant parameter or value for sorting** |
| AT-1, AT-3, AT-5, AT-7 – AT-12, AT-14, AT-15, AT-16, AT-20, AT-21, AT-23, AT-24, AT-26, AT-29, AT-30, AT-33, AT-37, AT-38, AT-40, AT-43, AT-46, BE-1, BE-5, BE-7, BE-8, BE-9, BE-11, BE-12, BE-13, BE-17, BE-18, BE-19, BE-21, BE-24, BE-28, BE-29, BE-32, BE-35, CZ-1, CZ-3, CZ-5, CZ-7 – CZ-12, CZ-14, CZ-15, CZ-16, CZ-20, CZ-21, CZ-22, CZ-24, CZ-26, CZ-27, CZ-29, CZ-32, CZ-33, CZ-36, CZ-40, CZ-41, CZ-43, CZ-46, CZ-49, DE-1, DE-3, DE-5, DE-7 – DE-12, DE-14, DE-15, DE-16, DE-20, DE-21, DE-23, DE-24, DE-26, DE-29, DE-30, DE-33, DE-37, DE-38, DE-40, DE-43, DE-46, NL-1, NL-3, NL-5, NL-7, NL-8, NL-10, NL-11, NL-12, NL-16, NL-17, NL-18, NL-20, NL-23, NL-27, NL-28, NL-31, PL-1, PL-3, PL-5, PL-7 – PL-12, PL-14, PL-15, PL-16, PL-20, PL-21, PL-23, PL-24, PL-26, PL-29, PL-30, PL-33, PL-37, PL-38, PL-40, PL-43, PL-46, SK-1, SK-3, SK-5, SK-7 – SK-11, SK-13, SK-17, SK-18, SK-20, SK-22, SK-25, SK-26, SK-29, SK-33, SK-35, SK-38, SK-41 | Leafy vegetables (lettuce, broccoli, brussels sprouts, cauliflower, chards and beet leaves, chicory, chives, common purslane, cress, curly kale, endive, escarole, head cabbage, kale, lamb's lettuce, leafy brassica, red mustard, parsley, pe-tsai, purple vein rocket, savoy cabbage, spinach, watercress, baby leaves, herbs and edible flowers, iceberg lettuce) | Crop group: leafy vegetables<br>Growth stage: BBCH 12 - 49<br>Application rate: 0.5 L A23109A/ha<br>Max. number of applications: 2<br>Min. application interval: 7 d | <u>Relevant scenario for:</u><br>Birds and mammal risk assessment<br>Aquatic organism risk assessment<br>Bees risk assessment<br>Non-target arthropods risk assessment<br>NTTP risk assessment |

| Grouping according to criterion | | | |
|---|---|---|---|
| **Group** | **Intended uses** | **relevant use parameters for grouping** | **relevant parameter or value for sorting** |
| AT-17, AT-18, AT-27, AT-28, AT-31, AT-32, AT-41, AT-42, AT-44, AT-45, BE-14, BE-15, BE-22, BE-23, BE-25, BE-26, BE-30, BE-31, BE-33, BE-34, CZ-17, CZ-18, CZ-30, CZ-31, CZ-34, CZ-35, CZ-44, CZ-45, CZ-47, CZ-48, DE-17, DE-18, DE-27, DE-28, DE-31, DE-32, DE-41, DE-42, DE-44, DE-45, NL-13, NL-14, NL-21, NL-22, NL-24, NL-25, NL-29, NL-30, NL-32, NL-33, PL-17, PL-18, PL-27, PL-28, PL-31, PL-32, PL-41, PL-42, PL-44, PL-45, SK-14, SK-15, SK-23, SK-24, SK-27, SK-28, SK-36, SK-37, SK-39, SK-40 | Bulb vegetables (garlic, onion, leek, shallot, spring onion, welsh onion, green onion) | Crop group: bulb and onion like crops<br>Growth stage: BBCH 12 - 48<br>Application rate: 0.5 L A23109A/ha<br>Max. number of applications: 2<br>Min. application interval: 7 d | Relevant scenario for:<br>Birds and mammal risk assessment<br>Aquatic organism risk assessment<br>Bees risk assessment<br>Earthworm and other soil macro-organism risk assessment<br>Soil micro-organism risk assessment<br>NTTP risk assessment |

## 9.1.3 Consideration of metabolites

A list of metabolites of metalaxyl-M found in environmental compartments is provided below. The need for conducting a metabolite-specific risk assessment in the context of the evaluation of A23109A is indicated in the table.

**Table 9.1-3: Metabolites of metalaxyl-M**

| Metabolite | Molar mass (g/mol) | Chemical structure | Maximum observed occurrence in compartments (%) | Risk assessment required? |
|---|---|---|---|---|
| NOA409045 (CGA62826) | 265.3 |  | Soil: > 10 % of a.s.<br>Water: > 10 % of a.s.<br>Sediment: > 10 % of a.s. | Yes, aquatic and soil organisms |
| CGA67868* | 193.2 |  | Soil: >5% of a.s. in 2 sequential measurements<br>Water: -**<br>Sediment: -** | No*** |
| CGA108906 (SYN546520)* | 295.3 |  | Soil: <5 % of a.s. and maximum of formation not yet reached at the end of the study<br>Water: -**<br>Sediment: -** | No |

* During the EU Review the metabolites CGA67868 and SYN546520 were not included in the definition of residues that require further assessment in surface water/sediment (Metalaxyl-M, EFSA Journal 2015;13(3):3999) and thus not considered in the $PEC_{SW/SED}$ risk assessment.

** No data available
*** According to Regulation 284/2013 PECs for metabolites >5% of a.s. in 2 sequential measurements are required, however the soil risk assessment for metabolites occurring at levels lower than 10 % only have to be considered in exceptional cases (SANCO 2002)[1]

The codenames for R-enantiomer parent metalaxyl-M and respective metabolites, and racemic parent metalaxyl and its metabolites are in the table below.

**Table 9.1-4: Code names for R-enantiomer and racemic parent metalaxyl-M and their respective metabolites**

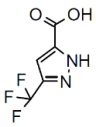
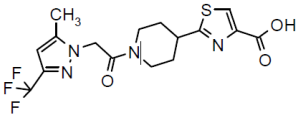
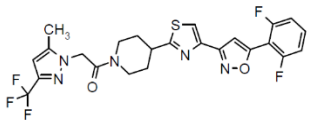
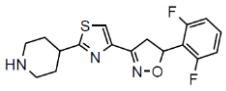
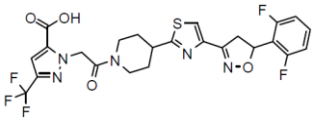
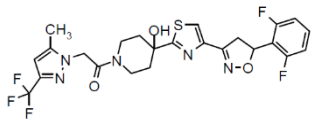
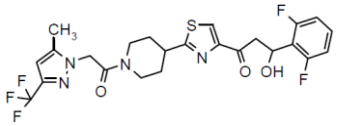
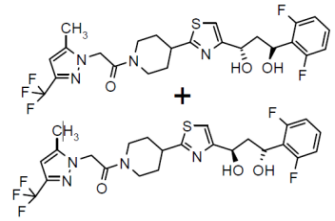
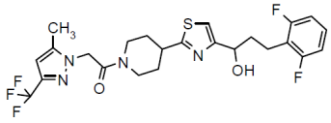
| Enantiomer composition | Parent | Acid metabolite | Diacid metabolite | Amide metabolite |
|---|---|---|---|---|
| R-enantiomer | Metalaxyl-M, CGA329351 | NOA409045 | SYN546520 | CGA67868[a] |
| Racemate (R/S) | Metalaxyl, CGA48988 | CGA62826 | CGA108906[b] | CGA67868[a] |

[a] Non-chiral CGA67868 is formed from both metalaxyl-M and metalaxyl
[b] CGA108906 was used historically as a reference material in metalaxyl-M dosed studies. More recently the R-enantiomer SYN546520 was synthesised and utilized in sorption and rate of degradation studies.

A list of metabolites of oxathiapiprolin found in environmental compartments is provided below. The need for conducting a metabolite-specific risk assessment in the context of the evaluation of A23109A is indicated in the table.

[1] SANCO/10329/2002. Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC

**Table 9.1-5: Metabolites of oxathiapiprolin potentially relevant for exposure assessment**

| Metabolite | Chemical structure | Molar mass | Maximum observed occurrence in compartments (% AR) | Risk assessment required? |
|---|---|---|---|---|
| IN-E8S72 |  | 180.09 | Soil: 10.3 [c]<br>Surface Water: not observed<br>Sediment: not observed | Soil = Yes<br><br>Surface water = Yes<br>Sediment = No |
| IN-P3X26 |  | 402.40 | Soil: not observed<br>Surface Water: 14.0<br>Sediment: not observed | Soil = No<br><br>Surface water = Yes<br>Sediment = No |
| IN-Q7D41 |  | 537.51 | Soil: 2.5<br>Surface Water: 1.5<br>Sediment: 10.5 | Soil = No<br><br>Surface water = Yes<br>Sediment = Yes |
| IN-QPS10 |  | 349.41 | Soil: 8.7 [a]<br>Surface Water: not observed<br>Sediment: not observed | Soil = Yes<br><br>Surface water = Yes<br>Sediment = No |
| IN-RAB06 |  | 569.51 | Soil: 13.5<br>Surface Water: 4.2<br>Sediment: 5.2 | Soil = Yes<br><br>Surface water = Yes<br>Sediment = No |
| IN-RDT31 |  | 555.53 | Soil: 9.4 [a]<br>Surface Water: not observed<br>Sediment: not observed | Soil = Yes<br><br>Surface water = Yes<br>Sediment = No |
| IN-RSE01 |  | 542.53 | Soil: not observed<br>Surface Water: 3.8<br>Sediment: 8.6 [a] | Soil = No<br><br>Surface water = Yes<br>Sediment = Yes |
| IN-RYJ52 |  | 544.54 | Soil: not observed<br>Surface Water: 7.9<br>Sediment: 14.7 | Soil = No<br><br>Surface water = Yes<br>Sediment = Yes |
| IN-S2K66 |  | 528.54 | Soil: not observed<br>Water: not observed<br>Sediment: 8.7 [b] | Soil = No<br><br>Surface water = Yes<br>Sediment = Yes |

AR: Applied radioactivity
[a] > 5 % at two consecutive sampling points.
[b] > 5 % and rising at the end of study.
[c] Maximum from the field study exceeds maximum amount observed in laboratory of 6.72 % reported in DAR, 2016[2], volume 1, List of endpoints.

[2] DAR, 2016: Draft Assessment Report for Oxathiapiprolin (February 2016).

## 9.2 Effects on birds (KCP 10.1.1)

### 9.2.1 Toxicity data

Avian toxicity studies have been carried out with metalaxyl-M and oxathiapiprolin. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on birds of A23109A were not evaluated as part of the EU assessment of metalaxyl-M and oxathiapiprolin. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

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

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| Bobwhite quail (*Colinus virginianus*) | Metalaxyl-M | Acute toxicity | $LD_{50}$ = 1419 mg a.s./kg bw/day | EFSA Journal 2015;13(3):3999; Johnson, 1995; CGA329351/0310 |
| Bobwhite quail (*Colinus virginianus*) | Metalaxyl-M | Acute toxicity | $LD_{50}$ = 981 mg a.s./kg bw/day | EFSA Journal 2015;13(3):3999; Palmer *et al*., 1995; CGA329351/0301 |
| Geometric mean (*Colinus virginianus*) | Metalaxyl-M | Acute toxicity | **$LD_{50}$ = 1180 mg a.s./kg bw/day** | EFSA Journal 2015;13(3):3999 |
| Mallard duck (*Anas platyrhychos)* | Metalaxyl | Acute toxicity | $LD_{50}$ = 1466 mg a.s./kg bw | EFSA Journal 2015;13(3):3999; Beavers, 1977; CGA48988/0149 |
| Geomean (overall) | Metalaxyl-M | Acute toxicity | $LD_{50}$ = 1315 mg a.s./kg bw/day | Refer to 9.2.1.1 |
| *Colinus virginianus* | A9651D (RIDOMIL GOLD) | Acute toxicity (extrapolated) | $LD_{50}$ = 3228[a] mg/kg bw | EFSA Journal 2015;13(3):3999; Gallagher & Beavers, 2005, report no. CGA329351/2154 |
| Bobwhite quail | Metalaxyl-M | Dietary 8 d Short-term | $LD_{50}$ > 5620 mg a.s./kg food (> 2631 mg a.s./kg bw/d) | EFSA Journal 2015;13(3):3999; Palmer, 1995a; CGA329351/0302 |
| Japanese quail (*Coturnix coturnix japanica*) | Metalaxyl | Dietary 8 d Short-term | $LD_{50}$ > 10000 mg a.s./kg food | EFSA Journal 2015;13(3):3999; Sachsse & Ullmann, 1976; CGA48988/0154 |
| Bobwhite quail (*Colinus virginianus*) | Metalaxyl | Dietary 8 d Short-term | $LD_{50}$ > 10000 mg a.s./kg food | EFSA Journal 2015;13(3):3999; Beavers, 1977a CGA48988/0147 |

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| Mallard duck (*Anas platyrhynchos*) | Metalaxyl | Dietary 8 d Short-term | $LD_{50}$ > 10000 mg a.s./kg food | EFSA Journal 2015;13(3):3999; Beavers, 1977b; CGA48988/1998 |
| Bobwhite quail | Metalaxyl-M | Dietary Reproductive toxicity | NOEL = 900 mg a.s./kg food (84 mg a.s./kg bw/d) | EFSA Journal 2015;13(3):3999; Taliaferro & Miller, 1998; CGA329351/1071 Refer also to 9.2.1.1 |
| Mallard duck *(Anas platyrhynchos)* | Metalaxyl-M | Dietary Reproductive toxicity | NOEL = 900 mg a.s./kg food (117.3 mg a.s./kg bw/d) | EFSA Journal 2015;13(3):3999; Taliaferro & Miller, 1998a; CGA329351/1072 |
| Bobwhite quail *(Colinus virginianus)* | Metalaxyl | Dietary Reproductive toxicity | NOEL = 900 mg a.s./kg food | EFSA Journal 2015;13(3):3999; Beavers, 1980 CGA48988/0151 |
| Mallard duck *(Anas platyrhynchos)* | Metalaxyl | Dietary Reproductive toxicity | **NOEL = 300 mg a.s./kg food (24.6 mg a.s./kg bw/d)** | EFSA Journal 2015;13(3):3999; Beavers, 1980a CGA48988/0152 |

Values in **bold** are used in the risk assessment
EFSA Journal 2015;13(3):3999. [105 pp.] doi:10.2903/j.efsa.2015.3999
[a] Acute oral $LD_{50}$ value for 'Ridomil Gold' extrapolated using the extrapolation factor given in EFSA/2009/1438

**Table 9.2-2: Endpoints and effect values relevant for the risk assessment for birds – oxathiapiprolin**

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| Bobwhite quail (*Colinus virginianus*) | Oxathiapiprolin | Oral 1 d Acute | $LD_{50}$ > 2250 mg a.s./kg bw Corrected $LD_{50}$ = **4248 mg a.s./kg bw** | EFSA 2016;14(7):4504, DuPont-31753 |
| Zebra finch (*Poephila guttata)* | Oxathiapiprolin | Oral 1 d Acute | $LD_{50}$ > 2250 mg a.s./kg bw Corrected $LD_{50}$ = **4248 mg a.s./kg bw** | EFSA 2016;14(7):4504, DuPont-31764 |
| Bobwhite quail (*Colinus virginianus*) | Oxathiapiprolin 100 g/L OD | Oral 1 d Acute | $LD_{50}$ > 2250 mg/kg bw | EFSA 2016;14(7):4504, DuPont-32706 |
| Bobwhite quail (*Colinus virginianus*) | Oxathiapiprolin | Dietary 8 d Short-term | $LC_{50}$ > 5620 mg a.s./kg feed (equivalent to > 1280 mg a.s./kg bw/day) | EFSA 2016;14(7):4504, DuPont-31754 |
| Mallard duck *(Anas platyrhynchos*) | Oxathiapiprolin | Dietary 8 d Short-term | $LC_{50}$ > 5620 mg a.s./ kg feed (equivalent to > 2728 mg a.s./kg bw/day) | EFSA 2016;14(7):4504, DuPont-31765 |

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| Bobwhite quail (*Colinus virginianus*) | Oxathiapiprolin 100 g/L OD | Dietary 8 d Short-term | $LC_{50}$ > 5620 mg/ kg feed | EFSA 2016;14(7):4504, DuPont-32703 |
| Bobwhite quail (*Colinus virginianus*) | Oxathiapiprolin | Dietary Reproductive toxicity | NOEL = 1200 mg a.s./kg feed (equivalent to **106.7 mg a.s./kg bw/day**) | EFSA 2016;14(7):4504, DuPont-31755 |
| Mallard duck (*Anas platyrhynchos*) | Oxathiapiprolin | Dietary Reproductive toxicity | NOEL = 1200 mg a.s./kg feed (equivalent to 156.3 mg a.s./kg bw/day) | EFSA 2016;14(7):4504, DuPont-31763 |

Values in **bold** are used in the risk assessment
EFSA Journal 2016;14(7):4504. [19 pp.] doi:10.2903/j.efsa.2016.4504

**Metalaxyl-M metabolites**

Characterization and identification of metalaxyl metabolites revealed that NOA409045 and CGA108906 were found at less than 10% in all tested crops. Also, from the plant metabolism study, it was concluded that NOA409045 and CGA108906 were minor metabolites, and therefore no further evaluation regarding avian risk is considered necessary.

**Oxathiapiprolin metabolites**

Studies performed with mammals indicated that plant metabolites of oxathiapiprolin are less toxic than the parent. Moreover, metabolites of oxathiapiprolin are not included in the definition of residues in plants and exposure *via* these food sources is not expected. For this reason, metabolites were not considered in the dietary risk assessment.

**A23109A**

**Table 9.2-3: Endpoints and effect values for birds - A23109A**

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| Bobwhite quail (*Colinus virginianus*) | A23109A | Oral 1 d Acute | $LD_{50}$ > 2000 mg formulation/kg bw (332 mg metalaxyl-M and 57 mg oxathiapiprolin/kg bw) | Hubbard & Temple, 2021; VV-891190 Study not evaluated by zRMS |

**Consideration of acute toxicity endpoint for A23109A used in the risk assessment**

In the acute oral toxicity study conducted with the bobwhite quail (***Hubbard & Temple, 2021; VV-891190***) no mortalities were observed and therefore the $LD_{50}$ was reported as > 2000 mg/kg bw. Under Point 2.1.2 of EFSA/2009/1438[3] a method has been proposed to extrapolate upwards the $LD_{50}$ value. The extrapolation is carried out assuming a 50% binomial probability bound that mortality could have occurred but had simply been missed by chance in the test. The extrapolation factors are presented in Table 1 of the guidance document and are dependent upon the number of animals tested and whether no, or a single mortality, was observed in the study. The acute toxicity value for the bobwhite quail has

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

been extrapolated and is presented in the table below.

**Table 9.2-4: Extrapolation of the acute oral toxicity values for A23109A**

| Study | Test species | Experimental $LD_{50}$ (mg/kg bw) | Number of animals tested | Number of mortalities | Extrapolation factor [a] | Corrected $LD_{50}$ (mg/kg bw) |
|---|---|---|---|---|---|---|
| Hubbard & Temple, 2021, VV-891190 | Bobwhite quail | > 2000 | 5 | 0 | 1.614 | 3228 |

[a] The extrapolation factor is presented in Table 1 of the guidance document (Point 2.1.2)

The extrapolated $LD_{50}$ value of 3228 mg/kg bw will therefore be used in the subsequent risk assessment.

**Review Comments:**

The metalaxyl-M is the risk driver in the formulation (95.4% of the toxicity). Therefore, the acute oral toxicity study conduct with A23109A is not required.

### 9.2.1.1 Justification for new endpoints

**Acute endpoint for metalaxyl-M**

~~The EFSA conclusion (2015) for metalaxyl-M proposes a geometric mean of 1 180 mg a.s./kg bw as endpoint for the bobwhite quail (*Colinus virginianus)*. The EFSA Guidance for birds and mammals (2009 – point 2.4.1 and 2.4.2)[4] proposes that when multiple studies are available an overall geometric mean is determined. In situations when more than one study is available with a single species (as is the situation for the bobwhite quail) a geometric mean is calculated for that species and used as a single input in determining an overall geometric mean for all species tested.~~

~~In addition to studies with the bobwhite quail a study was conducted with the mallard duck (*Anas platyrhynchos*) in which a $LD_{50}$ of 1466 mg a.s./kg bw was determined. Using these two endpoints (*i.e.* $LD_{50}$ of 1180 mg a.s./kg bw and $LD_{50}$ of 1 466 mg a.s./kg bw), and in accordance with the EFSA guidance (2009), an overall geometric mean of 1315 mg a.s./kg bw has been determined. However, for the acute risk assessment below~~ the EU agreed geomean $LD_{50}$ of **1180 mg a.s./kg bw** has been used.

**Consideration of reproductive endpoints for metalaxyl-M used in the risk assessment**

According to the EFSA Guidance (2009), an estimated reproductive endpoint should be obtained by using the acute oral $LD_{50}$ (from a single species or geometric mean) and divided by 10 to obtain an $LD_{50}$/10. This $LD_{50}$/10 is used as an endpoint in the reproductive assessment to take account of the possibility of reproductive impairment due to sub-lethal effects on pair formation and breeding site selection, incubation, parental care of nestlings, and survival of fledgling birds (in accordance with **Appendix J** of the EFSA Guidance). If the $LD_{50}$/10 is lower than the lowest reproductive endpoint, then this should be used as the reproductive endpoint.

For metalaxyl-M the $LD_{50}$ used in the acute risk assessment is 1180 mg a.s./kg bw, generating an $LD_{50}$/10 value of 118 mg a.s./kg bw.

EFSA in 2015 concluded a value for NOEL of 24.6 mg a.s./kg bw/day based on the mallard duck reproductive toxicity study with the racemic mixture metalaxyl (***Beavers, 1980a; CGA48988/0152***), ~~while, the previous EU evaluation (2002) and the current Applicant position support a value for NOEL of 84.0 mg a.s./kg bw/day based on the bobwhite quail study with metalaxyl-M (***Taliaferro and Miller, 1998; CGA329351/1071***). Both NOEL values are lower than the $LD_{50}$/10 value and thus~~ the NOEL

values will be used in the risk assessment.

During the original EU review (2002) it was concluded that the bird reproduction study conducted by ***Beavers (1980a; CGA48988/0152)*** with the racemic mixture metalaxyl was not suitable for use in a regulatory risk assessment due to a number of critical concerns over the conduct of the study, details of which are discussed below. Therefore, the Applicant conducted two additional studies looking at the effect of metalaxyl-M on the bobwhite quail (***Taliaferro and Miller, 1998; CGA329351/1071***) and the mallard duck (***Taliaferro and Miller, 1998a; CGA329351/1072***), which were included in the Supplementary Dossier for renewal (2012).

The Applicant maintains the position that the NOEL of 84 mg a.s./kg bw/day obtained from the bobwhite quail study ***(Taliaferro and Miller 1998a; CGA329351/1071)*** should be the appropriate endpoint to use in the regulatory risk assessment as it is a robust study and guideline compliant (OECD 206). This position was already evaluated and supported by the RMS (Belgium) during the evaluation for the renewal (peer review expert meeting in November 2014).

During the EU peer review expert meeting (2014) for the renewal of metalaxyl-M further consideration as to which endpoint was most appropriate for use in the regulatory risk assessment took place and further clarification on historical control data for hatchling survivorship in bobwhite quail was requested by some MS experts. However, the EU process at that stage of the evaluation did not allow the Applicant to provide additional information.

In this application the new information is reported which supports the use of the NOEL of 84 mg a.s./kg bw/day obtained with the bobwhite quail study ***(Taliaferro and Miller 1998; CGA329351/1071)***.

Robustness of the endpoint from the bobwhite quail study (***Taliaferro and Miller – 1998; CGA329351/1071***):

During the EU peer review (2014), it was noted that the hatchling survivor rate at 14 days was low (0.56) in the bobwhite quail study (***Taliaferro and Miller – 1998; CGA329351/1071)***. Studies conducted with bobwhite quail according to GLP and to OECD 206 guideline by the same authors in the same facility and in the same time frame as the above study were sourced. The hatchling survivorship from each study was collated to provide historical control data (HCD). These data showed hatchling survivor rates of 0.48-0.94 (Table 9.2-5). The data from the facility shows an inverse relationship between the number of eggs laid by control birds and 14 day old survivors / hatchlings (Figure 9.2-1). This position on the representativeness of the historical control data for the facility is further supported by the 14 day survivor values obtained in the test treatments within the study which were 0.74 (100 ppm), 0.66 (300 ppm) and 0.71 (900 ppm, the proposed endpoint). This confirms that 14 day survivors/hatchling for all the treatments were within the range of the facility HCD. Furthermore, it should be noted that there are no treatment related effects observed within the study (*i.e.* no dose response relationships). Therefore, it can be concluded that the NOEL of 84 mg a.s./kg bw/day obtained in the study is considered to be robust and suitable for use in a regulatory risk assessment.

**Table 9.2-5: Summary of HCD from the bobwhite quail studies observed in the controls of several reproductive toxicity tests from Taliaferro**

| | **Control group eggs laid per female** | **Control group 14-day old survivors / hatchlings** | **Study** |
|---|---|---|---|
| 14-day old survivors / hatchlings | 0.82 | 0.56 | Taliaferro and Miller, 1998 (CGA329351/1071).[A] |
| | 0.48 | 0.88 | Taliaferro and Brewer, 1996 (VV-352227; CGA277476/0292).[B] |
| | 0.87 | 0.48 | Taliaferro and Miller, 1998 (VV-376393; |

| | | | |
|---|---|---|---|
| | | | CGA293343/0653)[B] |
| | 0.71 | 0.72 | Taliaferro and Brewer, 1996 (VV 369024; CGA215944/0344)[B] |
| | 0.65 | 0.55 | Taliaferro et al., 1998 (VV 371001; CGA24705/2591)[B] |

[A] EU reviewed study with MFX-M and has been fully discussed EFSA Journal 2015;13(3):3999
[B] New data not EU reviewed. Full details provided in Appendix 1 and Appendix 2

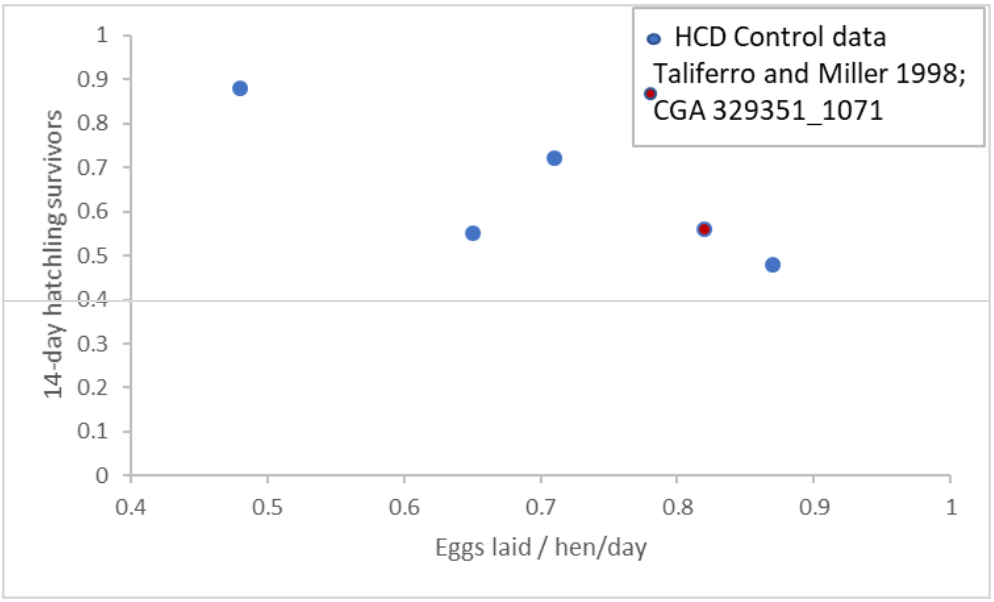



**Figure 9.2-1: Relationship between eggs laid per female per day and 14-day old survivors in control groups of several toxicity tests from Taliaferro**

With regards to the historical control (HCD) data the remaining endpoints are in line with both OECD 206 and published historical control data (***Valverde Garcia et al. 2018***[4]). It should be noted though that the number of eggs laid per hen per day is significantly higher compared to the published HCD and the OECD guideline 206 (Table 9.2 6: ). This is in fact the case for 4 out of the 5 studies conducted by Taliaferro which are summarised above. It is likely that the low percentage of 14 day old survivors is related to the high number of eggs laid per hen. In terms of number of 14 day old survivors per hen the control group has actually performed above average. The average number of 14 day old survivors per hen in the control group is well above 12 (26.5 respectively) which is the validity criteria for this endpoint in the OECD 206 Test Guideline. Table 3 of the OECD 206 Test Guideline states that for the bobwhite quail the normal value of the number of 14 day hatchlings per hen is 14 25. So, in the study conducted by ***Taliaferro and Miller (1998; CGA329351/1071)*** the number of 14 day old survivors per hen is actually slightly above the normal values according to OECD 206.

Based on the reasoning above the applicant is of the opinion that the NOEL of 84.0 mg a.s./kg bw/day from the study by ***Taliaferro and Miller (1998; CGA329351/1071)*** is robust and fit for purpose for long term avian risk assessment.

[4] Valverde-Garcia P., T. Springer, V. Kramer, M. Foudoulakis, J.R. Wheeler. 2018. An avian reproduction study historical control database: A tool for data interpretation. Regulatory Toxicology and Pharmacology 92:295-302.

**Table 9.2-6: Comparison of control data from *Taliaferro and Miller, 1998 (CGA329351/1071)* with published historical control data and OECD guideline 206**

| Endpoint | *Taliaferro and Miller, 1998 (CGA329351/1071)* | *Valverde-Garcia et al. 2018* | | | OECD 206 |
|---|---|---|---|---|---|
| | | Mean | 95% lower CL | 95% Upper CL | |
| No.eggs laid/hen/day | 0.82 | 0.52 | 0.44 | 0.61 | 0.4-0.54 |
| Eggshell thickness (mm) | 0.222 | 0.223 | 0.215 | 0.231 | 0.19-0.24 |
| Eggs cracked/eggs laid % | 1 | 2.84 | 0.93 | 8.33 | 0.6-2.0 |
| Viable embryos/eggs set % | 92 | 90.84 | 79.37 | 96.24 | 75-90 |
| Live 3-week embryos/viable embryos | 99 | 98.97 | 97.45 | 99.59 | |
| Hatchlings/live 3-week embryos % | 96 | 93.32 | 86.64 | 96.78 | |
| 14-day-old survivors/hatchlings % | 56 | 91.17 | 82.31 | 95.82 | 75-90 |
| Hatchlings/eggs set % | 88 | 84.02 | 67.58 | 92.98 | 50-90 |

Critical concerns over the mallard duck study (***Beavers, 1980a; CGA48988/0152***):

The Applicant maintains the position that the study conducted with the mallard duck and metalaxyl (***Beavers, 1980a; CGA48988/0152***) is not acceptable due to fundamental technical issues with the conduct of the study (*e.g.* age of birds, egg collection, *etc.*), which were identified in a previous EU review (2002), further than, it was not conducted according to GLP and some information (*e.g.* on purity) was missing. The above mentioned critical concerns have also been highlighted by the USA EPA. For this reason a more robust study on bobwhite quail ***Taliaferro and Miller (1998; CGA329351/1071)*** was conducted.

The fundamental issues raised by the Applicant are supported by the criteria set in the OECD 206 (1984) guideline published after the ***Beavers (1980a; CGA48988/0152)*** study was conducted. According to the OECD 206 guideline criteria, several technical deficiencies with the study design and environmental conditions for the birds during testing were highlighted, and these confounding variables likely invalidated the test results which were raised in the previous European regulatory review (SANCO/3037/99-final) resulting in using the study on bobwhite quail (***Taliaferro and Miller - 1998; CGA329351/1071)*** to establish the EU endpoint.

More details on the critical concerns of the ***Beavers (1980a; CGA48988/0152)*** study are provided hereafter:

Age of the birds: The study was conducted on ducks that were only 6 months old at the initiation of the study; the OECD 206 recommended age is 9-12 months. This, together with the shortened egg collection period (8 *vs.* 10 weeks) results in it being unclear that the birds effectively reached peak egg laying during the study. If the birds were not in the peak egg laying phase of their life cycle this may have impacted on either the number or quality of eggs produced.

Egg collection: In the study the eggs were only collected over an eight week period rather than the ten-week egg collection period recommended under OECD 206. This is likely to have contributed to the lower number of eggs laid in the control that treatment groups (666 in control *vs.* 715-818 in treatment groups) and supports that the birds may not have reached peak egg laying during the study as required in OECD guideline 206.

These data were compared to OECD Normal Values and OCSPP Validity Criteria (Table 9.2-7). Those values in red do not meet the criteria or normal values. The value in blue is low compared to the OECD normal value range:

**Table 9.2-7: Comparison of control data in *Beavers (1980a; CGA48988/0152)* study with OECD Normal Values and OCSPP Validity Criteria**

| OCSPP 850.2300 Guideline Parameter | OECD 206 Normal Values | OCSPP 850.2300 Validity Criteria | MLX Mallard Control | Source in Report |
|---|---|---|---|---|
| Assignment to treatments | | Random | Random | Text pg. 2 |
| Adult Mortality | ≤ 10% | ≤ 10% | 5.7% | Text pg. 11 |
| Average number of eggs laid / hen | 28-38 | ≥ 29 | **26.6** | Table 2 |
| Viable embryos / eggs set | 85-98 | ≥ 80% | 92.0% | Table 1A |
| Live 3-wk embryos / eggs set | | ≥ 94% | **90.9%** | Table 1A |
| Normal hatchlings / viable embryos | | ≥ 52% | 69.7% | Table 1A |
| Normal hatchlings / eggs set | 50-90 | ≥ 44% | **64.1%** | Table 1A |
| 14-d Survivors / normal hatchlings | 94-99 | ≥ 94% | **77.0%** | Table 1A |
| Egg shell thickness (mm) | ≥ 0.34 | ≥ 0.316 | 0.365 | Table 3B |
| Cracked eggs / eggs laid | 0.6-6 | ≤ 13% | 4.1% | Table 1A |

Test material dosing: It was noted in the expert meeting (2014) that there was no information on the purity of the test substance given. However, the lack of any chemical analysis of the amount of test material in the diet is of far greater concern. The OECD 206 requires test substance concentrations in diet to be confirmed during at least the first week of the test. In addition, there is no information on the amount of corn oil used as an inert carrier and there is no indication whether the same amount of corn oil was added to the control diet; OECD 206 requires no more than 2% of the weight of the basal diet and clearly the same level of carrier should be added to both treatment groups and control.

Environmental conditions: There are certain environmental conditions that are required to ensure an optimum test conduct as per OECD 206 (1984), however many of these conditions were not met. Again, this calls into question the validity of the test as the hatchlings and birds may have been of sub optimal health resulting in the lower endpoint than identified in the ***Taliaferro and Miller (1998a; CGA329351/1072)*** study conducted in the mallard in compliance with the OECD 206 guideline.

*"The temperature and humidity in the research facility were allowed to fluctuate with ambient temperature. Temperatures below 35.0°F and above 90.0°F (1.67-32.2°C) were prevented through the use of cooling ventilators, exhaust fans and ceiling insulation."*

Examples of these deviations include:

- The storage temperature deviated from the OECD 206 recommendation 13.3°C (test) compared to 14-16°C (OECD).
- Humidity was deviated outside the acceptable range at 87 % compared to 60-85 % (OECD).
- During brood development the temperatures were 37.8°C for days 0-7 compared with the OECD 206 requirement of a 32-35°C and 23.8°C, for days 7-14 compared with the OECD guideline requirement of 28-32°C.

The potential impact of these deviations from guidelines can be assessed by a comparison to background control data for mallard reproduction studies (***Valverde-Garcia et al. 2018***) (Table 9.2-8). Several control values from the metalaxyl mallard study are either outside the 95% confidence interval or differ from the mean value (shown in red).

~~**Table 9.2-8: Comparison of control data in *Beavers (1980a; CGA48988/0152)* study with Valverde-Garcia *et al.* 2018 background control data**~~

| Mallard Historical Control Values (Valverde-Garcia et al. 2018; Table 5) | MLX Mallard Study Control Values (Table 1B) | Control Mean | 95% Lower CL | 95% Upper CL | 95% Lower PL | 95% Upper PL | OECD TG206 | OSCPP 850.2300 |
|---|---|---|---|---|---|---|---|---|
| No. eggs laid / hen / d | 0.48 [a,b] | 0.58 | 0.48 | 0.69 | 0.44 | 0.73 | | |
| Eggshell thickness (mm) | 0.365 | 0.385 | 0.372 | 0.398 | 0.367 | 0.403 | 0.35–0.39 | 0.316–0.372 |
| Eggs cracked / eggs laid (%) | 4 | 1.57 | 0.52 | 4.66 | 0.33 | 7.2 | 0.6–7 | 0–4.0 |
| Viable embryos / eggs set (%) | 92 | 89.7 | 76.97 | 95.78 | 69.27 | 97.11 | 85–98 | |
| Live 3 week embryos / viable embryos (% | 99 | 98.27 | 96.26 | 99.21 | 94.89 | 99.43 | | 94–100 |
| Hatchlings / live 3 week embryos (%) | 71 | 78.1 | 60.67 | 89.18 | 52.23 | 92.08 | | 52–100 |
| 14-d old survivors / hatchlings (%) | 97 | 97.95 | 95.64 | 99.05 | 94.09 | 99.31 | 94–99 | 94–100 |
| Hatchlings / eggs set (%) | 64.1 | 69.31 | 46.86 | 85.26 | 37.46 | 89.49 | | 44–92 |

[a] Based on 8 weeks of exposure (56 d) as described on pg. 2 of the report.
[b] 666 eggs / 25 control females / 56 d

~~This comparison adds further evidence that the mallards used in the metalaxyl study may have had underlying issues that affected reproductive performance throughout the study. Thus, there is convincing evidence that NOECs generated from this study cannot be attributed solely to metalaxyl.~~

~~Replication: Five pens were established for each test treatment and control, with each pen containing 2 drakes and 5 hens compared to the 1 drake to three hens recommended in the guidance, although it is noted that other arrangements may be justified. However, the guidance document does explicitly state that when the mallard duck is tested in groups *i.e.* in ratios of other than 1 drake to 3 hens than at least 8 pens need to be established for each treatment rate and control. Given that only 5 pens were established for each test treatment rate and control then the statistical power of this study is open to question.~~

~~*Conclusions*~~

~~Based on the new information provided for the historical control data of the bobwhite quail study (***Taliaferro and Miller – 1998; CGA329351/1071***) and the critical concerns identified in the study of mallard duck (***Beavers – 1980a; CGA48988/0152***), the Applicant maintains the position that the **NOEL of 84 mg a.s./kg bw/day** (900 ppm) is the appropriate value to be used in the regulatory risk assessment.~~

~~The value of the NOEC of 300 ppm agreed during the EU peer review (2014) and used in the risk assessment (EFSA Conclusion 2015) was based on a weight of evidence and considering all the available studies at the time. However, the additional HCD provided in this application were not available at the time of the EU peer review evaluation, for this reason the Applicant considers that the endpoint needs further consideration during this evaluation.~~

~~The conclusion on the use of the **NOEL of 84 mg a.s./kg bw/day** in the regulatory risk assessment is further supported by another regulatory review for metalaxyl and mefenoxam (metalaxyl-M) conducted by the USA EPA[5] in 1996. This review concluded that the ***Beavers (1980a; CGA48988/0152)*** study was only suitable as supplemental data based on the short duration of the egg collection period, uncontrolled environmental conditions, lack of individual adult body weight data and a lack of treated diet analysis. Furthermore, a number of parameters in the control groups were lower than the test groups, *e.g.* total eggs laid resulted in lower numbers of eggs set and viable egg numbers for controls with only 27% of eggs laid surviving to 14 days.~~

~~In the USA EPA review the endpoints used for both active substances were those determined in the~~

[5] Anonymous (2016). Metalaxyl and Mefenoxam: Preliminary Ecological Risk Assessment for Registration Review of Metalaxyl and Mefenoxam (Metalaxyl-M) and Proposed Crop Group Conversion for Oilseed Group 20. **PC Code: 113501, 113502. DP Barcode: 433073**
https://archive.epa.gov/pesticides/chemicalsearch/chemical/foia/web/pdf/113501/113501-171.pdf;
https://archive.epa.gov/pesticides/chemicalsearch/chemical/foia/web/pdf/113501/113501-172.pdf

~~studies by ***Taliaferro and Miller (1998; CGA329351/1071; CGA329351/1072)*** with metalaxyl-M and the earlier studies conducted on metalaxyl (***Beavers, 1980; (CGA48988/0151; CGA48988/0152)*** were not considered in the dossier having been previously deemed as not being suitable for use in a regulatory risk assessment due to the issues described above.~~

~~For completeness, in this document, the endpoint from the racemic mixture of 24.6 mg a.s./kg bw/day will be used in the first-tier risk assessment as per current EFSA conclusion (2015) however the **84 mg a.s./kg bw/day NOEL** endpoint will be used in cases where a higher tier assessment is required, as per justification provided above.~~

**Consideration of acute mixture toxicity**

According to EFSA/2009/1438[6] combined action of several toxicants must be specifically considered in the risk assessment when it is obvious that such exposure situations will occur for animals.

For the assessment of acute effects (mortality), a surrogate $LD_{50}$ can be calculated. The EFSA Guidance Document indicates that the following equation should be used for deriving a surrogate $LD_{50}$ for a mixture of active substances with known toxicity assuming dose additivity:

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

*where:*
*$X\ (a.s._i)$* = *fraction of active substance (i) in the formulation mixture*
*$LD_{50}\ (a.s._i)$* = *acute toxicity for the active substance (i)*

The $LD_{50}$ of the mix is summarised in the table below.

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

**Table 9.2-9: Acute $LD_{50}$ for the mixture of metalaxyl-M and oxathiapiprolin**

| Test substance | Concentration of active substance in formulation A23109A (g a.s./L) | Fraction of active substance in the formulation mixture [A] | Acute toxicity endpoint (mg a.s./kg bw) | Fraction of active substance/$LD_{50}$ for the active substance | $LD_{50}$ mix (mg a.s./kg bw) |
|---|---|---|---|---|---|
| Metalaxyl-M | 178 | 0.853 | 1180 | 0.0007231 | 1320 |
| Oxathiapiprolin | 30.6 | 0.147 | 4248 | 0.0000345 | |
| Total | 208.6 | 1 | - | 0.0007577 | |

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

Note: Calculations undertaken using unrounded values consequently they may not be reproducible when using the figures given in the table

**Methodology to establish whether a single substance is driving the toxicity of a mixture**

**Table 9.2-10: Toxicity of metalaxyl-M and oxathiapiprolin in the mixture**

| Test substance | Concentration of active substance in formulation A23109A (g a.s./L) | Fraction of active substance in the formulation mixture [A] | Acute toxicity endpoint (mg a.s./kg bw) | Fraction of active substance/$LD_{50}$ for the active substance | Toxicity per fraction a.s. (mg/kg) | Toxicity per fraction quotient (%) | Single driver of toxicity |
|---|---|---|---|---|---|---|---|
| Metalaxyl-M | 178 | 0.853 | 1180 | 0.0007231 | 1383 | **95.4** | **yes** |
| Oxathiapiprolin | 30.6 | 0.147 | 4248 | 0.0000345 | 28959 | 4.6 | no |
| Total | 208.6 | 1 | - | 0.0007577 | - | 100 | - |

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

Note: Calculations undertaken using unrounded values consequently they may not be reproducible when using the figures given in the table

The table above shows that due to its higher fraction in the formulation metalaxyl-M is over 20 times more toxic in the mixture compared to oxathiapiprolin. Therefore, metalaxyl-M is the risk driver in the formulation (95.4% of the toxicity). Thus, an acute mixture toxicity risk assessment is not required according to the guidance. However, as a worst case approach the combination risk assessment has been presented.

According to the EFSA Guidance Document (2009; Appendix B, Step 2a), the surrogate $LD_{50}$ of 1320 mg a.s./kg bw for mixture toxicity should be compared to the acute oral toxicity of the formulation, using the following equation:

$$\sum_i \frac{\mathrm{X}(a.s._i)}{\mathrm{LD}_{50}(a.s._i)} = \frac{1}{\mathrm{LD}_{50}(prod.)}$$

*where:*
*$X(a.s._i)$ = fraction of active substance (i) in the formulation mixture*
*$LD_{50}(a.s._i)$ = acute toxicity for the active substance (i)*
*$LD_{50}(prod.)$ = measured acute toxicity value for the formulated mixture*

A comparison of measured and predicted toxicity of A23109A is provided in the table below.

**Table 9.2-11: Comparison of the measured formulation with the predicted mixture toxicity assuming dose additivity**

| Test substance | Metalaxyl-M | Oxathiapiprolin | Sum | $1/LD_{50}$ (prod.) (PPP as a.s.) |
|---|---|---|---|---|
| Fraction of a.s. in formulation | 0.853 | 0.147 | 1 | 1 |
| $LD_{50}$ (a.s.) | 1180 | 4248 | - | 628 [a] |
| Fraction of a.s. in formulation/ $LD_{50}$ for the individual active substance | 0.0007231 | 0.0000345 | 0.0007577 | 0.00159 |

[a] Formulation endpoint expressed as total a.s. (based on the total % w/w of metalaxyl-M (16.6%) and oxathiapiprolin (2.85%) in the formulation (19.45% w/w)

A greater value on the right side of the equation indicates that the formulation is more toxic than predicted from the toxicity of the individual components (active substances and co-formulants of known toxicity). This may be due to, *e.g*. further toxic co-formulants, toxicokinetic interaction or synergism/potentiation of effect. It may also reflect the inherent variability of toxicity testing. In all these cases, the use of the $LD_{50}$ for the formulation is recommended for the first-tier assessment, because it cannot be excluded that such effects would also occur after exposure of animals to residues in the environment.

If, in contrast, the measured toxicity of a formulation is lower than predicted, the predicted mixture toxicity according to Step 1 should be used in the first-tier risk assessment.

In the case of A23109A, this results in a value of 0.00076 on the left and 0.00159 on the right, indicating that the formulation is more toxic than predicted. However, this is not conclusive because the $LD_{50}$ for A23109A is above the highest dose tested. Nevertheless, the formulation endpoint will be used in the risk assessment.

**Combined reproductive toxicity**

As requested in the Working document on Risk Assessment of Plant Protection Products in the Central Zone – Ecotoxicology (May 2021), a calculation of long-term combitox risk according to the concentration addition (CA) model should be presented for Tier 1.

## 9.2.2 Risk assessment for spray applications

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

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessments for the uses in leafy vegetables and bulb and onion like crops cover the risk for birds from all intended uses (see 9.1.2).

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

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

**Table 9.2-12: Screening assessment of the acute and long-term/reproductive risk for birds due to the use of A23109A – metalaxyl-M**

| **Active substance** | | Metalaxyl-M | | | | |
|---|---|---|---|---|---|---|
| **Acute toxicity (mg a.s./kg bw)**<br>**TER criterion** | | 1180<br>10 | | | | |
| **Crop scenario**<br>**Growth stage** | **Application rate**<br>**(g a.s./ha)** | **Indicator species** | **$SV_{90}$** | **$MAF_{90}$** | **$DDD_{90}$**<br>**(mg/kg bw/d)** | **$TER_a$** |
| Leafy vegetables and Bulbs & onion like crops | 2 x 87.2<br>(7-d interval) | Small omnivorous bird | 158.8 | 1.4 | 19.4 | 60.9 |
| **Reprod. Toxicity (mg a.s./kg bw/d)**<br>**TER criterion** | | 24.6<br>5 | | | | |
| **Crop scenario**<br>**Growth stage** | **Application rate**<br>**(g a.s./ha)** | **Indicator species** | **$SV_m$** | **$MAF_m$ × TWA** | **$DDD_m$**<br>**(mg/kg bw/d)** | **$TER_{lt}$** |
| Leafy vegetables and Bulbs & onion like crops | 2 x 87.2<br>(7-d interval) | Small omnivorous bird | 64.8 | 1.6 × 0.53 | 4.79 | 5.13 |
| Leafy vegetables*<br>BBCH 10-19 | 2 x 87.2<br>(7-d interval) | Medium herbivorous/granivorous bird 'pigeon' | ~~37.0~~<br>22.6 | 1.6 × 0.53 | ~~2.74~~<br>1.67 | ~~8.99~~<br>14.7 |

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio

*Tier 1 the worst exposure scenario considered for the assessment of the combined reproductive toxicity

**Table 9.2-13: Screening assessment of the acute and long-term/reproductive risk for birds due to the use of A23109A – oxathiapiprolin**

| **Active substance** | | Oxathiapiprolin | | | | |
|---|---|---|---|---|---|---|
| **Acute toxicity (mg a.s./kg bw)**<br>**TER criterion** | | 4248<br>10 | | | | |
| **Crop scenario**<br>**Growth stage** | **Application rate**<br>**(g a.s./ha)** | **Indicator species** | **$SV_{90}$** | **$MAF_{90}$** | **$DDD_{90}$**<br>**(mg/kg bw/d)** | **$TER_a$** |
| Leafy vegetables and Bulbs & onion like crops | 2 x 15<br>(7-d interval) | Small omnivorous bird | 158.8 | 1.4 | 3.33 | 1274 |
| **Reprod. Toxicity (mg a.s./kg bw/d)**<br>**TER criterion** | | 106.7<br>5 | | | | |
| **Crop scenario**<br>**Growth stage** | **Application rate**<br>**(g a.s./ha)** | **Indicator species** | **$SV_m$** | **$MAF_m$ × TWA** | **$DDD_m$**<br>**(mg/kg bw/d)** | **$TER_{lt}$** |
| Leafy vegetables and Bulbs & onion like crops | 2 x 15<br>(7-d interval) | Small omnivorous bird | 64.8 | 1.6 × 0.53 | 0.824 | 129 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Leafy vegetables* BBCH 10-19 | 2 x ~~87.2~~ 15 (7-d interval) | Medium herbivorous/granivorous bird 'pigeon' | ~~37.0~~ 22.6 | 1.6 × 0.53 | ~~0.47~~ 0.287 | ~~227~~ 371 |

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio
*Tier 1 the worst case exposure scenario considered for the assessment of the combined reproductive toxicity

The acute and chronic screening assessment for metalaxyl-M and oxathiapiprolin, for all indicator species, concludes TER values greater than the trigger of 10 for acute risk and 5 for chronic risk, indicating that risk to birds is acceptable following use of A23109A according to the proposed use pattern.

**Metalaxyl-M/oxathiapiprolin mixture assessment**

**Acute risk**

The experimentally determined $LD_{50}$ of the formulation A23109A is used as the endpoint for the acute combination mixture risk assessment (see 9.1.2).

**Table 9.2-14: Screening assessment of the acute risk for birds due to the use of A23109A**

| **Product** | | A23109A | | | | |
|---|---|---|---|---|---|---|
| **Acute toxicity (mg total a.s./kg bw)**<br>**TER criterion** | | 628<br>10 | | | | |
| **Crop scenario**<br>**Growth stage** | **Application rate (g total a.s./ha)[a]** | **Indicator species** | **$SV_{90}$** | **$MAF_{90}$** | **$DDD_{90}$ (mg/kg bw/d)** | **$TER_a$** |
| Leafy vegetables and Bulbs & onion like crops | 2 x 102.2 (7-d interval) | Small omnivorous bird | 158.8 | 1.4 | 22.7 | 27.6 |

SV: shortcut value; MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio
[a] Based on a total application rate of 87.2 g/ha for metalaxyl-M and 15 g/ha for oxathiapiprolin

The $TER_a$ values for the mixture toxicity are greater than the relevant trigger value indicating that acute risk to birds is acceptable following use of A23109A according to the proposed use patterns.

**Review Comments:**

The metalaxyl-M is the risk driver in the formulation (95.4% of the toxicity). Therefore, the acute oral toxicity study conduct with A23109A is not required. The study by Hubbard & Temple, 2021; VV-891190, was not evaluated by zRMS, thus, the risk assessment presented above should be considered as "illustrative assessment".

**Chronic risk**

For assessment of chronic effects, according to EFSA/2009/1438[2], *'if a given formulation contains several active substances all known to cause similar effects via a similar biochemical mechanism (e.g. aromatase inhibition) and if this type of effect is actually driving the risk assessment, it is thus recommended to perform an assessment for combined effects on a case by case basis'.*

For A23109A the active substances, metalaxyl-M (a phenylamide with activity by inhibiting ribosomal

RNA synthesis) has a different mode of action in fungi than the active substance oxathiapiprolin (a piperidinyl thiazole isoxazoline with activity by inhibition of oxysterol binding protein (OSBP) homologue). Consequently, an assessment for combined chronic effects is not considered to be required.

**Combined reproductive toxicity**

As requested in the Working document on Risk Assessment of Plant Protection Products in the Central Zone – Ecotoxicology (May 2021), a calculation of long-term combitox risk according to the concentration addition (CA) model should be presented for Tier 1.

The $TER_{LT\ combi}$ values are calculated based on the screening step and Tier 1 TER values for the active substances. The calculations of the cumulative ecotoxicological effects are summarized in table below.

| Crop scenario and/or indicator species | | $TER_{LT}$ Metalaxyl-M | $TER_{LT}$ Oxathiapiprolin | $TER_{LT\ combi}$ | Trigger |
|---|---|---|---|---|---|
| **Reproductive (screening step)** | | | | | |
| Leafy vegetables and Bulbs & onion like crops | Small omnivorous bird | 5.13 | 129 | **4.93** | 5 |
| **Reproductive (tier 1)** | | | | | |
| Leafy vegetables BBCH 10-19 | Medium herbivorous/granivorous bird 'pigeon' | ~~8.99~~ 14.7 | ~~227~~ 371 | ~~8.65~~ 14.1 | 5 |

The $TER_{LT\ combi}$ value in the Tier 1 is above the trigger value of 5. Thus, it can be concluded that the reproductive risk for birds for the combined exposure to the two active substances in the application of A23109A according to good agricultural practice is low and acceptable.

### 9.2.2.2 Higher-tier risk assessment

A higher-tier risk assessment is not required.

### 9.2.2.3 Drinking water exposure

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

**Leaf scenario**

Since A23109A is intended to be applied on leafy vegetables forming heads or crop plants with comparable water collecting structures at principal growth stage 4 or later, the leaf scenario must be considered. The maximum single use rate for leafy vegetables of 0.5 L A23109A/ha equivalent to 87.2 g metalaxyl-M/ha and 15 g oxathiapiprolin/ha is used in combination with the minimum water volume of 200 L/ha to cover the risk to birds.

**Table 9.2-15: Assessment of the acute risk for birds due to exposure to metalaxyl-M *via* contaminated drinking water in leaf whorls**

| | | | | | | |
|---|---|---|---|---|---|---|
| **Active substance**<br>**Maximum application rate (g a.s./ha)** | | Metalaxyl-M<br>2 x 87.2 | | | | |
| **Acute toxicity (mg a.s./kg bw)**<br>**TER criterion** | | 1180<br>10 | | | | |
| **(Single) application rate (g/ha)** | **Water application rate (L/ha)** | **$C_{spray-sol.}$ (mg/L)** | **$PEC_{leaf-whorl}$ = $C_{spray-sol.}$/5 (mg/L)** | **DW uptake (L/kg bw/d)** | **Daily dose (mg/kg bw/d)** | **$TER_a$** |
| 87.2 | 200 | 436 | 87.2 | 0.46 | 40.1 | 29.4 |

$C_{spray-sol}$: concentration in spray solution; $PEC_{leaf-whorl}$: concentration in pools in leaf whorls; DW: drinking water; TER: toxicity to exposure ratio

**Table 9.2-16: Assessment of the acute risk for birds due to exposure to oxathiapiprolin *via* contaminated drinking water in leaf whorls**

| | | | | | | |
|---|---|---|---|---|---|---|
| **Active substance**<br>**Maximum application rate (g a.s./ha)** | | Oxathiapiprolin<br>2 x 15 | | | | |
| **Acute toxicity (mg a.s./kg bw)**<br>**TER criterion** | | 4248<br>10 | | | | |
| **(Single) application rate (g/ha)** | **Water application rate (L/ha)** | **$C_{spray-sol.}$ (mg/L)** | **$PEC_{leaf-whorl}$ = $C_{spray-sol.}$/5 (mg/L)** | **DW uptake (L/kg bw/d)** | **Daily dose (mg/kg bw/d)** | **$TER_a$** |
| 15 | 200 | 75.0 | 15.0 | 0.46 | 6.90 | 616 |

$C_{spray-sol}$: concentration in spray solution; $PEC_{leaf-whorl}$: concentration in pools in leaf whorls; DW: drinking water; TER: toxicity to exposure ratio

The $TER_a$ values for metalaxyl-M and oxathiapiprolin are greater than the trigger value of 10 indicating that acute risk to birds due to exposure *via* contaminated drinking water in leaf whorls is acceptable following use of A23109A on leafy vegetables according to the proposed use patterns.

**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 3 000 in the case of more sorptive substances (Koc ≥ 500 L/kg).

With a mean K(f)oc of 78.9 L/kg, metalaxyl-M belongs to the group of less sorptive substances and with a K(f)oc of 6243 L/kg, oxathiapiprolin belongs to the group of more sorptive substances.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the maximum use rate of 2 x 87.2 g metalaxyl-M/ha (7 d interval) and 2 x 15 g oxathiapiprolin/ha (7 d interval) is used to cover the risk to birds from all intended uses (see 9.1.2).

| **Metalaxyl-M** | | | |
|---|---|---|---|
| Effective application rate (g a.s./ha)* = | 161.32* | | |
| Acute toxicity (mg a.s./kg bw) = | 1180 | quotient = | 0.14 |
| Reprod. Toxicity (mg a.s./kg bw/d) = | 24.6 | quotient = | 6.56 |

* Effective application rate = Maximum application rate x MAF of 1.85 assuming a soil $DT_{50}$ of 30.9 days and 2 applications with a 7-day interval

| **Oxathiapiprolin** | | | |
|---|---|---|---|
| Effective application rate (g a.s./ha)* = | 29.4* | | |
| Acute toxicity (mg a.s./kg bw) = | 4248 | quotient = | 0.01 |
| Reprod. Toxicity (mg a.s./kg bw/d) = | 106.7 | quotient = | 0.28 |

* Effective application rate = Maximum application rate x MAF of 1.96 assuming a soil $DT_{50}$ of 121.2 days and 2 applications with a 7-day interval

The resulting ratios fall below the trigger of 50 for metalaxyl-M (less sorptive) and 3000 for oxathiapiprolin (more sorptive), indicating that further assessment of the acute and long-term risk to birds from drinking water from puddles is not required.

### 9.2.2.4 Effects of secondary poisoning

According to EFSA/2009/1438, substances with a log $P_{ow}$ of > 3 have a potential for bioaccumulation and as such consideration of the potential effects of secondary poisoning to birds and mammals are required.

The log $P_{OW}$ values for metalaxyl-M and its relevant metabolite (NOA409045) are 1.71 and -1.29, respectively. Therefore, the log $P_{ow}$ values do not exceed the trigger value of 3 and a risk assessment for effects due to secondary poisoning is not required. Although a paper previously indicated there might be potential for bioaccumulation in earthworms, metalaxyl-M review report (SANTE/11112/2019) indicates that concerns about bioaccumulation can be considered low.

The log $P_{ow}$ of oxathiapiprolin is 3.67 (at pH 7) and thus exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is required. The log $P_{ow}$ values of the oxathiapiprolin metabolites IN-Q7D41, IN-S2K66 and IN-RDT31 were reported to be 4.3, 3.4, and 4.1, respectively, and exceed the trigger value of 3. Therefore, a risk assessment for effects due to secondary poisoning is required. IN-S2K66 and IN-Q7D41 were not found in soil therefore the risk assessment was performed for fish-eating birds only.

**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.

**Oxathiapiprolin and its metabolites**

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the worst-case soil PEC following 2 x 15 g a.s./ha applications to onions is considered (see Section 8 - Environmental Fate, Chapter 8.7.2).

**Table 9.2-17: Assessment of the risk for earthworm-eating birds due to exposure to oxathiapiprolin *via* bioaccumulation in earthworms (secondary poisoning)**

| Parameter | Oxathiapiprolin | Comments |
|---|---|---|
| $PEC_{soil\ accumulation}$ (mg/kg soil) | 0.0392 | Onions, 2 x 15 g a.s./ha |
| log $P_{ow}$ / $K_{ow}$ | 3.67 / 4677 | EFSA, 2016 |
| $K_{oc}$ | 6243 | Arithmetic mean (n = 5) |
| $f_{oc}$ | 0.02 | Default |
| $BCF_{worm}$ | 1* | $BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times K_{ow}) / f_{oc} \times K_{oc}$ |
| $PEC_{worm}$ | 0.0392 | $PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$ |
| Daily dietary dose (mg/kg bw/d) | 0.0412 | $DDD = PEC_{worm} \times 1.05$ |
| NOEL (mg/kg bw/d) | 106.7 | Bobwhite quail (*Colinus virginianus*) |
| $TER_{lt}$ | 2592 | $\geq$ 5; acceptable risk |

* calculated BCF = 0.46. A BCF of 1 was used for $TER_{lt}$ estimation as a worst-case considering soil in gut of earthworms if relevant.

**Table 9.2-18: Assessment of the risk for earthworm-eating birds due to exposure to IN-RDT31 *via* bioaccumulation in earthworms (secondary poisoning)**

| Parameter | IN-RDT31 | Comments |
|---|---|---|
| $PEC_{soil\ accumulation}$ (mg/kg soil) | 0.0063 | Onions, 2 x 15 g a.s./ha |
| log $P_{ow}$ / $K_{ow}$ | 4.1 / 12589 | EFSA, 2016 |
| $K_{oc}$ | 1168 | Arithmetic mean (n = 5) |
| $f_{oc}$ | 0.02 | Default |
| $BCF_{worm}$ | 6.50 | $BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times K_{ow}) / f_{oc} \times K_{oc}$ |
| $PEC_{worm}$ | 0.0410 | $PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$ |
| Daily dietary dose (mg/kg bw/d) | 0.0430 | $DDD = PEC_{worm} \times 1.05$ |
| NOEL (mg/kg bw/d) | 10.67 | 10x parent toxicity assumed as a worst case [a] |
| $TER_{lt}$ | 248 | $\geq$ 5; acceptable risk |

[a] In accordance with the DAR, 2016, volume 3, Annex B.9 PPP

**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.

**Oxathiapiprolin and its metabolites**

To achieve a concise risk assessment, the risk envelope approach is applied. The worst-case 21-day time-weighted average surface water PEC (FOCUS Step 2) following 2 x 15 g a.s./ha applications to bulb vegetables was used for oxathiapiprolin and for metabolites IN-Q7D41, IN-S2K66 and IN-RDT31.

**Table 9.2-19: Assessment of the risk for fish-eating birds due to exposure to oxathiapiprolin *via* bioaccumulation in fish (secondary poisoning)**

| Parameter | Oxathiapiprolin | Comments |
|---|---|---|
| $PEC_{sw}$ (twa = 21 d) (mg/L) | 0.00036 | 21-day Step 2 $PEC_{sw, twa}$ (SEU) for bulb vegetables |
| $BCF_{fish}$ | 87 | EFSA, 2016 |
| BMF | - | Biomagnification factor (relevant for BCF ≥ 2000) |
| $PEC_{fish}$ | 0.0313 | $PEC_{fish} = PEC_{water} \times BCF_{fish}$ |
| Daily dietary dose (mg/kg bw/d) | 0.00498 | $DDD = PEC_{fish} \times 0.159$ |
| NOEL (mg/kg bw/d) | 106.7 | Bobwhite quail (*Colinus virginianus*) |
| $TER_{lt}$ | 21426 | ≥ 5; acceptable risk |

**Table 9.2-20: Assessment of the risk for fish-eating birds due to exposure to IN-Q7D41 *via* bioaccumulation in fish (secondary poisoning)**

| Parameter | IN-Q7D41 | Comments |
|---|---|---|
| $PEC_{sw}$ (maximum) (mg/L) | 0.00043 | 21-day Step 2 $PEC_{sw, twa}$ (SEU) for bulb vegetables |
| $BCF_{fish}$ | 533 | EFSA, 2016 |
| BMF | - | Biomagnification factor (relevant for BCF ≥ 2000) |
| $PEC_{fish}$ | 0.229 | $PEC_{fish} = PEC_{water} \times BCF_{fish}$ |
| Daily dietary dose (mg/kg bw/d) | 0.0364 | $DDD = PEC_{fish} \times 0.159$ |
| NOEL (mg/kg bw/d) | 10.67 | 10x parent toxicity assumed as a worst-case [a] |
| $TER_{lt}$ | 293 | ≥ 5; acceptable risk |

[a] In accordance with the DAR, 2016, volume 3, Annex B.9 PPP

**Table 9.2-21: Assessment of the risk for fish-eating birds due to exposure to IN-S2K66 *via* bioaccumulation in fish (secondary poisoning)**

| Parameter | IN-S2K66 | Comments |
|---|---|---|
| $PEC_{sw}$ (maximum) (mg/L) | 0.00031 | 21-day Step 2 $PEC_{sw, twa}$ (SEU) for bulb vegetables |
| $BCF_{fish}$ | 115 | EFSA, 2016 |
| BMF | - | Biomagnification factor (relevant for BCF ≥ 2000) |
| $PEC_{fish}$ | 0.0357 | $PEC_{fish} = PEC_{water} \times BCF_{fish}$ |
| Daily dietary dose (mg/kg bw/d) | 0.00567 | $DDD = PEC_{fish} \times 0.159$ |
| NOEL (mg/kg bw/d) | 10.67 | 10x parent toxicity assumed as a worst case [a] |
| $TER_{lt}$ | 1882 | ≥ 5; acceptable risk |

[a] In accordance with the DAR, 2016, volume 3, Annex B.9 PPP

**Table 9.2-22: Assessment of the risk for fish-eating birds due to exposure to IN-RDT31 *via* bioaccumulation in fish (secondary poisoning)**

| Parameter | IN-RDT31 | Comments |
|---|---|---|
| $PEC_{sw}$ (maximum) (mg/L) | 0.00013 | 21-day Step 2 $PEC_{sw, twa}$ (SEU) for bulb vegetables |
| $BCF_{fish}$ | 78 | EFSA, 2016 |
| BMF | - | Biomagnification factor (relevant for BCF ≥ 2000) |
| $PEC_{fish}$ | 0.0101 | $PEC_{fish} = PEC_{water} \times BCF_{fish}$ |
| Daily dietary dose (mg/kg bw/d) | 0.00161 | $DDD = PEC_{fish} \times 0.159$ |
| NOEL (mg/kg bw/d) | 10.67 | 10x parent toxicity assumed as a worst case [a] |
| $TER_{lt}$ | 6618 | ≥ 5; acceptable risk |

[a] In accordance with the DAR, 2016, volume 3, Annex B.9 PPP

Risk of secondary poisoning was assessed for oxathiapiprolin (log $P_{ow}$ = 3.67 at pH 7) and some of its metabolites (IN-Q7D41, IN-S2K66 and IN-RDT31 with log $P_{ow}$ values of 4.3, 3.4, and 4.1, respectively). All calculated $TER_{lt}$ values clearly exceed the trigger value of 5 indicating that the risk of secondary poisoning to birds is acceptable following use of A23109A according to the proposed use pattern.

### 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 acute and long-term risks of A23109A to birds were assessed from toxicity exposure ratios between toxicity endpoints, estimated from studies with A23109A, metalaxyl-M and oxathiapiprolin, and maximum residues occurring on food items following applications according to the proposed use pattern.

Risk of secondary poisoning has also been assessed, as oxathiapiprolin and some of its metabolites have log $P_{ow}$ values of > 3.0. The risk to birds from exposure *via* drinking water has also been assessed.

The TER values, calculated for recommended scenarios, all exceed the trigger values of 10 for acute risk and 5 for long-term risk (including secondary poisoning), indicating that the risk to birds is acceptable following use of A23109A according to the proposed use pattern.

**Review Comments:**

The acute and chronic risks of A23109A to birds 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 A23109A 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 is low.

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

### 9.3.1 Toxicity data

Mammalian toxicity studies have been carried out with metalaxyl-M and oxathiapiprolin. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on mammals of A23109A were not evaluated as part of the EU assessment of metalaxyl-M and oxathiapiprolin. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2 (see also section B6 (Toxicology) for study summary).

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

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

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| Rat | Metalaxyl-M | Oral<br>1 d<br>Acute | **$LD_{50}$ = 375 mg a.s./kg bw (female)**<br>$LD_{50}$ = 953 mg a.s./kg bw (male) | EFSA Journal 2015;13(3) :3999; Schoch, 1994; CGA329351/0002 |
| Rat | Metalaxyl | Long-term | **NOAEL = 96 mg a.s./kg bw/d** | EFSA Journal 2015;13(3) :3999; Cozens *et al*., 1980; CGA48988/0597 |
| Rat | A9642C (APRON) | Acute toxicity | > 3000 mg/kg bw (male)<br>> 1000 < 3000 mg/kg bw (female) | EFSA Journal 2015;13(3):3999; Winkler, 1997; CGA329351/0531 |
| Rat | A9651D (RIDOMIL GOLD) | Acute toxicity | > 5000 mg/kg bw | EFSA Journal 2015;13(3):3999; Straube, 2005; CGA329351/2095 |

Bold values are used for the risk assessment
EFSA Journal 2015;13(3):3999. [105 pp.] doi:10.2903/j.efsa.2015.3999

**Table 9.3-2: Endpoints and effect values relevant for the risk assessment for mammals – oxathiapiprolin**

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| Rat | Oxathiapiprolin | Acute toxicity | **$LD_{50}$ > 5000 mg a.s./kg bw** | EFSA 2016;14(7):4504, DuPont-29441 |
| Rat | Oxathiapiprolin | Long-term (2-generation study) | **NOAEL = 86.37 mg a.s./kg bw/day** | EFSA 2016;14(7):4504, DuPont-30258 |

Values in **bold** are used in the initial risk assessment
EFSA Journal 2016;14(7):4504. [19 pp.] doi:10.2903/j.efsa.2016.4504

**Metalaxyl-M metabolites**

Characterization and identification of metalaxyl metabolites revealed that NOA409045 and CGA108906

were found at less than 10% in all tested crops. Also, from the plant metabolism study, it was concluded that NOA409045 and CGA108906 were minor metabolites, and therefore no further evaluation regarding mammalian risk is considered necessary.

**Oxathiapiprolin metabolites**

Studies performed with mammals indicated that plant metabolites of oxathiapiprolin are less toxic than the parent. Moreover, metabolites of oxathiapiprolin are not included in the definition of residues in plants and exposure *via* these food sources is not expected. For this reason, metabolites were not considered in the dietary risk assessment.

**A23109A**

**Table 9.3-3: Endpoints and effect values relevant for the risk assessment for mammals - A23109A**

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| Rat | A23109A | Oral<br>1 d<br>Acute | $LD_{50}$ > 2000 mg formulation/kg bw (332 mg metalaxyl-M and 57 mg oxathiapiprolin/kg bw) | Rosos-Matting, 2021; VV-888460 |

### 9.3.1.1 Justification for new endpoints

**Consideration of acute mixture toxicity**

According to EFSA/2009/1438[7] combined action of several toxicants must be specifically considered in the risk assessment when it is obvious that such exposure situations will occur for animals.

For the assessment of acute effects (mortality), a surrogate $LD_{50}$ can be calculated. The EFSA Guidance Document indicates that the following equation should be used for deriving a surrogate $LD_{50}$ for a mixture of active substances with known toxicity assuming dose additivity:

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

*where:*
*$X\ (a.s._i)$ = fraction of active substance (i) in the formulation mixture*
*$LD_{50}\ (a.s._i)$ = acute toxicity for the active substance (i)*

The $LD_{50}$ of the mix is summarised in the table below.

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

**Table 9.3-4: Acute $LD_{50}$ for the mixture of metalaxyl-M and oxathiapiprolin**

| Test substance | Concentration of active substance in formulation A23109A (g a.s./L) | Fraction of active substance in the formulation mixture [A] | Acute toxicity endpoint (mg a.s./kg bw) | Fraction of active substance/$LD_{50}$ for the active substance | $LD_{50}$ mix (mg a.s./kg bw) |
|---|---|---|---|---|---|
| Metalaxyl-M | 178 | 0.853 | 375 | 0.002275 | 434 |
| Oxathiapiprolin | 30.6 | 0.147 | > 5000 | 0.000029 | |
| Total | 208.6 | 1 | - | 0.002305 | |

[A] Concentration of an active substance in the formulation, divided by the total concentration of all active substances in the formulation.
Note: Calculations undertaken using unrounded values consequently they may not be reproducible when using the figures given in the table

**Methodology to establish whether a single substance is driving the toxicity of a mixture**

**Table 9.3-5: Toxicity of metalaxyl-M and oxathiapiprolin in the mixture**

| Test substance | Concentration of active substance in formulation A23109A (g a.s./L) | Fraction of active substance in the formulation mixture [A] | Acute toxicity endpoint (mg a.s./kg bw) | Fraction of active substance/$LD_{50}$ for the active substance | Toxicity per fraction a.s. (mg/kg) | Toxicity per fraction quotient (%) | Single driver of toxicity |
|---|---|---|---|---|---|---|---|
| Metalaxyl-M | 178 | 0.853 | 375 | 0.00228 | 439 | **98.7** | **yes** |
| Oxathiapiprolin | 30.6 | 0.147 | > 5000 | 0.0000293 | 34085 | 1.3 | no |
| Total | 208.6 | 1 | - | 0.00230 | - | 100 | - |

[A] Concentration of an active substance in the formulation, divided by the total concentration of all active substances in the formulation
Note: Calculations undertaken using unrounded values consequently they may not be reproducible when using the figures given in the table

The table above shows that, due to its higher fraction in the formulation, metalaxyl-M is over 75 times more toxic in the mixture compared to oxathiapiprolin. Therefore, metalaxyl-M is the risk driver in the formulation (98.7% of the toxicity). Thus, an acute mixture toxicity risk assessment is not required according to the guidance. However, as a worst case approach the combination risk assessment will be presented.

According to the EFSA Guidance Document (2009; Appendix B, Step 2a), the surrogate $LD_{50}$ of 434 mg a.s./kg bw for mixture toxicity should be compared to the acute oral toxicity of the formulation, using the following equation:

$$\sum_i \frac{\mathrm{X}(a.s._i)}{\mathrm{LD}_{50}(a.s._i)} = \frac{1}{\mathrm{LD}_{50}(prod.)}$$

*where:*
*X (a.s.$_i$)* = *fraction of active substance (i) in the formulation mixture*
*$LD_{50}$ (a.s.$_i$)* = *acute toxicity for the active substance (i)*
*$LD_{50}$ (prod.)* = *measured acute toxicity value for the formulated mixture*

A comparison of measured and predicted toxicity of A23109A is provided in the table below.

**Table 9.3-6: Comparison of the measured formulation with the predicted mixture toxicity assuming dose additivity**

| Test substance | Metalaxyl-M | Oxathiapiprolin | Sum | $1/LD_{50}$ (prod.) (PPP as a.s.) |
|---|---|---|---|---|
| Fraction of a.s. in formulation | 0.853 | 0.147 | 1 | 1 |
| $LD_{50}$ (a.s.) | 375 | > 5000 | - | 389 [a] |
| Fraction of a.s. in formulation/ $LD_{50}$ for the individual active substance | 0.002275 | 0.000029 | 0.002305 | 0.00257 |

[a] Formulation endpoint expressed as total a.s. (based on the total % w/w of metalaxyl-M (16.6%) and oxathiapiprolin (2.85%) in the formulation (19.45% w/w)

A greater value on the right side of the equation indicates that the formulation is more toxic than predicted from the toxicity of the individual components (active substances and co-formulants of known toxicity). This may be due to, *e.g*. further toxic co-formulants, toxicokinetic interaction or synergism/potentiation of effect. It may also reflect the inherent variability of toxicity testing. In all these cases, the use of the $LD_{50}$ for the formulation is recommended for the first-tier assessment, because it cannot be excluded that such effects would also occur after exposure of animals to residues in the environment.

If, in contrast, the measured toxicity of a formulation is lower than predicted, the predicted mixture toxicity according to Step 1 should be used in the first-tier risk assessment.

In the case of A23109A, this results in a value of 0.0023 on the left and 0.0026 on the right, indicating that the formulation is more toxic than predicted. However, this is not conclusive because the $LD_{50}$ for A23109A is above the highest dose tested. Nevertheless, the formulation endpoint will be used in the risk assessment.

**Combined reproductive toxicity**

As requested in the Working document on Risk Assessment of Plant Protection Products in the Central Zone – Ecotoxicology (May 2021), a calculation of long-term combitox risk according to the concentration addition (CA) model should be presented for Tier 1.

## 9.3.2 Risk assessment for spray applications

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

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessments for the uses in leafy vegetables and bulb and onion like crops cover the risk for mammals from all intended uses (see 9.1.2).

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

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

**Table 9.3-7: Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of A23109A – metalaxyl-M**

| | | | | | | |
|---|---|---|---|---|---|---|
| **Active substance** | | Metalaxyl-M | | | | |
| **Acute toxicity (mg a.s./kg bw)**<br>**TER criterion** | | 375<br>10 | | | | |
| **Crop scenario**<br>**Growth stage** | **Application rate**<br>**(g a.s./ha)** | **Indicator species** | **$SV_{90}$** | **$MAF_{90}$** | **$DDD_{90}$**<br>**(mg/kg bw/d)** | **$TER_a$** |
| Leafy vegetables, BBCH 12 - 49 | 2 x 87.2 (7-d interval) | Small herbivorous mammal | 136.4 | 1.4 | 16.7 | 22.5 |
| Bulbs and onion like crops, BBCH 12 - 48 | 2 x 87.2 (7-d interval) | Small herbivorous mammal | 118.4 | 1.4 | 14.5 | 25.9 |
| **Reprod. Toxicity (mg a.s./kg bw/d)**<br>**TER criterion** | | 96<br>5 | | | | |
| **Crop scenario**<br>**Growth stage** | **Application rate**<br>**(g a.s./ha)** | **Indicator species** | **$SV_m$** | **$MAF_m$ × TWA** | **$DDD_m$**<br>**(mg/kg bw/d)** | **$TER_{lt}$** |
| Leafy vegetables, BBCH 12 - 49 | 2 x 87.2 (7-d interval) | Small herbivorous mammal | 72.3 | 1.6 × 0.53 | 5.35 | 18.0 |
| Bulbs and onion like crops, BBCH 12 - 48 | 2 x 87.2 (7-d interval) | Small herbivorous mammal | 48.3 | 1.6 × 0.53 | 3.57 | 26.9 |

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio

**Table 9.3-8: Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of A23109A – oxathiapiprolin**

| | | | | | | |
|---|---|---|---|---|---|---|
| **Active substance** | | Oxathiapiprolin | | | | |
| **Acute toxicity (mg a.s./kg bw)**<br>**TER criterion** | | > 5000<br>10 | | | | |
| **Crop scenario**<br>**Growth stage** | **Application rate**<br>**(g a.s./ha)** | **Indicator species** | **$SV_{90}$** | **$MAF_{90}$** | **$DDD_{90}$**<br>**(mg/kg bw/d)** | **$TER_a$** |
| Leafy vegetables, BBCH 12 - 49 | 2 x 15 (7-d interval) | Small herbivorous mammal | 136.4 | 1.4 | 2.86 | > 1 746 |
| Bulbs and onion like crops, BBCH 12 - 48 | 2 x 15 (7-d interval) | Small herbivorous mammal | 118.4 | 1.4 | 2.49 | > 2 011 |
| **Reprod. Toxicity (mg a.s./kg bw/d)**<br>**TER criterion** | | 86.37<br>5 | | | | |

| **Crop scenario**<br>**Growth stage** | **Application rate**<br>**(g a.s./ha)** | **Indicator species** | **$SV_m$** | **$MAF_m$ × TWA** | **$DDD_m$**<br>**(mg/kg bw/d)** | **$TER_{lt}$** |
|---|---|---|---|---|---|---|
| Leafy vegetables,<br>BBCH 12 - 49 | 2 x 15<br>(7-d interval) | Small herbivorous mammal | 72.3 | 1.6 × 0.53 | 0.920 | 93.9 |
| Bulbs and onion like crops,<br>BBCH 12 - 48 | 2 x 15<br>(7-d interval) | Small herbivorous mammal | 48.3 | 1.6 × 0.53 | 0.614 | 141 |

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio

The acute and chronic screening assessment for metalaxyl-M and oxathiapiprolin, for all indicator species, concludes TER values greater than the trigger of 10 for acute risk and 5 for chronic risk, indicating that risk to mammals is acceptable following use of A23109A according to the proposed use pattern.

**Metalaxyl-M/oxathiapiprolin mixture assessment**

**Acute risk**

The experimentally determined $LD_{50}$ of the formulation A23109A is used as the endpoint for the acute combination mixture risk assessment (see 9.3.1.1).

**Table 9.3-9: Screening assessment of the acute risk for mammals due to the use of A23109A**

| **Product** | | A23109A | | | | |
|---|---|---|---|---|---|---|
| **Acute toxicity (mg total a.s./kg bw)** | | 389 | | | | |
| **TER criterion** | | 10 | | | | |
| **Crop scenario**<br>**Growth stage** | **Application rate**<br>**(g total a.s./ha)**[a] | **Indicator species** | **$SV_{90}$** | **$MAF_{90}$** | **$DDD_{90}$**<br>**(mg/kg bw/d)** | **$TER_a$** |
| Leafy vegetables,<br>BBCH 12 - 49 | 2 x 102.2<br>(7-d interval) | Small herbivorous mammal | 136.4 | 1.4 | 19.5 | 19.9 |
| Bulbs and onion like crops,<br>BBCH 12 - 48 | 2 x 102.2<br>(7-d interval) | Small herbivorous mammal | 118.4 | 1.4 | 16.9 | 23.0 |

SV: shortcut value; MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio
[a] Based on a total application rate of 87.2 g/ha for metalaxyl-M and 15 g/ha for oxathiapiprolin

The $TER_a$ values for the mixture toxicity are greater than the relevant trigger value indicating that acute risk to mammals is acceptable following use of A23109A according to the proposed use patterns.

**Chronic risk**

For assessment of chronic effects, according to EFSA/2009/1438[2], *'if a given formulation contains several active substances all known to cause similar effects via a similar biochemical mechanism (e.g. aromatase inhibition) and if this type of effect is actually driving the risk assessment, it is thus recommended to perform an assessment for combined effects on a case by case basis'*.

For A23109A the active substances, metalaxyl-M (a phenylamide with activity by inhibiting ribosomal RNA synthesis) has a different mode of action in fungi than the active substance oxathiapiprolin (a

piperidinyl thiazole isoxazoline with activity by inhibition of oxysterol binding protein (OSBP) homologue). Consequently, an assessment for combined chronic effects is not considered to be required.

**Combined reproductive toxicity**

As requested in the Working document on Risk Assessment of Plant Protection Products in the Central Zone – Ecotoxicology (May 2021), a calculation of long-term combitox risk according to the concentration addition (CA) model should be presented for Tier 1.

The $TER_{LT\ combi}$ values are calculated based on the screening step TER values for the active substances. The calculations of the cumulative ecotoxicological effects are summarized in table below.

| Crop scenario and/or indicator species | | $TER_{LT}$ Metalaxyl-M | $TER_{LT}$ Oxathiapiprolin | $TER_{LT\ combi}$ | Trigger |
|---|---|---|---|---|---|
| **Reproductive (screening step)** | | | | | |
| Leafy vegetables | Small herbivorous mammal | 18.0 | 93.9 | 15.10 | 5 |
| Bulbs & onion like crops | Small herbivorous mammal | 26.9 | 141 | 21.95 | 5 |

The $TER_{LT\ combi}$ value in the screening step is above the trigger value of 5. Thus, it can be concluded that the reproductive risk for mammals for the combined exposure to the two active substances in the application of A23109A according to good agricultural practice is low and acceptable.

### 9.3.2.2 Higher-tier risk assessment

A higher-tier risk assessment is not required.

### 9.3.2.3 Drinking water exposure

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

**Puddle scenario**

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

With a mean K(f)oc of 78.9 L/kg, metalaxyl-M belongs to the group of less sorptive substances and with a K(f)oc of 6243 L/kg, oxathiapiprolin belongs to the group of more sorptive substances.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the maximum use rate of 2 x 87.2 g metalaxyl-M/ha (7 d interval) and 2 x 15 g oxathiapiprolin/ha (7 d interval) is used to cover the risk to birds from all intended uses (see 9.1.2).

| **Metalaxyl-M** | | | |
|---|---|---|---|
| Effective application rate (g a.s./ha)* = | 161.32* | | |
| Acute toxicity (mg a.s./kg bw) = | 375 | quotient = | 0.43 |
| Reprod. Toxicity (mg a.s./kg bw/d) = | 96 | quotient = | 1.68 |

* Effective application rate = Maximum application rate x MAF of 1.85 assuming a soil $DT_{50}$ of 30.9 days and 2 applications with a 7-day interval

| **Oxathiapiprolin** | | | |
|---|---|---|---|
| Effective application rate (g a.s./ha)* = | 29.4* | | |
| Acute toxicity (mg a.s./kg bw) = | > 5000 | quotient = | < 0.01 |
| Reprod. Toxicity (mg a.s./kg bw/d) = | 86.37 | quotient = | 0.34 |

* Effective application rate = Maximum application rate x MAF of 1.96 assuming a soil $DT_{50}$ of 121.2 days and 2 applications with a 7-day interval

The resulting ratios fall below the trigger of 50 for metalaxyl-M (less sorptive) and 3000 for oxathiapiprolin (more sorptive), indicating that further assessment of the acute and long-term risk to mammals from drinking water from puddles is not required.

### 9.3.2.4 Effects of secondary poisoning

According to EFSA/2009/1438, substances with a log $P_{ow}$ of > 3 have a potential for bioaccumulation and as such consideration of the potential effects of secondary poisoning to birds and mammals are required.

The log $P_{OW}$ values for metalaxyl-M and its relevant metabolite (NOA409045) are 1.71 and -1.29, respectively. Therefore, the log $P_{ow}$ values do not exceed the trigger value of 3 and a risk assessment for effects due to secondary poisoning is not required. Although a paper previously indicated there might be potential for bioaccumulation in earthworms, metalaxyl-M review report (SANTE/11112/2019) indicates that concerns about bioaccumulation can be considered low.

The log $P_{ow}$ of oxathiapiprolin is 3.67 (at pH 7) and thus exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is required. The log $P_{ow}$ values of the oxathiapiprolin metabolites IN-Q7D41, IN-S2K66 and IN-RDT31 were reported to be 4.3, 3.4, and 4.1, respectively, and exceed the trigger value of 3. Therefore, a risk assessment for effects due to secondary poisoning is required. IN-S2K66 and IN-Q7D41 were not found in soil therefore the risk assessment was performed for fish-eating mammals only.

**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.

**Oxathiapiprolin and its metabolites**

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the worst-case soil PEC following 2 x 15 g a.s./ha applications to onions is considered (see Section 8 - Environmental Fate, Chapter 8.7.2).

**Table 9.3-10: Assessment of the risk for earthworm-eating mammals due to exposure to oxathiapiprolin *via* bioaccumulation in earthworms (secondary poisoning)**

| Parameter | Oxathiapiprolin | Comments |
|---|---|---|
| $PEC_{soil,\ accumulation}$ (mg/kg soil) | 0.0392 | Onions, 2 x 15 g a.s./ha |
| log $P_{ow}$ / $K_{ow}$ | 3.67 / 4677 | EFSA, 2016 |
| $K_{oc}$ | 6243 | Arithmetic mean (n = 5) |
| $f_{oc}$ | 0.02 | Default |
| $BCF_{worm}$ | 1* | $BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times K_{ow}) / f_{oc} \times K_{oc}$ |
| $PEC_{worm}$ | 0.0392 | $PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$ |
| Daily dietary dose (mg/kg bw/d) | 0.0502 | $DDD = PEC_{worm} \times 1.28$ |
| NOEL (mg/kg bw/d) | 86.37 | Rat |
| $TER_{lt}$ | 1721 | $\geq$ 5; acceptable risk |

* calculated BCF = 0.46. A BCF of 1 was used for $TER_{lt}$ estimation as a worst-case.

**Table 9.3-11: Assessment of the risk for earthworm-eating mammals due to exposure to IN-RDT31 *via* bioaccumulation in earthworms (secondary poisoning)**

| Parameter | IN-RDT31 | Comments |
|---|---|---|
| $PEC_{soil,\ accumulation}$ (mg/kg soil) | 0.0063 | Onions, 2 x 15 g a.s./ha |
| log $P_{ow}$ / $K_{ow}$ | 4.1 / 12589 | EFSA, 2016 |
| $K_{oc}$ | 1168 | Arithmetic mean (n = 5) |
| $f_{oc}$ | 0.02 | Default |
| $BCF_{worm}$ | 6.50 | $BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times K_{ow}) / f_{oc} \times K_{oc}$ |
| $PEC_{worm}$ | 0.0410 | $PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$ |
| Daily dietary dose (mg/kg bw/d) | 0.0524 | $DDD = PEC_{worm} \times 1.28$ |
| NOEL (mg/kg bw/d) | 8.637 | 10x parent toxicity assumed as a worst case [a] |
| $TER_{lt}$ | 165 | $\geq$ 5; acceptable risk |

[a] In accordance with the DAR, 2016, volume 3, Annex B.9 PPP

**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.

**Oxathiapiprolin and its metabolites**

To achieve a concise risk assessment, the risk envelope approach is applied. The worst-case 21-day time-weighted average surface water PEC (FOCUS Step 2) following 2 x 15 g a.s./ha applications to bulb vegetables was used for oxathiapiprolin and for metabolites IN-Q7D41, IN-S2K66 and IN-RDT31.

**Table 9.3-12: Assessment of the risk for fish-eating mammals due to exposure to oxathiapiprolin *via* bioaccumulation in fish (secondary poisoning)**

| Parameter | Oxathiapiprolin | Comments |
|---|---|---|
| $PEC_{sw}$ (mg/L) | 0.00036 | 21-day Step 2 $PEC_{sw, twa}$ (SEU) for bulb vegetables |
| $BCF_{fish}$ | 87 | EFSA, 2016 |
| BMF | - | Biomagnification factor (relevant for BCF ≥ 2000) |
| $PEC_{fish}$ | 0.0313 | $PEC_{fish} = PEC_{water} \times BCF_{fish}$ |
| Daily dietary dose (mg/kg bw/d) | 0.00445 | $DDD = PEC_{fish} \times 0.142$ |
| NOEL (mg/kg bw/d) | 86.37 | Rat |
| $TER_{lt}$ | 19420 | ≥ 5; acceptable risk |

**Table 9.3-13: Assessment of the risk for fish-eating mammals due to exposure to IN-Q7D41 *via* bioaccumulation in fish (secondary poisoning)**

| Parameter | IN-Q7D41 | Comments |
|---|---|---|
| $PEC_{sw}$ (maximum) (mg/L) | 0.00043 | 21-day Step 2 $PEC_{sw, twa}$ (SEU) for bulb vegetables |
| $BCF_{fish}$ | 533 | EFSA, 2016 |
| BMF | - | Biomagnification factor (relevant for BCF ≥ 2000) |
| $PEC_{fish}$ | 0.229 | $PEC_{fish} = PEC_{water} \times BCF_{fish}$ |
| Daily dietary dose (mg/kg bw/d) | 0.0325 | $DDD = PEC_{fish} \times 0.142$ |
| NOEL (mg/kg bw/d) | 8.637 | 10x parent toxicity assumed as a worst case [a] |
| $TER_{lt}$ | 265 | ≥ 5; acceptable risk |

[a] In accordance with the DAR, 2016, volume 3, Annex B.9 PPP

**Table 9.3-14: Assessment of the risk for fish-eating mammals due to exposure to IN-S2K66 *via* bioaccumulation in fish (secondary poisoning)**

| Parameter | IN-S2K66 | Comments |
|---|---|---|
| $PEC_{sw}$ (maximum) (mg/L) | 0.00031 | 21-day Step 2 $PEC_{sw, twa}$ (SEU) for bulb vegetables |
| $BCF_{fish}$ | 115 | EFSA, 2016 |
| BMF | - | Biomagnification factor (relevant for BCF ≥ 2000) |
| $PEC_{fish}$ | 0.0357 | $PEC_{fish} = PEC_{water} \times BCF_{fish}$ |
| Daily dietary dose (mg/kg bw/d) | 0.00506 | $DDD = PEC_{fish} \times 0.142$ |
| NOEL (mg/kg bw/d) | 8.637 | 10x parent toxicity assumed as a worst case [a] |
| $TER_{lt}$ | 1706 | ≥ 5; acceptable risk |

[a] In accordance with the DAR, 2016, volume 3, Annex B.9 PPP

**Table 9.3-15: Assessment of the risk for fish-eating mammals due to exposure to IN-RDT31 *via* bioaccumulation in fish (secondary poisoning)**

| Parameter | IN-RDT31 | Comments |
|---|---|---|
| $PEC_{sw}$ (maximum) (mg/L) | 0.00013 | 21-day Step 2 $PEC_{sw, twa}$ (SEU) for bulb vegetables |
| $BCF_{fish}$ | 78 | EFSA, 2016 |
| BMF | - | Biomagnification factor (relevant for BCF ≥ 2000) |
| $PEC_{fish}$ | 0.0101 | $PEC_{fish} = PEC_{water} \times BCF_{fish}$ |
| Daily dietary dose (mg/kg bw/d) | 0.00144 | $DDD = PEC_{fish} \times 0.142$ |
| NOEL (mg/kg bw/d) | 8.637 | 10x parent toxicity assumed as a worst case [a] |
| $TER_{lt}$ | 5998 | ≥ 5; acceptable risk |

[a] In accordance with the DAR, 2016, volume 3, Annex B.9 PPP

Risk of secondary poisoning was assessed for oxathiapiprolin (log $P_{ow}$ = 3.67 at pH 7) and some of its metabolites (IN-Q7D41, IN-S2K66 and IN-RDT31 with log $P_{ow}$ values of 4.3, 3.4, and 4.1, respectively). All calculated $TER_{lt}$ values clearly exceed the trigger value of 5 indicating that the risk of secondary poisoning to mammals is acceptable following use of A23109A according to the proposed use pattern.

### 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 acute and long-term risks of A23109A to mammals were assessed from toxicity exposure ratios between toxicity endpoints, estimated from studies with A23109A, metalaxyl-M and oxathiapiprolin, and maximum residues occurring on food items following applications according to the proposed use pattern.

Risk of secondary poisoning has also been assessed, as oxathiapiprolin and some of its metabolites have log $P_{ow}$ values of > 3.0. The risk to mammals from exposure *via* drinking water has also been assessed.

The TER values, calculated for recommended scenarios, all exceed the trigger values of 10 for acute risk and 5 for long-term risk (including secondary poisoning), indicating that the risk to mammals is acceptable following use of A23109A according to the proposed use pattern.

**Review Comments:**

The acute and chronic risks of A23109A 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 A23109A does not pose an unacceptable risk to mammals 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 is low.

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

No relevant data on amphibians and reptiles are available for metalaxyl-M and oxathiapiprolin, consequently no further assessment of potential effects on reptiles and amphibians will be presented in this document.

Studies with terrestrial amphibian and reptile species are not data requirements under Regulation (EU) No 283/2013 and 284/2013. In addition, there is currently no guidance addressing terrestrial life stages of amphibians and reptiles in PPP risk assessments. Therefore, the risk assessment provided above for birds and mammals is considered to be protective of terrestrial amphibian and reptile species.

## 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 metalaxyl-M, oxathiapiprolin and their relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on aquatic organisms of A23109A were not evaluated as part of the EU assessment of metalaxyl-M or oxathiapiprolin. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

The selection of studies and endpoints for the risk assessment deviates from the results of the EU review process. Justifications are provided below.

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

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| *Oncorhynchus mykiss* | Metalaxyl-M | 96 h, s | **$LC_{50}$ >100 mg a.s./L $_{nom}$** | EFSA Journal 2015;13(3):3999; Rufli, 1994a; CGA329351/0025 |
| *Oncorhynchus mykiss* | Metalaxyl-M | 96 h, s | $LC_{50}$ >121 mg a.s./L $_{nom}$ | EFSA Journal 2015;13(3):3999; Drottar & Swigert, 1995; CGA329351/0299 |
| *Oncorhynchus mykiss* | A9642C (APRON) | 96 h, s | $LC_{50}$ > 100 mg/L $_{nom}$ (32.28 mg a.s./L) | EFSA Journal 2015;13(3):3999; Memmert,., 1997; CGA329351/0604 |
| *Cyprinus carpio* | A9651D (RIDOMIL GOLD) | 96 h, s | $LC_{50}$ = 23 mg/L $_{mm}$ (0.87 mg metalaxyl-M and 14 mg mancozeb/L) | EFSA Journal 2015;13(3):3999; Wheeler, 2005; CGA329351/2147 |

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| *Oncorhynchus mykiss* | NOA409045 (CGA62826) | 96 h, ss | **$LC_{50}$ >100 mg met./L $_{nom}$** | EFSA Journal 2015;13(3):3999; Memmert & Knoch, 1991; CGA62826/0003 |
| *Oncorhynchus mykiss* | CGA67868 | 96 h, ss | $LC_{50}$ >98.9 mg met./L $_{nom}$ | EFSA Journal 2015;13(3):3999; Memmert & Knoch, 1992; CGA67868/0002 |
| *Oncorhynchus mykiss* | CGA108906 (SYN546520) | 96 h, s | $LC_{50}$ >100 mg met./L $_{nom}$ | EFSA Journal 2015;13(3):3999; Rufli, 1994; CGA108906/0003 |
| *Oncorhynchus mykiss* | Metalaxyl-M | 28 d, f | NOEC = 50 mg a.s./L $_{mm}$ | EFSA Journal 2015;13(3):3999; Rufli, 2000; CGA329351/1305 |
| *Pimephales promelas* | Metalaxyl | 30 d ELS, f | **NOEC = 9.1 mg a.s./L $_{nom}$** | EFSA Journal 2015;13(3):3999; LeBlanc & Mastone, 1980; CGA48988/0157 |
| *Oncorhynchus mykiss* | NOA409045 (CGA62826) | 28 d, f | **NOEC = 100 mg met./L $_{nom}$** | EFSA Journal 2015;13(3):3999; Rufli, 2000a; CGA62826/0020 |
| *Daphnia magna* | Metalaxyl-M | 48 h, s | $EC_{50}$ >100 mg a.s./L $_{nom}$ | EFSA Journal 2015;13(3):3999; Grade, 1994a; CGA329351/0026 |
| *Daphnia magna* | Metalaxyl-M | 48 h, s | $EC_{50}$ >113 mg a.s./L $_{nom}$ | EFSA Journal 2015;13(3):3999; Drottar, 1995a; CGA329351/0298 |
| *Daphnia magna* | Metalaxyl-M | 48 h, s | $EC_{50}$ = 128.26 mg a.s./L [a] | EFSA Journal 2015;13(3):3999, Yao K. *et al*., 2009 |
| *Crassostrea virginica* | Metalaxyl-M | 96 h, f | Shell deposition, $EC_{50}$ = 9.7 mg a.s./L $_{nom}$ | EFSA Journal 2015;13(3):3999; Drottar, 1995b; CGA329351/0300 |
| *Crassostrea virginica* | Metalaxyl | 96 h, f | Shell deposition, **$EC_{50}$ = 5.6 mg a.s./L $_{nom}$** | EFSA Journal 2015;13(3):3999; Dionne (1989); CGA48988/0163 |
| *Daphnia magna* | A9642C (APRON) | 48 h, s | $EC_{50}$ = 54.4 mg/L $_{nom}$ (17.56 mg a.s./L) | EFSA Journal 2015;13(3):3999; Memmert, 1997a; CGA329351/0641 |

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| *Daphnia magna* | A9651D (RIDOMIL GOLD) | 48 h, s | $EC_{50}$ = 0.8 mg/L $_{nom}$ (0.030 mg metalaxyl-M and 0.48 mg mancozeb/L) | EFSA Journal 2015;13(3):3999; Ashwell, 2005; CGA329351/2141 |
| *Daphnia magna* | NOA409045 (CGA62826) | 48 h, s | **$EC_{50}$ = 852 mg met./L $_{nom}$** | EFSA Journal 2015;13(3):3999; Memmert & Knoch, 1991a; CGA62826/0001 |
| *Daphnia magna* | CGA67868 | 48 h, s | $EC_{50}$ = 158 mg met./L $_{nom}$ | EFSA Journal 2015;13(3):3999; Memmert *et al*., 1991b; CGA67868/0001 |
| *Daphnia magna* | CGA108906 (SYN546520) | 48 h, s | $EC_{50}$ >100 mg met./L $_{nom}$ | EFSA Journal 2015;13(3):3999; Grade, 1994; CGA108906/0002 |
| *Daphnia magna* | Metalaxyl-M | 21 d, ss | NOEC = 25 mg a.s./L $_{nom}$ | EFSA Journal 2015;13(3):3999; Pfeifle, 2000; CGA329351/1308 |
| *Daphnia magna* | Metalaxyl-M | 21 d, ss | **NOEC = 1.0 mg a.s./L $_{nom}$** [a] | EFSA Journal 2015;13(3):3999, Chen S., Liu W., 2008 |
| *Daphnia magna* | NOA409045 (CGA62826) | 21 d, ss | **NOEC = 100 mg met./L $_{nom}$** | EFSA Journal 2015;13(3):3999; Pfeifle, 2000a; CGA62826/0021 |
| *Desmodesmus subspicatus* | Metalaxyl-M | 72 h, s | $E_bC_{50}$ = 36 mg a.s./L $_{nom}$ $E_rC_{50}$ = 103 mg/L | EFSA Journal 2015;13(3):3999; Grade, 1994c; CGA329351/0027 |
| *Scenedesmus quadricanda* | Metalaxyl-M | 96 h, s | **$E_rC_{50}$ = 19.95 mg a.s./L $_{nom}$** [a] | EFSA Journal 2015;13(3):3999, Yao K. *et al.*, 2009 |
| *Desmodesmus subspicatus* | A9642C (APRON) | 72 h, s | $E_rC_{50}$ = > 100 mg/L $_{nom}$ (32.28 mg a.s./L) $E_bC_{50}$ = 103 mg/L $_{nom}$ (33.25 mg a.s./L) | EFSA Journal 2015;13(3):3999; Memmert, 1997b; CGA329351/0641 |

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| *Pseudokirchneriella subcapitata* | A9651D (RIDOMIL GOLD) | 96 h, s | $E_rC_{50}$ = 0.248 mg/L $_{nom}$ (0.009 mg metalaxyl-M and 0.15 mg mancozeb/L)<br>$E_bC_{50}$ = 0.102 mg/L $_{nom}$ (0.004 mg metalaxyl-M and 0.062 mg mancozeb/L) | EFSA Journal 2015;13(3):3999; Maynard, 2005; CGA329351/2150 |
| *Desmodesmus subspicatus* | NOA409045 (CGA62826) | 72 h, s | $E_bC_{50}$ >1000 mg met./L $_{nom}$ | EFSA Journal 2015;13(3):3999; Memmert & Knoch, 1991c; CGA62826/0002 |
| *Desmodesmus subspicatus* | CGA67868 | 72 h, s | $E_bC_{50}$ = 195.4 mg met./L $_{nom}$ | EFSA Journal 2015;13(3):3999; Memmert *et al*., 1992a; CGA67868/0003 |
| *Desmodesmus subspicatus* | CGA108906 (SYN546520) | 72 h, s | $E_bC_{50}$ = 74 mg met./L $_{nom}$ | EFSA Journal 2015;13(3):3999; Grade, 1994b; CGA108906/0001 |
| *Pseudokirchneriella subcapitata* | NOA409045 (CGA62826) | 7 d, s | **$E_rC_{50}$ = 81 mg met./L $_{nom}$**<br>$E_yC_{50}$ = 65.01 mg met./L $_{nom}$<br>$E_bC_{50}$ = 63.25 mg met./L $_{nom}$ | EFSA Journal 2015;13(3):3999; Weber, 2011; NOA409045/10002 |
| Higher-tier studies (micro- or mesocosm studies): Not conducted | | | | |

s: static; ss: semi-static; f: flow-through; nom: based on nominal concentrations; mm: based on mean measured concentrations
[a] = Endpoint from literature referenced in the EFSA Conclusion 2015
Bold values are used for the risk assessment
EFSA Journal 2015;13(3):3999. [105 pp.] doi:10.2903/j.efsa.2015.3999

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

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| **Fish** | | | | |
| *Oncorhynchus mykiss* | Oxathiapiprolin | 96 h, s | $LC_{50}$ > 0.69 mg a.s./L $_{mm}$[a] | EFSA Journal 2016;14(7):4504; DuPont-32481 |
| *Lepomis macrochirus* | Oxathiapiprolin | 96 h, s | $LC_{50}$ > 0.72 mg a.s./L $_{mm}$[a] | EFSA 2016;14(7):4504, DuPont-32818 |
| *Cyprinodon variegatus* | Oxathiapiprolin | 96 h, s | **$LC_{50}$ > 0.65 mg a.s./L** $_{mm}$[a] | EFSA 2016;14(7):4504, DuPont-32819 |
| *Oncorhynchus mykiss* | Oxathiapiprolin 100 g/L OD | 96 h, s | $LC_{50}$ > 0.51 mg/L $_{mm}$ | EFSA 2016;14(7):4504, DuPont-32702 |
| *Cyprinodon variegatus* | Oxathiapiprolin | 35 d ELS, f | **$NOEC_{growth\ and\ survival}$ = 0.34 mg a.s./L** $_{mm}$ | EFSA 2016;14(7):4504, DuPont-32820 |
| *Oncorhynchus mykiss* | Oxathiapiprolin | 88 d ELS, f | $NOEC_{growth}$ = 0.46 mg a.s./L $_{mm}$ | EFSA 2016;14(7):4504, DuPont-32482 |
| *Oncorhynchus mykiss* | IN-E8S72 | 96 h, s | **$LC_{50}$ > 100 mg met./L** $_{nom}$ | EFSA 2016;14(7):4504, DuPont-34396 |
| *Oncorhynchus mykiss* | IN-P3X26 | 96 h, s | **$LC_{50}$ > 67.72 mg met./L $_{mm}$[a]** | EFSA 2016;14(7):4504, DuPont-32662 |
| *Oncorhynchus mykiss* | IN-Q7D41 | 96 h, ss | **$LC_{50}$ > 0.18 mg met./L** $_{mm}$[a] | EFSA 2016;14(7):4504, DuPont-32660 |
| *Oncorhynchus mykiss* | IN-QFD61 [b] | 96 h, s | $LC_{50}$ > 7.38 mg met./L $_{mm}$[a] | EFSA 2016;14(7):4504, DuPont-34403 |
| *Oncorhynchus mykiss* | IN-QPS10 | 96 h, s | **$LC_{50}$ = 6.96 mg met./L** $_{mm}$ | EFSA 2016;14(7):4504, DuPont-34395 |
| *Oncorhynchus mykiss* | IN-RAB06 | 96 h, s | **$LC_{50}$ > 50.0 mg met./L $_{nom}$** | EFSA 2016;14(7):4504, DuPont-34401 |
| *Oncorhynchus mykiss* | IN-RDT31 | 96 h, s | **$LC_{50}$ > 11.56 mg met./L $_{mm}$[a]** | EFSA 2016;14(7):4504, DuPont- 34397 |
| *Oncorhynchus mykiss* | IN-RSE01 | 96 h, ss | **$LC_{50}$ > 9.84 mg met./L $_{mm}$[a]** | EFSA 2016;14(7):4504, DuPont-32661 |
| *Oncorhynchus mykiss* | IN-RYJ52 | 96 h, s | **$LC_{50}$ > 13.8 mg met./L $_{mm}$[a]** | EFSA 2016;14(7):4504, DuPont-32659 |

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| *Oncorhynchus mykiss* | IN-S2K66 | 96 h, s | **$LC_{50}$ > 7.48 mg met./L $_{mm}$[a]** | EFSA 2016;14(7):4504, DuPont-34394 |
| *Oncorhynchus mykiss* | IN-S2K67 [b] | 96 h, s | $LC_{50}$ > 82.5 mg met./L $_{mm}$[a] | EFSA 2016;14(7):4504, DuPont-34410 |
| **Aquatic invertebrate** | | | | |
| *Daphnia magna* | Oxathiapiprolin | 48 h, s | $EC_{50}$ = 0.67 mg a.s./L $_{mm}$ | EFSA 2016;14(7):4504, DuPont-32484 |
| *Americamysis bahia* | Oxathiapiprolin | 96 h, s | $EC_{50}$ > 0.64 mg a.s./L $_{mm}$[a] | EFSA 2016;14(7):4504, DuPont-32485 |
| *Crassostrea virginica* | Oxathiapiprolin | 96 h, f | **$EC_{50}$ > 0.33 mg a.s./L $_{mm}$[a]** | EFSA 2016;14(7):4504, DuPont-32453 |
| *Daphnia magna* | Oxathiapiprolin 100 g/L OD | 48 h, s | $EC_{50}$ > 0.82 mg/L $_{mm}$ | EFSA 2016;14(7):4504, DuPont-30561 |
| *Daphnia magna* | Oxathiapiprolin | 21 d, ss | $NOEC_{reproduction}$ = 0.75 mg a.s./L $_{mm}$[a] | EFSA 2016;14(7):4504, DuPont-32455 |
| *Americamysis bahia* | Oxathiapiprolin | 32 d, f | **$NOEC_{reproduction}$ = 0.058 mg a.s./L** $_{mm}$ | EFSA 2016;14(7):4504, DuPont-32456 |
| *Daphnia magna* | IN-E8S72 | 48 h, s | **$EC_{50}$ > 100.0 mg met./L** $_{nom}$ | EFSA 2016;14(7):4504, DuPont-34400 |
| *Daphnia magna* | IN-P3X26 | 48 h, s | **$EC_{50}$ > 67.74 mg met./L** $_{mm}$[a] | EFSA 2016;14(7):4504, DuPont-32653 |
| *Daphnia magna* | IN-Q7D41 | 48 h, ss | **$EC_{50}$ > 0.15 mg met./L** $_{mm}$[a] | EFSA 2016;14(7):4504, DuPont-32651 |
| *Daphnia magna* | IN-QFD61 [b] | 48 h, s | $EC_{50}$ = 6.29 mg met./L $_{mm}$ | EFSA 2016;14(7):4504, DuPont-34404 |
| *Daphnia magna* | IN-QPS10 | 48 h, s | **$EC_{50}$ = 15.87 mg met./L** $_{mm}$ | EFSA 2016;14(7):4504, DuPont-34399 |
| *Daphnia magna* | IN-RAB06 | 48 h, s | **$EC_{50}$ > 100.0 mg met./L** $_{nom}$ | EFSA 2016;14(7):4504, DuPont-33941 |
| *Daphnia magna* | IN-RDT31 | 48 h, s | **$EC_{50}$ > 10.49 mg met./L** $_{mm}$[a] | EFSA 2016;14(7):4504, DuPont-34398 |
| *Daphnia magna* | IN-RSE01 | 48 h, s | **$EC_{50}$ > 10.16 mg met./L** $_{mm}$[a] | EFSA 2016;14(7):4504, DuPont-32652 |

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| *Daphnia magna* | IN-RYJ52 | 48 h, s | **$EC_{50}$ > 16.21 mg met./L** $_{mm}$[a] | EFSA 2016;14(7):4504, DuPont-32663 |
| *Daphnia magna* | IN-S2K66 | 48 h, s | **$EC_{50}$ = 0.86 mg met./L** $_{mm}$ | EFSA 2016;14(7):4504, DuPont-34409 |
| *Daphnia magna* | IN-S2K67 [b] | 48 h, s | $EC_{50}$ = 66.91 mg met./L $_{mm}$ | EFSA 2016;14(7):4504, DuPont-34594 |
| **Sediment dwelling organisms** | | | | |
| *Chironomus riparius* | Oxathiapiprolin | 48 h, s | $EC_{50}$ > 0.56 mg a.s./L $_{mm}$[a] | EFSA 2016;14(7):4504, DuPont-32454 |
| *Chironomus riparius* | Oxathiapiprolin | 28 d, s, water spiked | **NOEC = 0.11 mg a.s./L** $_{mm}$ | EFSA 2016;14(7):4504, DuPont-36043 |
| *Chironomus riparius* | Oxathiapiprolin | 28 d, spiked sediment | **NOEC = 2.80 mg a.s./kg sediment** $_{mm}$ | EFSA 2016;14(7):4504, DuPont-35835 |
| *Chironomus riparius* | IN-Q7D41 | 28 d, spiked sediment | **NOEC = 72.0 mg met./kg sediment** $_{mm}$ | EFSA 2016;14(7):4504, DuPont-35845 |
| *Chironomus riparius* | IN-RYJ52 | 10x parent toxicity | **NOEC = 0.011 mg met./L** [c] | EFSA 2016;14(7):4504 |
| *Chironomus riparius* | IN-RYJ52 | 10x parent toxicity | **NOEC = 0.280 mg met./kg sediment** [c] | EFSA 2016;14(7):4504 |
| *Chironomus riparius* | IN-S2K66 | 10x parent toxicity | **NOEC = 0.011 mg met./L** [c] | EFSA 2016;14(7):4504 |
| *Chironomus riparius* | IN-S2K66 | 10x parent toxicity | **NOEC = 0.280 mg met./kg sediment** [c] | EFSA 2016;14(7):4504 |
| *Chironomus riparius* | IN-RSE01 | 10x parent toxicity | **NOEC = 0.011 mg met./L** [c] | EFSA 2016;14(7):4504 |
| *Chironomus riparius* | IN-RSE01 | 10x parent toxicity | **NOEC = 0.280 mg met./kg sediment** [c] | EFSA 2016;14(7):4504 |
| **Algae** | | | | |
| *Pseudokirchneriella subcapitata* | Oxathiapiprolin | 96 h, s | $E_rC_{50}$ > 0.142 mg a.s./L $_{mm}$<br>$E_yC_{50}$ > 0.142 mg a.s./L $_{mm}$<br>NOEC = 0.142 mg a.s./L $_{mm}$ | EFSA 2016;14(7):4504, DuPont-29275 |
| *Anabaena flos-aquae* | Oxathiapiprolin | 96 h, s | $E_rC_{50}$ > 0.193 mg a.s./L $_{mm}$<br>$E_yC_{50}$ > 0.193 mg a.s./L $_{mm}$<br>NOEC = 0.193 mg a.s./L $_{mm}$ | EFSA 2016;14(7):4504, DuPont-29320 |
| *Skeletonema costatum* | Oxathiapiprolin | 72 h, s | $E_rC_{50}$ > 0.351 mg a.s./L $_{mm}$<br>$E_yC_{50}$ = 0.348 mg a.s./L $_{mm}$<br>$E_bC_{50}$ > 0.351 mg a.s./L $_{mm}$<br>NOEC = 0.141 mg a.s./L $_{mm}$ | EFSA 2016;14(7):4504, DuPont-35834 |

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| *Navicula pelliculosa* | Oxathiapiprolin | 72 h, s | $E_rC_{50}$ > 0.163 mg a.s./L $_{mm}$<br>$E_yC_{50}$ > 0.163 mg a.s./L $_{mm}$<br>$E_bC_{50}$ > 0.163 mg a.s./L $_{mm}$<br>NOEC = 0.163 mg a.s./L $_{mm}$ | EFSA 2016;14(7):4504, DuPont-35843 |
| *Pseudokirchneriella subcapitata* | Oxathiapiprolin 100 g/L OD | 72 h, s | $E_rC_{50}$ > 0.36 mg/L $_{mm}$<br>$E_yC_{50}$ > 0.36 mg/L $_{mm}$<br>$E_bC_{50}$ > 0.36 mg/L $_{mm}$<br>NOEC = 0.18 mg/L $_{mm}$ | EFSA 2016;14(7):4504, DuPont-32701 |
| *Pseudokirchneriella subcapitata* | IN-E8S72 | 72 h, s | **$E_rC_{50}$ > 100.0 mg met./L** $_{nom}$<br>$E_yC_{50}$ > 100.0 mg met./L $_{nom}$<br>NOEC = 100.0 mg met./L $_{nom}$ | EFSA 2016;14(7):4504, DuPont-32817 |
| *Pseudokirchneriella subcapitata* | IN-P3X26 | 72 h, s | **$E_rC_{50}$ > 66.64 mg met./L** $_{mm}$<br>$E_yC_{50}$ > 66.64 mg met./L $_{mm}$<br>$E_bC_{50}$ > 66.64 mg met./L $_{mm}$<br>NOEC = 66.64 mg met./L $_{mm}$ | EFSA 2016;14(7):4504, DuPont-32657 |
| *Pseudokirchneriella subcapitata* | IN-Q7D41 | 72 h, s | **$E_rC_{50}$ > 0.21 mg met./L** $_{mm}$<br>$E_yC_{50}$ > 0.21 mg met./L $_{mm}$<br>$E_bC_{50}$ > 0.21 mg met./L $_{mm}$<br>NOEC = 0.21 mg met./L $_{mm}$ | EFSA 2016;14(7):4504, DuPont-32654 |
| *Pseudokirchneriella subcapitata* | IN-QFD61 [b] | 72 h, s | $E_rC_{50}$ > 7.53 mg met./L $_{mm}$<br>$E_yC_{50}$ > 7.53 mg met./L $_{mm}$<br>$E_bC_{50}$ > 7.53 mg met./L $_{mm}$<br>NOEC = 1.29 mg met./L $_{mm}$ | EFSA 2016;14(7):4504, DuPont-34402 |
| *Pseudokirchneriella subcapitata* | IN-QPS10 | 72 h, s | **$E_rC_{50}$ = 2.32 mg met/L** $_{mm}$<br>$E_yC_{50}$ = 0.86 mg met./L $_{mm}$<br>$E_bC_{50}$ = 0.86 mg met./L mm<br>$NOE_rC$ = 0.48 mg met./L $_{mm}$<br>$NOE_{by}C$ = 0.20 mg met./L $_{mm}$ | EFSA 2016;14(7):4504, DuPont-32816 |
| *Pseudokirchneriella subcapitata* | IN-RAB06 | 72 h, s | **$E_rC_{50}$ > 100.0 mg met./L** $_{nom}$<br>$E_yC_{50}$ > 100.0 mg met./L $_{nom}$<br>NOEC = 100.0 mg met./L $_{nom}$ | EFSA 2016;14(7):4504, DuPont-32825 |
| *Pseudokirchneriella subcapitata* | IN-RDT31 | 72 h, s | **$E_rC_{50}$ > 11.43 mg met./L** $_{mm}$<br>$E_yC_{50}$ > 11.43 mg met./L $_{mm}$<br>$E_bC_{50}$ > 11.43 mg met./L $_{mm}$<br>NOEC = 1.97 mg met./L $_{mm}$ | EFSA 2016;14(7):4504, DuPont-32826 |
| *Pseudokirchneriella subcapitata* | IN-RSE01 | 72 h, s | **$E_rC_{50}$ > 10.80 mg met./L** $_{mm}$<br>$E_yC_{50}$ > 10.80 mg met./L $_{mm}$<br>$E_bC_{50}$ > 10.80 mg met./L $_{mm}$<br>NOEC = 0.31 mg met./L $_{mm}$ | EFSA 2016;14(7):4504, DuPont-32656 |
| *Pseudokirchneriella subcapitata* | IN-RYJ52 | 72 h, s | **$E_rC_{50}$ > 15.34 mg met./L** $_{mm}$<br>$E_yC_{50}$ > 15.34 mg met./L $_{mm}$<br>$E_bC_{50}$ > 15.34 mg met./L $_{mm}$<br>NOEC = 0.14 mg met./L $_{mm}$ | EFSA 2016;14(7):4504, DuPont-32655 |
| *Pseudokirchneriella subcapitata* | IN-S2K66 | 72 h, s | **$E_rC_{50}$ > 7.56 mg met./L** $_{mm}$<br>$E_yC_{50}$ > 7.56 mg met./L $_{mm}$<br>$E_bC_{50}$ > 7.56 mg met./L $_{mm}$<br>NOEC = 4.71 mg met./L $_{mm}$ | EFSA 2016;14(7):4504, DuPont-34507 |
| *Pseudokirchneriella subcapitata* | IN-S2K67 [b] | 72 h, s | $E_rC_{50}$ > 83.51 mg met./L $_{mm}$<br>$E_yC_{50}$ > 83.51 mg met./L $_{mm}$<br>$E_bC_{50}$ > 83.51 mg met./L $_{mm}$<br>NOEC = 0.28 mg met./L $_{mm}$ | EFSA 2016;14(7):4504, DuPont-32658 |

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| **Aquatic macrophyte** | | | | |
| *Lemna gibba* | Oxathiapiprolin | 7 d, ss | $EC_{50}$ (frond count) > 0.79 mg a.s./L $_{mm}$[a]<br>**$E_rC_{50}$ (frond count) > 0.79 mg a.s./L $_{mm}$**<br>$E_yC_{50}$ (frond count) > 0.79 mg a.s./L $_{mm}$<br>$EC_{50}$ (biomass) > 0.79 mg a.s./L $_{mm}$<br>$E_rC_{50}$ (biomass) > 0.79 mg a.s./L $_{mm}$<br>$E_yC_{50}$ (biomass) > 0.79 mg a.s./L $_{mm}$<br>NOEC = 0.79 mg a.s./L $_{mm}$ | EFSA 2016;14(7):4504, DuPont-32480 |
| **Higher-tier studies (micro- or mesocosm studies)** | | | | |
| Not conducted | | | | |

s: static; ss: semi-static; f: flow-through; nom: based on nominal concentrations; mm: based on mean measured concentrations
Values in **bold** are used in the risk assessment
EFSA Journal 2016;14(7):4504. [19 pp.] doi:10.2903/j.efsa.2016.4504.

[a] Endpoints above the highest tested concentration; nominal concentrations of the test item were chosen with consideration of water solubility limit.

[b] IN-QFD61 and IN-S2K67 are considered minor aquatic metabolites and their endpoints are given in the table for reasons of completeness. Taking into account their low toxicity to aquatic organisms compared to the parent compound, evaluation was deemed not necessary (refer to DAR, 2016, Vol. 3, Annex B.9 PPP and EFSA, 2016).

[c] Since no endpoint is available the metabolite is assumed to be 10 times more toxic than the parent (in accordance with EFSA, 2016).

### Table 9.5-3: Endpoints and effect values relevant for the risk assessment for aquatic organisms – A23109A

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| *Oncorhynchus mykiss* | A23109A | 96 h, s | **$LC_{50}$ >100 mg/L $_{nom}$** (>16.6 mg metalaxyl-M and 2.85 mg oxathiapiprolin/L) | Schuler 2021, VV-893371 |
| *Daphnia magna* | A23109A | 48 h, s | **$EC_{50}$ = 79.4 mg/L $_{nom}$** (13.2 mg metalaxyl-M and 2.26 mg oxathiapiprolin/L) | Schuler 2021, VV-893390 |

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| *Raphidocelis subcapitata* (formerly known as *Pseudokirchneriella subcapitata*) | A23109A | 96 h, s | 72 h $E_rC_{10}$ = 43.6 mg/L $_{nom}$<br>72 h $E_yC_{10}$ = 58.4 mg/L $_{nom}$<br><br>72 h $E_rC_{20}$ = 80.6 mg/L $_{nom}$<br>72 h $E_yC_{20}$ = 65.6 mg/L $_{nom}$<br><br>**72 h $E_rC_{50}$ >100 mg/L $_{nom}$**<br>(>16.6 mg metalaxyl-M and 2.85 mg oxathiapiprolin/L)<br>72 h $E_yC_{50}$ = 81.8 mg/L $_{nom}$<br><br>96 h $E_rC_{10}$ = 41.8 mg/L $_{nom}$<br>96 h $E_yC_{10}$ = 52.3 mg/L $_{nom}$<br><br>96 h $E_rC_{20}$ = 75.0 mg/L $_{nom}$<br>96 h $E_yC_{20}$ = 59.0 mg/L $_{nom}$<br><br>**96 h $E_rC_{50}$ >100 mg/L $_{nom}$**<br>(>16.6 mg metalaxyl-M and 2.85 mg oxathiapiprolin/L)<br>96 h $E_yC_{50}$ = 74.1 mg/L $_{nom}$ | Schuler 2021, VV-898484 |

s: static; nom: based on nominal concentrations
Bold values are used for the risk assessment

### 9.5.1.1 Justification for new endpoints

New studies are available for formulation A23109A which are required to fulfil the data requirements for plant protection products in accordance with Regulation (EC) No 1107/2009. The endpoints are summarised in Table 9.5-3.

**Consideration of mixture toxicity of metalaxyl-M/oxathiapiprolin in A23109A**

According to the EFSA Aquatic Guidance (2013) it is recommended to compare the measured acute endpoint of the formulation derived from experimental testing ($LC/EC_{xPPP}$) and the acute calculated mixture toxicity by concentration addition ($LC/EC_{x\ mix\text{-}CA}$). This is to determine whether there is any synergism or antagonism between the active substances. This comparison may also indicate any relevant toxicity contributions of co-formulants not included in the calculation.

Equation 13 of the EFSA Aquatic Guidance (2013, page 148) details the calculated mixture toxicity by concentration addition.

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

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

The deviation between calculated and measured toxicity is termed Model Deviation Ratio (MDR) and is calculated using equation 15 of the EFSA Aquatic Guidance (page 149).

$$MDR = \frac{ECx_{mix-CA}\ \text{(calculated mixture toxicity)}}{ECx_{PPP}\ \text{(measured mixture toxicity)}}$$

The observed and calculated mixture toxicity are considered in agreement if the model deviation ratio (MDR) is between 0.2 and 5.

The acute toxicity of A23109A to fish, aquatic invertebrates and algae expected according to the assumption of concentration addition are given in the table below.

**Table 9.5-4: Toxicity of A23109A to aquatic organisms, measured and calculated according to assumption of concentration addition, together with and MDR analysis**

| Species | Test substance | Concentration of active substance in formulation A23109A (g/L) | Fraction of active substance in the formulation mixture | $LC/EC_{50}$ for active substance (µg/L) | Fraction of active substance / $LC/EC_{50}$ for the active substance | Calculated $LC/EC_{50\ mix\text{-}CA}$ (µg a.s./L) [a] | Measured $LC/EC_{50\ ppp}$ (µg form./L) | Measured $LC/EC_{50\ ppp}$ (µg a.s./L) [b] | MDR [c] $LC/EC_{50mix\text{-}CA}$ / $LC/EC_{50\ ppp}$ |
|---|---|---|---|---|---|---|---|---|---|
| Fish (*Oncorhynchus mykiss*) | Metalaxyl-M | 178 | 0.853 | >100000 | 0.00000853 | 4522 | >100000 | >19450 | 0.233 |
| | Oxathiapiprolin | 30.6 | 0.147 | >690 | 0.000213 | | | | |
| Total | - | 208.6 | 1.000 | - | 0.000221 | - | - | - | |
| Aquatic invertebrates (*Daphnia magna*) | Metalaxyl-M | 178 | 0.853 | >100000 | 0.00000853 | 4396 | 79400 | 15443 | 0.285 |
| | Oxathiapiprolin | 30.6 | 0.147 | 670 | 0.000219 | | | | |
| Total | - | 208.6 | 1.000 | - | 0.000227 | - | - | - | |
| Algae [d] | Metalaxyl-M | 178 | 0.853 | 19950 | 0.0000428 | 930 | >100000 | >19450 | 0.0478 * |
| | Oxathiapiprolin | 30.6 | 0.147 | >142 | 0.00103 | | | | |
| Total | - | 208.6 | 1.000 | - | 0.00108 | - | - | - | - |

Note: Calculations undertaken using unrounded values consequently they may not be reproducible when using the figures given in the table

[a] Predicted mixture toxicity under assumption of concentration-addition

[b] Formulation endpoint expressed as total a.s. (based on the total % w/w of metalaxyl-M (16.6%) and oxathiapiprolin (2.85%) in the formulation (19.45% w/w)

[c] In accordance with EFSA Aquatic Guidance (2013), mixture toxicity conforms to assumptions of concentration-addition when model deviation ratio (MDR; $ECx_{mix\text{-}CA}/ECx_{ppp}$) is between 0.2 and 5

[d] Metalaxyl-M endpoint generated using *Scenedesmus quadricanda* whereas the oxathiapiprolin endpoint was generated using *Raphidocelis subcapitata*, formerly known as *Pseudokirchneriella subcapitata* and *Selenastrum capricornutum.* Endpoints based on 96h $E_rC_{50}$

* MDR is below 0.2 suggesting antagonism, however, this is not considered to be a reliable prediction of mixture toxicity as different species of algae have been used in the studies from which the active substance endpoints were derived

The model deviation ratio (MDR) values in the table above indicate that toxicity of A23109A to fish and aquatic invertebrates is as predicted on the assumption of concentration addition (0.2<MDR<5). The MDR for the algal data is not within the range of 0.2 - 5 however this is considered to be a consequence of the fact that the predicted toxicity endpoint has been generated using algal data from different species and therefore is not considered to allow for a meaningful comparison with the experimentally determined toxicity endpoint. The principles of concentration addition are therefore considered to have been demonstrated for A23109A on the basis of the fish and aquatic invertebrate data.

**Consideration of mixture toxicity of metalaxyl-M/oxathiapiprolin in A23109A according to the Central Zone Tool**

The central zone tool (AGD_AquaMix_v1.22 accessed October 2023), was used to address the mixture toxicity. The central zone tool uses a stepwise approach to address the mixture toxicity 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) No 1107/2009".

Toxicity data for both active substances metalaxyl-M and oxathiapiprolin, and the formulated product A23109A on the acute risk to fish, aquatic invertebrates, and algae were assessed. The same data as presented in the MDR analysis above were considered. Maximum FOCUS Step 1 and 2 PECsw resulted from the proposed use in bulb and onion like crops were also considered.

At screening step 1 the tool states that "if no synergistic effects are indicated and the ETR values of the individual a.s. (ETRi) contained in the formulation are below the relevant trigger value, the mixture RA can follow a simplified approach: if all ETRi ≤ ETR trigger/n (n= number of a.s.) the mixture also fulfils the authorisation criteria and the procedure can be stopped. Care must be taken that the PECi values considered in ETRi are identical to those defined relevant for the mixture RA (i.e. PECmix = sum of PECi)." The results of screening step 1 are shown below.

**Table 9.5-5: Screening Step 1 results for the evaluation of mixture toxicity of A32109A according to the central zone tool**

| Organism | PECsw | ETR | | Any a.s. above ETRtrigger? | ETRtrigger/n (if needed) | All ETRi ≤ ETRtrigger/n? |
|---|---|---|---|---|---|---|
| | | Metalaxyl-M | Oxathiapiprolin | | | |
| Fish (*Oncorhynchus mykiss*) | Step 1 | 0.000568 | 0.00196 | No | 0.005 | Yes |
| | Step 2 | | | | | |
| | N-Europe | 0.0000601 | 0.000319 | No | 0.005 | Yes |
| | S-Europe | 0.000108 | 0.000594 | No | 0.005 | Yes |
| Aquatic invertebrates (*Daphnia magna*) | Step 1 | 0.000568 | 0.00201 | No | 0.005 | Yes |
| | Step 2 | | | | | |
| | N-Europe | 0.0000601 | 0.000328 | No | 0.005 | Yes |
| | S-Europe | 0.000108 | 0.000612 | No | 0.005 | Yes |
| Algae [a] | Step 1 | 0.00285 | 0.00951 | No | 0.05 | Yes |
| | Step 2 | | | | | |
| | N-Europe | 0.000301 | 0.00155 | No | 0.05 | Yes |
| | S-Europe | 0.000541 | 0.00289 | No | 0.05 | Yes |

[a] Metalaxyl-M endpoint generated using *Scenedesmus quadricanda* whereas the oxathiapiprolin endpoint was generated using *Raphidocelis subcapitata*, formerly known as *Pseudokirchneriella subcapitata* and *Selenastrum capricornutum*. Endpoints based on 96h $E_rC_{50}$

As shown in the table above all ETRi values are greater than the ETR trigger/n trigger demonstrating that no further consideration is necessary.

## 9.5.2 Risk assessment

The evaluation of the risk for aquatic and sediment-dwelling organisms was performed in accordance with the recommendations of the "Guidance document on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters in the context of Regulation (EC) No

1107/2009", as provided by the Commission Services (SANTE-2015-00080, 15 January 2015).

The relevant global maximum FOCUS Step 1 and 2 $PEC_{SW}$ for risk assessments covering the proposed use pattern have been used in the risk assessment. For further details refer to Part B Section 8 Environmental Fate.

From the endpoints and effect values relevant for the risk assessment for aquatic organisms the following Regulatory Acceptable Concentrations (RAC) are derived for use in the Tier 1 risk assessment.

**Table 9.5-6: Derivation of RAC values used in the Tier 1 risk assessment – metalaxyl-M and relevant metabolite (NOA409045)**

| Species | Substance | Exposure system | Results (µg/L) | Assessment safety factor | RAC (µg/L) |
|---|---|---|---|---|---|
| *Oncorhynchus mykiss* | Metalaxyl-M | 96 h, s | $LC_{50}$ >100000 | 100 | >1000 |
| *Oncorhynchus mykiss* | NOA409045 | 96 h, ss | $LC_{50}$ >100000 | 100 | >1000 |
| *Pimephales promelas* | Metalaxyl | 30 d (ELS), f | NOEC = 9100 | 10 | 910 |
| *Oncorhynchus mykiss* | NOA409045 | 28 d, f | NOEC = 100000 | 10 | 10000 |
| *Crassostrea virginica* | Metalaxyl | 96 h, f | $EC_{50}$ = 5600 | 100 | 56 |
| *Daphnia magna* | NOA409045 | 48 h, s | $LC_{50}$ = 852000 | 100 | 8520 |
| *Daphnia magna* | Metalaxyl-M | 21 d, s | NOEC = 1000 | 10 | 100 |
| *Daphnia magna* | NOA409045 | 21 d, s | NOEC = 100000 | 10 | 10000 |
| *Scenedesmus quadricanda* | Metalaxyl-M | 96 h, s | $E_rC_{50}$ = 19950 | 10 | 1995 |
| *Pseudokirchneriella subcapitata* | NOA409045 | 72 h, s | $E_rC_{50}$ = 81000 | 10 | 8100 |

**Table 9.5-7: Derivation of RAC values used in the risk assessment – Oxathiapiprolin and relevant metabolites**

| Species | Substance | Exposure System | Results (µg/L) | Assessment Safety factor | RAC (µg/L) |
|---|---|---|---|---|---|
| *Cyprinodon variegatus* | Oxathiapiprolin | 96 h, s | $LC_{50}$ >650 | 100 | >6.5 |
| *Cyprinodon variegatus* | Oxathiapiprolin | 35-day, f, ELS | NOEC = 340 | 10 | 34 |
| *Oncorhynchus mykiss* | IN-E8S72 | 96 h, s | $LC_{50}$ >100000 | 100 | >1000 |
| *Oncorhynchus mykiss* | IN-P3X26 | 96 h, s | $LC_{50}$ >67720 | 100 | >677.2 |
| *Oncorhynchus mykiss* | IN-Q7D41 | 96 h, ss | $LC_{50}$ >180 | 100 | >1.8 |
| *Oncorhynchus mykiss* | IN-QPS10 | 96 h, s | $LC_{50}$ = 6960 | 100 | 69.6 |
| *Oncorhynchus mykiss* | IN-RAB06 | 96 h, s | $LC_{50}$ >50000 | 100 | >500 |
| *Oncorhynchus mykiss* | IN-RDT31 | 96 h, s | $LC_{50}$ >11560 | 100 | >115.6 |
| *Oncorhynchus mykiss* | IN-RSE01 | 96 h, ss | $LC_{50}$ >9840 | 100 | >98.4 |
| *Oncorhynchus mykiss* | IN-RYJ52 | 96 h, s | $LC_{50}$ >13800 | 100 | >138 |

| Species | Substance | Exposure System | Results (µg/L) | Assessment Safety factor | RAC (µg/L) |
|---|---|---|---|---|---|
| *Oncorhynchus mykiss* | IN-S2K66 | 96 h, s | $LC_{50}$ >7480 | 100 | >74.8 |
| *Crassostrea virginica* | Oxathiapiprolin | 96 h, f | $EC_{50}$ >330 | 100 | >3.3 |
| *Americamysis bahia* | Oxathiapiprolin | 32 d, f | NOEC = 58 | 10 | 5.8 |
| *Daphnia magna* | IN-E8S72 | 48 h, s | $EC_{50}$ >100000 | 100 | >1000 |
| *Daphnia magna* | IN-P3X26 | 48 h, s | $EC_{50}$ >67740 | 100 | >677.4 |
| *Daphnia magna* | IN-Q7D41 | 48 h, ss | $EC_{50}$ >150 | 100 | >1.5 |
| *Daphnia magna* | IN-QPS10 | 48 h, s | $EC_{50}$ = 15870 | 100 | 158.7 |
| *Daphnia magna* | IN-RAB06 | 48 h, s | $EC_{50}$ >100000 | 100 | >1 000 |
| *Daphnia magna* | IN-RDT31 | 48 h, s | $EC_{50}$ >10490 | 100 | >104.9 |
| *Daphnia magna* | IN-RSE01 | 48 h, s | $EC_{50}$ >10160 | 100 | >101.6 |
| *Daphnia magna* | IN-RYJ52 | 48 h, s | $EC_{50}$ >16210 | 100 | >162.1 |
| *Daphnia magna* | IN-S2K66 | 48 h, s | $EC_{50}$ = 860 | 100 | 8.6 |
| *Chironomus riparius* | Oxathiapiprolin | 28 d, s, water spiked | NOEC = 110 | 10 | 11 |
| *Chironomus riparius* | Oxathiapiprolin | 28 d, spiked sediment | NOEC = 2800 µg/kg sediment | 10 | 280 µg/kg |
| *Chironomus riparius* | IN-Q7D41 | 28 d, spiked sediment | NOEC = 72000 µg/kg sediment | 10 | 7200 µg/kg |
| *Chironomus riparius* | IN-RYJ52 | 10x parent toxicity | NOEC = 11 | 10 | 1.1 |
| *Chironomus riparius* | IN-RYJ52 | 10x parent toxicity | NOEC = 280 µg/kg sediment | 10 | 28 µg/kg |
| *Chironomus riparius* | IN-S2K66 | 10x parent toxicity | NOEC = 11 | 10 | 1.1 |
| *Chironomus riparius* | IN-S2K66 | 10x parent toxicity | NOEC = 280 µg/kg sediment | 10 | 28 µg/kg |
| *Chironomus riparius* | IN-RSE01 | 10x parent toxicity | NOEC = 11 | 10 | 1.1 |
| *Chironomus riparius* | IN-RSE01 | 10x parent toxicity | NOEC = 280 µg/kg sediment | 10 | 28 µg/kg |
| *Pseudokirchneriella subcapitata* | Oxathiapiprolin | 96 h, s | $E_rC_{50}$ >142 | 10 | >14.2 |
| *Pseudokirchneriella subcapitata* | IN-E8S72 | 72 h, s | $E_rC_{50}$ >100000 | 10 | >10000 |
| *Pseudokirchneriella subcapitata* | IN-P3X26 | 72 h, s | $E_rC_{50}$ >66640 | 10 | >6664 |
| *Pseudokirchneriella subcapitata* | IN-Q7D41 | 72 h, s | $E_rC_{50}$ >210 | 10 | >21 |
| *Pseudokirchneriella subcapitata* | IN-QPS10 | 72 h, s | $E_rC_{50}$ = 2320 | 10 | 232 |
| *Pseudokirchneriella subcapitata* | IN-RAB06 | 72 h, s | $E_rC_{50}$ >100000 | 10 | >10000 |
| *Pseudokirchneriella subcapitata* | IN-RDT31 | 72 h, s | $E_rC_{50}$ >11430 | 10 | >1143 |

| Species | Substance | Exposure System | Results (µg/L) | Assessment Safety factor | RAC (µg/L) |
|---|---|---|---|---|---|
| *Pseudokirchneriella subcapitata* | IN-RSE01 | 72 h, s | $E_rC_{50}$ >10800 | 10 | >1080 |
| *Pseudokirchneriella subcapitata* | IN-RYJ52 | 72 h, s | $E_rC_{50}$ >15340 | 10 | >1534 |
| *Pseudokirchneriella subcapitata* | IN-S2K66 | 72 h, s | $E_rC_{50}$ >7560 | 10 | >756 |
| *Lemna gibba* | Oxathiapiprolin | 7 d, ss | $E_rC_{50}$ (frond count) >790 | 10 | >79 |

**Table 9.5-8: Derivation of RAC values used in the risk assessment – A23109A**

| Species | Substance | Exposure System | Results (µg/L) | Assessment Safety factor | RAC (µg/L) |
|---|---|---|---|---|---|
| *Oncorhynchus mykiss* | A23109A | 96 h, s | $LC_{50}$ >100000 | 100 | >1000 |
| *Daphnia magna* | A23109A | 48 h, s | $EC_{50}$ = 79400 | 100 | 794 |
| *Raphidocelis subcapitata* (formerly known as *Pseudokirchneriella subcapitata*) | A23109A | 96 h, s | $E_rC_{50}$ >100000 | 10 | >10000 |

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

**Metalaxyl-M**

**Table 9.5-9: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metalaxyl-M for each organism group based on the maximum FOCUS Step 1 and 2 calculations for the use of A23109A in leafy vegetables (BBCH 12-49, 2 x 87.2 g a.s./ha, application interval of 7 days)**

| Group | | Fish acute | Fish prolonged | Inverteb. acute | Inverteb. prolonged | Algae |
|---|---|---|---|---|---|---|
| **RAC (µg/L)** | | >1000 | 910 | 56 | 100 | 1995 |
| **FOCUS Scenario** | **$PEC_{sw, max}$ (µg/L)** | **PEC/RAC ratios** | | | | |
| **Step 1** | | | | | | |
| | 56.79 | 0.0568 | 0.0624 | **1.01** | 0.568 | 0.0285 |
| **Step 2** | | | | | | |
| N-Europe | 5.21 | * | * | 0.0930 | * | * |
| S-Europe | 9.19 | * | * | 0.164 | * | * |

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**
* No further assessment was required as an acceptable risk has been demonstrated at FOCUS Step 1

**Table 9.5-10: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metalaxyl-M for each organism group based on the maximum FOCUS Step 1 and 2 calculations for the use of A23109A in bulb & onion like crops (BBCH 12-48, 2 x 87.2 g a.s./ha, application interval of 7 days)**

| Group | | Fish acute | Fish prolonged | Inverteb. acute | Inverteb. prolonged | Algae |
|---|---|---|---|---|---|---|
| **RAC (µg/L)** | | >1000 | 910 | 56 | 100 | 1995 |
| **FOCUS Scenario** | **$PEC_{sw, max}$ (µg/L)** | **PEC/RAC ratios** | | | | |
| **Step 1** | | | | | | |
| | 56.79 | 0.0568 | 0.0624 | **1.01** | 0.568 | 0.0285 |
| **Step 2** | | | | | | |
| N-Europe | 6.01 | * | * | 0.107 | * | * |
| S-Europe | 10.79 | * | * | 0.193 | * | * |

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**
* No further assessment was required as an acceptable risk has been demonstrated at FOCUS Step 1

**Metalaxyl-M metabolite**

**Table 9.5-11: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metalaxyl-M metabolite NOA409045 for each organism group based on the maximum FOCUS Step 1 and 2 calculations for the use of A23109A in leafy vegetables (BBCH 12-49, 2 x 87.2 g a.s./ha, application interval of 7 days)**

| Group | | Fish acute | Fish prolonged | Inverteb. acute | Inverteb. prolonged | Algae |
|---|---|---|---|---|---|---|
| **RAC (µg/L)** | | >1000 | 10000 | 8520 | 10000 | 8100 |
| **FOCUS Scenario** | **$PEC_{sw, max}$ (µg/L)** | **PEC/RAC ratios** | | | | |
| **Step 1** | | | | | | |
| | 88.29 | 0.0883 | 0.00883 | 0.0104 | 0.00883 | 0.0109 |
| **Step 2** | | | | | | |
| N-Europe | 9.58 | * | * | * | * | * |
| S-Europe | 18.00 | * | * | * | * | * |

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration
* No further assessment is required as an acceptable risk has been demonstrated at FOCUS Step 1

**Table 9.5-12: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metalaxyl-M metabolite NOA409045 for each organism group based on the maximum FOCUS Step 1 and 2 calculations for the use of A23109A in bulb & onion like crops (BBCH 12-48, 2 x 87.2 g a.s./ha, application interval of 7 days)**

| Group | | Fish acute | Fish prolonged | Inverteb. acute | Inverteb. prolonged | Algae |
|---|---|---|---|---|---|---|
| **RAC (µg/L)** | | >1000 | 10000 | 8520 | 10000 | 8100 |
| **FOCUS Scenario** | **$PEC_{sw, max}$ (µg/L)** | **PEC/RAC ratios** | | | | |
| **Step 1** | | | | | | |
| | 88.29 | 0.0883 | 0.00883 | 0.0104 | 0.00883 | 0.0109 |
| **Step 2** | | | | | | |
| N-Europe | 11.27 | * | * | * | * | * |
| S-Europe | 21.36 | * | * | * | * | * |

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration
* No further assessment is required as an acceptable risk has been demonstrated at FOCUS Step 1

**Table 9.5-13: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for oxathiapiprolin for each organism group based on the maximum FOCUS Step 1 and 2 calculations for the use of A23109A in leafy vegetables (BBCH 12-49, 2 x 15 g a.s./ha, application interval of 7 days)**

| **Group** | | **Fish acute** | **Fish prolonged** | **Inverteb. acute** | **Inverteb. prolonged** | **Algae** | **Aquatic macrophytes** | **Sed. dwell. prolonged** | | **Sed. dwell. prolonged (spiked sediment)** |
|---|---|---|---|---|---|---|---|---|---|---|
| **RAC (µg/L)** | | >6.5 | 34 | >3.3 | 5.8 | >14.2 | >79 | 11 | **RAC (µg/kg)** | 280 |
| **FOCUS Scenario** | **$PEC_{sw, max}$ (µg/L)** | **PEC/RAC ratios** | | | | | | | **$PEC_{sed, max}$ (µg/kg)** | **PEC/RAC ratios** |
| **Step 1** | | | | | | | | | | |
| | 1.35 | 0.208 | 0.0397 | 0.409 | 0.233 | 0.0951 | 0.0171 | 0.123 | 75.08 | 0.268 |
| **Step 2** | | | | | | | | | | |
| N-Europe | 0.19 | * | * | 0.0576 [a] | * | * | * | * | 12.25 | * |
| S-Europe | 0.34 | * | * | 0.103 [a] | * | * | * | * | 22.86 | * |

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration
* No further assessment was required as an acceptable risk has been demonstrated at FOCUS Step 1
[a] PEC/RAC ratios presented as values required for the mixture toxicity risk assessment

**Table 9.5-14: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for oxathiapiprolin for each organism group based on the maximum FOCUS Step 1 and 2 calculations for the use of A23109A in bulb & onion like crops (BBCH 12-48, 2 x 15 g a.s./ha, application interval of 7 days)**

| **Group** | | **Fish acute** | **Fish prolonged** | **Inverteb. acute** | **Inverteb. prolonged** | **Algae** | **Aquatic macrophytes** | **Sed. dwell. prolonged** | | **Sed. dwell. prolonged (spiked sediment)** |
|---|---|---|---|---|---|---|---|---|---|---|
| **RAC (µg/L)** | | >6.5 | 34 | >3.3 | 5.8 | >14.2 | >79 | 11 | **RAC (µg/kg)** | 280 |
| **FOCUS Scenario** | **$PEC_{sw, max}$ (µg/L)** | **PEC/RAC ratios** | | | | | | | **$PEC_{sed, max}$ (µg/kg)** | **PEC/RAC ratios** |
| **Step 1** | | | | | | | | | | |
| | 1.35 | 0.208 | 0.0397 | 0.409 | 0.233 | 0.0951 | 0.0171 | 0.123 | 75.08 | 0.268 |
| **Step 2** | | | | | | | | | | |
| N-Europe | 0.22 | * | * | 0.0667 [a] | * | * | * | * | 14.37 | * |
| S-Europe | 0.41 | * | * | 0.124 [a] | * | * | * | * | 27.10 | * |

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration
* No further assessment was required as an acceptable risk has been demonstrated at FOCUS Step 1
[a] PEC/RAC ratios presented as values required for the mixture toxicity risk assessment

**Oxathiapiprolin metabolites**

**Table 9.5-15: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for oxathiapiprolin metabolite IN-E8S72 for each organism group based on maximum FOCUS Step 1 and 2 calculations for the use of A23109A in leafy vegetables (BBCH 12-49, 2 x 15 g a.s./ha, application interval of 7 days)**

| **Group** | | **Fish acute** | **Inverteb. acute** | **Algae** |
|---|---|---|---|---|
| **RAC (µg/L)** | | >1000 | >1000 | >10000 |
| **FOCUS Scenario** | **$PEC_{sw, max}$ (µg/L)** | **PEC/RAC ratios** | | |
| **Step 1** | | | | |
| | 0.34 | 0.000340 | 0.000340 | 0.0000340 |
| **Step 2** | | | | |
| N-Europe | 0.05 | * | * | * |
| S-Europe | 0.10 | * | * | * |

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration
* No further assessment is required as an acceptable risk has been demonstrated at FOCUS Step 1

**Table 9.5-16: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for oxathiapiprolin metabolite IN-E8S72 for each organism group based on maximum FOCUS Step 1 and 2 calculations for the use of A23109A in bulb & onion like crops (BBCH 12-48, 2 x 15 g a.s./ha, application interval of 7 days)**

| **Group** | | **Fish acute** | **Inverteb. acute** | **Algae** |
|---|---|---|---|---|
| **RAC (µg/L)** | | >1000 | >1000 | >10000 |
| **FOCUS Scenario** | **$PEC_{sw, max}$ (µg/L)** | **PEC/RAC ratios** | | |
| **Step 1** | | | | |
| | 0.34 | 0.000340 | 0.000340 | 0.0000340 |
| **Step 2** | | | | |
| N-Europe | 0.06 | * | * | * |
| S-Europe | 0.12 | * | * | * |

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration
* No further assessment is required as an acceptable risk has been demonstrated at FOCUS Step 1

**Table 9.5-17: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for oxathiapiprolin metabolite IN-P3X26 for each organism group based on maximum FOCUS Step 1 and 2 calculations for the use of A23109A in leafy vegetables (BBCH 12-49, 2 x 15 g a.s./ha, application interval of 7 days)**

| **Group** | | **Fish acute** | **Inverteb. acute** | **Algae** |
|---|---|---|---|---|
| **RAC (µg/L)** | | >677.2 | >677.4 | >6664 |
| **FOCUS Scenario** | **$PEC_{sw, max}$ (µg/L)** | **PEC/RAC ratios** | | |
| **Step 1** | | | | |
| | 1.07 | 0.00158 | 0.00158 | 0.000161 |
| **Step 2** | | | | |
| N-Europe | 0.18 | * | * | * |
| S-Europe | 0.33 | * | * | * |

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration
* No further assessment is required as an acceptable risk has been demonstrated at FOCUS Step 1

**Table 9.5-18: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for oxathiapiprolin metabolite IN-P3X26 for each organism group based on maximum FOCUS Step 1 and 2 calculations for the use of A23109A in bulb & onion like crops (BBCH 12-48, 2 x 15 g a.s./ha, application interval of 7 days)**

| **Group** | | **Fish acute** | **Inverteb. acute** | **Algae** |
|---|---|---|---|---|
| **RAC (µg/L)** | | >677.2 | >677.4 | >6664 |
| **FOCUS Scenario** | **$PEC_{sw, max}$ (µg/L)** | **PEC/RAC ratios** | | |
| **Step 1** | | | | |
| | 1.07 | 0.00158 | 0.00158 | 0.000161 |
| **Step 2** | | | | |
| N-Europe | 0.21 | * | * | * |
| S-Europe | 0.39 | * | * | * |

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration
* No further assessment is required as an acceptable risk has been demonstrated at FOCUS Step 1

**Table 9.5-19: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for oxathiapiprolin metabolite IN-Q7D41 for each organism group based on maximum FOCUS Step 1 and 2 calculations for the use of A23109A in leafy vegetables (BBCH 12-49, 2 x 15 g a.s./ha, application interval of 7 days)**

| **Group** | | **Fish acute** | **Inverteb. acute** | **Algae** | | **Sed. dwell. prolonged** |
|---|---|---|---|---|---|---|
| **RAC (µg/L)** | | >1.8 | >1.5 | >21 | | 7200 |
| **FOCUS Scenario** | **$PEC_{sw, max}$ (µg/L)** | **PEC/RAC ratios** | | | **$PEC_{sed, max}$ (µg/kg)** | **PEC/RAC ratios** |
| **Step 1** | | | | | | |
| | 1.19 | 0.661 | 0.793 | 0.0567 | 8.42 | 0.00117 |
| **Step 2** | | | | | | |
| N-Europe | 0.20 | * | * | * | 1.38 | * |
| S-Europe | 0.36 | * | * | * | 2.55 | * |

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration
* No further assessment is required as an acceptable risk has been demonstrated at FOCUS Step 1

**Table 9.5-20: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for oxathiapiprolin metabolite IN-Q7D41 for each organism group based on maximum FOCUS Step 1 and 2 calculations for the use of A23109A in bulb & onion like crops (BBCH 12-48, 2 x 15 g a.s./ha, application interval of 7 days)**

| **Group** | | **Fish acute** | **Inverteb. acute** | **Algae** | | **Sed. dwell. prolonged** |
|---|---|---|---|---|---|---|
| **RAC (µg/L)** | | >1.8 | >1.5 | >21 | | 7200 |
| **FOCUS Scenario** | **$PEC_{sw, max}$ (µg/L)** | **PEC/RAC ratios** | | | **$PEC_{sed, max}$ (µg/kg)** | **PEC/RAC ratios** |
| **Step 1** | | | | | | |
| | 1.19 | 0.661 | 0.793 | 0.0567 | 8.42 | 0.00117 |
| **Step 2** | | | | | | |
| N-Europe | 0.23 | * | * | * | 1.61 | * |
| S-Europe | 0.43 | * | * | * | 3.03 | * |

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration
* No further assessment is required as an acceptable risk has been demonstrated at FOCUS Step 1

**Table 9.5-21: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for oxathiapiprolin metabolite IN-QPS10 for each organism group based on maximum FOCUS Step 1 and 2 calculations for the use of A23109A in leafy vegetables (BBCH 12-49, 2 x 15 g a.s./ha, application interval of 7 days)**

| **Group** | | **Fish acute** | **Inverteb. acute** | **Algae** |
|---|---|---|---|---|
| **RAC (µg/L)** | | 69.6 | 158.7 | 232 |
| **FOCUS Scenario** | **$PEC_{sw, max}$ (µg/L)** | **PEC/RAC ratios** | | |
| **Step 1** | | | | |
| | 0.08 | 0.00115 | 0.000504 | 0.000345 |
| **Step 2** | | | | |
| N-Europe | 0.01 | * | * | * |
| S-Europe | 0.02 | * | * | * |

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration
* No further assessment is required as an acceptable risk has been demonstrated at FOCUS Step 1

**Table 9.5-22: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for oxathiapiprolin metabolite IN-QPS10 for each organism group based on maximum FOCUS Step 1 and 2 calculations for the use of A23109A in bulb & onion like crops (BBCH 12-48, 2 x 15 g a.s./ha, application interval of 7 days)**

| **Group** | | **Fish acute** | **Inverteb. acute** | **Algae** |
|---|---|---|---|---|
| **RAC (µg/L)** | | 69.6 | 158.7 | 232 |
| **FOCUS Scenario** | **$PEC_{sw, max}$ (µg/L)** | **PEC/RAC ratios** | | |
| **Step 1** | | | | |
| | 0.08 | 0.00115 | 0.000504 | 0.000345 |
| **Step 2** | | | | |
| N-Europe | 0.01 | * | * | * |
| S-Europe | 0.03 | * | * | * |

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration
* No further assessment is required as an acceptable risk has been demonstrated at FOCUS Step 1

**Table 9.5-23: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for oxathiapiprolin metabolite IN-RAB06 for each organism group based on maximum FOCUS Step 1 and 2 calculations for the use of A23109A in leafy vegetables (BBCH 12-49, 2 x 15 g a.s./ha, application interval of 7 days)**

| **Group** | | **Fish acute** | **Inverteb. acute** | **Algae** |
|---|---|---|---|---|
| **RAC (µg/L)** | | >500 | >1000 | >10000 |
| **FOCUS Scenario** | **$PEC_{sw, max}$ (µg/L)** | **PEC/RAC ratios** | | |
| **Step 1** | | | | |
| | 1.49 | 0.00298 | 0.00149 | 0.000149 |
| **Step 2** | | | | |
| N-Europe | 0.22 | * | * | * |
| S-Europe | 0.43 | * | * | * |

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration
* No further assessment is required as an acceptable risk has been demonstrated at FOCUS Step 1

**Table 9.5-24: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for oxathiapiprolin metabolite IN-RAB06 for each organism group based on maximum FOCUS Step 1 and 2 calculations for the use of A23109A in bulb & onion like crops (BBCH 12-48, 2 x 15 g a.s./ha, application interval of 7 days)**

| **Group** | | **Fish acute** | **Inverteb. acute** | **Algae** |
|---|---|---|---|---|
| **RAC (µg/L)** | | >500 | >1000 | >10000 |
| **FOCUS Scenario** | **$PEC_{sw, max}$ (µg/L)** | **PEC/RAC ratios** | | |
| **Step 1** | | | | |
| | 1.49 | 0.00298 | 0.00149 | 0.000149 |
| **Step 2** | | | | |
| N-Europe | 0.26 | * | * | * |
| S-Europe | 0.51 | * | * | * |

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration
* No further assessment is required as an acceptable risk has been demonstrated at FOCUS Step 1

**Table 9.5-25: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for oxathiapiprolin metabolite IN-RDT31 for each organism group based on maximum FOCUS Step 1 and 2 calculations for the use of A23109A in leafy vegetables (BBCH 12-49, 2 x 15 g a.s./ha, application interval of 7 days)**

| **Group** | | **Fish acute** | **Inverteb. acute** | **Algae** |
|---|---|---|---|---|
| **RAC (µg/L)** | | >115.6 | >104.9 | >1143 |
| **FOCUS Scenario** | **$PEC_{sw, max}$ (µg/L)** | **PEC/RAC ratios** | | |
| **Step 1** | | | | |
| | 0.38 | 0.00329 | 0.00362 | 0.000332 |
| **Step 2** | | | | |
| N-Europe | 0.05 | * | * | * |
| S-Europe | 0.11 | * | * | * |

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration
* No further assessment is required as an acceptable risk has been demonstrated at FOCUS Step 1

**Table 9.5-26: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for oxathiapiprolin metabolite IN-RDT31 for each organism group based on maximum FOCUS Step 1 and 2 calculations for the use of A23109A in bulb & onion like crops (BBCH 12-48, 2 x 15 g a.s./ha, application interval of 7 days)**

| **Group** | | **Fish acute** | **Inverteb. acute** | **Algae** |
|---|---|---|---|---|
| **RAC (µg/L)** | | >115.6 | >104.9 | >1143 |
| **FOCUS Scenario** | **$PEC_{sw, max}$ (µg/L)** | **PEC/RAC ratios** | | |
| **Step 1** | | | | |
| | 0.38 | 0.00329 | 0.00362 | 0.000332 |
| **Step 2** | | | | |
| N-Europe | 0.07 | * | * | * |
| S-Europe | 0.13 | * | * | * |

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration
* No further assessment is required as an acceptable risk has been demonstrated at FOCUS Step 1

**Table 9.5-27: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for oxathiapiprolin metabolite IN-RSE01 for each organism group based on maximum FOCUS Step 1 and 2 calculations for the use of A23109A in leafy vegetables (BBCH 12-49, 2 x 15 g a.s./ha, application interval of 7 days)**

| **Group** | | **Fish acute** | **Inverteb. acute** | **Algae** | **Sed. dwell. prolonged** | | **Sed. dwell. prolonged** |
|---|---|---|---|---|---|---|---|
| **RAC (µg/L)** | | >98.4 | >101.6 | >1080 | 1.1 | **RAC (µg/kg)** | 28 |
| **FOCUS Scenario** | **$PEC_{sw, max}$ (µg/L)** | **PEC/RAC ratios** | | | | **$PEC_{sed, max}$ (µg/kg)** | **PEC/RAC ratios** |
| **Step 1** | | | | | | | |
| | 1.06 | 0.0108 | 0.0104 | 0.000981 | 0.964 | 7.49 | 0.268 |
| **Step 2** | | | | | | | |
| N-Europe | 0.17 | * | * | * | * | 1.22 | * |
| S-Europe | 0.32 | * | * | * | * | 2.27 | * |

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration
* No further assessment is required as an acceptable risk has been demonstrated at FOCUS Step 1

**Table 9.5-28: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for oxathiapiprolin metabolite IN-RSE01 for each organism group based on maximum FOCUS Step 1 and 2 calculations for the use of A23109A in bulb & onion like crops (BBCH 12-48, 2 x 15 g a.s./ha, application interval of 7 days)**

| **Group** | | **Fish acute** | **Inverteb. acute** | **Algae** | **Sed. dwell. prolonged** | | **Sed. dwell. prolonged** |
|---|---|---|---|---|---|---|---|
| **RAC (µg/L)** | | >98.4 | >101.6 | >1080 | 1.1 | **RAC (µg/kg)** | 28 |
| **FOCUS Scenario** | **$PEC_{sw, max}$ (µg/L)** | **PEC/RAC ratios** | | | | **$PEC_{sed, max}$ (µg/kg)** | **PEC/RAC ratios** |
| **Step 1** | | | | | | | |
| | 1.06 | 0.0108 | 0.0104 | 0.000981 | 0.964 | 7.49 | 0.268 |
| **Step 2** | | | | | | | |
| N-Europe | 0.20 | * | * | * | * | 1.43 | * |
| S-Europe | 0.38 | * | * | * | * | 2.69 | * |

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration
* No further assessment is required as an acceptable risk has been demonstrated at FOCUS Step 1

**Table 9.5-29: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for oxathiapiprolin metabolite IN-RYJ52 for each organism group based on maximum FOCUS Step 1 and 2 calculations for the use of A23109A in leafy vegetables (BBCH 12-49, 2 x 15 g a.s./ha, application interval of 7 days)**

| **Group** | | **Fish acute** | **Inverteb. acute** | **Algae** | **Sed. dwell. prolonged** | | **Sed. dwell. prolonged** |
|---|---|---|---|---|---|---|---|
| **RAC (µg/L)** | | >138 | >162.1 | >1534 | 1.1 | **RAC (µg/kg)** | 28 |
| **FOCUS Scenario** | **$PEC_{sw, max}$ (µg/L)** | **PEC/RAC ratios** | | | | **$PEC_{sed, max}$ (µg/kg)** | **PEC/RAC ratios** |
| **Step 1** | | | | | | | |
| | 1.64 | 0.0119 | 0.0101 | 0.00107 | **1.49** | 11.57 | 0.413 |
| **Step 2** | | | | | | | |
| N-Europe | 0.27 | * | * | * | 0.245 | 1.89 | * |
| S-Europe | 0.50 | * | * | * | 0.455 | 3.51 | * |

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration
PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**
* No further assessment is required as an acceptable risk has been demonstrated at FOCUS Step 1

**Table 9.5-30: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for oxathiapiprolin metabolite IN-RYJ52 for each organism group based on maximum FOCUS Step 1 and 2 calculations for the use of A23109A in bulb & onion like crops (BBCH 12-48, 2 x 15 g a.s./ha, application interval of 7 days**

| **Group** | | **Fish acute** | **Inverteb. acute** | **Algae** | **Sed. dwell. prolonged** | | **Sed. dwell. prolonged** |
|---|---|---|---|---|---|---|---|
| **RAC (µg/L)** | | >138 | >162.1 | >1534 | 1.1 | **RAC (µg/kg)** | 28 |
| **FOCUS Scenario** | **$PEC_{sw, max}$ (µg/L)** | **PEC/RAC ratios** | | | | **$PEC_{sed, max}$ (µg/kg)** | **PEC/RAC ratios** |
| **Step 1** | | | | | | | |
| | 1.64 | 0.0119 | 0.0101 | 0.00107 | **1.49** | 11.57 | 0.413 |
| **Step 2** | | | | | | | |
| N-Europe | 0.31 | * | * | * | 0.282 | 2.21 | * |
| S-Europe | 0.59 | * | * | * | 0.536 | 4.16 | * |

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration
PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**
* No further assessment is required as an acceptable risk has been demonstrated at FOCUS Step 1

**Table 9.5-31: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for oxathiapiprolin metabolite IN-S2K66 for each organism group based on maximum FOCUS Step 1 and 2 calculations for the use of A23109A in leafy vegetables (BBCH 12-49, 2 x 15 g a.s./ha, application interval of 7 days)**

| **Group** | | **Fish acute** | **Inverteb. acute** | **Algae** | **Sed. dwell. prolonged** | | **Sed. dwell. prolonged** |
|---|---|---|---|---|---|---|---|
| **RAC (µg/L)** | | >74.8 | 8.6 | >756 | 1.1 | **RAC (µg/kg)** | 28 |
| **FOCUS Scenario** | **$PEC_{sw, max}$ (µg/L)** | **PEC/RAC ratios** | | | | **$PEC_{sed, max}$ (µg/kg)** | **PEC/RAC ratios** |
| **Step 1** | | | | | | | |
| | 0.86 | 0.0115 | 0.100 | 0.00114 | 0.782 | 6.11 | 0.218 |
| **Step 2** | | | | | | | |
| N-Europe | 0.14 | * | * | * | * | 1.00 | * |
| S-Europe | 0.26 | * | * | * | * | 1.85 | * |

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration
* No further assessment is required as an acceptable risk has been demonstrated at FOCUS Step 1

**Table 9.5-32: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for oxathiapiprolin metabolite IN-S2K66 for each organism group based on maximum FOCUS Step 1 and 2 calculations for the use of A23109A in bulb & onion like crops (BBCH 12-48, 2 x 15 g a.s./ha, application interval of 7 days**

| **Group** | | **Fish acute** | **Inverteb. acute** | **Algae** | **Sed. dwell. prolonged** | | **Sed. dwell. prolonged** |
|---|---|---|---|---|---|---|---|
| **RAC (µg/L)** | | >74.8 | 8.6 | >756 | 1.1 | **RAC (µg/kg)** | 28 |
| **FOCUS Scenario** | **$PEC_{sw, max}$ (µg/L)** | **PEC/RAC ratios** | | | | **$PEC_{sed, max}$ (µg/kg)** | **PEC/RAC ratios** |
| **Step 1** | | | | | | | |
| | 0.86 | 0.0115 | 0.100 | 0.00114 | 0.782 | 6.11 | 0.218 |
| **Step 2** | | | | | | | |
| N-Europe | 0.17 | * | * | * | * | 1.17 | * |
| S-Europe | 0.31 | * | * | * | * | 2.19 | * |

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration
* No further assessment is required as an acceptable risk has been demonstrated at FOCUS Step 1

The PEC/RAC ratios, using worst-case $PEC_{SW}$ values for metalaxyl-M, oxathiapiprolin and their metabolites are less than the trigger value of 1, indicating that the risk to aquatic organisms is acceptable following use of A23109A according to the proposed use patterns. Therefore, no further assessment is necessary.

**Formulation A23109A**

As mentioned in Section B8, the formulation components are expected to dissipate rapidly after application; therefore, only one application and drift entry are taken into consideration. The maximum $PEC_{SW}$ for formulation A23109A was determined to be for leafy vegetables (field crops) and this value has therefore been used below to cover all proposed uses of A23109A.

**Table 9.5-33: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for A23109A for each organism group based on the maximum instantaneous $PEC_{SW}$ following application of A23109A in field crops**

| **Group** | | | **Fish acute** | **Inverteb. acute** | **Algae** |
|---|---|---|---|---|---|
| **RAC (µg/L)** | | | >1000 | 794 | >10000 |
| **Buffer Distance (m)** | **Drift Reducing Nozzle (%)** | **PEC $_{gl-max}$ (µg/L)** | **PEC/RAC ratios** | | |
| 1 m | - | 4.958 | 0.00496 | 0.00624 | 0.000496 |

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration

For the formulation A23109A, the calculated PEC/RAC ratios were <1 thereby demonstrating an acceptable risk to aquatic organisms without the need for any application mitigation measures.

**Combined risk assessment for A23109A based on calculated mixture toxicity**

The EFSA Aquatic Guidance (2013) requires assessment of the risk from a combination of active substances where a product contains more than one active substance. As stated above, comparison of measured and calculated mixture toxicity of A23109A indicates no deviation from that expected on the basis of concentration addition (see 9.5.1.1).

Methodology to establish whether a single substance is driving the toxicity of a mixture

The EFSA Aquatic Guidance (2013), states that where concentration addition gives a reliable estimate of the toxicity of a mixture, when one active substance represents >90% of the risk, it drives the overall mixture risk and no further assessment is required. The MDR calculation (see Table 9.5-4) shows that concentration addition does give a reliable estimate of toxicity, thus this simplified approach is applicable. To evaluate this, toxic units can be calculated at comparable exposures (*i.e.* FOCUS Steps 1 and 2 which have been modelled for both active substances).

Equation 14 of the EFSA aquatic guidance highlights how to calculate the sum of the toxic units for each component of a mixture:

*Equation 14*: $$\sum_{i=1}^{n} TU_i = \sum_{i=1}^{n} \frac{c_i}{ECx_i}$$

**Table 9.5-34: Aquatic organisms: Toxic Units (TU) approach to identify risk driver – fish acute**

| **Active substance** | | **Metalaxyl-M** | | **Oxathiapiprolin** | | **SUM Toxic Units** | **Toxic Unit as % of total** | |
|---|---|---|---|---|---|---|---|---|
| **Endpoint** | | ***Oncorhynchus mykiss*** $LC_{50}$ **>100 000 µg/L** | | ***Cyprinodon variegatus*** $LC_{50}$ **>650 µg/L** | | | **Metalaxyl-M** | **Oxathiapiprolin** |
| **FOCUS Scenario** | **Use pattern** | **PECsw** | **Toxic Unit** | **PECsw** | **Toxic Unit** | | | |
| Step 1 | Leafy vegetables | 56.79 | 0.000568 | 1.35 | 0.00208 | 0.00264 | 21.5 | 78.5 |
| Step 2 - NEU | | 5.21 | 0.0000521 | 0.19 | 0.000292 | 0.000344 | 15.1 | 84.9 |
| Step 2 - SEU | | 9.19 | 0.0000919 | 0.34 | 0.000523 | 0.000615 | 14.9 | 85.1 |
| Step 1 | Onion and bulb like crops | 56.79 | 0.000568 | 1.35 | 0.00208 | 0.00264 | 21.5 | 78.5 |
| Step 2 - NEU | | 6.01 | 0.0000601 | 0.22 | 0.000338 | 0.000399 | 15.1 | 84.9 |
| Step 2 - SEU | | 10.79 | 0.000108 | 0.41 | 0.000631 | 0.000739 | 14.6 | 85.4 |

TU: Toxic Units

**Table 9.5-35: Aquatic organisms: Toxic Units (TU) approach to identify risk driver – fish chronic**

| Active substance | | Metalaxyl-M | | Oxathiapiprolin | | SUM Toxic Units | Toxic Unit as % of total | |
|---|---|---|---|---|---|---|---|---|
| Endpoint | | *Pimephales promelas* *NOEC = 9 100 µg/L* | | *Cyprinodon variegatus* *NOEC = 340 µg/L* | | | Metalaxyl-M | Oxathiapiprolin |
| FOCUS Scenario | Use pattern | PECsw | Toxic Unit | PECsw | Toxic Unit | | | |
| Step 1 | Leafy vegetables | 56.79 | 0.00624 | 1.35 | 0.00397 | 0.0102 | 61.1 | 38.9 |
| Step 2 - NEU | | 5.21 | 0.000573 | 0.19 | 0.000559 | 0.00113 | 50.6 | 49.4 |
| Step 2 - SEU | | 9.19 | 0.00101 | 0.34 | 0.00100 | 0.00201 | 50.2 | 49.8 |
| Step 1 | Onion and bulb like crops | 56.79 | 0.00624 | 1.35 | 0.00397 | 0.0102 | 61.1 | 38.9 |
| Step 2 - NEU | | 6.01 | 0.000660 | 0.22 | 0.000647 | 0.00131 | 50.5 | 49.5 |
| Step 2 - SEU | | 10.79 | 0.00119 | 0.41 | 0.00121 | 0.00239 | 49.6 | 50.4 |

TU: Toxic Units

**Table 9.5-36: Aquatic organisms: Toxic Units (TU) approach to identify risk driver – invertebrate acute**

| Active substance | | Metalaxyl-M | | Oxathiapiprolin | | SUM Toxic Units | Toxic Unit as % of total | |
|---|---|---|---|---|---|---|---|---|
| Endpoint | | *Crassostrea virginica* *$EC_{50}$ = 5 600 µg/L* | | *Crassostrea virginica* *$EC_{50}$ >330 µg/L* | | | Metalaxyl-M | Oxathiapiprolin |
| FOCUS Scenario | Use pattern | PECsw | Toxic Unit | PECsw | Toxic Unit | | | |
| Step 1 | Leafy vegetables | 56.79 | 0.0101 | 1.35 | 0.00409 | 0.0142 | 71.3 | 28.7 |
| Step 2 - NEU | | 5.21 | 0.000930 | 0.19 | 0.000576 | 0.00151 | 61.8 | 38.2 |
| Step 2 - SEU | | 9.19 | 0.00164 | 0.34 | 0.00103 | 0.00267 | 61.4 | 38.6 |
| Step 1 | Onion and bulb like crops | 56.79 | 0.0101 | 1.35 | 0.00409 | 0.0142 | 71.3 | 28.7 |
| Step 2 - NEU | | 6.01 | 0.00107 | 0.22 | 0.000667 | 0.00174 | 61.7 | 38.3 |
| Step 2 - SEU | | 10.79 | 0.00193 | 0.41 | 0.00124 | 0.00317 | 60.8 | 39.2 |

TU: Toxic Units

**Table 9.5-37: Aquatic organisms: Toxic Units (TU) approach to identify risk driver – invertebrate prolonged**

| Active substance | | Metalaxyl-M | | Oxathiapiprolin | | SUM Toxic Units | Toxic Unit as % of total | |
|---|---|---|---|---|---|---|---|---|
| Endpoint | | *Daphnia magna* NOEC = 1 000 µg/L | | *Americamysis bahia* NOEC = 58 µg/L | | | Metalaxyl-M | Oxathiapiprolin |
| FOCUS Scenario | Use pattern | PECsw | Toxic Unit | PECsw | Toxic Unit | | | |
| Step 1 | Leafy vegetables | 56.79 | 0.0568 | 1.35 | 0.0233 | 0.0801 | 70.9 | 29.1 |
| Step 2 - NEU | | 5.21 | 0.00521 | 0.19 | 0.00328 | 0.00849 | 61.4 | 38.6 |
| Step 2 - SEU | | 9.19 | 0.00919 | 0.34 | 0.00586 | 0.0151 | 61.1 | 38.9 |
| Step 1 | Onion and bulb like crops | 56.79 | 0.0568 | 1.35 | 0.0233 | 0.0801 | 70.9 | 29.1 |
| Step 2 - NEU | | 6.01 | 0.00601 | 0.22 | 0.00379 | 0.00980 | 61.3 | 38.7 |
| Step 2 - SEU | | 10.79 | 0.0108 | 0.41 | 0.00707 | 0.0179 | 60.4 | 39.6 |

TU: Toxic Units

**Table 9.5-38: Aquatic organisms: Toxic Units (TU) approach to identify risk driver – algae**

| Active substance | | Metalaxyl-M | | Oxathiapiprolin | | SUM Toxic Units | Toxic Unit as % of total | |
|---|---|---|---|---|---|---|---|---|
| Endpoint | | *Scenedesmus quadricanda* $E_rC_{50}$ = 19 950 µg/L | | *Pseudokirchneriella subcapitata* $E_rC_{50}$ >142 µg/L | | | Metalaxyl-M | Oxathiapiprolin |
| FOCUS Scenario | Use pattern | PECsw | Toxic Unit | PECsw | Toxic Unit | | | |
| Step 1 | Leafy vegetables | 56.79 | 0.00285 | 1.35 | 0.00951 | 0.0124 | 23.0 | 77.0 |
| Step 2 - NEU | | 5.21 | 0.000261 | 0.19 | 0.00134 | 0.00160 | 16.3 | 83.7 |
| Step 2 - SEU | | 9.19 | 0.000461 | 0.34 | 0.00239 | 0.00286 | 16.1 | 83.9 |
| Step 1 | Onion and bulb like crops | 56.79 | 0.00285 | 1.35 | 0.00951 | 0.0124 | 23.0 | 77.0 |
| Step 2 - NEU | | 6.01 | 0.000301 | 0.22 | 0.00155 | 0.00185 | 16.3 | 83.7 |
| Step 2 - SEU | | 10.79 | 0.000541 | 0.41 | 0.00289 | 0.00343 | 15.8 | 84.2 |

TU: Toxic Units

The analyses presented in the tables above indicate that neither metalaxyl-M nor oxathiapiprolin is driving the risk assessment for any organism group (<90% of the sum of the toxic units for the mixture). Therefore, the assessment of the combined risk of metalaxyl-M and oxathiapiprolin is presented in the tables below.

Combination risk assessment

One method to evaluate the combination risk assessment is to sum the single substance risk quotients (*i.e.* PEC/RAC ratios), as described in Step 8 of the EFSA Aquatic GD (Equation 21).

*Equation 21*: $$RQ_{mix} = \sum_{i=1}^{n} \frac{PEC_i}{RAC_i}$$

If $RQ_{mix} < 1$, the combination risk is considered acceptable.

**Table 9.5-39: Combined risk assessment for metalaxyl-M and oxathiapiprolin following application of A23109A – Leafy vegetables (BBCH 12-49, 2 x 102.2 g total a.s./ha, application interval of 7 days)**

| Organism group | Substance | Maximum $PEC_{SW}$ µg/L | | RAC µg/L | PEC/RAC ratio | $RQ_{mix}$ |
|---|---|---|---|---|---|---|
| Acute fish | Metalaxyl-M | Step 1 | 56.79 | >1000 | 0.0568 | 0.265 |
| | Oxathiapiprolin | Step 1 | 1.35 | >6.5 | 0.208 | |
| Chronic fish | Metalaxyl-M | Step 1 | 56.79 | 910 | 0.0624 | 0.102 |
| | Oxathiapiprolin | Step 1 | 1.35 | 34 | 0.0397 | |
| Acute invertebrate | Metalaxyl-M | Step 1 | 56.79 | 56 | 1.01 | **1.42** |
| | Oxathiapiprolin | Step 1 | 1.35 | >3.3 | 0.409 | |
| | Metalaxyl-M | Step 2 NEU | 5.21 | 56 | 0.0930 | 0.151 |
| | Oxathiapiprolin | Step 2 NEU | 0.19 | >3.3 | 0.0576 | |
| | Metalaxyl-M | Step 2 SEU | 9.19 | 56 | 0.164 | 0.267 |
| | Oxathiapiprolin | Step 2 SEU | 0.34 | >3.3 | 0.103 | |
| Chronic invertebrate | Metalaxyl-M | Step 1 | 56.79 | 100 | 0.568 | 0.801 |
| | Oxathiapiprolin | Step 1 | 1.35 | 5.8 | 0.233 | |
| Algae | Metalaxyl-M | Step 1 | 56.79 | 1995 | 0.0285 | 0.124 |
| | Oxathiapiprolin | Step 1 | 1.35 | >14.2 | 0.0951 | |

Sum of PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

**Table 9.5-40: Combined risk assessment for metalaxyl-M and oxathiapiprolin following application of A23109A – Bulb & onion like crops (BBCH 12-48, 2 x 102.2 g total a.s./ha, application interval of 7 days)**

| Organism group | Substance | Maximum $PEC_{SW}$ µg/L | | RAC µg/L | PEC/RAC ratio | $RQ_{mix}$ |
|---|---|---|---|---|---|---|
| Acute fish | Metalaxyl-M | Step 1 | 56.79 | >1000 | 0.0568 | 0.265 |
| | Oxathiapiprolin | Step 1 | 1.35 | >6.5 | 0.208 | |
| Chronic fish | Metalaxyl-M | Step 1 | 56.79 | 910 | 0.0624 | 0.102 |
| | Oxathiapiprolin | Step 1 | 1.35 | 34 | 0.0397 | |
| Acute invertebrate | Metalaxyl-M | Step 1 | 56.79 | 56 | 1.01 | **1.42** |
| | Oxathiapiprolin | Step 1 | 1.35 | >3.3 | 0.409 | |
| | Metalaxyl-M | Step 2 NEU | 6.01 | 56 | 0.107 | 0.174 |
| | Oxathiapiprolin | Step 2 NEU | 0.22 | >3.3 | 0.0667 | |
| | Metalaxyl-M | Step 2 SEU | 10.79 | 56 | 0.193 | 0.317 |
| | Oxathiapiprolin | Step 2 SEU | 0.41 | >3.3 | 0.124 | |
| Chronic invertebrate | Metalaxyl-M | Step 1 | 56.79 | 100 | 0.568 | 0.801 |
| | Oxathiapiprolin | Step 1 | 1.35 | 5.8 | 0.233 | |
| Algae | Metalaxyl-M | Step 1 | 56.79 | 1 995 | 0.0285 | 0.124 |
| | Oxathiapiprolin | Step 1 | 1.35 | >14.2 | 0.0951 | |

Sum of PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

The $RQ_{mix}$ values using worst-case RAC and Step 1 $PEC_{SW}$ values are less than the trigger value of 1 for the fish, chronic invertebrate and algal risk assessments. For acute invertebrates the $RQ_{mix}$ values were <1 when Step 2 $PEC_{SW}$ values were considered. Thus, the risks to aquatic organisms from the combined effects of metalaxyl-M and oxathiapiprolin are acceptable following use of A23109A according to the proposed use pattern.

### 9.5.3 Overall conclusions

The PEC/RAC ratios, using worst-case Step 1 or Step 2 $PEC_{SW}$ values for metalaxyl-M, oxathiapiprolin and their metabolites are less than the trigger value of 1, for all aquatic organisms.

The PEC/RAC ratios, using worst-case $PEC_{SW}$ values for A23109A, are also less than the trigger value of

1, for all aquatic organisms.

The toxic unit analysis indicated that neither metalaxyl-M nor oxathiapiprolin was driving the toxicity of the mixture therefore a quantitative mixture toxicity risk assessment was conducted. The $RQ_{mix}$ values for all organism groups are below the trigger value of 1 when worst case Step 1 or Step 2 $PEC_{SW}$ values are considered.

Acceptable risks to aquatic organisms following the proposed uses of A23109A have therefore been demonstrated without the need for any application mitigation.

**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 initial risk assessment was based on the worst case $PEC_{sw}$ values and the results of laboratory toxicity testing.

For active substances and relevant metabolites $PEC_{SW}$ calculations were performed with FOCUS STEPS 1-2 (active substances and metabolites).

The calculated PEC/RAC ratios indicate an acceptable risk for all groups of aquatic organisms without the need for any mitigation measures.

## 9.6 Effects on bees (KCP 10.3.1)

### 9.6.1 Toxicity data

Studies on the toxicity to bees have been carried out with metalaxyl-M and oxathiapiprolin. Full details of these studies are provided in the respective EU DAR and related documents as well as in Appendix 2 of this document (for new studies).

Effects on bees of A23109A were not evaluated as part of the EU assessment of metalaxyl-M and oxathiapiprolin. New data submitted with this application are listed in (see Appendix 1) and summarised in Appendix 2.

The selection of studies and endpoints for the acute oral and contact adult bee risk assessments is in line with the results of the EU review process, whilst new studies have been conducted to address the potential chronic exposure of adult honeybees and honeybee larvae.

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

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| *Apis mellifera* | Metalaxyl-M | Oral | **$LD_{50}$ >97.3 µg a.s./bee** | EFSA Journal 2015;13(3):3999; Kling 2011; CGA329351/11459 |
| *Apis mellifera* | Metalaxyl-M | Contact | **$LD_{50}$ >100 µg a.s./bee** | EFSA Journal 2015;13(3):3999; Kling 2011; CGA329351/11459 |
| *Apis mellifera* | Metalaxyl-M | Contact | $LD_{50}$ >25 µg a.s./bee | EFSA Journal 2015; 13(3):3999 Palmer & Beavers, 1995b |

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| | | | | CGA329351/0303 |
| *Bombus terrestris* | Metalaxyl-M | Oral | $LD_{50}$ >88.5 µg consumed a.s./bumble bee | Amsel 2021; VV-939735 |
| *Bombus terrestris* | Metalaxyl-M | Contact | $LD_{50}$ >200 µg a.s./bumble bee | Amsel 2021; VV-939735 |
| *Apis mellifera* | A9642C (APRON) | Oral | $LD_{50}$ = >334 µg/bee | EFSA Journal 2015;13(3):3999; Kling, 2012; A9642C_10040 |
| *Apis mellifera* | A9642C (APRON) | Contact | $LD_{50}$ = 675 µg/bee | EFSA Journal 2015;13(3):3999; Bocksch, 2005; CGA329351/2151 |
| *Apis mellifera* | A9651D (RIDOMIL GOLD) | Oral | $LD_{50}$ = >613 µg/bee | EFSA Journal 2015;13(3):3999; Kling, , 2012; A9642C_10040 |
| *Apis mellifera* | A9651D (RIDOMIL GOLD) | Contact | $LD_{50}$ = >1471 µg/bee | EFSA Journal 2015;13(3):3999; Bocksch, 2005; CGA329351/2151 |
| *Apis mellifera* | Metalaxyl-M (tested as A13947A) | 10 d Oral | $LDD_{50}$ = 67.8 µg A13947A/bee/day (31 µg a.s./bee/day [a]) | Kling A. 2015;VV414721 Study not evaluated by zRMS |
| *Apis mellifera* | Metalaxyl-M | 10 d Oral | **$LDD_{50}$ = 20.3 µg a.s./bee/day** | Ruhland S. 2021; VV-932880 Study not evaluated by zRMS |
| *Apis mellifera* | Metalaxyl-M (tested as A13947A) | 8 d Larval | NOED = 346.5 µg A13947A/larva (157 µg a.s./larva/development period [a]) | Eckert J. 2016; VV-415529 Study not evaluated by zRMS |
| *Apis mellifera* | Metalaxyl-M | 22 d Larval | **NOED = 40.0 µg a.s./larva/development period** | Schmidt K. 2021; VV-930784 Study not evaluated by zRMS |
| **Higher-tier studies (tunnel test, field studies):** not required | | | | |

[a] converted to active substance consumption considering nominal active substance content of 45.2% w/w
Bold values are used for the risk assessment
EFSA Journal 2015;13(3):3999. [105 pp.] doi:10.2903/j.efsa.2015

**Table 9.6-2: Endpoints and effect values relevant for the risk assessment for bees – oxathiapiprolin and its relevant metabolites**

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| *Apis mellifera* | Oxathiapiprolin | Oral | **$LD_{50}$ >40.26 µg a.s./bee** | EFSA 2016;14(7):4504, DuPont-32476 |

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| *Apis mellifera* | Oxathiapiprolin | Contact | **$LD_{50}$ >100.0 µg a.s./bee** | EFSA 2016;14(7):4504, DuPont-32476 |
| *Apis mellifera* | Metabolite IN-E8S72 | Oral | $LD_{50}$ >109.0 µg met./bee | EFSA 2016;14(7):4504, DuPont-37896 |
| *Apis mellifera* | Metabolite IN-E8S72 | Contact | $LD_{50}$ >100.0 µg met./bee | EFSA 2016;14(7):4504, DuPont-37896 |
| *Apis mellifera* | Metabolite IN-WR791 | Oral | $LD_{50}$ >56.2 µg met./bee | EFSA 2016;14(7):4504, DuPont-37897 |
| *Apis mellifera* | Metabolite IN-WR791 | Contact | $LD_{50}$ >100.0 µg met./bee | EFSA 2016;14(7):4504, DuPont-37897 |
| *Apis mellifera* | Oxathiapiprolin 100 g/L OD | Oral | $LD_{50}$ >137.4 µg a.s./bee | EFSA 2016;14(7):4504, DuPont-31006 |
| *Apis mellifera* | Oxathiapiprolin 100 g/L OD | Contact | $LD_{50}$ >100 µg a.s./bee | EFSA, 2 EFSA 2016;14(7):4504016, DuPont-31006 |
| *Apis mellifera* | Oxathiapiprolin 100 g/L OD | Oral Adult Chronic (10-day) | **$LDD_{50}$ = 34.7 µg a.s./bee/day** NOED = 24.1 µg a.s./bee/day | Tänzler, 2015, DuPont-41989 Study not evaluated by zRMS |
| *Apis mellifera* | Oxathiapiprolin | Oral Larval Development (22-day, repeat dose) | **NOED = 7.02 µg a.s./larva/ development period** NOEC = 45.6 mg a.s./kg diet | Oberrauch, 2017, DuPont-48606 Study not evaluated by zRMS |
| **Higher-tier studies (tunnel test, field studies)** | | | | |
| *Apis mellifera* | Oxathiapiprolin 100 g/L OD | Bee brood, semi-field | NOEC = 180.0 g a.s./ha | EFSA 2016;14(7):4504, DuPont-34268 |

Values in **bold** are used in the initial risk assessment
EFSA Journal 2016;14(7):4504. [19 pp.] doi:10.2903/j.efsa.2016.4504.

**Table 9.6-3: Endpoints and effect values relevant for the risk assessment for bees – A23109A**

| Species | Substance | Exposure system | Results | Reference |
|---|---|---|---|---|
| *Apis mellifera* | A23109A | Oral | $LD_{50}$ >959 µg product/bee<br>**$LD_{50}$ >187 µg total a.s./bee** [a] | Franke 2021; VV-892684 |
| *Apis mellifera* | A23109A | Contact | $LD_{50}$ >1000 µg product/bee<br>**$LD_{50}$ >195 µg total a.s./bee** [a] | Franke 2021; VV-892684 |
| *Bombus terrestris* | A23109A | Oral | $LD_{50}$ >357.9 µg consumed product/bumble bee<br>$LD_{50}$ >69.6 µg consumed total a.s./bumble bee [a] | Amsel 2022; VV-936483 |
| *Bombus terrestris* | A23109A | Contact | $LD_{50}$ >1000 µg product/bumble bee<br>$LD_{50}$ >194.5 µg total a.s./bumble bee [a] | Amsel 2022; VV-936483 |
| *Apis mellifera* | A23109A | Oral Adult Chronic (10-day) | $LDD_{50}$ = 117 µg product/bee/day<br>NOED = 57.4 µg product/bee/day<br>**$LDD_{50}$ = 22.8 µg total a.s./bee/day**<br>NOED = 11.2 µg total a.s./bee/day | Dreßler 2020; VV-896929 |
| *Apis mellifera* | A23109A | Oral Larval Development (22-day, repeat dose) | NOED = 24 µg product/larva/development period<br>NOEC = 155 mg product/kg diet<br>**NOED = 4.67 µg total a.s./larva/development period**<br>NOEC = 30.1 mg total a.s./kg diet | Schmidt 2020; VV-895633 |

Endpoints used for risk assessment are shown in **bold**.

[a] Formulation endpoint expressed as total a.s. (based on the total % w/w of metalaxyl-M (16.6%) and oxathiapiprolin (2.85%) in the formulation (19.45% w/w)

### 9.6.1.1 Justification for new endpoints

Since active substance approval, new studies with metalaxyl-M, oxathiapiprolin and A23109A have been performed in accordance with Regulation (EC) No 1107/2009 to address the data requirements. New endpoints are used in the risk assessment. The results of the studies are summarised in Table 9.6-1 to Table 9.6-3. Study summaries are provided in Appendix 2.

## 9.6.2 Risk assessment

The evaluation of the risk for bees was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services[8] (hereafter referred to as SANCO/10329/2002 rev.2) referring to the Environmental risk assessment scheme for plant

[8] SANCO/10329/2002 rev.2: Anonymous, 2002. Guidance Document on terrestrial ecotoxicology under Council Directive 91/414/EEC. SANCO/10329/2002. 17 October 2002.

protection products, Chapter 10: Honeybees (PP 3/10(3), Bulletin OEPP/EPPO Bulletin 40: 323-331, 2010 (hereafter referred to as EPPO, 2010) as proposed in the list of Guidance Documents relevant to the implementation of Regulation (EC) No 1107/2009, published in the official EU Journal 2013/C 95/01 and 95/02.

The applicant considers that risk assessment to the EFSA guidance[9] is not appropriate for regulatory decision making at EU level as the guidance is not agreed by all Member States and as such has not been noted. However, given recent requests by EFSA and many Members States an assessment has been provided by the applicant below. Areas where a lot of uncertainty in approach still exist (*e.g.*, water exposure, HPG assessment and bumble and solitary bee assessments) have not been addressed.

The EFSA risk assessment guidance[9] is structured in a stepwise manner beginning with a screening step assessment. Those scenarios which pass the screening step are considered to demonstrate acceptable risk and as such will not be considered at higher tiers of the assessment.

All calculations are performed using the EFSA Bee calculator Tool (Bee-Tool v.3; Date accessed 07/01/2022)[10]. Where the Screening Step indicates a potential risk for acute or chronic exposure to bees and/or bee brood a Tier 1 risk assessment is performed.

The treated crop is considered by the applicant to represent the worst-case[11] exposure as all other scenarios are considered to have lower exposure *e.g.* field margins, adjacent crop *etc*. Therefore, only contact and oral exposure in the treated crop is considered at Tier 1 and where this indicates ETR values below the triggers, acceptable risk to bees is demonstrated.

**Table 9.6-4: Crop groupings and critical use patterns relevant to the use of A23109A**

| Test substance | GAP crop species | Application category | Critical use pattern | | |
|---|---|---|---|---|---|
| | | | Rate (kg a.s./ha) | No. of apps | App. Interval (days) |
| Metalaxyl-M | Leafy vegetables, BBCH 12 - 49 | Downward spray | 0.0872 | 2 | 7 |
| Metalaxyl-M | Bulbs and onion like crops, BBCH 12 - 48 | Downward spray | 0.0872 | 2 | 7 |
| Oxathiapiprolin | Leafy vegetables, BBCH 12 - 49 | Downward spray | 0.015 | 2 | 7 |
| Oxathiapiprolin | Bulbs and onion like crops, BBCH 12 - 48 | Downward spray | 0.015 | 2 | 7 |
| A23109A | Leafy vegetables, BBCH 12 - 49 | Downward spray | 0.1022 [a] | 2 | 7 |
| A23109A | Bulbs and onion like crops, BBCH 12 - 48 | Downward spray | 0.1022 [a] | 2 | 7 |

[a] Based upon an application rate of 87.2 g a.s./ha for metalaxyl-M and 15 g a.s./ha for oxathiapiprolin

[9] EFSA Bee Guidance Document, 2013: European Food Safety Authority, 2013. EFSA Guidance Document on the risk assessment of plant protection products on bees (Apis mellifera, Bombus spp. and solitary bees). EFSA Journal 2013;11(7):3295, 268 pp., doi:10.2903/j.efsa.2013.3295 – updated 2014.

[10] EFSA Bee calculator Tool (Bee-Tool v.3) available at http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2013.3295/full

[11] Exposure to treated weeds is not considered a relevant exposure scenario according to the guidance as evidence is available to demonstrate that in arable crops flowering attractive weeds are not present at >10% of the area of use: Last *et. al*., 2019; Regulatory report on the occurrence of flowering weeds in agricultural fields. ERM, Harrogate, North Yorkshire, UK. The full regulatory report is available through ECPA on request.

### 9.6.2.1 Hazard quotients for bees

**Risk assessment according to SANCO/10329/2002 rev.2**

Acute honeybee studies have been conducted with the active substances metalaxyl-M and oxathiapiprolin and the formulated product A23109A according to the data requirements under 1107/2009. The potential risk to honeybees from use of A23109A is assessed using the maximum single application rate and the $LD_{50}$ values to calculate hazard quotients in accordance with SANCO/10329/2002 rev.2.

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

Compounds with hazard quotients below 50 are considered low risk to bees.

**Table 9.6-5: First-tier assessment of the risk for bees due to the use of A23109A at maximum single use rate – metalaxyl-M**

| **Active substance**<br>**Application rate (g a.s./ha)** | Metalaxyl-M<br>87.2 | | |
|---|---|---|---|
| **Test design** | **$LD_{50}$ (lab.)**<br>**(µg a.s./bee)** | **Single application rate**<br>**(g a.s./ha)** | **$Q_{HO}$, $Q_{HC}$**<br>**criterion: $Q_H \leq 50$** |
| Oral toxicity | >97.3 | 87.2 | <0.90 |
| Contact toxicity | >100 | 87.2 | <0.87 |

$Q_{HO}$, $Q_{HC}$: Hazard quotients for oral and contact exposure

**Table 9.6-6: First-tier assessment of the risk for honeybees due to the use of A23109A at maximum single use rate – oxathiapiprolin**

| **Active substance**<br>**Application rate (g a.s./ha)** | Oxathiapiprolin<br>15 | | |
|---|---|---|---|
| **Test design** | **$LD_{50}$ (lab.)**<br>**(µg a.s./bee)** | **Single application rate**<br>**(g a.s./ha)** | **$Q_{HO}$, $Q_{HC}$**<br>**criterion: $Q_H \leq 50$** |
| Oral toxicity | >40.26 | 15 | <0.37 |
| Contact toxicity | >100 | 15 | <0.15 |

$Q_{HO}$, $Q_{HC}$: Hazard quotients for oral and contact exposure

**Table 9.6-7: First-tier assessment of the risk for bees due to the use of A23109A at maximum single use rate – A23109A**

| **Product**<br>**Application rate (g total a.s./ha)[a]** | A23109A<br>102.2 | | |
|---|---|---|---|
| **Test design** | **$LD_{50}$ (lab.)**<br>**(µg total a.s./bee)** | **Single application rate**<br>**(g total a.s./ha)** | **$Q_{HO}$, $Q_{HC}$**<br>**criterion: $Q_H \leq 50$** |
| Oral toxicity | >187 | 102.2 [a] | <0.55 |
| Contact toxicity | >195 | 102.2 [a] | <0.52 |

$Q_{HO}$, $Q_{HC}$: Hazard quotients for oral and contact exposure
[a] Based upon an application rate of 87.2 g a.s./ha for metalaxyl-M and 15 g a.s./ha for oxathiapiprolin

Overall, the acute oral and contact hazard quotients are less than the relevant trigger of 50, indicating that the risk to bees is acceptable following use of A23109A according to the proposed use pattern.

**Risk assessment according to EFSA Bee Guidance Document (2013)**

**Screening step - Acute and Chronic Risk Assessment**

Acute, chronic adult and larval honeybee studies have been conducted with metalaxyl-M and oxathiapiprolin (a.s. or formulated products) according to the data requirements under 1107/2009. The endpoints from these studies have been assessed by using EFSA Bee Guidance Document (2013)[9] and EFSA Bee calculator Tool[10].

**Table 9.6-8: Screening Step assessment of the risk for honeybees due to the use of formulation A23109A on leafy vegetables and bulb & onion like crops – metalaxyl-M**

| | | | | | |
|---|---|---|---|---|---|
| **Intended use** | Downward Spray | | | | |
| **Active substance** | Metalaxyl-M | | | | |
| **Application rate (g a.s./ha)** | 2 × 87.2 | | | | |
| **Test design** | **Endpoint (lab.)** | **Single application rate** | **Calculation factor (Ef x SV)** | **HQ / ETR** | **Trigger** |
| Acute contact toxicity $LD_{50}$ | >100 µg a.s./bee | 87.2 g a.s./ha | 1 | <0.9 | ≤42 |
| Acute oral toxicity $LD_{50}$ | >97.3 µg a.s./bee | 0.0872 kg a.s./ha | 7.6 | < 0.01 | ≤0.2 |
| Chronic adult oral toxicity $LDD_{50}$ | 20.3 µg a.s./bee/day | 0.0872 kg a.s./ha | 7.6 | **0.033** | ≤0.03 |
| Larval development NOED | 40.0 µg a.s./larva | 0.0872 kg a.s./ha | 4.4 | 0.01 | ≤0.2 |

HQ (hazard quotients) and ETR (exposure toxicity ratio) for oral and contact exposure. HQ/ETR values shown in **bold** breach the relevant trigger.

The HQ / ETR values for metalaxyl-M are less than the screening step trigger values for downward spray indicating that the acute and larval risk to honeybees is acceptable following use of A23109A in leafy vegetables and bulb & onion like crops according to the proposed use pattern. However, the screening step risk assessment has indicated a potential chronic risk for adult honeybees following chronic oral exposure and therefore a Tier 1 risk assessment for the treated crop is provided.

**Table 9.6-9: Screening Step assessment of the risk for honeybees due to the use of formulation A23109A on leafy vegetables and bulb & onion like crops – oxathiapiprolin**

| | | | | | |
|---|---|---|---|---|---|
| **Intended use** | Downward Spray | | | | |
| **Active substance** | Oxathiapiprolin | | | | |
| **Application rate (g a.s./ha)** | 2 × 15 | | | | |
| **Test design** | **Endpoint (lab.)** | **Single application rate** | **Calculation factor (Ef x SV)** | **HQ / ETR** | **Trigger** |
| Acute contact toxicity $LD_{50}$ | >100 µg a.s./bee | 15 g a.s./ha | 1 | <0.2 | ≤42 |
| Acute oral toxicity $LD_{50}$ | >40.26 µg a.s./bee | 0.015 kg a.s./ha | 7.6 | 0.0028 | ≤0.2 |
| Chronic adult oral toxicity $LDD_{50}$ | 34.7 µg a.s./bee/day | 0.015 kg a.s./ha | 7.6 | 0.0033 | ≤0.03 |
| Larval development NOED | 7.02 µg a.s./larva | 0.015 kg a.s./ha | 4.4 | 0.0094 | ≤0.2 |

HQ (hazard quotients) and ETR (exposure toxicity ratio) for oral and contact exposure

The HQ / ETR values for oxathiapiprolin are less than the screening step trigger values for downward spray indicating that the acute, chronic and larval risk to honeybees is acceptable following use of A23109A in leafy vegetables and bulb & onion like crops according to the proposed use pattern.

**Combination mixture assessment**

**Acute and chronic mixture toxicity**

According to the EFSA Bee Guidance Document, 2013[9], combined action of mixture toxicity and toxicity of formulated products with two or more active substances should be considered in the risk assessment when it is obvious that such exposure situations will occur for bees.

For the assessment of acute and chronic effects, surrogate endpoints ($LD_{50}$, $LDD_{50}$, NOED) can be calculated. The EFSA Bee Guidance Document, 2013[9], indicates that the following equation should be used for deriving a surrogate endpoint ($LD_{50}$, $LDD_{50}$, NOED) for a mixture of active substances with known toxicity assuming dose additivity:

$$1/EC_x \text{ (mix) or } 1/NOEC \text{ (mix)} = \left( \sum_i \frac{X(a.s._i)}{EC_x\_or\_NOEC(a.s._i)} \right)$$

*where:*
*$X(a.s._i)$ = fraction of active substance [i] in the mixture (please note that the sum $\Sigma X(a.s._i)$ must be 1)*
*ECx or $NOEC(a.s._i)$ = toxicity value for active substance [i] (for the same endpoint)*

Where the toxicity value of a formulated product with more than one active substance is available (acute and chronic studies), this value should be compared with the predicted mixture toxicity assuming dose additivity. A different form of the equation is used.

$$\sum_i \frac{X(a.s._i)}{EC_x\_or\_NOEC(a.s._i)} = \frac{1}{EC_x\_or\_NOEC(mix)}$$

*$X(a.s._i)$ = fraction of active substance [i] in the mixture*

*ECx or NOEC(a.s.$_i$)* = *acute toxicity value for active substance [i]*
*ECx or NOEC(mix)* = *measured acute toxicity value for the mixture.*

A greater value on the right side of the equation indicates that the formulation is more toxic than predicted from the toxicity of the individual components (active substances and co-formulants of known toxicity). If, in contrast, the measured toxicity of a formulation is lower than predicted, the predicted mixture toxicity should be used in the first-tier risk assessment.

The calculated mixture endpoints are summarised in the following table.

**Table 9.6-10: Calculated mixture toxicity endpoints for A23109A and comparison to measured endpoints**

| Exposure system (endpoint) | Test substance | Concentration of active substance in formulation A23109A (g a.s./L) | Fraction of active substance in the formulation mixture [a] | Toxicity endpoint [b] | Predicted endpoint for mixture (µg total a.s.)[c] | Measured endpoint for mixture (based on total a.s.) | Endpoint used for risk assessment based on comparison |
|---|---|---|---|---|---|---|---|
| **Acute oral** | Metalaxyl-M | 178 | 0.853 | >97.3 | >80.6 | >187 | use predicted endpoint |
| | Oxathiapiprolin | 30.6 | 0.147 | >40.26 | | | |
| | Total | 208.6 | 1 | - | | | |
| **Acute contact** | Metalaxyl-M | 178 | 0.853 | >100 | >100 | >195 | use predicted endpoint |
| | Oxathiapiprolin | 30.6 | 0.147 | >100 | | | |
| | Total | 208.6 | 1 | - | | | |
| **Chronic oral, adult** | Metalaxyl-M | 178 | 0.853 | 20.3 | 21.6 | 22.8 | use predicted endpoint |
| | Oxathiapiprolin | 30.6 | 0.147 | 34.7 | | | |
| | Total | 208.6 | 1 | - | | | |
| **Larval development** | Metalaxyl-M | 178 | 0.853 | 40.0 | 23.7 | 4.67 | use measured endpoint |
| | Oxathiapiprolin | 30.6 | 0.147 | 7.02 | | | |
| | Total | 208.6 | 1 | - | | | |

[a] Concentration of an active substance in the formulation divided by the total concentration of all active substances in the formulation.
[b] $LD_{50}$ in µg a.s./bee, $LDD_{50}$ in µg a.s./bee/day for adult honeybees, NOED for bee larvae in µg a.s./larva/development period
[c] Used for comparison with measured toxicity of product. Predicted endpoint for the mixture (µg total a.s.) is calculated based on total content of the active substances (208.6 g a.s./L) with 178 g/L of metalaxyl-M and 30.6 g/L of oxathiapiprolin

In accordance with the above presented calculation, toxicity endpoints for metalaxyl-M and oxathiapiprolin and the formulation endpoint are used in the acute risk assessment according to SANCO/10329/2002 rev.2. In accordance with the EFSA Bee Guidance Document, 2013[10], predicted mixture endpoints are used in the acute and chronic adult risk assessments as the predicted endpoints are lower than the measured endpoints. However, for the larval risk assessment the measured endpoint has been used as this is lower than the predicted value.

**Acute and Chronic Mixture Assessment – Screening Step**

Lowest endpoints of metalaxyl-M, oxathiapiprolin and formulation A23109A (based on total a.s.) were used to calculate the mixture toxicity endpoints (
Table 9.6-10). A comparison of the available formulation data and the calculated endpoints based on toxicity of the active substances indicated that the measured acute and chronic adult toxicity is lower than the predicted toxicity therefore predicted endpoints for the formulation are used as a worst-case in the acute and chronic adult risk assessments. However, the measured larval toxicity was greater than the

predicted toxicity therefore the measured endpoint for the formulation has been used for the larval risk assessment.

**Table 9.6-11: Screening step assessment of the risk for honeybees due to the use of formulation A23109A on leafy vegetables and bulb & onion like crops – metalaxyl-M / oxathiapiprolin mixture**

| | | | | | |
|---|---|---|---|---|---|
| **Intended use** | Downward spray | | | | |
| **Product** | A23109A | | | | |
| **Application rate (g total a.s./ha)** | 2 x 102.2 [a] | | | | |
| **Test design** | **Endpoint** | **Single application rate** | **Calculation factor (Ef x SV)** | **HQ / ETR** | **Trigger** |
| Acute contact toxicity $LD_{50}$ | >100 µg total a.s./bee | 102.2 g total a.s./ha [a] | 1 | <1.0 | ≤42 |
| Acute oral toxicity $LD_{50}$ | >80.6 µg total a.s./bee | 0.1022 kg total a.s./ha [a] | 7.6 | < 0.0096 | ≤0.2 |
| Chronic adult oral toxicity $LDD_{50}$ | 21.6 µg total a.s./bee/day | 0.1022 kg total a.s./ha [a] | 7.6 | **0.036** | ≤0.03 |
| Larval development NOED | 4.67 µg total a.s./larva | 0.1022 kg total a.s./ha [a] | 4.4 | 0.096 | ≤0.2 |

HQ (hazard quotients) and ETR (exposure toxicity ratio) for oral and contact exposure. HQ/ETR values shown in **bold** breach the relevant trigger.

[a] metalaxyl-M 87.2 g/ha + oxathiapiprolin 15 g/ha

The HQ / ETR values for the metalaxyl-M/oxathiapiprolin mixture are less than the screening step trigger values for downward spray indicating that the acute oral and contact risk to adult bees and chronic risk to honeybee larvae is acceptable following the proposed uses of A23109A. However, the screening step risk assessment has indicated a potential chronic risk for adult honeybees following chronic oral exposure and therefore a Tier 1 risk assessment for the treated crop is provided.

**Tier 1 - Chronic Risk Assessment**

The screening step risk assessments have indicated potential chronic risk for adult honeybees from both metalaxyl-M and A23109A exposure based on the use of A23109A in leafy vegetables and bulb & onion like crops. Therefore, a Tier 1 assessment is provided here.

**Methodology to establish whether a single substance is driving the toxicity of a mixture**

**Table 9.6-12: Toxicity of metalaxyl-M and oxathiapiprolin in the mixture**

| Exposure system (endpoint) | Test substance | Concentration of active substance in formulation A23109A (g a.s./L) | Fraction of active substance in the formulation mixture [a] | Toxicity endpoint [b] | Fraction of active substance/$LDD_{50}$ for the active substance | Toxicity per fraction a.s. (mg/kg) | Toxicity per fraction quotient (%) | Single driver of toxicity |
|---|---|---|---|---|---|---|---|---|
| **Chronic oral, adult** | Metalaxyl-M | 178 | 0.853 | 20.3 | 0.0420 | 23.8 | 90.9 | Yes |
| | Oxathiapiprolin | 30.6 | 0.147 | 34.7 | 0.00423 | 237 | 9.1 | No |
| | Total | 208.6 | 1 | - | - | - | 100 | - |

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

[b] $LDD_{50}$ in µg a.s./bee/day for adult honeybees

Note: Calculations undertaken using unrounded values consequently they may not be reproducible when using the figures given in the table

Metalaxyl-M has a higher toxicity per fraction quotient (90.9%) in the formulation compared to oxathiapiprolin (9.1%). As the toxicity per fraction quotient for metalaxyl-M is >90% it can be concluded that metalaxyl-M is the single driver (≥90%) of the toxicity of the mixture. Thus, the chronic Tier 1 risk assessment has been conducted only for metalaxyl-M and is considered to also cover the chronic risk assessment for the mixture.

For leafy vegetables and bulb & onion like crops the treated crop scenario represents the worst case exposure situation, however, for lettuce the weeds in the treated field exposure scenario represents the worst case in terms of exposure therefore this exposure route has been included in the risk assessment below for completeness.

**Table 9.6-13: First-tier assessment of the chronic adult risk for honeybees due to the use of formulation A23109A in lettuce <u>for the treated crop</u> – metalaxyl-M**

| **Intended use** | | Downward spray (lettuce, BBCH 12 - 49) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Active substance | | Metalaxyl-M | | | | | | |
| Application rate (g a.s./ha) | | 2 × 87.2 | | | | | | |
| **Test design** | **Endpoint (lab.)** | **Single application rate** | **BBCH** | **SV (downward spray)** | **TWA** | **Ef** | **ETR** | **Trigger** |
| Chronic adult oral toxicity $LDD_{50}$ | 20.3 µg a.s./bee/day | 0.0872 kg a.s./ha | 10 - 49 | 0.92 | 0.72 | 1 | 0.003 | ≤0.03 |

SV: Shortcut value; TWA: Time-weighted average factor; Ef: Exposure factor; ETR (exposure toxicity ratio) for oral exposure

**Table 9.6-14: First-tier assessment of the chronic adult risk for honeybees due to the use of formulation A23109A in lettuce <u>for weeds</u> – metalaxyl-M**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Intended use** | | Downward spray (lettuce, BBCH 12 - 49) | | | | | | |
| Active substance | | Metalaxyl-M | | | | | | |
| Application rate (g a.s./ha) | | 2 × 87.2 | | | | | | |
| **Test design** | **Endpoint (lab.)** | **Single application rate** | **BBCH** | **SV (downward spray)** | **TWA** | **Ef** | **ETR** | **Trigger** |
| Chronic adult oral toxicity $LDD_{50}$ | 20.3 µg a.s./bee/day | 0.0872 kg a.s./ha | 10 - 49 | 2.9 | 0.72 | 1 | 0.009 | ≤0.03 |

SV: Shortcut value; TWA: Time-weighted average factor; Ef: Exposure factor; ETR (exposure toxicity ratio) for oral exposure

**Table 9.6-15: First-tier assessment of the chronic adult risk for honeybees due to the use of formulation A23109A in leafy vegetables <u>for the treated crop</u> – metalaxyl-M**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Intended use** | | Downward spray (leafy vegetables, BBCH 12 - 49) | | | | | | |
| Active substance | | Metalaxyl-M | | | | | | |
| Application rate (g a.s./ha) | | 2 × 87.2 | | | | | | |
| **Test design** | **Endpoint (lab.)** | **Single application rate** | **BBCH** | **SV (downward spray)** | **TWA** | **Ef** | **ETR** | **Trigger** |
| Chronic adult oral toxicity $LDD_{50}$ | 20.3 µg a.s./bee/day | 0.0872 kg a.s./ha | 10 - 49 | 5.8 | 0.72 | 1 | 0.018 | ≤0.03 |

SV: Shortcut value; TWA: Time-weighted average factor; Ef: Exposure factor; ETR (exposure toxicity ratio) for oral exposure;

**Table 9.6-16: First-tier assessment of the chronic adult risk for honeybees due to the use of formulation A23109A in bulb & onion like crops <u>for the treated crop</u> – metalaxyl-M**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Intended use** | | Downward spray (bulb vegetables, BBCH 12 - 48) | | | | | | |
| Active substance | | Metalaxyl-M | | | | | | |
| Application rate (g a.s./ha) | | 2 × 87.2 | | | | | | |
| **Test design** | **Endpoint (lab.)** | **Single application rate** | **BBCH** | **SV (downward spray)** | **TWA** | **Ef** | **ETR** | **Trigger** |
| Chronic adult oral toxicity $LDD_{50}$ | 20.3 µg a.s./bee/day | 0.0872 kg a.s./ha | 10 - 39 | 5.8 | 0.72 | 1 | 0.018 | ≤0.03 |
| | | | 40 - 69 | 5.8 | 0.72 | 1 | 0.018 | |

SV: Shortcut value; TWA: Time-weighted average factor; Ef: Exposure factor; ETR (exposure toxicity ratio) for oral exposure;

The Tier 1 ETR values for the chronic risk for metalaxyl-M are below the trigger value for downward spray, indicating that the chronic oral risk to honeybees is acceptable following the proposed uses of A23109A according to the proposed use pattern.

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

Not relevant.

### 9.6.3 Effects on bumble bees

The EFSA Bee Guidance document (2013)[9] has not yet been noted at the time of the submission of this dossier. In consideration of the recommendations of the "Technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology" (2015)[12] currently no risk assessment for bumble bees is required, given that the EFSA Bee Guidance document (2013)[9] has not yet been noted. Furthermore, EFSA stated that it cannot be recommended to routinely perform a risk assessment for bumble bees. Data are available for bumble bees and have been presented in Table 9.6-3.

### 9.6.4 Effects on solitary bees

In consideration of the recommendations of the "Technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology" (2015)[13] currently no risk assessment for solitary bees is required, given that the EFSA Bee Guidance document (2013)[10] has not yet been noted and no OECD technical guidance is available for acute tests on solitary bees. Furthermore, EFSA stated that it cannot be recommended to routinely perform a risk assessment for solitary bees, this is also in line with the feedback of different authorities. Therefore, no data or information is currently available for solitary bees.

### 9.6.5 Overall conclusions

The risk to honeybees was assessed following SANCO/10329/2002 rev.2 and EPPO, 2010 as proposed in the list of guidance documents relevant to the implementation of Regulation 1107/2009, published in the official EU Journal 2013/C 95/01 and 95/02.

The risk of A23109A to honeybees was assessed from hazard quotients, estimated from acute oral and contact studies with metalaxyl-M, oxathiapiprolin and A23109A. The acute oral and contact hazard quotients were less than the relevant trigger of 50, indicating that the risk to honeybees is acceptable following use of A23109A according to the proposed use pattern.

In addition, the acute risk to honeybees was assessed from hazard quotients (HQ) and Exposure Toxicity Ratios (ETRs) following EFSA Bee Guidance Document (2013) using endpoints from acute oral and contact studies with metalaxyl-M and oxathiapiprolin. Acute contact HQ and oral ETR values were less than the relevant triggers at the screening step, indicating acceptable acute risk to adult honeybees.

The chronic adult and larval risk of A23109A to honeybees was assessed from ETRs following EFSA Bee Guidance Document (2013) using endpoints from chronic adult oral and larval studies with metalaxyl-M and oxathiapiprolin. At the screening step assessment the ETR values for chronic adult oral and larval toxicity were below the relevant trigger values for oxathiapiprolin, as was the larval ETR for metalaxyl-M, thereby demonstrating acceptable risks to honeybees following the proposed uses of A23109A.

A Tier 1 chronic adult oral risk assessment for metalaxyl-M in the treated crop and for lettuce also in weeds, representing the worst case exposure scenario, was conducted. The ETR values for all proposed uses were below the relevant trigger values thereby demonstrating an acceptable chronic risk to adult honeybees following the proposed uses of A23109A.

The screening step assessment for the combined effects of metalaxyl-M and oxathiapiprolin indicated an

[12] EFSA (European Food Safety Authority), 2015. Technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology. EFSA supporting publication 2015:EN-924. 62 pp.

acceptable risk to honeybees for acute oral and contact exposure and larval exposure but a Tier I risk assessment was necessary for chronic adult oral exposure. However, an assessment of the contribution of each active substance to the mixture toxicity showed that metalaxyl-M was driving the toxicity (>90%) therefore the Tier I chronic adult oral risk assessment for the mixture is covered by the Tier I risk assessment for metalaxyl-M which demonstrated acceptable risks to honeybees following the proposed uses of A23109A.

**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 submitted risk assessment, based on laboratory studies, has been accepted. It can therefore be concluded that there will be negligible acute risk associated with the exposure of *Apis mellifera* to A23109A.

The data requirements in accordance with Commission Regulation (EU) No 284/2013 for the chronic toxicity to adult honeybees and honeybee larvae are fulfilled.

There is not harmonized approach for the chronic risk assessment for bees, therefore, Concerned Member States must decide on the acceptability of EFSA Bee Guidance Document (2013) approach at national level. New studies with active substances were not evaluated by zRMS.

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

### 9.7.1 Toxicity data

Studies on the toxicity to non-target arthropods have been carried out with the representative formulations of metalaxyl-M and oxathiapiprolin. Full details of these studies are provided in the respective EU DAR and related documents, and a summary of the endpoints from laboratory tests is given below.

Effects on non-target arthropods of A23109A were not evaluated as part of the EU assessment of metalaxyl-M and oxathiapiprolin. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

The selection of studies and endpoints for the risk assessment deviates from the results of the EU review process. Justifications are provided below.

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

| Species | Substance | Exposure System | Results | Reference [a] |
|---|---|---|---|---|
| *Aleochara bilineata* (adults) | APRON (A9642C) | Laboratory test, treated sunflower seeds in quartz sand (2D) | $LR_{50}$ = 1.68 mg A9642C/kg dw<br><br>< 50% effect in mortality and reproduction at 300 g/100 kg seeds (equivalent to 1.68 mg A9642C/kg dw [B]) | EFSA Journal 2015;13(3):3999 Candolfi, 1997 CGA329351/0580 |

| Species | Substance | Exposure System | Results | Reference [a] |
|---|---|---|---|---|
| *Poecilus cupreus* (adults) | APRON (A9642C) | Laboratory test, treated sunflower seeds in quartz sand (2D) | $LR_{50}$ = 1.29 mg A9642C/kg dw<br><br>< 50% effect at 300 g/100 kg seeds (equivalent to 1.29 mg A9642C/kg dw [B]) | EFSA Journal 2015; 13(3):3999<br>Reber, 1997<br>CGA329351/0589 |
| *Typhlodromus pyri* (protonymphs) | RIDOMIL GOLD (A9651D) | Laboratory test glass plates (2D) | 187.5 < $LR_{50}$ < 2500 | EFSA Journal 2015; 13(3):3999<br>Grimm, 1999<br>CGA329351/1086 |
| *Aleochara bilineata* (adults) | RIDOMIL GOLD (A9651D) | Laboratory test, quartz sand (2D) | $LR_{50}$ > 7500 g/ha<br><br>$ER_{50}$ > 3700 g/ha | EFSA Journal 2015; 13(3):3999<br>Hoogendoorn, 2000<br>CGA329351/1238 |
| *Poecilus cupreus* (adults) | RIDOMIL GOLD (A9651D) | Laboratory test, quartz sand (2D) | $LR_{50}$ > 7500 g/ha<br><br>$ER_{50}$ > 7500 g /ha | EFSA Journal 2015; 13(3):3999<br>Hoogendoorn, 1999<br>CGA329351/1214 |
| *Chrysoperla carnea* (larvae) | RIDOMIL GOLD (A9651D) | Laboratory test, glass plates (2D) | $LR_{50}$ > 7000 g/ha<br><br>$ER_{50}$ > 7000 g /ha | EFSA Journal 2015; 13(3):3999<br>Wientjens, 2000<br>CGA329351/1250 |
| *Coccinella septempunctata* (larvae) | RIDOMIL GOLD (A9651D) | Laboratory test, glass plates (2D) | $LR_{50}$ > 7000 g/ha<br><br>$ER_{50}$ > 7000 g /ha | EFSA Journal 2015; 13(3):3999<br>Wientjens, 2000a<br>CGA329351/1251 |
| **Extended laboratory tests** | | | | |
| *Aleochara bilineata* (adults) | APRON (A9642C) | Extended laboratory test, incorporated into soil (2D) | < 50% effect in mortality and reproduction at 6000 ml A9642C/ha (equivalent to 8.75 mg A9642C/kg soil dw)[b]<br><br>NOEC = 4000 mg A9642C/L (equivalent to 5.83 mg A9642C/kg soil dw)[b] | EFSA Journal 2015; 13(3):3999<br>Röhlig, 2011<br>A9642C_10039 |
| *Aphidius rhopalosiphi* (adults) | RIDOMIL GOLD (A9651D) | Extended laboratory test barley plants (3D) | $LR_{50}$ > 7000 g/ha<br><br>$ER_{50}$ > 7000 g /ha | EFSA Journal 2015; 13(3):3999<br>Aldersho, 1999<br>CGA329351/1215 |
| *Aleochara bilineata* (adults) | RIDOMIL GOLD (A9651D) | Extended laboratory test sandy soil (2D) | $LR_{50}$ > 3750 g/ha<br><br>$ER_{50}$ > 3750 g /ha | EFSA Journal 2015; 13(3):3999<br>Taruza, 2002<br>CGA329351/1550 |
| *Typhlodromus pyri* (adults) | RIDOMIL GOLD (A9651D) | Extended laboratory test leaf discs (2D) | $LR_{50}$ > 381.9 g/ha<br>$ER_{50}$ > 64 g/ha | EFSA Journal 2015; 13(3):3999<br>Fallowfield, 2011<br>A9651D_10070 |

| Species | Substance | Exposure System | Results | Reference [a] |
|---|---|---|---|---|
| **Field or semi-field tests** | | | | |
| Semi-field tests on *Aphidius rhopalosiphi* 277.5 & 3700 g A9651D (RIDOMIL GOLD)/ha applied 4 times with 7-day spray interval. Reduction in reproduction compared to the control of 17.5 and 30.4% at 277.5 & 3 700 g A9651D (RIDOMIL GOLD)/ha (not statistically significant) immediately after 4$^{th}$ application. Reduction in reproduction compared to the control of 54.8% at 3700 g A9651D/ha (not statistically significant) 14 days after 4$^{th}$ application. Reduction in reproduction compared to the control of 23.7% at 3700 g A9651D/ha (not statistically significant) 28 days after 4$^{th}$ application. 9990 g A9651D/ha applied once. Reduction in reproduction compared to the control of 36.4% at 9.99 g A9651D (RIDOMIL GOLD)/ha (statistically significant) immediately after the single application. | | | | EFSA Journal 2015;13(3):3999; Grimm, C., 2000; CGA329351/1422 |
| Field test on Predatory mite:190 & 2 500 g A9651D (RIDOMIL GOLD)/ha applied 4 times with 10-14 day spray interval The formulation A9651D (RIDOMIL GOLD) had effects on the predatory mite population. | | | | EFSA Journal 2015;13(3):3999; Bakker, F.M., 1999; CGA329351/1235 |
| Field test on Predatory mite:187.5 & 2 500 g A9651D (RIDOMIL GOLD)/ha applied 4 times with 9-12 day spray interval;4 applications at 2500 g A9651D/ha significantly affected the predatory mite population until the last sampling at 83 days after the 4th application.;4 applications at 187.5 g A9651D (RIDOMIL GOLD)/ha caused significant effects on the predatory mite population after the 2nd application until 4 weeks after the 4th and last application where the effects were apparent but not statistically significant. | | | | EFSA Journal 2015;13(3):3999; Reber, B., 1999; CGA329351/1206 |
| Field test on Predatory mite: A9651D was applied either 2 or 3 times at 2 500 g A9651D (RIDOMIL GOLD) /ha with a 14-day spray interval;Following 2 applications at 2500 g A9651D (RIDOMIL GOLD)/ha no statistically significant effects on the predatory mite population were observed. No statistically significant effects of up to 18.99% were observed.;Following 3 applications at 2500 g A9651D (RIDOMIL GOLD)/ha there was a statistically significant reduction in predatory mites 6 days after the last treatment application (maximal 38.98%). The reduction compared to the control on the last sampling date (28DAA3) was 27.43% which was not statistically significant. | | | | EFSA Journal 2015;13(3):3999; Muther-Paul, 2009; A9651D_10042 |

EFSA Journal 2015;13(3):3999. [105 pp.] doi:10.2903/j.efsa.2015.3999

**Table 9.7-2: Endpoints and effect values relevant for the risk assessment for non-target arthropods - oxathiapiprolin**

| Species | Formulation | Exposure System | Results | Reference |
|---|---|---|---|---|
| *Typhlodromus pyri* (protonymphs) | Oxathiapiprolin 100 g/L OD | Laboratory test glass plates (2D) | $LR_{50}$ > 200 g a.s./ha | EFSA 2016;14(7):4504, DuPont-33193 |
| *Aphidius rhopalosiph*i (adults) | Oxathiapiprolin 100 g/L OD | Laboratory test glass plates (2D) | $LR_{50}$ = 116.1 g a.s./ha | EFSA 2016;14(7):4504, DuPont-32696 |
| **Extended laboratory tests** | | | | |
| *Aphidius rhopalosiphi* (adults) | Oxathiapiprolin 100 g/L OD | Extended laboratory test barley plants (3D) | $LR_{50}$ > 200 g/ha<br>$ER_{50}$ > 200 g /ha | EFSA 2016;14(7):4504, DuPont-34267 |
| *Chrysoperla carnea* (larvae) | Oxathiapiprolin 100 g/L OD | Extended laboratory test bean leaves (2D) | $LR_{50}$ > 200 g/ha<br>$ER_{50}$ > 200 g /ha | EFSA 2016;14(7):4504, DuPont-34111 |
| **Field or semi-field tests** | | | | |

| Species | Formulation | Exposure System | Results | Reference |
|---|---|---|---|---|
| Effects on population of predatory mites in vineyards in Germany (2012):<br>Formulation DPX-QGU42 100 g/L OD was applied 3 times at single application rate of 60.0 g a.s./ha with 9 days interval to grapes at BBCH stages 57, 64 and 69. Observations of number of mites (adults and nymphs) on leaf samples were performed 2 days before first application (2 DBA1), 5 days after first application (5 DAA1), 6 DAA2, 7 DAA3 and 28 DAA3. No statistically significant reduction in predatory mites populations greater than 50% comparing to control was observed (maximum reduction was 20.4% comparing to control). | | | | EFSA 2016;14(7):4504, DuPont-34209 |
| Effects on population of predatory mites in vineyards in Southern France (2012):<br>Formulation DPX-QGU42 100 g/L OD was applied 3 times at single application rate of 60.0 g a.s./ha with 9 and 8 days interval to grapes at BBCH stages 15, 17 and 18. Observations of number of mites (adults and nymphs) on leaf samples were performed 2 days before first application (2 DBA1), 9 days after first application (9 DAA1), 8 DAA2, 7 DAA3 and 30 DAA3. No statistically significant reduction in predatory mites populations greater than 50% comparing to control was observed (maximum reduction was 11.9% comparing to control). | | | | EFSA 2016;14(7):4504, DuPont-34210 |
| Effects on population of predatory mites in vineyards in Italy (2012):<br>Formulation DPX-QGU42 100 g/L OD was applied 3 times at single application rate of 60.0 g a.s./ha with 10 days interval to grapes at BBCH stages 69, 73 and 75. Observations of number of mites (adults and nymphs) on leaf samples were performed 2 days before first application (2 DBA1), 7 days after first application (7 DAA1), 7 DAA2, 8 DAA3 and 29 DAA3. No statistically significant reduction in predatory mites populations greater than 50% comparing to control was observed (maximum reduction was 13.1% comparing to control). | | | | EFSA 2016;14(7):4504, DuPont-34211 |

EFSA Journal 2016;14(7):4504. [19 pp.] doi:10.2903/j.efsa.2016.4504.

**Table 9.7-3: Endpoints and effect values relevant for the risk assessment for non-target arthropods – A23109A**

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| *Typhlodromus pyri* (protonymphs) | A23109A | Laboratory test glass plates (2D) | **$LR_{50}$ = 252.4 mL/ha** | Fallowfield 2020; VV-887718 |
| *Aphidius rhopalosiphi* (adults) | A23109A | Laboratory test glass plates (2D) | **$LR_{50}$ = 257.73 mL/ha** | Stevens 2020; VV-887720 |
| *Typhlodromus pyri* (adults) | A23109A | Extended laboratory test leaf discs (2D) | **$LR_{50}$ > 2000 mL/ha**<br>**$ER_{50}$ > 2000 mL/ha** | Fallowfield 2021; VV-895497 |
| *Aphidius rhopalosiphi* (adults) | A23109A | Extended laboratory test barley plants (3D) | **$LR_{50}$ > 2000 mL/ha**<br>**$ER_{50}$ > 2000 mL/ha** | Stevens 2021; VV-895493 |
| *Chrysoperla carnea* (larvae) | A23109A | Extended laboratory test bean leaves (2D) | **$LR_{50}$ > 2000 mL/ha**<br>**$ER_{50}$ > 2000 mL/ha** | Vaughan 2021; VV-902193 |
| *Aleochara bilineata* (adults) | A23109A | Extended laboratory test loamy sand (2D) | **$LR_{50}$ > 2000 mL/ha**<br>**$ER_{50}$ > 2000 mL/ha** | Tew 2021; VV-905353 |
| **Field or semi-field tests** | | | | |
| Not applicable | | | | |

Endpoints used for risk assessment are shown in **bold**

### 9.7.1.1 Justification for new endpoints

Studies with non-target arthropods are always conducted with a formulated product and no testing is carried out with unformulated technical material. Therefore, it may not be appropriate to rely on the data from the individual formulations, submitted as representative formulations for the EU review, for the risk assessment for non-target arthropods.

The toxicity of A23109A to non-target arthropods has been investigated by carrying out a Tier I test on the representative non-target arthropods *Aphidius rhopalosiphi* and *Typhlodromus pyri*, in accordance with ESCORT 2, and Tier II tests on *Aphidius rhopalosiphi*, *Typhlodromus pyri*, *Chrysoperla carnea* and *Aleochara bilineata*.

## 9.7.2 Risk assessment

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

### 9.7.2.1 Risk assessment for in-field exposure

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the application of 2 x 500 mL A23109A/ha for leafy vegetables covers the risk for non-target arthropods from all intended uses of A23109A (see 9.1.2).

The $PER_{in\text{-}field}$ values according to ESCORT 2 were calculated as:

Application rate × MAF.

**Table 9.7-4: First-tier and higher-tier assessment of the in-field risk for non-target arthropods due to the use of A23109A**

| | | | |
|---|---|---|---|
| **Intended use** | Leafy vegetables | | |
| **Product** | A23109A | | |
| **Application rate** | 2 × 500 mL prod./ha | | |
| **MAF** | 1.7 (foliar) / 1.9 (soil) | | |
| **Test species<br>Tier I** | **$LR_{50}$ (lab.)<br>(mL prod./ha)** | **$PER_{in\text{-}field}$<br>(mL prod./ha)** | **$HQ_{in\text{-}field}$<br>criterion: HQ ≤ 2** |
| *Typhlodromus pyri* | 252.4 | 850 (foliar)[a]<br>950 (soil)[a] | **3.37** (foliar)<br>**3.76** (soil) |
| *Aphidius rhopalosiphi* | 257.73 | | **3.30** (foliar)<br>**3.69** (soil) |
| **Test species<br>Higher-tier** | **Rate with ≤ 50 % effect<br>(mL prod./ha)** | **$PER_{in\text{-}field}$<br>(mL prod./ha)** | **$PER_{in\text{-}field}$ below rate with ≤ 50 % effect?** |
| *Typhlodromus pyri* | 2000 | 850 (foliar)[a]<br>950 (soil)[a] | Yes |
| *Aphidius rhopalosiphi* | 2000 | | Yes |
| *Chrysoperla carnea* | 2000 | | Yes |
| *Aleochara bilineata* | 2000 | | Yes |

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient
Criteria values shown in bold breach the relevant trigger.
[a] As a worst-case risk assessment, 100% and 0% crop interception are considered for foliar and soil substrates, respectively.

Using the Tier I laboratory data the $HQ_{in\text{-}field}$ values are not ≤2 therefore a Tier II risk assessment using the extended laboratory test data for four species has been conducted and demonstrates an acceptable in-field risk to non-target arthropods following the proposed uses of A23109A.

### 9.7.2.2 Risk assessment for off-field exposure

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the application of 2 x 500 mL A23109A/ha for leafy vegetables covers the risk for non-target arthropods from all intended uses of A23109A (see 9.1.2).

The $PER_{off\text{-}field}$ value according to ESCORT 2 was calculated as:

Application rate × MAF × (drift factor/vegetation distribution factor).

The corrected $PER_{off\text{-}field}$ values according to ESCORT 2 was calculated as:

corr. $PER_{off\text{-}field}$ = $PER_{off\text{-}field}$ x correction factor.

**Table 9.7-5: First-tier and higher-tier assessment of the off-field risk for non-target arthropods due to the use of A23109A**

| | | | | | | |
|---|---|---|---|---|---|---|
| **Intended use** | | Leafy vegetables | | | | |
| **Active substance/product** | | A23109A | | | | |
| **Application rate** | | 2 × 500 mL prod./ha | | | | |
| **MAF** | | 1.7 | | | | |
| **Drift rate (%)** | | 2.38 (at a distance of 1 m) [a] | | | | |
| **vdf** | | 10/5 (2D), 1 (3D) | | | | |
| **Test species Tier I** | **$LR_{50}$ (lab.) (mL prod./ha)** | **Drift factor** | **$PER_{off\text{-}field}$ (mL prod./ha)** | **CF** | **corr. $PER_{off\text{-}field}$ (mL prod./ha)** | **$HQ_{off\text{-}field}$ criterion: HQ ≤ 2** |
| *Typhlodromus pyri* | 252.4 | 0.0238 | 2.02 / 4.05 | 10 | 20.2 / 40.5 | 0.0800 / 0.16 |
| *Aphidius rhopalosiphi* | 257.73 | | | | | 0.0784 / 0.157 |
| ~~**Test species Higher-tier**~~ | ~~**Rate with ≤ 50 % effect (mL prod./ha)**~~ | ~~**Drift factor**~~ | ~~**$PER_{off\text{-}field}$ (mL prod./ha)**~~ | ~~**CF**~~ | ~~**corr. $PER_{off\text{-}field}$ (mL prod./ha)**~~ | ~~**corr. $PER_{off\text{-}field}$ below rate with ≤ 50 % effect?**~~ |
| ~~*Typhlodromus pyri*~~ | ~~2000~~ | ~~0.0238~~ | ~~2.02~~ | ~~5~~ | ~~10.1~~ | ~~Yes~~ |
| ~~*Aphidius rhopalosiphi*~~ | ~~2000~~ | | ~~20.2~~ [b] | ~~5~~ | ~~101~~ | ~~Yes~~ |
| ~~*Chrysoperla carnea*~~ | ~~2000~~ | | ~~2.02~~ | ~~5~~ | ~~10.1~~ | ~~Yes~~ |
| ~~*Aleochara bilineata*~~ | ~~2000~~ | | ~~2.02~~ | ~~5~~ | ~~10.1~~ | ~~Yes~~ |

MAF: Multiple application factor; vdf: Vegetation distribution factor; (corr.) PER: (corrected) Predicted environmental rate; CF: Correction factor; HQ: Hazard quotient.

[a] An 82nd percentile drift value was considered to cover the two applications

[b] Study incorporated 3-Dimensional exposure therefore no vegetation distribution factor (VDF) applied

Using the Tier I laboratory data the $HQ_{off\text{-}field}$ values are ≤2 thereby demonstrating an acceptable off-field risk to non-target arthropods following the proposed uses of A23109A. A Tier II risk assessment was not necessary ~~but, as extended laboratory test data for four species are available, the risk assessment has also been presented above and confirms an acceptable off-field risk to non-target arthropods following the proposed uses of A23109A.~~

#### 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

At Tier 1, the in-field HQ values based on the $LR_{50}$ were greater than the trigger value for the worst-case intended use scenarios. However, extended laboratory test data for four species, including *Typhlodromus pyri* and *Aphidius rhopalosiphi*, confirmed that the in-field risks following the proposed uses of A23109A were acceptable.

At Tier 1, the off-field HQ values based on the $LR_{50}$ were below the trigger value for the worst-case intended use scenarios thereby demonstrating that the off-field risks following the proposed uses of A23109A were acceptable.

**Review Comments:**

Based on the results of the conducted risk assessment, it can be concluded that low risk for non-target arthropods is expected from the use of A23109A 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 metalaxyl-M, oxathiapiprolin, their representative formulations and their relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of A23109A were not evaluated as part of the EU assessment of metalaxyl-M or oxathiapiprolin. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

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

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| *Eisenia fetida* | Metalaxyl-M | Mixed into substrate<br>14 d, acute<br>10% peat content | **$LC_{50}$ = 830 mg a.s./kg dw** | EFSA Journal 2015;13(3):3999; Rufli, 1997; CGA329351/0689 |
| *Eisenia fetida* | Metalaxyl-M | Mixed into substrate<br>14 d, acute | $LC_{50}$ >1000 mg a.s./kg dw | EFSA Journal 2015;13(3):3999; |

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| | | 10% peat content | | Vial, 1990; CGA48988/0168 |
| *Eisenia fetida* | RIDOMIL GOLD (A9651D) | Mixed into substrate 14 d, acute 10 % peat content | $LC_{50}$ > 1000 mg/kg dw (0.98 mg metalaxyl-M and 15 mg mancozeb/kg dw) | EFSA Journal 2015;13(3):3999; Schmidt, 2005; CGA329351/2153 |
| *Eisenia fetida* | NOA409045 (CGA62826) | Mixed into substrate 14 d, acute 10% peat content | **$LC_{50}$ >1000 mg met./kg dw** | EFSA Journal 2015;13(3):3999; Bätscher, 1999; ; NOA409045/0002 |
| *Eisenia fetida* | CGA108906 (SYN546520) | Mixed into substrate 14 d, acute 10% peat content | $LC_{50}$ >1000 mg met./kg dw | EFSA Journal 2015;13(3):3999; Bätscher, 1999a; ; CGA108906/0010 |
| *Eisenia fetida* | Metalaxyl-M | Mixed into substrate 56 d, chronic 10% peat content | **NOEC = 35.63 mg a.s./kg dw** | EFSA Journal 2015;13(3):3999; Friedrich, 2011; CGA329351_11468 |
| *Eisenia fetida* | APRON (A9642C) | Mixed into substrate 56 d, chronic 10 % peat content | NOEC = 250 mg/kg dw (89 mg a.s./kg dw) | EFSA Journal 2015;13(3):3999; Friedrich, 2011a; A9642C/10035 |
| *Eisenia fetida* | RIDOMIL GOLD (A9651D) | Mixed into substrate 56 d, chronic 10 % peat content | NOEC = 39.06 mg/kg dw (1.56 mg metalaxyl-M and 25 mg mancozeb/kg dw) | EFSA Journal 2015;13(3):3999; Friedrich, 2007; CGA329351/2397 |
| *Eisenia fetida* | NOA409045 (CGA62826) | Mixed into substrate 56 d, chronic 10% peat content | **NOEC = 500 mg met./kg dw** | EFSA Journal 2015;13(3):3999; Friedrich, 2011b; NOA409045_10001 |
| *Eisenia fetida* | CGA108906 (SYN546520) | Mixed into substrate 56 d, chronic 10% peat content | NOEC = 500 mg met./kg dw | EFSA Journal 2015;13(3):3999; Friedrich, 2011c; CGA108906_10000 |
| *Folsomia candida* | Metalaxyl-M | Mixed into substrate 28 d, chronic 5% peat content | **NOEC = 125 mg a.s./kg dw** | EFSA Journal 2015;13(3):3999; Friedrich, 2011d; CGA329351_11456 |
| *Folsomia candida* | APRON (A9642C) | Mixed into substrate 28 d, chronic 5 % peat content | NOEC = 250 mg/kg dw (89 mg a.s./kg dw) | EFSA Journal 2015;13(3):3999; Friedrich, 2011e; A9642C/10037 |
| *Folsomia candida* | RIDOMIL GOLD (A9651D) | Mixed into substrate 28 d, chronic 5 % peat content | NOEC = 125 mg/kg dw (4.71 mg metalaxyl-M and 81 mg mancozeb/kg dw) | EFSA Journal 2015;13(3):3999; Friedrich, 2011f; A9651D/10075 |
| *Hypoaspis aculeifer* | APRON (A9642C) | Mixed into substrate | **$EC_{10}$ = 16.6 mg** | EFSA Journal |

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| | | 14 d, chronic<br>5 % peat content | **a.s./kg dw** | 2015;13(3):3999; Vinall, 2011; A9642C/10038 |
| **Field studies** | | | | |
| Not conducted | | | | |
| **Litter bag test** | | | | |
| Not conducted | | | | |

Bold values are used for the risk assessment
EFSA Journal 2015;13(3):3999. [105 pp.] doi:10.2903/j.efsa.2015.3999

Endpoints have been presented for all relevant soil metabolites of metalaxyl-M. However, risk assessment is required for NOA409045 only.

**Table 9.8-2: Endpoints and effect values relevant for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) – oxathiapiprolin and relevant metabolites**

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| **Acute** | | | | |
| *Eisenia fetida* | Oxathiapiprolin | Mixed into substrate<br>14 d, acute<br>5 % peat content | $LC_{50}$ > 1000 mg a.s./kg dw<br>**$LC_{50corr}$ > 500 mg a.s./kg dw** | EFSA 2016;14(7):4504, DuPont-45261 |
| **Chronic** | | | | |
| *Eisenia fetida* | Oxathiapiprolin | Mixed into substrate<br>56 d, chronic<br>5 % peat content | NOEC = 1000 mg a.s./kg dw<br>**$NOEC_{corr}$ = 500 mg a.s./kg dw** | EFSA 2016;14(7):4504, DuPont-32457 |
| *Eisenia fetida* | Oxathiapiprolin 100 g/L OD | Mixed into substrate<br>56 d, chronic<br>5 % peat content | NOEC = 15.43 mg a.s./kg dw<br>$NOEC_{corr}$ = 7.72 mg a.s./kg dw | EFSA 2016;14(7):4504, DuPont-35836 |
| *Eisenia fetida* | IN-E8S72 | Mixed into substrate<br>56 d, chronic<br>5 % peat content | **NOEC = 100.0 mg met./kg dw** | EFSA 2016;14(7):4504, DuPont-32720 |
| *Eisenia fetida* | IN-QPS10 | Mixed into substrate<br>56 d, chronic<br>5 % peat content | **NOEC = 100.0 mg met./kg dw** | EFSA 2016;14(7):4504, DuPont-32134 |
| *Eisenia fetida* | IN-RAB06 | Mixed into substrate<br>56 d, chronic<br>5 % peat content | **NOEC = 100.0 mg met./kg dw** | EFSA 2016;14(7):4504, DuPont-32470 |
| *Eisenia fetida* | IN-RDT31 | Mixed into substrate<br>56 d, chronic<br>5 % peat content | NOEC = 100.0 mg met./kg dw<br>**$NOEC_{corr}$ = 50.0 mg met./kg dw** | EFSA 2016;14(7):4504, DuPont-32461 |
| *Folsomia candida* | Oxathiapiprolin | Mixed into substrate<br>28 d, chronic<br>5 % peat content | NOEC = 25 mg a.s./kg dw<br>**$NOEC_{corr}$ = 12.5 mg a.s./kg dw** | EFSA 2016;14(7):4504, DuPont-32458 |

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| *Folsomia candida* | Oxathiapiprolin 100 g/L OD | Mixed into substrate 28 d, chronic 5 % peat content | NOEC = 25 mg a.s./kg dw $NOEC_{corr}$ = 12.5 mg a.s./kg dw | EFSA 2016;14(7):4504, DuPont-35840 |
| *Folsomia candida* | IN-E8S72 | Mixed into substrate 28 d, chronic 5 % peat content | **NOEC = 100.0 mg met./kg dw** | EFSA 2016;14(7):4504, DuPont-32721 |
| *Folsomia candida* | IN-QPS10 | Mixed into substrate 28 d, chronic 5 % peat content | **NOEC = 100.0 mg met./kg dw** | EFSA 2016;14(7):4504, DuPont-32135 |
| *Folsomia candida* | IN-RAB06 | Mixed into substrate 28 d, chronic 5 % peat content | **NOEC = 100.0 mg met./kg dw** | EFSA 2016;14(7):4504, DuPont-32462 |
| *Folsomia candida* | IN-RDT31 | Mixed into substrate 28 d, chronic 5 % peat content | NOEC = 100 mg met./kg dw **$NOEC_{corr}$ = 50.0 mg met./kg dw** | EFSA 2016;14(7):4504, DuPont-32463 |
| *Hypoaspis aculeifer* | Oxathiapiprolin | Mixed into substrate 14 d, chronic 5 % peat content | NOEC = 1000 mg a.s./kg dw **$NOEC_{corr}$ = 500 mg a.s./kg dw** | EFSA 2016;14(7):4504, DuPont-33723 |
| *Hypoaspis aculeifer* | Oxathiapiprolin 100 g/L OD | Mixed into substrate 14 d, chronic 5 % peat content | NOEC = 50.0 mg a.s./kg dw $NOEC_{corr}$ = 25.0 mg a.s./kg dw | EFSA 2016;14(7):4504, DuPont-35839 |
| *Hypoaspis aculeifer* | IN-E8S72 | Mixed into substrate 14 d, chronic 5 % peat content | **NOEC = 100.0 mg met./kg dw** | EFSA 2016;14(7):4504, DuPont-32719 |
| *Hypoaspis aculeifer* | IN-QPS10 | Mixed into substrate 14 d, chronic 5 % peat content | **NOEC = 50.0 mg met./kg dw** | EFSA 2016;14(7):4504, DuPont-32136 |
| *Hypoaspis aculeifer* | IN-RAB06 | Mixed into substrate 14 d, chronic 5 % peat content | **NOEC = 25.0 mg met./kg dw** | EFSA 2016;14(7):4504, DuPont-32464 |
| *Hypoaspis aculeifer* | IN-RDT31 | Mixed into substrate 14 d, chronic 5 % peat content | NOEC = 12.5 mg met./kg dw **$NOEC_{corr}$ = 6.25 mg met./kg dw** | EFSA 2016;14(7):4504, DuPont-32465 |
| **Field studies** | | | | |
| Not conducted | | | | |
| **Litter bag test** | | | | |
| Not conducted | | | | |

Values in **bold** are used in the initial risk assessment
EFSA Journal 2016;14(7):4504. [19 pp.] doi:10.2903/j.efsa.2016.4504.

**Table 9.8-3: Endpoints and effect values relevant for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) – A23109A**

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| **Acute** | | | | |
| *Eisenia andrei* | A23109A | Mixed into substrate<br>14 d, acute<br>10 % peat content | $LC_{50}$ > 1000 mg/kg dw<br>**$LC_{50corr}$ > 500 mg/kg dw*** (>83.0 mg metalaxyl-M/kg dw and >14.3 mg oxathiapiprolin/kg dw) | Friedrich 2021; VV-892721<br>Study not evaluated by zRMS |
| **Chronic** | | | | |
| *Eisenia andrei* | A23109A | Mixed into substrate<br>56 d, chronic<br>10% peat content | NOEC = 309 mg/kg dw;<br>$NOEC_{corr}$ = 155 mg/kg dw<br><br>$EC_{10}$ = 302 mg/kg dw;<br>**$EC_{10corr}$ = 151 mg/kg dw*** (25.1 mg metalaxyl-M/kg dw and 4.30 mg oxathiapiprolin/kg dw);<br><br>$EC_{20}$ = 413 mg/kg dw;<br>$EC_{20corr}$ = 207 mg/kg dw | Friedrich 2021; VV-892682 |
| *Folsomia candida* | A23109A | Mixed into substrate<br>28 d, chronic<br>5% peat content | **NOEC = 556 mg/kg dw** (92.3 mg metalaxyl-M/kg dw and 15.8 mg oxathiapiprolin/kg dw);<br><br>**$NOEC_{corr}$ = 278 mg/kg dw**<br><br>$EC_{10}$ = 675 mg/kg dw;<br><br>$EC_{20}$ = 811 mg/kg dw | Friedrich 2021; VV-891167 |
| *Hypoaspis aculeifer* | A23109A | Mixed into substrate<br>14 d, acute<br>5% peat content | **NOEC = 1000 mg/kg dw** (166 mg metalaxyl-M/kg dw and 28.5 mg oxathiapiprolin/kg dw);<br><br>**$NOEC_{corr}$ = 500 mg/kg dw**<br><br>$EC_{10}$ > 1000 mg/kg dw;<br><br>$EC_{20}$ > 1000 mg/kg dw | Schulz 2021; VV-890311 |

Bold values are used for the risk assessment
* Corrected value derived by dividing the endpoint by a factor of 2 in accordance with the EPPO earthworm scheme 2002. Only applicable to studies which used artificial soil with a 10% peat content

It is the view of Syngenta that when assessing the acute and long-term risk to earthworms from A23109A, the active substances and metabolites, it is not appropriate to divide the $LC_{50}$ or NOEC by 2 in the case that the endpoint was generated in an artificial soil that contained reduced (5 %) organic matter rather

than the 10 % organic matter of standard test soils. Current Guidance (EU Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002 rev 2; EPPO, 2003[13]) states that the correction of endpoints is only necessary when chemicals have a log Pow > 2 *and* the artificial soil used in the tests contains 10 % peat. When the peat level is reduced to 5 % it is considered more comparable to agricultural soils (EPPO, 2003) and thus the correction is not required.

In addition, the view of Syngenta is that when assessing the long-term risk from A23109A, the active substances and metabolites to *Folsomia candida* and *Hypoaspis* sp. it is not appropriate to divide the NOEC by 2 as the relationship between organic matter and toxicity has only been demonstrated for a small number of substances and only for earthworms (van Gestel, 1992[14]). Other soil macro-organisms such as *Hypoaspis* sp. and collembolans are not directly involved in organic matter breakdown and thus the correction conservatively applied to earthworms should not be applied here. Furthermore, the endpoints were generated in an artificial soil that contained 5 % organic matter. Current Guidance (EU Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002 rev 2; EPPO 2003) states that the correction of endpoints is only necessary *for earthworms* when chemicals have a logPow > 2 *and* the artificial soil used in the tests contains 10 % peat. When the peat level is reduced to 5 % it is considered more comparable to agricultural soils (EPPO, 2003) and thus the correction is not required.

Nevertheless, the correction factor in the endpoints of the active substance oxathiapiprolin and its metabolite IN-RDT31 was included as a worst-case approach in line with EFSA Journal 2016;14(7):4504, respectively.

#### 9.8.1.1 Justification for new endpoints

New studies are available for A23109A which are required to fulfil the data requirements for plant protection products in accordance with Regulation (EC) No 1107/2009. The endpoints are summarised in Table 9.8-3 and study summaries are provided in Appendix 2 to this document.

### 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, multi-annual accumulation in soil needs to be considered for oxathiapiprolin and its metabolites IN-RDT31, IN-E8S72, IN-QPS10 and IN-RAB06 and for the metalaxyl-M metabolite NOA409045. According to the assessment of environmental-fate data, multi-annual accumulation in soil does not need to be considered for metalaxyl-M.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, for A23109A, metalaxyl-M, oxathiapiprolin and their relevant metabolites, the relevant endpoints are compared to the maximum $PEC_{soil}$ (onions) ensuring that the risk for earthworms and other non-target soil organisms from all intended uses is covered (see 9.1.2).

[13] EPPO, 2003: Anonymous, 2003. Environmental risk assessment scheme for plant protection products Chapter 8: Soil organisms and functions. OEPP/EPPO, Bulletin OEPP/EPPO Bulletin 33, 147–149

[14] van Gestel CAM (1992) The influence of soil characteristics on the toxicity of chemicals for earthworms; a review. In: Ecotoxicology of Earthworms (Ed. Becker, H, Edwards, PJ, Greig-Smith, PW & Heimbach, F). Intercept Press, Andover (GB)

**Table 9.8-4: 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 A23109A - worst-case**

| **Intended use** | All intended uses | | |
|---|---|---|---|
| ~~**Acute effects on earthworms**~~ | | | |
| ~~**Test substance**~~ | ~~**$LC_{50}$ (mg/kg dw)**~~ | ~~**$PEC_{soil}$ (mg/kg dw)**~~ | ~~**$TER_a$ (criterion TER ≥ 10)**~~ |
| ~~Metalaxyl-M~~ | ~~830~~ | ~~0.1941 [b]~~ | ~~4276~~ |
| ~~NOA409045~~ | ~~>1000~~ | ~~0.1350 [c]~~ | ~~>7407~~ |
| ~~Oxathiapiprolin~~ | ~~>500*~~ | ~~0.0392 [c]~~ | ~~>12755~~ |
| ~~A23109A~~ | ~~>500*~~ | ~~0.644~~ | ~~776~~ |
| **Chronic effects on earthworms** | | | |
| **Test substance** | **NOEC / $EC_{10}$ (mg/kg dw)** | **$PEC_{soil}$ (mg/kg dw)** | **$TER_{lt}$ (criterion TER ≥ 5)** |
| Metalaxyl-M | 35.63 | 0.1941 [b] | 184 |
| NOA409045 | 500 | 0.1350 [c] | 3704 |
| Oxathiapiprolin | 500* | 0.0392 [c] | 12755 |
| IN-E8S72 | 100 | 0.0017 [c] | 58824 |
| IN-QPS10 | 100 | 0.0025 [c] | 40000 |
| IN-RAB06 | 100 | 0.0054 [c] | 18519 |
| IN-RDT31 | 50* | 0.0063 [c] | 7937 |
| A23109A | 151* | 0.644 | 234 |
| **Chronic effects on other soil macro- and mesofauna - *Folsomia candida*** | | | |
| **Test substance** | **NOEC (mg/kg dw)** | **$PEC_{soil}$ (mg/kg dw)** | **$TER_{lt}$ (criterion TER ≥ 5)** |
| Metalaxyl-M | 125 | 0.1941 [b] | 644 |
| NOA409045 | 12.5 [a] | 0.1350 [c] | 92.6 |
| Oxathiapiprolin | 12.5* | 0.0392 [c] | 319 |
| IN-E8S72 | 100 | 0.0017 [c] | 58824 |
| IN-QPS10 | 100 | 0.0025 [c] | 40000 |
| IN-RAB06 | 100 | 0.0054 [c] | 18519 |
| IN-RDT31 | 50* | 0.0063 [c] | 7937 |
| A23109A | 556 /278* | 0.644 | 863 /432 |
| **Chronic effects on other soil macro- and mesofauna - *Hypoaspis aculeifer*** | | | |
| **Test substance** | **NOEC / $EC_{10}$ (mg/kg dw)** | **$PEC_{soil}$ (mg/kg dw)** | **$TER_{lt}$ (criterion TER ≥ 5)** |
| Metalaxyl-M | 16.6 | 0.1941 [b] | 85.5 |
| NOA409045 | 1.66 [a] | 0.1350 [c] | 12.3 |
| Oxathiapiprolin | 500* | 0.0392 [c] | 12755 |
| IN-E8S72 | 100 | 0.0017 [c] | 58824 |

| | | | |
|---|---|---|---|
| IN-QPS10 | 50 | 0.0025 [c] | 20000 |
| IN-RAB06 | 25 | 0.0054 [c] | 4630 |
| IN-RDT31 | 6.25* | 0.0063 [c] | 992 |
| A23109A | 1 000 /500* | 0.644 | 1553 /776 |

* Corrected value derived by dividing the endpoint by a factor of 2 in accordance with the EPPO earthworm scheme 2002
[a] Toxicity endpoints are not available for this metabolite so as a worst case it is assumed that it is 10 x more toxic than parent
[b] Maximum $PEC_{soil}$ from use on onions ($PEC_{soil, initial}$)
[c] Maximum $PEC_{soil}$ from use on onions ($PEC_{soil, accumulation}$)

The TER values for metalaxyl-M, oxathiapiprolin, their relevant metabolites and A23109A are all above the respective trigger values thereby demonstrating an acceptable risk to earthworms and other soil macro-organisms following application of A23109A according to the proposed use pattern.

### 9.8.2.2 Higher-tier risk assessment

Not relevant.

### 9.8.3 Overall conclusions

The risk of A23109A to earthworms was assessed from ~~acute and~~ long-term toxicity exposure ratios (TERs) between the selected toxicity endpoints for A23109A, metalaxyl-M, oxathiapiprolin and their relevant metabolites, and the maximum $PEC_{soil}$. The ~~acute and~~ long-term TER values derived are greater than the Regulation (EU) 546/2011 triggers of ~~10 and~~ 5, respectively, indicating that the risk to earthworms is acceptable following use of A23109A according to the proposed use pattern.

The risk of A23109A to other non-target soil macro-organisms, as represented by *Folsomia* and *Hypoaspis* was assessed from long-term toxicity exposure ratios (TERs) between the selected no-effect concentrations or $EC_{10}$ values, derived from laboratory tests on A23109A, metalaxyl-M, oxathiapiprolin and their relevant metabolites, and the maximum $PEC_{soil}$. The long-term TER values are all greater than the recommended trigger value of 5, indicating that the risk to soil macro-organisms is acceptable following use of A23109A according to the proposed use pattern.

**Review Comments:**

The long-term risks of A23109A 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 use of A23109A was confirmed based on $TER_{LT}$ calculations for formulation, metalaxyl-M, oxathiapiprolin and their relevant metabolites.

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

### 9.9.1 Toxicity data

Studies on effects on soil microorganisms have been carried out with metalaxyl-M, oxathiapiprolin, their representative formulations and their relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on soil microorganisms of A23109A were not evaluated as part of the EU assessment of

metalaxyl-M and oxathiapiprolin. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

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

| Endpoint | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| N-mineralisation | Metalaxyl-M | 28 – 90 d, aerobic sand, sandy silty loam | **<25% effects at 6.6 mg a.s./kg** | EFSA Journal 2015;13(3):3999; Völk, 1992; CGA48988/3122 |
| C-mineralisation | Metalaxyl-M | 28 - 90 d, aerobic sandy silty loam | <25% effects at 6.6 mg a.s./kg | EFSA Journal 2015;13(3):3999; Völk, 1992; CGA48988/3122 |
| N-mineralisation | NOA409045 (CGA62826) | 28 days, aerobic Sandy loam, silty loam | **<25% effects at 0.76 mg met./kg** | EFSA Journal 2015;13(3):3999; Völkel, 1999; NOA409045/0009 |
| C-mineralisation | NOA409045 (CGA62826) | 28 days, aerobic Sandy loam, silty loam | <25% effects at 7.6 mg met./kg | EFSA Journal 2015;13(3):3999; Völkel, 1999; NOA409045/0009 |
| N-mineralisation | CGA108906 (SYN546520) | 28 days, aerobic Sandy loam, silty loam | <25% effects at 1.4 mg met./kg | EFSA Journal 2015;13(3):3999; Völkel, 1999a; CGA108906/0011 |
| C-mineralisation | CGA108906 (SYN546520) | 28 days, aerobic Sandy loam, silty loam | <25% effects at 1.4 mg met./kg | EFSA Journal 2015;13(3):3999; Völkel, 1999a; CGA108906/0011 |
| N-mineralisation | RIDOMIL GOLD (A9651D) | 28 d, aerobic Agricultural sandy loam soil | < 25 % effects at 1.35 mg metalaxyl-M/kg dw and 21.47 mg mancozeb/kg dw | EFSA Journal 2015;13(3):3999; Volkel, 2005; CGA329351/2127 |
| C-mineralisation | RIDOMIL GOLD (A9651D) | 28 d, aerobic Agricultural sandy loam soil | < 25 % effects at 1.35 mg metalaxyl-M/kg dw and 21.47 mg mancozeb/kg dw | EFSA Journal 2015;13(3):3999; Volkel, 2005; CGA329351/2127 |

Values in **bold** are used in the risk assessment
EFSA Journal 2015;13(3):3999. [105 pp.] doi:10.2903/j.efsa.2015.3999

**Table 9.9-2: Endpoints and effect values relevant for the risk assessment for soil micro-organisms – oxathiapiprolin and relevant metabolites**

| Endpoint | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| N-mineralisation | Oxathiapiprolin | 28 d, aerobic<br>Silty loamy sand | day 28:<br>2.2% effect at **15.59 mg a.s./kg dw** | EFSA 2016;14(7):4504, DuPont-32477 |
| C-mineralisation | | | day 28:<br>-2.91% effect at 15.59 mg a.s./kg dw | |
| N-mineralisation | Oxathiapiprolin 100 g/L OD | 28 d, aerobic | day 28:<br>-4.35% effect at 1.308 mg a.s./kg dw | EFSA 2016;14(7):4504, DuPont-32698 |
| C-mineralisation | | | day 28:<br>0.65% effect at 1.308 mg a.s./kg dw | |
| N-mineralisation | IN-E8S72 | 28 d, aerobic<br>Silty loamy sand | day 28:<br>9.19% effect at **5.38 mg met./kg dw** | EFSA 2016;14(7):4504, DuPont-32824 |
| C-mineralisation | | | day 28:<br>0.76% effect at 5.38 mg met./kg dw | |
| N-mineralisation | IN-QPS10 | 28 d, aerobic<br>Loamy sand | day 42:<br>14.76% effect at **0.68 mg met./kg dw** | EFSA 2016;14(7):4504, DuPont-32823 |
| C-mineralisation | | | day 42:<br>-9.93% effect at 0.68 mg met./kg dw | |
| N-mineralisation | IN-RAB06 | 28 d, aerobic<br>Silty loamy sand | day 28:<br>0.67% effect at **5.50 mg met./kg dw** | EFSA 2016;14(7):4504, DuPont-32821 |
| C-mineralisation | | | day 28:<br>-1.74% effect at 5.50 mg met./kg dw | |
| N-mineralisation | IN-RDT31 | 42/28 d, aerobic<br>Loamy sand | day 42:<br>23.38% effect at **0.71 mg met./kg dw** | EFSA 2016;14(7):4504, DuPont-32822 |
| C-mineralisation | | | day 28:<br>14.81% effect at 0.71 mg met./kg dw | |

Values in **bold** are used in the risk assessment
EFSA Journal 2016;14(7):4504. [19 pp.] doi:10.2903/j.efsa.2016.4504.

**Table 9.9-3: Endpoints and effect values relevant for the risk assessment for soil microorganisms – A23109A**

| Endpoint | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| N-mineralisation | A23109A | 28 days, aerobic soil type | <25% effects at **9.59 mg/kg soil** (1.59 mg metalaxyl-M/kg soil and 0.273 mg oxathiapiprolin/kg soil) | Schulz 2021; VV-894279 |
| C-mineralisation | A23109A | 28 days, aerobic soil type | <25% effects at 9.59 mg/kg soil (1.59 mg metalaxyl-M/kg soil and 0.273 mg oxathiapiprolin/kg soil) | Schulz 2021; VV-894279 |

Values in **bold** are used in the risk assessment

#### 9.9.1.1 Justification for new endpoints

New studies are available for A23109A, which are required to fulfil the data requirements for plant protection products in accordance with Regulation (EC) No 1107/2009. The endpoints are summarised in Table 9.9-3.

### 9.9.2 Risk assessment

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

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

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment based on the maximum $PEC_{soil}$ (onions) covers the risk for the soil microorganisms from all intended uses (see 9.1.2).

**Table 9.9-4: Assessment of the risk for effects on soil micro-organisms due to the use of A23109A – worst case**

| **Intended use** | All intended uses | | |
|---|---|---|---|
| **N-mineralisation** | | | |
| **Product/active substance** | **Max. conc. with effects ≤ 25% (mg/kg dw)** | **$PEC_{soil}$ (mg/kg dw)** | **Risk acceptable?** |
| Metalaxyl-M | 6.6 | 0.1941 [a] | Yes |
| NOA409045 | 0.76 | 0.1350 [b] | Yes |
| Oxathiapiprolin | 15.59 | 0.0392 [b] | Yes |
| IN-E8S72 | 5.38 | 0.0017 [b] | Yes |
| IN-QPS10 | 0.68 | 0.0025 [b] | Yes |
| IN-RAB06 | 5.50 | 0.0054 [b] | Yes |
| IN-RDT31 | 0.71 | 0.0063 [b] | Yes |
| A23109A | 9.59 | 0.644 | Yes |

[a] Maximum $PEC_{soil}$ from use on onions ($PEC_{soil, initial}$)
[b] Maximum $PEC_{soil}$ from use on onions ($PEC_{soil, accumulation}$)

For metalaxyl-M, oxathiapiprolin, their relevant metabolites and A23109A the $PEC_{soil}$ values are well below the concentrations at which there were ≤25% effects on nitrogen transformation in the respective studies. Thus, an acceptable risk to soil micro-organisms, following application of A23109A according to the proposed use pattern, can be demonstrated.

### 9.9.3 Overall conclusions

The risk of A23109A, metalaxyl-M, oxathiapiprolin and their relevant metabolites to soil micro-organisms was evaluated by comparison of the maximum concentrations with effects <25% derived from laboratory tests, with the maximum $PEC_{soil}$.

All the effect levels exceeded the relevant $PEC_{soil}$ values, indicating that the risk to soil micro-organisms is acceptable following the use of A23109A according to the proposed use pattern.

**Review Comments:**

The use of A23109A at the proposed rates poses no unacceptable risk to 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 metalaxyl-M (formulated as RIDOMIL GOLD) and with oxathiapiprolin (formulated as a 100 g/L OD). Full details of these studies are provided in the respective EU DAR and related documents.

Effects on non-target terrestrial plants of A23109A were not evaluated as part of the EU assessment of metalaxyl-M or oxathiapiprolin. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

The selection of studies and endpoints for the risk assessment deviates from the results of the EU review

process. Justifications are provided below.

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

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| *Brassica napus* d<br>*Avena fatua* m<br>*Beta vulgaris* d<br>*Zea mays* m<br>*Glycine max* d<br>*Allium cepa* m | RIDOMIL GOLD (A9651D) | Screening study | No effects on seedling emergence or vegetative vigour at 4.5 kg/ha | EFSA Journal 2015;13(3):3999; Walder, L., 2001, report no. CGA329351/1457 |

m: monocotyledonous; d: dicotyledonous
EFSA Journal 2015;13(3):3999. [105 pp.] doi:10.2903/j.efsa.2015.3999

**Table 9.10-2: Endpoints and effect values relevant for the risk assessment for non-target terrestrial plants – oxathiapiprolin**

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| *Zea mays* m<br>*Avena sativa* m<br>*Allium cepa* m<br>*Lolium perenne* m<br>*Cucumis sativus* d<br>*Pisum sativum* d<br>*Brassica napus* d<br>*Glycine max* d<br>*Beta vulgaris* d<br>*Lycopersicon esculentum* d | Oxathiapiprolin (formulated as 100 g/L OD) | 21 d<br>Seedling emergence | $ER_{50}$ > 600 g a.s./ha for all species tested | EFSA 2016;14(7):4504, DuPont-32478 |
| *Zea mays* m<br>*Avena sativa* m<br>*Allium cepa* m<br>*Lolium perenne* m<br>*Cucumis sativus* d<br>*Pisum sativum* d<br>*Brassica napus* d<br>*Glycine max* d<br>*Beta vulgaris* d<br>*Lycopersicon esculentum* d | Oxathiapiprolin (formulated as 100 g/L OD) | 21 d<br>Vegetative vigour | $ER_{50}$ > 600 g a.s./ha for all species tested | EFSA 2016;14(7):4504, DuPont-32479 |

m: monocotyledonous; d: dicotyledonous
EFSA Journal 2016;14(7):4504. [19 pp.] doi:10.2903/j.efsa.2016.4504.

**Table 9.10-3: Endpoints and effect values relevant for the risk assessment for non-target terrestrial plants – A23109A**

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| *Allium cepa*$_m$<br>*Triticum aestivum*$_m$<br>*Beta vulgaris*$_d$<br>*Brassica napus*$_d$<br>*Cucumis sativus*$_d$<br>*Glycine max*$_d$ | A23109A | 28 d phytotoxicity screen, seedling emergence | No phytotoxic effects at rates up to and including 1 000 mL/ha, the highest rate tested | Jones 2020; VV-890173 |
| *Allium cepa*$_m$<br>*Triticum aestivum*$_m$<br>*Beta vulgaris*$_d$<br>*Brassica napus*$_d$<br>*Cucumis sativus*$_d$<br>*Glycine max*$_d$ | A23109A | 21 d phytotoxicity screen, vegetative vigour | No phytotoxic effects at rates up to and including 1 000 mL/ha, the highest rate tested | Jones 2020; VV-890173 |

m: monocotyledonous; d: dicotyledonous

### 9.10.1.1 Justification for new endpoints

Studies with non-target terrestrial plants are always conducted with a formulated product and no testing is carried out with unformulated technical material. Therefore, it may not be appropriate to rely on the data from the individual solo formulations, submitted as representative formulations for the EU review, for the risk assessment for non-target terrestrial plants.

A screening study with formulated product A23109A has been conducted. The data are listed in Appendix 1 and summarised in Appendix 2.

## 9.10.2 Risk assessment

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

Screening test rates up to and including 1000 mL/ha were tested with formulation A23109A and effects were below the critical threshold as defined by the "Guidance Document on Terrestrial Ecotoxicology", (SANCO/10329/2002 rev.2 final, 2002). There were no phytotoxic effects based on seedling emergence or vegetative vigour up to and including the highest test rate of 1000 mL A23109A/ha.

The test rates exceed the highest field application rate of 500 mL A23109A/ha and are thus considered an indicator for an acceptable risk. For completeness a quantitative risk assessment has been presented below.

**Table 9.10-4: Assessment of the risk for non-target plants due to the use of A23109A in leafy vegetables and bulbs & onion like crops**

| | | | | |
|---|---|---|---|---|
| **Intended use** | | Arable crops | | |
| **Product** | | A23109A | | |
| **Application rate (mL prod./ha)** | | 2 × 500* | | |
| **Drift rate (%)** | | 2.77 | | |
| **Test species** | **$ER_{50}$ (mL prod./ha)** | **Drift factor** | **$PER_{off\text{-}field}$ (mL prod./ha)** | **TER criterion: TER ≥ 5** |
| All species | >1000 | 0.0277 | 13.85 | 72.2 |

PER: Predicted environmental rate; TER: toxicity to exposure ratio
* MAF is not considered due to dilution related to growth of plants

The TER is greater than the trigger value of 5 therefore an acceptable risk to non-target plants following the proposed uses of A23109A can be concluded.

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

Not relevant.

#### 9.10.2.3 Higher-tier risk assessment

Not relevant.

#### 9.10.2.4 Risk mitigation measures

No risk mitigation needed.

### 9.10.3 Overall conclusions

Screening test rates up to and including 1000 mL/ha were tested for six plant species with formulation A23109A. Less than 50% effect on seedling emergence and vegetative vigour on all six species was observed at the maximum test rate of 1000 mL A23109A/ha. This indicates that the risk to non-target terrestrial plants in off-crop areas is acceptable following use of A23109A according to the proposed use pattern.

**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 A23109A 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 A23109A 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 or 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:

| | |
|---|---|
| Hazard class(es), categories | Chronic aquatic toxicity, Category 2 |
| Hazard pictograms or Code(s) for hazard pictogram(s) |  GHS09 |
| Signal word | No signal word is used |
| Hazard statements | H411: Toxic to aquatic life with long lasting effects |
| Label elements for labelling | Pictogram GHS09<br>Signal word: No signal word is used<br>H411: Toxic to aquatic life with long lasting effects<br>P391: Collect spillage<br>P501: Dispose of contents/container to an approved waste disposal plant<br>EUH401: To avoid risks to human health and the environment, comply with the instructions for use. |

The classification of the product is derived from data on the ingredients and the summation method is applied.
Metalaxyl-M has a harmonized classification : not classified.
Based on the classification of oxathiapiprolin as Aquatic Chronic 1 and the corresponding M-factor of 1, this active substance forms >2.5% of the composition of the product A23109A (actual content of oxathiapiprolin in A23109A of 2.79% w/w). Therefore, the product is classified as Aquatic Chronic 2 according to Regulation (EC) No 1272/2008.

Precautionary statements: P391 (Collect spillage) and P501 (Dispose of contents/container to an approved waste disposal plant) are applied. P273 (Avoid release to the environment) is not required as this product is for use in the environment as a fungicide.

**Oxathiapiprolin**

Aquatic Chronic 1, M-factor 1.

C&L driving data:
Chronic toxicity to *Daphnia* and other aquatic invertebrates: NOEC *Americamysis bahia* (Mysid shrimp): 0.058 mg/L, Exposure time: 32 d
The substance is not rapidly degradable.

**Review Comments:**

The proposed classification is accepted.

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

**List of data submitted by the applicant and relied on (A23109A formulation data)**

| **Data point** | **Author(s)** | **Year** | **Title**<br>**Company Report No.**<br>**Source (where different from company)**<br>**GLP or GEP status**<br>**Published or not** | **Vertebrate study**<br>**Y/N** | **Owner** |
|---|---|---|---|---|---|
| ~~KCP 10.1.1~~ | ~~[redacted]~~ | ~~28/01/2021~~ | ~~Oxathiapiprolin/Metalaxyl-M DC (A23109A) – An Acute Oral Toxicity Study with the Northern Bobwhite using a Sequential Testing Procedure~~<br>~~Report No. 528B-605~~<br>~~Document No. VV-891190~~<br>~~Test Facility Eurofins EAG Agroscience LCC~~<br>~~GLP~~<br>~~Unpublished~~ | ~~Y~~ | ~~SYN~~ |
| KCP 10.1.2<br>(KCP 7.1.1) | [redacted] | 12/01/2021 | Metalaxyl-M / Oxathiapiprolin<br>Metalaxyl-M / Oxathiapiprolin DC (A23109A) - Acute Oral Toxicity Study in Rats (Up and Down Procedure)<br>Report No. 20/131-001P<br>Document No. VV-888460<br>xxxxxxxxxxxxxxxx<br>GLP<br>Unpublished | Y | SYN |
| KCP 10.2.1 | [redacted] | 26/02/2021 | Oxathiapiprolin/Metalaxyl-M DC (A23109A) - Toxicity to the Rainbow Trout Oncorhynchus mykiss under Laboratory Conditions (Acute Toxicity Test – Static)<br>Report No. S20-06894<br>Document No. VV-893371<br>xxxxxxxxxxxxx<br>GLP<br>Unpublished | Y | SYN |

| 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 | Schuler, L. | 26/02/2021 | Oxathiapiprolin/metalaxyl-M DC (A23109A) - Toxicity to the Water Flea Daphnia magna Straus under Laboratory Conditions (Acute Immobilisation Test – Static)<br>Report No. S20-06895<br>Document No. VV-893390<br>Test Facility Eurofins Agroscience Services EcoTox GmbH<br>GLP<br>Unpublished | N | SYN |
| KCP 10.2.1 | Schuler, L. | 12/04/2021 | Oxathiapiprolin/Metalaxyl-M DC (A23109A) - Toxicity to the Single Cell Green Alga Raphidocelis subcapitata Korshikov under Laboratory Conditions<br>Report No. S20-06896<br>Document No. VV-898484<br>Test Facility Eurofins Agroscience Services EcoTox GmbH<br>GLP<br>Unpublished | N | SYN |
| KCP 10.3.1.1 | Franke, M. | 25/01/2021 | Oxathiapiprolin/Metalaxyl-M DC (A23109A) - Acute Toxicity to the Honeybee Apis mellifera L. under Laboratory Conditions<br>Report No. 21 48 BAA 0002<br>Document No. VV-892684<br>Test Facility BioChem agrar GmbH<br>GLP<br>Unpublished | N | SYN |
| KCP 10.3.1.1.1 | Amsel, K. | 10/01/2022 | Oxathiapiprolin/Metalaxyl-M DC (A23109A) - Acute toxicity to the bumblebee Bombus terrestris L. under laboratory conditions<br>Report No. 21 48 BBA 0033<br>Document No. VV-936483<br>Test Facility BioChem agrar GmbH<br>GLP<br>Unpublished | N | SYN |

| 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 10.3.1.2 | Dressler, K. | 11/11/2020 | Oxathiapiprolin/Metalaxyl-M DC (A23109A) - Chronic toxicity to the honey bee Apis mellifera L. in a 10-day continuous laboratory feeding study<br>Report No. 20 48 BAC 0044<br>Document No. VV-896929<br>Test Facility BioChem agrar GmbH<br>GLP<br>Unpublished | N | SYN |
| KCP 10.3.1.3 | Schmidt, K. | 19/03/2021 | Oxathiapiprolin/Metalaxyl-M DC (A23109A) – Repeated Exposure of the Honey Bee Larvae (Apis mellifera L.) under Laboratory Conditions (until Adult Emergence up to Day 22)<br>Report No. 20 48 BLC 0044<br>Document No. VV-895633<br>Test Facility BioChem agrar GmbH<br>GLP<br>Unpublished | N | SYN |
| ~~KCP 10.3.2.2~~ | ~~Fallowfield, L.~~ | ~~08/04/2014~~ | ~~Metalaxyl-M ES (A9642C) – A rate-response extended laboratory bioassay of the effects of fresh residues on the predatory mite Typhlodromus pyri (Acari: Phytoseiidae)~~<br>~~Report No. SYN-14-5~~<br>~~Document No. VV-407366 , A9642C_10289~~<br>~~Test Facility Mambo-Tox, Ltd.~~<br>~~GLP~~<br>~~Unpublished~~ | ~~N~~ | ~~SYN~~ |
| KCP 10.3.2.1 | Stevens, J. | 03/12/2020 | Oxathiapiprolin/Metalaxyl-M DC (A23109A) – A Rate-Response Laboratory Study to Determine the Effects of Fresh Residues on the Parasitic Wasp Aphidius rhopalosiphi (Hymenoptera, Braconidae)<br>Report No. SYN-20-72<br>Document No. VV-887720<br>Test Facility Mambo-Tox, Ltd.<br>GLP<br>Unpublished | N | SYN |

| 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 10.3.2.2 | Fallowfield, L. | 16/03/2021 | Oxathiapiprolin/metalaxyl-M DC (A23109A) – A Rate-Response Extended Laboratory Study to Determine the Effects of Fresh Residues on the Predatory Mite Typhlodromus pyri (Acari: Phytoseiidae)<br>Report No. SYN-20-77<br>Document No. VV-895497<br>Test Facility Mambo-Tox, Ltd.<br>GLP<br>Unpublished | N | SYN |
| KCP 10.3.2.2 | Stevens, J. | 16/03/2021 | Oxathiapiprolin/Metalaxyl-M DC (A23109A) – A Rate-Response Extended Laboratory Study of the Effects of Fresh Residues on the Parasitic Wasp Aphidius rhopalosiphi (Hymenoptera, Braconidae)<br>Report No. SYN-20-78<br>Document No. VV-895493<br>Test Facility Mambo-Tox, Ltd.<br>GLP<br>Unpublished | N | SYN |
| KCP 10.3.2.2 | Tew, G. | 01/06/2021 | Oxathiapiprolin/metalaxyl-M DC (A23109A) - A Rate-Response Extended Laboratory Study of the Effects of Freshly Treated Substrate on the Rove Beetle,<br>Aleochara bilineata (Coleoptera, Staphylinidae)<br>Report No. SYN-20-79<br>Document No. VV-905353<br>Test Facility Mambo-Tox, Ltd.<br>GLP<br>Unpublished | N | SYN |

| 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 10.3.2.2 | Vaughan, R. | 05/05/2021 | Oxathiapiprolin/Metalaxyl-M DC (A23109A) – A Rate-Response Extended Laboratory Study to Evaluate the Effects of Fresh Residues on the Green Lacewing, Chrysoperla carnea (Neuroptera, Chrysopidae)<br>Report No. SYN-20-80<br>Document No. VV-902193<br>Test Facility Mambo-Tox, Ltd.<br>GLP<br>Unpublished | N | SYN |
| ~~KCP 10.4.1~~ | ~~Friedrich, S.~~ | ~~17/02/2021~~ | ~~Oxathiapiprolin/metalaxyl-M DC (A23109A) – Acute Toxicity to the Earthworm Eisenia andrei in Artificial Soil~~<br>~~Report No. 21 48 TEA 0001~~<br>~~Document No. VV-892721~~<br>~~Test Facility BioChem agrar GmbH~~<br>~~GLP~~<br>~~Unpublished~~ | ~~N~~ | ~~SYN~~ |
| KCP 10.4.1.1 | Friedrich, S. | 19/02/2021 | Oxathiapiprolin/metalaxyl-M DC (A23109A) - Sublethal Effects on the Reproduction of the Earthworm Eisenia andrei in Artificial Soil<br>Report No. 21 48 TEC 0005<br>Document No. VV-892682<br>Test Facility BioChem agrar GmbH<br>GLP<br>Unpublished | N | SYN |
| KCP 10.4.1.2 | Friedrich, S. | 04/02/2021 | Oxathiapiprolin/metalaxyl-M DC (A23109A) - Effects on the Reproduction of the Collembolan Folsomia candida<br>Report No. 21 48 TCC 0003<br>Document No. VV-891167<br>Test Facility BioChem agrar GmbH<br>GLP<br>Unpublished | N | SYN |

| **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 10.4.1.2 | Schulz, L. | 15/01/2021 | Oxathiapiprolin/metalaxyl-M DC (A23109A) - Effects on the Reproduction of the Predatory Mite Hypoaspis aculeifer<br>Report No. 21 48 THC 0003<br>Document No. VV-890311<br>Test Facility BioChem agrar GmbH<br>GLP<br>Unpublished | N | SYN |
| KCP 10.5 | Schulz, L. | 24/02/2021 | Oxathiapiprolin/Metalaxyl-M DC (A23109A) – Effects on the Activity of Soil Microflora (Nitrogen and Carbon Transformation Tests)<br>Report No. 21 48 SMO 0002<br>Document No. VV-894279<br>Test Facility BioChem agrar GmbH<br>GLP<br>Unpublished | N | SYN |
| KCP 10.6.2 | Jones, K. | 26/01/2021 | Oxathiapiprolin/metalaxyl-M DC (A23109A) - Phytotoxicity to Non-Target Plants Screening Test<br>Report No. ACE-20-109<br>Document No. VV-890173<br>Test Facility AgroChemex, Ltd<br>GLP<br>Unpublished | N | SYN |

**List of data submitted by the applicant and relied on (metalaxyl-M)**

| 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 |
|---|---|---|---|---|---|
| KCA1 8.1.1.3 | [redacted] | 15/01/1996 | The reproductive toxicity test of CGA 277476 technical in northern bobwhite, colinus virginianus<br>Report No. 029401<br>Document No. VV-352227 , CGA277476/0292<br>Test Facility ETL_undetermined<br>GLP<br>Unpublished | Y | SYN |
| KCA1 8.1.1.3 | [redacted] | 15/01/1996 | The reproductive toxicity test of CGA 215944 technical in northern bobwhite, Colinus virginianus<br>Report No. 029502<br>Document No. VV-369024 , CGA215944/0344<br>Test Facility EBA, Inc.<br>GLP<br>Unpublished | Y | SYN |
| KCA1 8.1.1.3 | [redacted] | 09/07/1998 | The reproductive toxicity test of CGA 293343 technical with the northern bobwhite (Colinus virginianus)<br>Report No. 029518<br>Document No. VV-376393 , CGA293343/0653<br>Test Facility EBA, Inc.<br>GLP<br>Unpublished | Y | SYN |
| KCA1 8.1.1.3 | [redacted] | 07/05/1996 | The reproductive toxicity test of CGA 24705 in Northern Bobwhite (Colinus virginianus)<br>Report No. 029508<br>Document No. VV-371001 , CGA24705/2591<br>Test Facility EBA, Inc.<br>GLP<br>Unpublished | Y | SYN |

| 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 |
|---|---|---|---|---|---|
| KCA1 8.3.1.1 | Amsel, K. | 17/12/2021 | Metalaxyl-M - Acute toxicity to the bumblebee Bombus terrestris L. under laboratory conditions<br>Report No. 21-48 BBA 0011<br>Document No. VV-939735<br>Test Facility BioChem agrar GmbH<br>GLP<br>Unpublished | N | SYN |
| KCA1 8.3.1.2 | Ruhland, S. | 09/12/2021 | Metalaxyl-M – Chronic toxicity to the honey bee Apis mellifera L. in a 10-day continuous laboratory feeding study<br>Report No. 21-48 BAC 0027<br>Document No. VV-932880<br>Test Facility BioChem agrar GmbH<br>GLP<br>Unpublished | N | SYN |
| KCA1 8.3.1.2 | Kling, A. | 06/06/2018 | Metalaxyl-M SL (A13947A) – Assessment of Effects on the Adult Honey Bee, Apis mellifera L., in a 10-Day Chronic Feeding Test under Laboratory Conditions<br>Report No. S15-00380<br>Document No. VV-414721 , A13947A_11449<br>Test Facility Eurofins Agroscience Services EcoChem GmbH<br>GLP<br>Unpublished | N | SYN |
| KCA1 8.3.1.3 | Schmidt, K. | 18/11/2021 | Metalaxyl-M – Repeated Exposure of the Honey Bee Larvae (Apis mellifera L.) under Laboratory Conditions (until Adult Emergence up to Day 22)<br>Report No. 21-48 BLC 0026<br>Document No. VV-930784<br>Test Facility BioChem agrar GmbH<br>GLP<br>Unpublished | N | SYN |

| 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 |
|---|---|---|---|---|---|
| KCA1 8.3.1.3 | Eckert, J. | 14/01/2016 | Metalaxyl-M SL (A13947A) – Honey Bee (Apis mellifera L.) Larval Toxicity Test (Repeated Exposure)<br>Report No. S15-02457<br>Document No. VV-415529 , A13947A_11455<br>Test Facility Eurofins Agroscience Services GmbH<br>GLP<br>Unpublished | N | SYN |

**List of data submitted by the applicant and relied on (oxathiapiprolin)**

| 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 |
|---|---|---|---|---|---|
| KCA2 8.3.1.2 | Tänzler V | 2015 | Oxathiapiprolin (DPX-QGU42) 100 g/L OD: Chronic oral toxicity to the honey bee, *Apis mellifera* L. (Hymenoptera, Apidae)<br>Report Number 94441136<br>Institut für Biologische Analytik und Consulting IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany<br>GLP<br>Unpublished<br>DuPont Study No. DuPont-41989 | N | Corteva (Syngenta access) |

| Data point | Author(s) | Year | Title<br>Company Report No.<br>Source (where different from company)<br>GLP or GEP status<br>Published or not | Vertebrate study<br>Y/N | Owner |
|---|---|---|---|---|---|
| KCA2 8.3.1.3 | Oberrauch S | 2017 | Oxathiapiprolin (DPX-QGU42) technical: Honey bee (*Apis mellifera* L.) 22-day larval toxicity test (repeated exposure)<br>Report Number S17-01639<br>Eurofins Agroscience Services EcoChem GmbH / Eurofins Agroscience Services Ecotox GmbH, Eutinger Str. 24, 75223 Niefern-Öschelbronn, Germany<br>GLP<br>Unpublished<br>DAS Study No. DuPont-48606 | N | Corteva (Syngenta access) |

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<br>Y/N | Owner |
|---|---|---|---|---|---|
| KCP 10.1.1 | [redacted] | 28/01/2021 | Oxathiapiprolin/Metalaxyl-M DC (A23109A) – An Acute Oral Toxicity Study with the Northern Bobwhite using a Sequential Testing Procedure<br>Report No. 528B-605<br>Document No. VV-891190<br>Test Facility Eurofins EAG Agroscience LCC<br>GLP<br>Unpublished | Y | SYN |
| KCA1 8.1.1.3 | [redacted] | 15/01/1996 | The reproductive toxicity test of CGA 277476 technical in northern bobwhite, colinus virginianus<br>Report No. 029401<br>Document No. VV-352227 , CGA277476/0292<br>Test Facility ETL_undetermined<br>GLP<br>Unpublished | Y | SYN |
| KCA1 8.1.1.3 | [redacted] | 15/01/1996 | The reproductive toxicity test of CGA 215944 technical in northern bobwhite, Colinus virginianus<br>Report No. 029502<br>Document No. VV-369024 , CGA215944/0344<br>Test Facility EBA, Inc.<br>GLP<br>Unpublished | Y | SYN |
| KCA1 8.1.1.3 | [redacted] | 09/07/1998 | The reproductive toxicity test of CGA 293343 technical with the northern bobwhite (Colinus virginianus)<br>Report No. 029518<br>Document No. VV-376393 , CGA293343/0653<br>Test Facility EBA, Inc.<br>GLP<br>Unpublished | Y | SYN |

| 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 |
|---|---|---|---|---|---|
| KCA1 8.1.1.3 | [redacted] | 07/05/1996 | The reproductive toxicity test of CGA 24705 in Northern Bobwhite (Colinus virginianus)<br>Report No. 029508<br>Document No. VV-371001 , CGA24705/2591<br>Test Facility EBA, Inc.<br>GLP<br>Unpublished | Y | SYN |
| KCA1 8.3.1.1 | Amsel, K. | 17/12/2021 | Metalaxyl-M - Acute toxicity to the bumblebee Bombus terrestris L. under laboratory conditions<br>Report No. 21 48 BBA 0011<br>Document No. VV-939735<br>Test Facility BioChem agrar GmbH<br>GLP<br>Unpublished | N | SYN |
| KCA1 8.3.1.2 | Ruhland, S. | 09/12/2021 | Metalaxyl-M - Chronic toxicity to the honey bee Apis mellifera L. in a 10-day continuous laboratory feeding study<br>Report No. 21 48 BAC 0027<br>Document No. VV-932880<br>Test Facility BioChem agrar GmbH<br>GLP<br>Unpublished | N | SYN |
| KCA1 8.3.1.2 | Kling, A. | 06/06/2018 | Metalaxyl-M SL (A13947A) – Assessment of Effects on the Adult Honey Bee, Apis mellifera L., in a 10 Day Chronic Feeding Test under Laboratory Conditions<br>Report No. S15-00380<br>Document No. VV-414721 , A13947A_11449<br>Test Facility Eurofins Agroscience Services EcoChem GmbH<br>GLP<br>Unpublished | N | SYN |
| KCA1 8.3.1.3 | Schmidt, K. | 18/11/2021 | Metalaxyl-M – Repeated Exposure of the Honey Bee Larvae (Apis mellifera L.) under Laboratory Conditions (until Adult Emergence up to Day 22)<br>Report No. 21 48 BLC 0026<br>Document No. VV-930784 | N | SYN |

| 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 |
|---|---|---|---|---|---|
| | | | Test Facility BioChem agrar GmbH<br>GLP<br>Unpublished | | |
| KCA1 8.3.1.3 | Eckert, J. | 14/01/2016 | Metalaxyl-M SL (A13947A) – Honey Bee (Apis mellifera L.) Larval Toxicity Test (Repeated Exposure)<br>Report No. S15-02457<br>Document No. VV-415529 , A13947A_11455<br>Test Facility Eurofins Agroscience Services GmbH<br>GLP<br>Unpublished | N | SYN |
| KCA2 8.3.1.2 | Tänzler V | 2015 | Oxathiapiprolin (DPX-QGU42) 100 g/L OD: Chronic oral toxicity to the honey bee, Apis mellifera L. (Hymenoptera, Apidae)<br>Report Number 94441136<br>Institut für Biologische Analytik und Consulting IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany<br>GLP<br>Unpublished<br>DuPont Study No. DuPont-41989 | N | Corteva (Syngenta access) |
| KCA2 8.3.1.3 | Oberrauch S | 2017 | Oxathiapiprolin (DPX-QGU42) technical: Honey bee (Apis mellifera L.) 22 day larval toxicity test (repeated exposure)<br>Report Number S17-01639<br>Eurofins Agroscience Services EcoChem GmbH / Eurofins Agroscience Services Ecotox GmbH, Eutinger Str. 24, 75223 Niefern-Öschelbronn, Germany<br>GLP<br>Unpublished<br>DAS Study No. DuPont-48606 | N | Corteva (Syngenta access) |
| KCP 10.3.2.2 | Fallowfield, L. | 08/04/2014 | Metalaxyl-M ES (A9642C) – A rate-response extended laboratory bioassay of the effects of fresh residues on the predatory mite Typhlodromus pyri (Acari: Phytoseiidae)<br>Report No. SYN-14-5<br>Document No. VV-407366 , A9642C_10289<br>Test Facility Mambo-Tox, Ltd. | | |

| 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>Unpublished | | |
| KCP 10.4.1 | Friedrich, S. | 17/02/2021 | Oxathiapiprolin/metalaxyl-M DC (A23109A) - Acute Toxicity to the Earthworm Eisenia andrei in Artificial Soil<br>Report No. 21 48 TEA 0001<br>Document No. VV-892721<br>Test Facility BioChem agrar GmbH<br>GLP<br>Unpublished | N | SYN |

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

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

# Appendix 2 Detailed evaluation of the new studies

**Review Comments:**

Additional active substance data submitted by the applicant were consider by zRMS as not necessary for authorisation. Therefore, according to SANCO/10328/2004 – rev 9 (21.10.2021) point 4.2.1, those studies were not evaluated by zRMS.

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

### A 2.1.1 KCP 10.1.1 Effects on birds

| Comments of zRMS: | Study not evaluated by zRMS. |
|---|---|

| | |
|---|---|
| Reference: | KCA1 8.1.1.3 |
| Report | Taliaferro L., Brewer L. (1996), The Reproductive Toxicity Test of CGA-277476 Technical In northern bobwhite *(Colinus virginianus)*, Report Number 029401. Ecotoxicology & Biosystems Associates, Inc. 2900 Quakenbush Rd. Snow Camp, NC 27349 (Syngenta File No. CGA277476/0292, VV-352227) |
| Guideline(s): | EPA Guideline No.: 71-4 |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication (if vertebrate study) | No |

**Executive Summary**

No mortality or toxicity was observed in Northern bobwhite exposed to CGA277476 at dietary concentrations of 10, 35 and 100 ppm for 22 weeks. There were no significant differences detected at any level of treatment when statistically compared against the control group. The no observable effect concentration (NOEC) during the study was therefore 100 ppm.

**Materials**

| | |
|---|---|
| **Test Material** | CGA277476 Technical |
| **Lot/Batch #:** | FL-930038 |
| **Purity:** | 95.4% |
| **Stability of test compound:** | Stable under standard conditions |
| **Reanalysis/Expiry date:** | 27 July 1997 |
| **Treatments** | |
| **Test rates:** | Control, 10, 35 and 100 ppm |
| **Food:** | Purina Layina® game bird ration |
| **Water:** | Water was provided *ad libitum* |
| **Chemical analysis:** | Yes, at 10, 35 and 100 ppm by LC-UV |
| **Test organisms** | |
| **Species:** | Northern bobwhite *(Colinus virginianus)* |
| **Source:** | Whistling Wings, Inc., 113 Washington St., Hanover, Illinois 61041 |

| | |
|---|---|
| **Acclimatisation period:** | 2 weeks |
| **Treatment for disease:** | None |
| **Weight :** | 150-250 g |
| **Test design** | |
| **Test cage description:** | weld-wire cages measuring 50.7 cm (d) x 26 cm (w) x 25 cm (h) |
| **Replication:** | 16 |
| **No. of birds/pen :** | 2 (one male and one female) |
| **Duration of test:** | 22 weeks |
| **Environmental test conditions** | |
| **Temperature:** | 23.3°C ± 2.6°C |
| **Humidity:** | 54.5% ± 7.9% |
| **Photoperiod:** | 7 hours light per 24-hours for weeks and 17 hours light per day from week 9 |

**Study Design and Methods**

Experimental dates: 7 March 1994 – 14 September 1994

Mature Northern bobwhite received CGA277476 technical at nominal dietary concentrations of 10, 35 and 100 ppm for 22 weeks. A control group receiving basal diet was maintained concurrently with the treatment groups.

The test item was provided as a treated diet. Purina bird ration was used for feed and was provided *ad libitum* during acclimation and the test period.

Birds were kept in pairs one male and one female per cage. The cage dimensions were 50.7 cm deep, 26 cm wide and 25 cm in height, with a floor slope so eggs roll forward and out of the cage on to a collection tray.

Body weight was measured during acclimation, week 11 and at adult termination but not measured during egg laying due to the possible adverse effects handling of the test birds may have on the egg production and to avoid injury to the eggs. Feed consumption was measured weekly for each pair of birds. Birds were observed daily for signs of behavioural abnormality and mortality. Post-mortem examinations were performed on all birds upon completion of the exposure.

Eggs were collected on a daily basis and marked. Cracked eggs were recorded as cracked and discarded. Weekly throughout the egg laying period, one egg was collected, when available, from each of the odd numbered cages during even numbered weeks and from each of the even numbered changes on odd numbered weeks. The eggs were cleaned of their contents, eggshells were then allowed to air dry and measured at 5 points around the equator.

On day 10-12 of incubation, eggs were candled for fertility. Those that were not fertile were discarded. On day 21 of incubation eggs were candled for viability, those deemed not viable were discarded.

Upon hatching chicks were weighed and banded with a unique wing tag. Chicks were housed in caging units measuring 71 cm x 91 cm x 27 cm. Chicks were fed Purina Startena game ration *ad libitum*. Chicks were observed daily for behaviour and mortality. Surviving chicks were euthanized on the 14$^{th}$ day and weighed.

Statistical analysis was performed using TOXSTAT (1994). Data sets were tested for normality using a chi-square test and for homogeneity of variance using a Bartlett's test or Levene's test. Proportional data were arcsine transformed. If the data were normal and variances were homogenous the parameters were analysed with ANOVA Dunnett's (equal sized groups) or an ANOVA and then a Tukey's post-hoc test for pair-wise comparisons. If data was not normal or were heterogeneous, they were analysed with a Kruskal Wallis' ANOVA by ranks followed by a Dunn's multiple comparison.

**Results and Discussion**

Results are summarised in the tables below.

**Table A 1: Summary of bodyweight of Northern bobwhite quail with CGA277476**

| Experimental group (ppm) | Sex | Body weight (g) | | |
|---|---|---|---|---|
| | | Start of test feed | Start of egg lay | Study finish |
| Control | Male | 214.3 | 218.1 | 231.5 |
| | Female | 214.3 | 230.5 | 252.3 |
| 10 | Male | 211.8 | 216.5 | 225.4 |
| | Female | 210.4 | 230.9 | 254.0 |
| 35 | Male | 211.7 | 216.8 | 229.1 |
| | Female | 202.6 | 222.2 | 243.6 |
| 100 | Male | 217.8 | 223.0 | 242.4 |
| | Female | 221.4 | 238.5 | 263.7 |

No significant difference between Control group and Treatment groups ($p > 0.05$)

**Table A 2: Summary of reproductive performance from Northern bobwhite quail reproduction with CGA277476**

| Parameter | Experimental group (ppm) | | | |
|---|---|---|---|---|
| | Control | 10 | 35 | 100 |
| Total eggs laid | 552 | 561 | 727 | 649 |
| Eggs cracked | 7 | 6 | 20 | 9 |
| Fertile eggs | 477 | 475 | 595 | 533 |
| Viable embryos | 469 | 469 | 571 | 523 |
| Hatchlings | 447 | 457 | 546 | 510 |
| 14 day survivors | 404 | 414 | 497 | 452 |

**Conclusions**

No mortality or toxicity was observed in Northern bobwhite exposed to CGA277476 at dietary concentrations of 10, 35 and 100 ppm for 22 weeks. There were no significant differences detected at any level of treatment when statistically compared against the control group. The no observable effect concentration (NOEC) during the study was therefore 100 ppm.

(Taliaferro L., Brewer L. 1996)

| Comments of zRMS: | Study not evaluated by zRMS. |
|---|---|

**Reference:** KCA1 8.1.1.3

**Report** Taliaferro LC. Brewer LW. (1996) The Reproductive Toxicity Test of CGA-215944 Technical in Northern Bobwhite, *Colinus Virginianus.* Report Number 029502. Ecotoxicology and Biosystems Associates, Inc. 2900 Quakenbush Rd., Snow Camp, North Carolina 27349 (Syngenta File No. CGA215944/0344 / VV-369024)

**Guideline(s):** EPA Guideline No.: 71-4

**Deviations:** No

**GLP:** Yes

**Acceptability:** Yes

**Duplication (if vertebrate study)** No

**Executive Summary**

The effects of CGA-215944 on adult northern bobwhites (*Colinus virginianus*) were determined in a 20-week reproduction toxicity test. Mortality, body weight, food consumption, reproductive parameters, and any other overt signs of toxicity, were assessed at nominal dietary test concentrations of 30, 100 and 300 ppm CGA-215944, alongside an untreated control group.

There were no treatment related mortalities or treatment-related effects observed in any of the parameters tested. The NOEC for dietary exposure to CGA-215944 was determined to be 300 ppm CGA-215944, the highest concentration tested.

**Materials**

| | |
|---|---|
| **Test Material** | CGA-215944 technical |
| **Lot/Batch #:** | P102002 |
| **Purity:** | 98.5% |
| **Description:** | Yellowish powder |
| **Stability of test compound:** | Stable under standard conditions |
| **Reanalysis/Expiry date:** | 8 July 1996 |
| **Density:** | Not applicable |
| **Treatments** | |
| **Test rates:** | 30, 100 and 300 ppm CGA 215944, alongside an untreated control |
| **Food:** | Basal diet (minimum 20% protein and 2.5% fat; maximum 7 % fibre for adults and minimum 30% protein and 2.5 % fat; maximum 6.5 % fibre for the offspring) |
| **Water:** | From deep well located on the EBA, Inc. Snow Camp, NC site. |
| **Analysis of test concentrations** | Homogeneity tested day 0 of week 1; stability tested on days 0, 7 and 14. Additional samples collected from each treatment group each time the feed was mixed to measure/verify test concentrations. Samples taken from control and treated diets on each occasion. |
| **Test organisms** | |
| **Species:** | Northern bobwhite (*Colinus virginianus*) |
| **Source:** | Birds from the same hatch, obtained from Buffalo Creek Quail Farms, Ellerbe, North Carolina. |
| **Acclimatisation period:** | 3.5 weeks |
| **Treatment for disease:** | None |
| **Weight:** | 219.5 – 228.0 g at test initiation |
| **Test design** | |
| **Replication:** | 19 |
| **No. of birds/pen :** | 2 (1 male and 1 female) |
| **Duration of test:** | Study phases:<br>Acclimation – approximately 3.5 weeks<br>Pre-photostimulation – approximately 8 weeks<br>Pre-egg laying (with photostimulation) – approximately 2 weeks<br>Treated Feed – approximately20 weeks<br>Egg laying – approximately 10 weeks<br>Post-adult termination (final incubation, hatching, and 14-day offspring rearing period) – approximately 5 weeks |
| **Parameters monitored:** | Adult male and female body weight, adult feed consumption, number eggs laid per day, number eggs set into incubator, number eggs cracked/number eggs laid, number fertile eggs/number eggs set, number viable embryos/number fertile eggs, number of hatchlings/number of fertile eggs, number 14-day survivors/number hatchlings, hatchling weights, 14-day survivor weights, and eggshell thickness. |

**Environmental test conditions**

| | |
|---|---|
| **Temperature:** | 18.0 – 30.0°C (Mean = 23.8 °C) |
| **Humidity:** | 40 – 84 % RH (Mean = 62.1 % (RH)) |
| **Photoperiod:** | Adult birds: 7 hours light from test initiation to Week 8<br>17 hours light from Week 8 to adult birds were euthanized (photoperiod was increased over a 3-day period of time to 17 hours of light per day)<br>Fluorescent light, approximately 13.1 foot candles.<br>Hatchlings: 17 hours light per day, however, the brooder heat lamps did provide intermittent infra-red light throughout the 24 hour period. |

**Study Design and Methods**

Experimental dates: 18 April 1995 – 12 October 1995
Adult northern bobwhite (76 males and 76 females) in good health and approaching first breeding season, were randomly assigned into one control group and three treatment groups. The test substance doses were prepared using a premix of appropriate volumes of CGA-215944, such that, once incorporated into the final diet, treatment levels of 30, 100 and 300 ppm CGA-215944 were achieved. The premix for the control groups consisted of ration only. The birds were housed in male/female pairs in batteries of pens measuring approximately 50.7 x 26 x 25 cm high, and had slanted floors to facilitate eggs rolling to the front collection tray. The pens were constructed of epoxy-coated wire mesh and steel sheeting and were equipped with food and automatic watering system.

During the test all birds were given feed and water *ad libitum*. Adult birds were observed daily for signs of toxicity or abnormal behaviour. Offspring were observed daily from hatching until 14 days old. Adult body weights were measured at test initiation, end of week 10 and at adult termination. Body weights were not measured during egg-laying. Feed consumption per pen was measured weekly. Spillage was measured from each cage by use of a catch pan under the feeding trays. At the conclusion of the exposure period all adult birds were euthanized with $CO_2$ and subjected to a gross necropsy.

Eggs were collected daily, marked with the pen number, and stored in weekly interval lots in a cold room until incubation (~ 50 °F). At the end of each weekly interval eggs were counted, indiscriminately selected for measurement of eggshell thickness[15], and candled to detect cracked or abnormal eggs which were discarded. All eggs not discarded or used for eggshell thickness measurements were placed in an incubator. Embryo viability and survival were determined during incubation by candling on days 10-12 and day 20, respectively.

On Day 20 of incubation, eggs were transferred to an incubator configured for hatching, and all hatchlings, unhatched eggs, and eggshells were removed on Day 22 - 24 of incubation. Surviving hatchlings were leg-banded for identification and their body weights recorded. They were transferred to batteries of brooding pens and fed untreated diet until 14 days old, when the body weights of survivors were recorded.

Data sets were tested for normality using a Chi-Square test and for homogeneity of variance using Bartlett's test or Levene's test. Proportional data was Arcsine transformed and any data set departing from the normal distribution was transformed if the transformation results in a normal distribution. Statistical analyses used were ANOVA with Tukey's post-hoc test for paired comparisons if the data exhibited homogeneity of variance and a normal distribution. If data were heterogeneous or non-normal, they were analysed with a Kruskal-Wallis test followed by Dunn's multiple pair comparisons test. Sample units were individual pens within experimental groups, apart from adult body weight where the sample unit was the individual bird.

[15] One egg, when available, collected from odd numbered pens during odd numbered weeks, and even numbered pens during even numbered weeks

**Results and Discussion**

Samples collected during the test to verify test substance concentrations for the 30, 100 and 300 ppm CGA-2159444 diets were found to be 27.68 ± 3.56, 100.99 ± 12.22 and 293.35 ± 20.41 ppm CGA-215944, respectively.

Adult mortality, growth and feed consumption are summarised in the table below.

**Table A 3: Summary of effects of CGA-215944 on mortality, growth and feed consumption on adult northern bobwhite (Colinus virginianus) following dietary exposure**

| Nominal dose (ppm CGA-215944) | Mortality after 20 weeks (%) | Mean body weight [g] | | Mean Feed Consumption (g/bird/day) | |
|---|---|---|---|---|---|
| | | Week 10 (Pre-Egg Production) | Week 20 (Overall) | Week 10 | Week 20 |
| Control | etc0[1] | Male: 232.4 | Male: 246.0 | 19.1 | 23.3 |
| | | Female; 245.7 | Female; 261.0 | | |
| 30 | 0 | Male: 235.8 | Male: 239.9 | 19.3 | 23.5 |
| | | Female; 250.7 | Female; 263.0 | | |
| 100 | 0 | Male: 227.1 | Male: 236.3 | 19.1 | 23.2 |
| | | Female; 250.9 | Female; 261.2 | | |
| 300 | 0[2] | Male: 239.6 | Male: 248.7 | 19.4 | 26.9 |
| | | Female; 253.6 | Female; 263.9 | | |

[1] 3 mortalities found in the Control group in Test week 14, and 1 in test week 17. Post-mortem examinations were performed on all of the mortalities. There were no treatment-related findings.
[2] 1 mortality found in 300 ppm CGA-215944 group in test week 13. A post-mortem examination was performed. There were no treatment-related findings.

There were no treatment related mortalities in the control group or any of the treatment groups, and all birds in all groups were normal in appearance and behaviour for the duration of the test. There were no statistically significant differences in adult body weight or feed consumption between the treatment groups and the control group. No significant pathological changes were observed in any of the surviving birds examined at termination.

Reproductive effects are summarised in the table below.

**Table A 4: Summary of effects of CGA-215944 on reproductive parameters for northern bobwhite (*Colinus virginianus*) following dietary exposure**

| Nominal dose (ppm CGA-215944)[1] | Total eggs laid | Fertile eggs/Eggs incubated (normalized as %)[2] | Viable embryos / fertile eggs (normalized as %)[2] | Hatchlings / viable embryos (normalized as %)[2] | 14-Day old survivors / hatchlings (normalized as %)[2] | Hatchlings / Eggs Incubated (normalized as %)[2] | 14-day old survivors / Eggs incubated (normalized as %)[2] |
|---|---|---|---|---|---|---|---|
| Control | 797 | 0.96 | 0.99 | 0.97 | 0.72 | 0.93 | 0.67 |
| 30 | 932 | 0.92 | 0.98 | 0.95 | 0.83 | 0.86 | 0.71 |
| 100 | 976 | 0.96 | 0.99 | 0.98 | 0.72 | 0.93 | 0.67 |
| 300 | 886 | 0.87 | 0.96 | 0.98 | 0.66 | 0.82 | 0.54 |

[1] number of replicates = 16, 19, 19 and 18 for the control, 30, 100 and 300 ppm groups, respectively
[2]Mean value for all pens in treatment group
Differences between the control and treatment groups were not statistically significant.

There were no treatment-related effects on eggshell thickness, or on any of the reproductive performance parameters tested.

**Table A 5: Summary of effects of CGA-215944 on Northern Bobwhite (*Colinus virginianus*) hatchling growth following adult dietary exposure**

| Nominal dose (ppm CGA-215944) | Hatchlings | | 14-day old survivors | |
|---|---|---|---|---|
| | Number | Mean (± SD) body weight of (g) | Number | Mean (± SD) body weight of (g) |
| Control | 663 | 7.7 (± 0.643) | 479 | 24.4 (± 7.981) |
| 30 | 722 | 7.6 (± 0.660) | 599 | 23.4 (± 7.251) |
| 100 | 823 | 7.4 (± 0.639) | 596 | 20.8 (± 6.651) |
| 300 | 648 | 7.5 (± 0.625) | 428 | 24.6 (± 7.611) |

Differences between the control and treatment groups were not statistically significant.

**Conclusions**

There were no treatment-related mortalities or treatment-related effects observed in any of the parameters tested. The NOEC for dietary exposure to CGA-215944 was determined to be 300 ppm CGA-215944, the highest concentration tested.

(Taliaferro LC, Brewer LW., 1996)

| Comments of zRMS: | Study not evaluated by zRMS. |
|---|---|

| | |
|---|---|
| **Reference:** | KCA1 8.1.1.3 |
| **Report** | Taliaferro *et al*. (1996), The Reproductive Toxicity Test of CGA-24705 In Northern Bobwhite (*Colinus virginianus*). EBA, Inc., 2900 Quakenbush Rd., P.O. Box 554, Snow Camp, North Carolina 27349, USA. Unpublished. Report Number 029508 (Syngenta File No. CGA24705/2591 / VV-371001) |
| **Guideline(s):** | EPA Guideline No.: 71-4 |
| **Deviations:** | No |
| **GLP:** | Yes |
| **Acceptability:** | Yes |
| **Duplication (if vertebrate study)** | No |

**Executive Summary**

The effects of CGA 24705 on the northern bobwhite (*Colinus virginianus*) were determined in a 24-week reproduction toxicity test. Mortality, body weight, food consumption, reproductive parameters, and any other overt signs of toxicity, were assessed at nominal dietary concentrations of 50, 200 or 800 ppm CGA 24705.

No treatment-related effects were observed for adult mortality, signs of toxicity, adult body weight or feed consumption, reproductive performance, eggshell thickness or offspring body weight. The no observed effect level was considered to be 800 ppm CGA 24705, the highest rate tested.

**Materials**

| | |
|---|---|
| **Test Material** | Metolachlor Technical<br>CGA-24705<br>FL 930326 |
| **Lot/Batch #:** | P.111072 |
| **Purity** | 97.3 % |

| | |
|---|---|
| **Description:** | Brown liquid |
| **Stability of test compound:** | Stable under standard conditions |
| **Reanalysis/Expiry date:** | 01 March 1997 |
| **Density:** | Not reported |
| **Treatments** | |
| **Test rates:** | 50 ppm, 200 ppm and 800 ppm CGA 24705 (adjusted for purity), alongside an untreated control |
| **Food:** | Basal diet for the adult birds and their offspring was Purina Layena (minimum 20 % protein, minimum 2.5 % fat and maximum 7 % fibre) and Purina Startena (minimum 30 % protein, minimum 2.5 % fat and maximum 6.5 % fibre), respectively |
| **Water:** | Well water from a deep well located on the test facility site |
| **Analysis of test concentrations:** | Homogeneity was tested in four samples each from the 50 ppm and 800 ppm diet mix at the beginning of the study. Additional samples collected from the freshly prepared test diet batches to verify test substance concentrations. Samples for analysis of stability were analysed on day 0, 7 and 14 and at 4 months for ambient samples and on days 0, 14, 28 and at 4 months for frozen samples. |
| **Test organisms** | |
| **Species:** | Northern bobwhite (*Colinus virginianus*), 15 weeks old |
| **Source:** | Laboratory stock hatched and raised at test facility (Colony 022). Eggs from Strickland Quail Farm, Pooler Georgia, USA |
| **Acclimatisation period:** | 2 weeks |
| **Treatment for disease:** | None reported |
| **Weight:** | 162.0 to 229.3 g at start of acclimation period |
| **Test design** | |
| **Replication:** | 18 |
| **No. of birds/pen:** | Two (1 male and 1 female) |
| **Duration of test:** | 24 weeks |
| **Parameters monitored:** | Adult male bodyweight, adult female body weight, adult feed consumption, number eggs laid, number eggs set into incubator, number eggs cracked / number eggs laid, number fertile eggs / number eggs set, number viable embryos/ number fertile eggs, number hatchlings / number viable embryos, number 14-day survivors / number hatchlings, hatchling weights, 14-day survivor weights, eggshell thickness |
| **Environmental test conditions** | |
| **Temperature:** | Adult birds (study room range): 61 to 85 ºF<br>Egg storage prior to incubation: 50 ºF<br>Incubation and hatching (brooding pen): 102 ºF |
| **Humidity:** | Adult birds, average relative humidity: 62.1 % (range 40 to 84 %) |
| **Photoperiod:** | Adult birds: 7 hours of light per day during acclimation and the first 8 weeks, increasing over 13-day period to 17 hours of light per day from week 9 until the adult birds were euthanised. Mean light intensity was approximately 11.1 footcandles.<br>Incubation and hatching: 17 hours of light and 7 hours of dark, intermittent infra-red light was provided by the brooder heat lamps throughout the 24-hour period |

### Study Design and Methods

Experimental dates: 22 August 1995 to 28 March 1996
Adult northern bobwhite quail (72 males and 72 females), 15 weeks old and in good health, approaching first breeding season, were randomly assigned to one control group and three treatment groups. A pre-mix of suitable strength was prepared on an as-needed basis within the stability result parameters, by mixing the required quantities of test substance with the basal diet. The test substance doses were prepared by mixing appropriate quantities of pre-mix and basal ration, to achieve treatment levels of 50, 200 and 800 ppm CGA 24705. The mix for the control groups consisted of basal ration only.

Adult mating pairs were housed in epoxy-coated wire mesh battery cages measuring 50.7 x 26 x 25 cm. During the test birds were given food and water *ad libitum*. For the first eight weeks of treated feed, the

test birds were held under a photoperiod of 7 hours of light per day. At the end of week 8, the photoperiod was increased to 17 hours of light per day to induce egg laying. Adult continued on a photoperiod of 17 hours light per day until termination. The first eggs were set for incubation at the end of week 14.

Adult birds were observed daily for mortality, signs of toxicity or abnormal behaviour. Offspring were observed daily from hatching until 14 days old. Adult body weights were measured at the beginning of acclimatisation, at exposure initiation, at the end of week10 and test termination. Feed consumption per pen was recorded weekly throughout the treatment period. At the conclusion of the experiment, all adult birds and hatchlings were examined following euthanasia by carbon dioxide asphyxiation.

Eggs were collected daily and stored in a cold room until incubation. Following each seven-day period, eggs were removed from the cold rooms prior to incubation and candling. The total number of eggs collected and the number of un-cracked and unblemished eggs were recorded. Non-defective eggs were placed in an incubator. On approximately day 21 of incubation, the eggs were placed in a hatcher and allowed to hatch.

Weekly during the egg-laying period, one egg laid per treatment group was examined for shell thickness. Embryo viability and survival, unhatched eggs, hatchlings, and weight and viability of chicks, were recorded for each hatch. Chicks were not examined *postmortem* upon study completion.

Data sets were tested for normality using a Chi-Square test and for homogeneity of variance using Bartlett's test or Levene's test. Proportional data was arcsine transformed and any data set departing from the normal distribution was transformed if the transformation resulted in a normal distribution. Data parameters were analysed by ANOVA with Tukey's post-hoc test for paired comparisons if the data exhibited homogeneity of variance and a normal distribution. If data were heterogeneous or non-normal, they were analysed with Kruskal-Wallis test followed by Dunn's multiple pair comparisons test.

**Results and Discussion**

Fecundity is summarised in the table below.

**Table A 6: Summary of effects of CGA 24705 on fecundity of northern bobwhite (*Colinus viginianus*) following oral exposure**

| Parameter | Control | 50 ppm CGA 24705 | 200 ppm CGA 24705 | 800 ppm CGA 24705 |
|---|---|---|---|---|
| Number of replicates | 13 | 15 | 14* | 14 |
| Total eggs laid | 668 | 587 | 433 | 597 |
| Eggs cracked | 24 | 33 | 16 | 29 |
| Eggs for eggshell thickness | 62 | 66 | 57 | 56 |
| Eggs set | 582 | 488 | 360 | 512 |
| Fertile eggs | 540 | 417 | 320 | 477 |
| Viable embryos | 533 | 398 | 304 | 455 |
| Hatchlings | 497 | 354 | 262 | 391 |
| 14-day old survivors | 274 | 170 | 130 | 216 |
| Number of eggs laid** | 0.65 | 0.50 | 0.37*** | 0.54 |
| Number of eggs set into incubator** | 0.57 | 0.41 | 0.33*** | 0.46 |
| Eggs cracked / Eggs laid | 0.04 | 0.06 | 0.04 | 0.05 |
| Fertile eggs / Eggs set | 0.93 | 0.85 | 0.89 | 0.93 |
| Viable embryos / Fertile eggs | 0.99 | 0.95 | 0.95 | 0.95 |
| Hatchlings / Viable embryos | 0.93 | 0.89 | 0.86 | 0.86 |
| 14-day old survivors / | 0.55 | 0.48 | 0.50 | 0.55 |

| | | | | |
|---|---|---|---|---|
| Hatchlings | | | | |

* Cage number 19 in the 200 ppm group produced 0 eggs. As a result, it was included only as a statistical replicated for the comparison of eggs laid and not for any of the subsequent reproductive parameters, because no real data values exist for those parameters. Therefore, the number of statistical replicates for eggs laid in the 200 ppm group is 15 and for the remaining comparisons it is 14.
** These parameters computed as number per day per hen. All others computed as total / total (for example: total eggs cracked / total eggs laid)
*** Significantly different from the control group ($p < 0.05$). The effects observed do not reflect the expected dose response, and therefore are probably not treatment related.

There were no treatment-related mortalities or signs of toxicity during the experimental period or in the *postmortem* examination. There were no apparent treatment related effects upon adult body weight or feed consumption at any of the concentrations tested.

There were no apparent treatment-related effects upon reproductive performance, eggshell thickness or offspring body weights at any of the concentrations tested. Birds in the 200 ppm CGA 24705 test concentration showed decrease in number of eggs laid that was statistically significant ($p < 0.05$); however, there were no indications of concentration-dependant effects, it was not considered to be treatment related.

**Validity criteria**

No validity criteria were reported.

**Conclusions**

No treatment-related effects were observed for northern bobwhites exposed to CGA 24705 for adult mortality, signs of toxicity, adult body weight or feed consumption, reproductive performance, eggshell thickness or offspring body weight. The no observed effect level was considered to be 800 ppm CGA 24705.

(Taliaferro LC, Taliaferro MC, Miller VC, 1996)

| | |
|---|---|
| Comments of zRMS: | Study not evaluated by zRMS. |

| | |
|---|---|
| Reference: | KCA1 8.1.1.3 |
| Report | Taliaferro M.C. & Miller V. C. (1998), The reproductive toxicity test of CGA293343 Technical with the Northern bobwhite (*Colinus virginianus*), Report Number 029518. EBA Inc. 2900 Quakenbush Rd., Snow Camp, NC27349 (Syngenta File No. CGA293343/0653, VV-376393) |
| Guideline(s): | EPA Guideline No.: 71-4 |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication (if vertebrate study) | No |

**Executive Summary**

No mortality or toxicity was observed in Northern bobwhite quail exposed to CGA293343 at a dietary concentration of 100 ppm, 300 ppm or 900 ppm for 23 weeks and five days. There were no significant differences detected at any level of treatment when statistically compared against the control group.

The no observable effect concentration (NOEC) during the study was therefore 900 ppm.

## Materials

| | |
|---|---|
| **Test Material** | CGA293343 |
| **Lot/Batch #:** | FL961679 |
| **Purity:** | 99.2% |
| **Stability of test compound:** | Stable under standard conditions. |
| **Expiration date:** | 31 July 1997 |
| **Density:** | n/a |
| **Treatments** | |
| **Test rates:** | 100 ppm, 300 ppm and 900 ppm |
| **Test organisms** | |
| **Species:** | Northern bobwhite *(Colinus virginianus)* |
| **Source:** | Buffalo Creek Quail Farm, PO Box 579, Ellerbe, NC, USA |
| **Acclimatisation period:** | 28 days |
| **Treatment for disease:** | None |
| **Weight:** | 169– 237 g |
| **Test design** | |
| **Replication:** | 18 |
| **No. of birds/pen :** | 2 (one male, one female) |
| **Duration of test:** | 204 days |
| **Environmental test conditions** | |
| **Temperature:** | 14.4 to 24.4°C |
| **Humidity:** | 24 to 82% (mean 47.8% SD 12.1%) |
| **Photoperiod:** | 7 hours light for 8 weeks, increased over a period of 5 days to 17 hours light for remainder (14.8 footcandles) |

## Study Design and Methods

Experimental dates: 5 September 1996 to 28 March 1997
Mature Northern bobwhite received CGA293343 technical at nominal dietary concentrations of 100, 300 and 900 ppm for 23 weeks and 5 days. A control group receiving basal diet was maintained concurrently with the treatment groups.

The test item was provided at a treated diet. Purina game bird ration was used for feed and was provided *ad libitum* during acclimation and the test period.

Birds were kept in pairs one male and one female per cage. The cage dimensions were 51 cm deep, 25cm wide and 20.5-25 cm in height, with a floor slope so eggs roll forward and out of the cage on to a collection tray.

Body weight was measured 4 times during acclimation and the definitive test. Feed consumption was measured weekly for each pair of birds. Birds where observed daily for signs of behavioural abnormality and mortality. Gross pathological examinations were performed on all birds succumbing prior to adult termination and on all birds surviving the test.

Eggs were collected on a daily basis and marked. Eggs were candled prior to being placed in an incubator. Cracked eggs were recorded as cracked and discarded. Weekly throughout the egg laying period, one egg was collected, when available, from each of the odd numbered cages during even numbered weeks and from each of the even numbered changes on odd numbered weeks. The eggs were cleaned of their contents, eggshells were then allowed to air dry measured at 5 points around the equator.

On day 14 of incubation, eggs were candled for fertility. Those that were not fertile were discarded. On day 21 of incubation eggs were candled for viability, those deemed not viable were discarded.

Upon hatch chicks were weighed and banded with a unique wing tag. Chicks were housed in caging units measuring 21 cm long x 21 cm wide x 27 cm high, chicks from no more than two parental pairs were housed in one unit. Chicks were fed Purina Startena game ration *ad libitum*. Chicks were observed daily for behaviour and mortality. Surviving chicks were euthanized on the 14$^{th}$ day and weighed.

Statistical analysis was performed using TOXSTAT (1994). Data sets were tested for normality using a chi-square test and for homogeneity of variance using a Bartlett's test or Levene's test. Proportional data were arcsine transformed. If the data were normal and variances were homogenous the parameters were analysed with ANOVA Dunnett's post hoc test or an ANOVA and then a Tukey's post-hoc test for pair-wise comparisons. If data was not normal or were heterogeneous, they were analysed with a Steel's Many One Rank Test (equal size groups) or a Kruskal Wallis' ANOVA by ranks followed by a Dunn's multiple comparison (unequal size groups).

**Results and Discussion**

Results are summarised in the tables below.

**Table A 7: Summary of bodyweight of Northern bobwhite quail with CGA293343**

| Experimental group (ppm) | Sex | Body weight (g) | | |
|---|---|---|---|---|
| | | Start of test feed | Start of photostimulation | Adult termination |
| Control | Male | 200.1 | 213.9 | 219.8 |
| | Female | 198.4 | 212.2 | 250.2 |
| 100 | Male | 197.7 | 215.2 | 228.5 |
| | Female | 197.4 | 215.4 | 254.9 |
| 300 | Male | 197.8 | 212.8 | 219.4 |
| | Female | 196.7 | 213.3 | 238.4 |
| 900 | Male | 197.0 | 210.8 | 214.0 |
| | Female | 195.3 | 208.8 | 233.7 |

No significant difference between the control group and treatment groups ($p<0.05$)

**Table A 8: Summary of reproductive performance from Northern bobwhite quail reproduction with CGA293343**

| Parameter | Experimental group (ppm) | | | |
|---|---|---|---|---|
| | Control | 100 | 300 | 900 |
| Total eggs laid | 990 | 1139 | 918 | 876 |
| Eggs cracked | 9 | 10 | 9 | 8 |
| Fertile eggs | 815 | 986 | 787 | 706 |
| Viable eggs | 794 | 972 | 777 | 690 |
| Hatchlings | 755 | 933 | 745 | 650 |
| 14 day survivors | 429 | 501 | 497 | 412 |

total egg laying days = 71

**Conclusions**

No mortality or toxicity was observed in Northern bobwhite exposed to CGA293343 technical at a dietary concentration of 100, 300 or 900 ppm for 23 weeks and five days. There were no significant differences detected at any level of treatment when statistically compared against the control group. The NOEC during the study was therefore 900 ppm.

(Taliaferro and Miller, 1998)

## A 2.1.1.1 KCP 10.1.1.1 Acute oral toxicity

| | |
|---|---|
| Comments of zRMS: | The metalaxyl-M is the risk driver in the formulation (95.4% of the toxicity). Therefore, the acute oral toxicity study conduct with A23109A is not required. Study not evaluated by zRMS. |

| | |
|---|---|
| Reference: | KCP 10.1.1.1 |
| Report | Hubbard, P.M., Temple, D.T., (2021), Oxathiapiprolin/Metalaxyl-M DC (A23109A) – An Acute Oral Toxicity Study with the Northern Bobwhite using a Sequential Testing Procedure. Report Number 528B-605. Eurofins EAG Agrosciences, LLC. (Syngenta file no VV-891190) |
| Guideline(s): | OECD Guidelines for Testing of Chemicals, Method 223: Avian Acute Oral Toxicity Test (2016) |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication (if vertebrate study) | No |

**Executive Summary**

The acute oral $LD_{50}$ value for northern bobwhite exposed to A23109A as a single oral dose was determined to be greater than 2000 mg/kg body weight, the highest dosage tested. The no-mortality level was 2000 mg/kg body weight.

**Materials**

| | |
|---|---|
| **Test material** | Metalaxyl-M / oxathiapiprolin DC |
| **Lot/Batch #:** | JHU003-036-001 (1094822) |
| **Purity:** | Metalaxyl-M: 17.2 % w/w corresponding to 185 g/L<br>CGA351920 (S-enantiomer): 0.57% w/w corresponding to 6.1 g/L<br>CGA329351 (R-enantiomer): 16.6 % w/w corresponding to 178 g/L<br>Oxathiapiprolin: 2.85 % w/w corresponding to 30.6 g/L |
| **Description:** | Brownish liquid |
| **Stability of test compound:** | < 30 ºC |
| **Reanalysis/expiry date:** | End of June 2021 |
| **Treatments** | |
| **Test concentrations:** | 0 and 2000 mg /kg body weight |
| **Control:** | Reverse osmosis deionized water |
| **Test vehicle:** | Reverse osmosis deionized water |
| **Test organisms** | |
| **Species:** | Northern Bobwhite (*Colinus virginianus*) |
| **Age at Dosing:** | 62 weeks |
| **Source:** | Trace Pheasantry, Inc. Douglassville, PA |
| **Acclimatisation period:** | 37 weeks to facility and 7 weeks to test caging |
| **Treatment for disease:** | Water soluble antibiotics for 8 days during the first 2 weeks of acclimation |
| **Weight at exposure:** | 180-221 grams. |
| **Feed:** | Basal game bird ration (containing at least 27% protein and 3.7% crude fat, and no more than 3.5% crude fiber), *ad libitum* |
| **Water:** | Municipal tap water, *ad libitum* |
| **Test design** | |
| **Test caging:** | Wire mesh pen with galvanized sheet sides, approximate 25 x 51 cm floor, approximate 20 to 26 cm height |
| **Replication:** | 5 birds per level |
| **Duration:** | Single dose with 14 day observation period |
| **Environmental conditions** | |

**Temperature:** 18.5 ºC (16.4-20.7 ºC)
**Humidity:** 38% (24-62%)
**Photoperiod:** 8 hour light per day (average of 152 lux)

**Study Design and Methods**

Experimental dates: 04 December 2020 to 18 December 2020
A limit dose of 2000 mg/kg was used in this study. Five northern bobwhite were randomly assigned to each of the control group and test group pens. Based on the results of the limit test no further testing was needed.

Birds were acclimated to the study facility for 37 weeks and to the caging for seven weeks prior to dosing. The birds were fasted for approximately 16.5 hours prior to dosing. From test initiation until termination, all birds were observed at least twice daily. A record was maintained of all mortality, signs of toxicity, and abnormal behavior. Body weights were measured on the day of dosing (Day 0) and on Days 3, 7, and 14 of the test. Feed consumption was determined by pen for approximately 24-hour intervals from Day 0 to 1, Day 1 to 2, and Day 2 to 3. Average daily feed consumption was then determined from Days 3 to 7 and from Days 7 to 14.

**Results and Discussion**

No mortalities were observed. No regurgitation was observed. Signs of toxicity were observed for all treatment birds. When compared to the control group, there were apparent treatment-related effects on body weight at the 2000 mg/kg bw dosage level. When compared to the control group, there were apparent effects on feed consumption at the 2000 mg/kg bw dosage level.

Results are summarised in the tables that follow.

**Table A 9: Acute oral toxicity of A23109A to northern bobwhite**

| Nominal (mg/kg bw) | Toxicological results[a] | Duration of clinical signs [b] | Time of death [b] |
|---|---|---|---|
| 0 | 0/0/5 | — | — |
| 2000 | 0/5/5 | — | — |

[a] Number of animals which died/number of animals with clinical signs/number of animals used (per treatment level)
[b] '—' indicates not applicable

**Table A 10: Acute oral toxicity to northern bobwhite - Summary of endpoints**

| | |
|---|---|
| Test item | A23109A |
| Test object | Northern bobwhite |
| $LD_{50}$ | >2000 mg/kg bw |
| Lowest observed adverse effect level (LOAEL) | 2000 mg/kg bw |
| Highest tested dose without adverse toxic effect (NOAEL) | Not determined |

**Validity Criteria**

The test was considered valid;

- There was no mortality in the control (must be ≤ 10 %)

**Conclusions**

The acute oral $LD_{50}$ value for northern bobwhite exposed to A23109A as a single oral dose was determined to be greater than 2000 mg/kg body weight, the highest dosage tested. The no-mortality level was 2000 mg/kg body weight.

(Hubbard, P.M., Temple, D.T., 2021)

### 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

Please refer to Section B6 (Toxicology) for study summary.

| | |
|---|---|
| Reference: | KCP 10.1.2.1 (KCP 7.1.1) |
| Report | xxxxxxx (2021), Metalaxyl-M / Oxathiapiprolin DC (A23109A) - Acute Oral Toxicity Study in Rats (Up and Down Procedure), Report Number 20/131-001P. xxxxxxxxxxxx. (Syngenta File No. VV-888460) |
| Guideline(s): | Acute Oral Toxicity (rat): OECD Test Guideline 425 (2008): EPA OPPTS 870.1100 (2002) |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication (if vertebrate study) | No |

### 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

| | |
|---|---|
| Comments of zRMS: | The study was conducted to OECD guideline 203 and according to the principles of GLP. All validity criteria were met. It should be noted that analytical verification of A23109A concentrations in test medium was done by analysing the content of metalaxyl-M, only. This approach was accepted due to low water solubility limit of oxathiapiprolin.<br>The study is considered to be reliable and suitable for the risk assessment. |

| | |
|---|---|
| Reference: | KCP 10.2.1 |
| Report | xxxxxxxxx (2021), Oxathiapiprolin/Metalaxyl-M DC (A23109A) - Toxicity to the Rainbow Trout *Oncorhynchus mykiss* under Laboratory Conditions (Acute Toxicity Test – Static), Report Number S20-06894. xxxxxxxxxxxxxxxxxxx (Syngenta File No. VV-893371) |
| Guideline(s): | OECD Guidelines for Testing of Chemicals, Method 203: Fish, Acute Toxicity Testing (2019) |

| | |
|---|---|
| Deviations: | ~~None~~ There were no major deviations.<br>The pH of the test solution in the control and at the test item concentrations of 15.3 mg/L and 100 mg/L was ≥ 8.5 after 96 h. This did not influence the integrity of the study, since no adverse effects in control fish were observed and the validity criteria in the study were all achieved. |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication (if vertebrate study) | No |

**Executive Summary**

The acute toxicity of Oxathiapiprolin/Metalaxyl-M DC (A23109A) to rainbow trout *Oncorhynchus mykiss* was determined under static conditions. The test was performed as a concentration-response test and fish were exposed to a nominal concentration of 15.3, 24.4, 39.1, 62.5, 100 mg/L alongside a water control.

**Materials**

| | |
|---|---|
| **Test material** | |
| **Name/Code:** | Oxathiapiprolin/Metalaxyl-M DC (A23109A) |
| **Lot/Batch #:** | JHU003-036-001 |
| **Purity:** | Metalaxyl-M: 17.2 % w/w corresponding to 185 g/L<br>CGA351920 (S-enantiomer): 0.57% w/w corresponding to 6.1 g/L<br>CGA329351 (R-enantiomer): 16.6 % w/w corresponding to 178 g/L<br>Oxathiapiprolin: 2.85 % w/w corresponding to 30.6 g/L |
| **Description:** | Liquid / brownish |
| **Stability of test compound:** | Sufficient for the test purpose (at least 1 h) |
| **Reanalysis/expiry date:** | 30 June 2022 |
| **Density:** | 1.074 g/cm³ |
| **Treatments** | |
| **Test concentrations:** | 100, 62.5, 39.1, 24.4 and 15.3 mg A23109A/L and control |
| **Solvent:** | None |
| **Analysis of test concentrations:** | Analysis of metalaxyl-M in all test concentrations and the control at 0 h fresh and 96 hours (aged) from samples by HPLC-MS/MS detection. |
| **Test organisms** | |
| **Species:** | *Oncorhynchus mykiss* |
| **Source:** | Forellenzucht Peter Störk, D-88348 Bad Saulgau, Germany |
| **Acclimatisation period:** | > 9 days |
| **Treatment for disease:** | None |
| **Weight and length of dilution water control fish at end of exposure period:** | 48 – 58 mm; 0.87 – 1.58 g |
| **Feeding:** | None |
| **Test design** | |
| **Test vessels:** | 18 L glass aquaria, filled with 15 L test solution |
| **Test medium:** | Mix of dechlorinated drinking water and deionised water |
| **Replication:** | None |
| **No of fish per tank:** | 7 |
| **Exposure regime:** | Static |
| **Aeration:** | After 48 hours |
| **Duration:** | 96 h |
| **Environmental conditions** | |
| **Test temperature:** | 13.0 – 13.7 °C |
| **pH:** | 7.73 – 8.80 |
| **Dissolved oxygen:** | ≥ 62 % of air saturation |

**Hardness of dilution water:** 10°dH corresponding to approx. 178 mg/L (as $CaCO_3$)
**Lighting:** 16 hours daily / 8 hours darkness daily, with 30 minutes transition

**Study Design and Methods**

Test facility: xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx
Experimental dates: 07 December 2020 to 22 January 2021
At the start of the test, the necessary amount of test item for preparing the stock solution S1 was weighed on a weighing scoop and transferred to a volumetric flask. Test medium was added up to the benchmark and the stock solution was treated with five minutes of ultrasonication. Afterwards, was observed to be turbid. Lower solutions were prepared by dilution of the appropriate solution with test medium. Dilution solutions V1 to V4 appeared to be turbid. Defined volumes of the stock solution and dilution solutions were transferred into the respective aquarium filled with test medium in order to achieve the following nominal test concentrations: 15.3, 24.4, 39.1, 62.5 and 100 mg/L. The test solutions were homogenized by stirring with a whisk directly in the aquarium.

At the start of the test seven fish were randomly allocated to the test concentration and the dilution water control. Observations for mortalities and symptoms of toxicity were made at 0 h, 4-6 h, day 1 (24 h), day 2 (48 h, 51-54 h), day 3 (72 h, 75-78 h) and day 4 (96 h) after exposure. Also, the appearance of the test solutions was recorded daily.

Daily measurements of the test solutions were undertaken throughout the 96 hours period for pH, temperature and dissolved oxygen concentration. Water hardness of the untreated control and light intensity was determined at the beginning of the test.

The test concentration was verified by chemical analysis of metalaxyl-M at 0 and 96 hours using high performance liquid chromatography with UV detection.

The NOEC was determined directly from the raw data and was established based on the highest test concentration at which no mortality was observed within the allowed control mortality. The $LC_{50}$ at the observation times could not be quantified due to the absence of a toxic effect of the test item at the tested concentrations.

**Results**

The initial measured concentrations of metalaxyl-M were between 82 % and 94 % of nominal. The measured concentrations after 96 hours were between 80 % and 99 % of nominal (see table below). The limit of quantification was 0.103 mg a.s./L.

Since all measured concentrations for metalaxyl-M at test start were between 80 – 120 % of nominal, the results were calculated using the nominal concentrations of A23109A.

**Table A 11: Analytical results**

| Nominal concentrations of A23109A (mg/L) | % of nominal measured at 0 hours | % of nominal measured at 96 hours |
|---|---|---|
| Control | n.a. | n.a. |
| 15.3 | 82 | 84 |
| 24.4 | 92 | 80 |
| 39.1 | 88 | 91 |
| 62.5 | 90 | 91 |
| 100 | 94 | 99 |

n.a.: not applicable
LOQ: Limit of quantification: 0.258 mg/L metalaxyl-M
LOD: Limit of detection: 0.0001 mg/L metalaxyl-M

The mortality data and estimated $LC_{50}$ values are shown in the table below:

**Table A 12: Effects of A23109A on the survival of *Oncorhynchus mykiss***

| Nominal concentrations of A23109A (mg/L) | Mortality observed (cumulative number of dead fish) (n = 7) | | | | |
|---|---|---|---|---|---|
| | 4 - 6 hours | 24 hours | 48 hours | 72 hours | 96 hours |
| Control | 0 | 0 | 0 | 0 | 0 |
| 15.3 | 0 | 0 | 0 | 0 | 0 |
| 24.4 | 0 | 0 | 0 | 0 | 0 |
| 39.1 | 0 | 0 | 0 | 0 | 0 |
| 62.5 | 0 | 0 | 0 | 0 | 0 |
| 100 | 0 | 0 | 0 | 0 | 0 |
| **$LC_{50}$ (mg/L)** | > 100 | > 100 | > 100 | > 100 | > 100 |
| **95% confidence interval** | n.d. | n.d | n.d | n.d | n.d |
| **NOEC (mg/L)** | 100 | | | | |

n.d.: could not be determined - 95% confidence limits could not be calculated with the mortality data obtained.

**Validity Criteria**

The test was considered valid;

- There was no mortality in the control (must be ≤ 1 fish)
- Oxygen saturation was ≥ 62% (must be ≥ 60%)
- analytical measurements of test concentrations were conducted

**Conclusion**

The acute toxicity of A23109A to rainbow trout *Oncorhynchus mykiss* was determined under static conditions. The test was performed as a concentration-response test and fish were exposed to a nominal concentration of 15.3, 24.4, 39.1, 62.5 and 100 mg/L alongside a water control.

(Schuler, L., 2021)

| | |
|---|---|
| Comments of zRMS: | The study was conducted to OECD guideline 202 and according to the principles of GLP. All validity criteria were met. It should be noted that analytical verification of A23109A concentrations in test medium was done by analysing the content of metalaxyl-M, only. This approach was accepted due to low water solubility limit of oxathiapiprolin.<br>The study is considered to be reliable and suitable for the risk assessment. |

| | |
|---|---|
| Reference: | KCP 10.2.1 |
| Report | Schuler, L., (2021), Oxathiapiprolin/metalaxyl-M DC (A23109A) - Toxicity to the Water Flea *Daphnia magna* Straus under Laboratory Conditions (Acute Immobilisation Test – Static), Report Number S20-06895. Eurofins Agroscience Services Ecotox GmbH, Eutinger Str. 24, 75223 Niefern-Öschelbronn, Germany. (Syngenta File No. VV-893390) |
| Guideline(s): | OECD Guidelines for Testing of Chemicals, Method 202: *Daphnia* sp., Acute Immobilisation Test (2004) |
| Deviations: | None |

| | |
|---|---|
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication (if vertebrate study) | N/A |

### Executive Summary

The acute toxicity of Oxathiapiprolin/metalaxyl-M DC (A23109A) to *Daphnia magna* was determined under static conditions. Daphnids were exposed to nominal concentrations of 6.25, 12.5, 25.0, 50.0 and 100 mg a.s/L alongside a dilution water control.

### Materials

**Test Material**

| | |
|---|---|
| **Name/code:** | Oxathiapiprolin/metalaxyl-M DC (A23109A) |
| **Lot/Batch #:** | JHU003-036-001 |
| **Purity:** | Metalaxyl-M: 17.2 % w/w corresponding to 185 g/L<br>CGA351920 (S-enantiomer): 0.57% w/w corresponding to 6.1 g/L<br>CGA329351 (R-enantiomer): 16.6 % w/w corresponding to 178 g/L<br>Oxathiapiprolin: 2.85 % w/w corresponding to 30.6 g/L |
| **Description:** | Liquid / brownish |
| **Stability of test compound:** | Sufficient for the test purpose (at least 1 h) |
| **Reanalysis/Expiry date:** | 30 June 2022 |
| **Density:** | 1.074 g/cm³ |

**Treatments**

| | |
|---|---|
| **Test concentrations:** | 100, 50.0, 25.0, 12.5 and 6.25 mg A23109A/L and control |
| **Solvent:** | None |
| **Positive control:** | Two concentrations of the reference item potassium dichromate were tested around the same time period as the study. |
| **Analysis of test concentrations:** | Analysis of metalaxyl-M in all test concentrations and the control at 0 h fresh and 48 hours (aged) from samples by HPLC-MS/MS detection. |

**Test organisms**

| | |
|---|---|
| **Species:** | *Daphnia magna* STRAUS, Clone V |
| **Source:** | Continuously bred in the laboratory, originally obtained from Federal Environment Agency in Berlin/Germany |
| **Feeding:** | The animals were fed on single cell green algae (*Desmodesmus subspicatus*, formerly *Scenedesmus subspicatus*) at least three times a week. No feeding during testing |

**Test design**

| | |
|---|---|
| **Test vessels:** | 100 mL glass beaker, filled with ≥ 50 mL test solution |
| **Test medium:** | Elendt M4 |
| **Replication:** | 4 test vessels each with 5 test organisms at all test concentrations and the control (individual organisms were randomly assigned to the treatments and test vessels) |
| **Exposure regime:** | Static |
| **Duration:** | 48 hours |

**Environmental conditions**

| | |
|---|---|
| **Test temperature:** | 19.4 – 20.0 °C |
| **pH range:** | 7.71 – 8.04 |
| **Dissolved oxygen:** | 7.5 – 8.9 mg/L |
| **Total hardness of dilution water:** | 232 mg/L as $CaCO_3$ |
| **Lighting:** | 16 hours photoperiod / 8 hours darkness daily (mean 1170 lux) |

**Study Design and Methods**

Test facility: Eurofins Agroscience Services Ecotox GmbH
Experimental dates: 08 December 2020 to 25 Jan 2021
The test medium of the highest nominal concentration of 100 mg A23109/L was prepared by dissolving 100 mg of the test item completely in 1000 mL of test medium. The stock solution was homogenised by shaking and treated with 5 minutes of ultrasonication. Using this stock solution, the remaining nominal test concentrations as stated above were prepared by serial dilution. The control consisted of dilution water only. Test solutions were added to the test vessels and the *Daphnia* added without conscious bias.

The immobility of the daphnids was determined by visual observations after 24 and 48 hours of exposure. Organisms unable to swim within 15 seconds after gentle agitation of the test beaker were considered to be immobile.

The pH, temperature and dissolved oxygen were measured at the start, after 24 hours and end of the test in each test concentration and the control.

The test concentrations were verified by chemical analysis of metalaxyl-M at 0 and 48 hours using high performance liquid chromatography with MS/MS detection.
The median effect concentration ($EC_{50}$) was defined as the concentration resulting in 50 % immobilisation of the *Daphnia* in the time period specified and was calculated by Probit analysis at 24 and 48 hours. The NOEC (No Observed Effect Concentration) is defined as the highest tested concentration which did not produce an adverse effect when compared to the control and was determined directly from the raw data.

**Results**

At the start of the test, the analytically determined concentrations of metalaxyl-M were in the range 97 % to 110 % of the nominal values and at the end of the test were in the range 92 % to 113 % (see table below). The limit of quantification in this study was 0.103 mg a.s./L.

**Table A 13: Analytical results**

| Nominal concentrations A23109A (mg/L) | Determined Concentration at 0 hours (% of nominal) | Determined Concentration at 48 hours (% of nominal) |
|---|---|---|
| Control | n.a. | n.a. |
| 6.25 | 105 | 105 |
| 12.5 | 100 | 113 |
| 25.0 | 110 | 107 |
| 50.0 | 97 | 101 |
| 100 | 101 | 92 |

n.a.: not applicable
LOQ: Limit of quantification: 0.103 mg/L metalaxyl-M
LOD: Limit of detection: 0.0001 mg/L metalaxyl-M

There was no immobility observed in the dilution water control. Immobility data and estimated $EC_{50}$ values are shown in the table below:

**Table A 14: Effects of A23109A on *Daphnia magna* following exposure for 48-hours in a static test**

| Nominal Concentration of A23109A (mg a.s./L) | Immobilised daphnids after 24 hours | | Immobilised daphnids after 48 hours | |
|---|---|---|---|---|
| | Number | % | Number | % |
| Control | 0 | 0 | 0 | 0 |

| Nominal Concentration of A23109A | Immobilised daphnids after 24 hours | | Immobilised daphnids after 48 hours | |
|---|---|---|---|---|
| 6.25 | 0 | 0 | 0 | 0 |
| 12.5 | 0 | 0 | 0 | 0 |
| 25.0 | 0 | 0 | 1 | 5 |
| 50.0 | 0 | 0 | 6 | 30 |
| 100 | 0 | 0 | 12 | 60 |
| $EC_{50}$ (mg./L) | >100 | | 79.4 | |
| 95% Confidence limits | - | | 60.9 – 123 | |
| NOEC (mg/L) | 100 | | 25.0 | |

n.d. = not determined, (x) = number of test animals with adverse effects, F = reduced swimming activity

**Validity criteria**

The test was considered valid;

- There was no immobilization or other signs of disease or stress in the control (must be ≤ 10%)
- Oxygen concentration at the end of the test were 7.5 - 8.9 mg/L in the control and test vessels (must be ≥ 3 mg/L)

**Conclusion**

The acute toxicity of Oxathiapiprolin/metalaxyl-M DC (A23109A) to *Daphnia magna* was determined under static conditions. Daphnids were exposed to nominal concentrations of 6.25, 12.5, 25, 50 and 100 mg/L alongside a dilution water control.

(Schuler, L., 2021)

| | |
|---|---|
| Comments of zRMS: | The study was conducted to OECD guideline 201 and according to the principles of GLP. All validity criteria were met. It should be noted that analytical verification of A23109A concentrations in test medium was done by analysing the content of metalaxyl-M, only. This approach was accepted due to low water solubility limit of oxathiapiprolin.<br>The initial measured concentrations were between 81 % and 98 % of nominal. The measured concentrations after 96 hours were between 84 % and 99 % of nominal.<br>The study is considered to be reliable and suitable for the risk assessment. |

| | |
|---|---|
| Reference: | KCP 10.2.1 |
| Report | Schuler, L., (2021), Oxathiapiprolin/Metalaxyl-M DC (A23109A) - Toxicity to the Single Cell Green Alga *Raphidocelis subcapitata* Korshikov under Laboratory Conditions, Report Number S20-06896. Eurofins Agroscience Services Ecotox GmbH, Eutinger Str. 24, 75223 Niefern-Öschelbronn, Germany. (Syngenta File No. VV-898484) |
| Guideline(s): | OECD Guidelines for Testing of Chemicals, Method 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test (2011) |
| Deviations: | None |
| GLP: | Yes |

| | |
|---|---|
| Acceptability: | Yes |
| Duplication (if vertebrate study) | N/A |

**Executive Summary**

The toxicity of A23109A to the green alga *Raphidocelis subcapitata* was investigated in a 96-hour static test. Algae were exposed to nominal concentrations of 9.57, 17.1, 30.9, 55.6 and 100 mg A23109A/L alongside a culture medium control. Based on nominal concentrations, the 72-hour $E_rC_{50}$ was > 100 mg A23109A/L, the $E_yC_{50}$ was 81.8 mg A23109A/L and the $E_bC_{50}$ was 92.2 mg A23109A/L. The 96-hour $E_rC_{50}$ was > 100 mg A23109A/L, the $E_yC_{50}$ was 74.1 mg A23109A/L and the $E_bC_{50}$ was 79.2 mg A23109A/L.

**Materials**

| | |
|---|---|
| **Test Material** | |
| **Name/Code:** | Oxathiapiprolin/Metalaxyl-M DC (A23109A) |
| **Lot/Batch #:** | JHU003-036-001 |
| **Purity** | Metalaxyl-M: 17.2 % w/w corresponding to 185 g/L<br>CGA351920 (S-enantiomer): 0.57% w/w corresponding to 6.1 g/L<br>CGA329351 (R-enantiomer): 16.6 % w/w corresponding to 178 g/L<br>Oxathiapiprolin: 2.85 % w/w corresponding to 30.6 g/L |
| **Description:** | Liquid / brownish |
| **Stability of test compound:** | Sufficient for the test purpose (at least 1 h) |
| **Reanalysis/expiry date:** | 30 June 2022 |
| **Density:** | 1.074 g/cm³ |
| **Treatments** | |
| **Test concentrations:** | 100, 55.6, 30.9, 17.1 and 9.57 mg A23109A/L and control |
| **Solvent:** | None |
| **Positive control:** | None |
| **Analysis of test concentrations:** | Analysis of metalaxyl-M in all test concentrations and the control at 0 h fresh and 96 hours (aged) from samples by HPLC-MS/MS detection. |
| **Test organism** | |
| **Species:** | *Raphidocelis subcapitata* Korshikov, Strain SAG 61.81 |
| **Source:** | MBM Sciencebridge GmbH, Hans-Adolf-Krebs-Weg 1, D-37077 Göttingen, Germany |
| **Test design** | |
| **Test vessels:** | 100 mL Erlenmeyer flasks with aluminum caps |
| **Test medium:** | AAP medium |
| **Replication:** | The control was prepared with six replicates and the concentrations were prepared with three replicates at each concentration. |
| **Starting cell density:** | 5000 cells per mL (nominal) |
| **Exposure regime:** | Static |
| **Aeration:** | By continuous agitation: Test vessels were placed in an incubator on a pivoted bogie which turns around and induces shaking by regular sudden stops |
| **Duration:** | 96 h |
| **Environmental conditions** | |
| **Test temperature:** | 22.4 – 23.3 °C |
| **pH of control:** | 7.20 – 8.07 within 72 h, up to 8.85 after 96 h |
| **Lighting:** | Continuously, 88.6 – 100.0 µE $m^{-2}$ $s^{-1}$ |

**Study Design and Methods**

Test facility: Eurofins Agroscience Services Ecotox GmbH
Experimental dates: 18 January 2021 to 10 Mar 2021
A stock solution with a nominal concentration of 1000 mg A23109A/L was prepared by dissolving 250 mg of the test item in 250 mL of test media by shaking. 50 mL of the stock solution was diluted with

450 mL of test media to prepare the highest test concentration of 100 mg A23109A/L. Appropriate volumes of the highest test concentration were diluted to give the test concentration series. The control consisted of culture medium only.

An aliquot of approximately 60 ml test solution was placed into each test vessel and the test was started by inoculation of 5,000 algal cells per mL of test medium. The test flasks were continuously agitated in a temperature controlled light incubator.

Small volumes of all test concentrations and controls were taken from all test flasks after 24, 48, 72 and 96 hours of exposure. The algal cell densities in these samples were determined by fluorescence measurements. In addition, after 96 hours exposure, a sample was taken from the control and all test item concentrations and the shape of the algal cells was examined microscopically in these samples.

The pH was measured at the start, after 72 hours and at the end of the test. The water temperature was measured continuously in a flask incubated under the same conditions as the test flasks and recorded daily. The appearance of the test media was also recorded daily.

The test concentrations were verified by chemical analysis of metalxyl-M at 0 and 96 hours, using high performance liquid chromatography with LC-MS/MS.

The algal cell densities were measured at 24, 48, 72 and 96 hours and the mean biomass, growth rate and yield calculated. The 72-hour and 96-hour $EC_{10}$, $EC_{20}$ and $EC_{50}$ values (defined as the concentration resulting in 10, 20 and 50% reduction of each parameter) and their 95% confidence intervals were calculated using by probit analysis using maximum likelihood regression. For determination of the LOEC (Lowest Observed Effect Concentration) and NOEC (No Observed Effect Concentration) values, the Williams multiple sequential t-test procedure and multiple sequentially-rejective Welsh t-test after Bonferroni-Holm were used to identify significant differences in the calculated mean biomass integral, growth rate and yield of test item treatments compared to the control.

**Results**

At the start of the test, the measured concentrations were in the range 81 % and 98 % of the nominal values and at the end of the test were in the range 84 % and 99 % (see table below). The limit of quantification in this study was 0.163 mg metalaxyl-M/L. Nominal concentrations of the test item were used for the calculation and reporting of results.

**Table A 15: Analytical results**

| Nominal concentrations (mg A23109A/L) | Determined concentration as % of nominal measured at 0 hours | Determined concentration as % of nominal measured at 96 hours |
|---|---|---|
| Control | n.a. | n.a. |
| 9.57 | 81 | 86 |
| 17.1 | 93 | 90 |
| 30.9 | 94 | 87 |
| 55.6 | 91 | 84 |
| 100 | 98 | 99 |

n.a.: not applicable
LOQ: Limit of quantification: 0.163 mg/L metalaxyl-M
LOD: Limit of detection: 0.00250 mg/L metalaxyl-M

The morphology of the algae cells was observed microscopically after 96 hours. The cells were considered normal for the control and up to a test item concentration of 55.6 mg/L. At the highest test item concentration of 100 mg/L deformation like spherical, slender or agglomerated cells were partly visible.

***Algal Biomass***

The algal biomass at 0, 24, 48, 72 and 96 hours were calculated for each replicate culture and the means are shown below.

**Table A 16: Mean values for the control and test item treatment of A23109A for the density of algal cultures at 24, 48, 72 and 96 hours for *Pseudokirchneriella subcapitata***

| Nominal concentrations (mg A23109A /L) | Density of algal cells[a] | | | |
|---|---|---|---|---|
| | 24 h | 48 h | 72 h | 96 h |
| Control | 1.38 | 6.99 | 33.90 | 122.11 |
| 9.57 | 2.33 | 13.05 | 56.50 | 177.67 |
| 17.1 | 2.13 | 12.55 | 51.26 | 160.88 |
| 30.9 | 1.87 | 10.27 | 42.82 | 145.53 |
| 55.6 | 1.60 | 8.46 | 31.66 | 104.47 |
| 100 | 1.21 | 3.47 | 7.85 | 16.69 |

[a] The biomass was determined by particle counting (at least duplicate measurements per replicate) and is given as number of cells (x $10^4$) per milliliter. At the start of the test, the initial cell density was 4000 algal cells/mL.

***Growth rate, yield and biomass (area under the growth curve)***

**Table A 17: Mean values for the control and test item treatment of A23109A for the percent inhibition of growth rate, yield and AUC at 72 hours for Pseudokirchneriella subcapitata**

| Nominalconcentrations (mg A23109A /L) | 0 to 72 h | | | | | |
|---|---|---|---|---|---|---|
| | AUC ($10^4$*day) | Percentage inhibition of AUC | Growth rate (1/day) | Percentage inhibition of growth rate | Yield (x $10^4$) | Percentage inhibition of yield |
| Control | 24.35 | 0.0 | 1.48178 | 0.0 | 33.51 | 0.0 |
| 9.57 | 42.64 | -75.1 | 1.65014 | -11.4 | 56.10 | -67.4 |
| 17.1 | 39.32 | -61.5 | 1.61624 | -9.1 | 50.86 | -51.8 |
| 30.9 | 32.55 | -33.7 | 1.55758 | -5.1 | 42.42 | -26.6 |
| 55.6 | 24.90 | -2.3 | 1.45651 | 1.7 | 31.26 | 6.7 |
| 100 | 7.60 | 68.8* | 0.99170 | 33.1* | 7.45 | 77.8* |

* Statistically significant different to the control following Williams multiple sequential t-test procedure / multiple sequentially-rejective Welsh-t-test after Bonferroni-Holm/ multiple sequentially-rejective Welsh-t-test after Bonferroni-Holm (left-sided, $p<0.05$) for growth rate / yield / biomass integral

**Table A 18: Mean values for the control and test item treatment of A23109A for the percent inhibition of growth rate, yield and AUC at 96 hours for *Pseudokirchneriella subcapitata***

| Nominalconcentrations (mg A23109A /L) | 0 to 96 h | | | | | |
|---|---|---|---|---|---|---|
| | AUC ($10^4$*day) | Percentage inhibition of AUC | Growth rate (1/day) | Percentage inhibition of growth rate | Yield (x $10^4$) | Percentage inhibition of yield |
| Control | 101.96 | 0.0 | 1.43347 | 0.0 | 121.71 | 0.0 |
| 9.57 | 159.32 | -56.3 | 1.52401 | -6.3 | 177.27 | -45.6 |
| 17.1 | 144.99 | -42.2 | 1.49875 | -4.6 | 160.48 | -31.9 |
| 30.9 | 126.33 | -23.9 | 1.47416 | -2.8 | 145.13 | -19.2 |
| 55.6 | 92.57 | 9.2 | 1.39117 | 3.0* | 104.07 | 14.5* |
| 100 | 19.48 | 80.9* | 0.93279 | 34.9* | 16.29 | 86.6* |

* Statistically significant different to the control following multiple sequentially-rejective Welsh-t-test after Bonferroni-Holm / Williams multiple sequential t-test procedure / multiple sequentially-rejective Welsh-t-test after Bonferroni-Holm (left-sided, p<0.05) for growth rate / yield / biomass integral

**Table A** 19**: Summary of biological results for toxicity of A23109A to Pseudokirchneriella subcapitata after 72 and 96 hours**

| | 72 h | | | 96 h | | |
|---|---|---|---|---|---|---|
| Parameter | Growth rate[1)] | Yield[1)] | Biomass integral[1)] | Growth rate[1)] | Yield[1)] | Biomass integral[1)] |
| | A23109A [mg/L] | | | | | |
| $EC_{10}$ | 43.6 | 58.4 | 35.6 | 41.8 | 52.3 | 56.3 |
| 95% CL | 32.6 – 57.5 | 54.7 – 61.6 | 25.5 – 44.7 | 31.8 – 53.6 | 51.2 – 53.4 | 54.0 – 58.4 |
| $EC_{20}$ | 80.6 | 65.6 | 49.3 | 75.0 | 59.0 | 63.3 |
| 95% CL | 60.7 – 126 | 62.3 – 68.4 | 38.6 – 61.8 | 58.0 - 109 | 57.9 – 59.9 | 61.2 – 65.2 |
| $EC_{50}$ | > 100[2)] | 81.8 | 92.2 | > 100[2)] | 74.1 | 79.2 |
| 95% CL | - | 79.3 – 84.1 | 72.3 - 135 | - | 73.1 – 75.1 | 77.5 – 80.9 |
| NOEC | 55.6[3)] | 55.6[4)] | 55.6[4)] | 30.9[4)] | 30.9[3)] | 55.6[4)] |
| LOEC | 100[3)] | 100[4)] | 100[4)] | 55.6[4)] | 55.6[3)] | 100[4)] |

1) Probit analysis using linear max likelihood regression
2) no $EC_{50}$ value available, inhibition below 50%. The $EC_{50}$-value is estimated to be higher than 100 mg/L (nominal concentration)
3) following Williams multiple sequential t-test procedure (left-sided, $p \leq 0.05$)
4) following Multiple sequentially-rejective Welsh t-test after Bonferroni-Holm (left-sided, $p \leq 0.05$)
- not determined due to mathematical reasons or inappropriate data
CL confidence limits of $EC_x$

**Validity criteria**

The test was considered valid;

- The algal biomass in the control increased by a factor of 84.75 over 72 hours and 305.28 over 96 hours (must be at least a factor of 16).
- The mean coefficient of variation of the daily growth rates in the control was 15 and 15 % over 72 and 96 hours, respectively (must be ≤ 35%).
- The coefficient of variation of average specific growth rates in replicate control cultures was 3.5 and 1.8 % after 72 and 96 hours, respectively (must be <7%).

**Conclusion**

The toxicity of A23109A to the green alga *Pseudokirchneriella subcapitata* was investigated in a 96-hour static test. Algae were exposed to nominal concentrations of 9.57, 17.1, 30.9, 55.6 and 100 mg A23109A/L alongside a culture medium control. Based on nominal concentrations, the 72-hour $E_rC_{50}$ was > 100 mg A23109A/L, the $E_yC_{50}$ was 81.8 mg A23109A/L and the $E_bC_{50}$ was 92.2 mg A23109A/L. The 96-hour $E_rC_{50}$ was > 100 mg A23109A/L, the $E_yC_{50}$ was 74.1 mg A23109A/L and the $E_bC_{50}$ was 79.2 mg A23109A/L.

(Schuler, L., 2021)

## A 2.2.2 KCP 10.2.2 Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms

## A 2.2.3 KCP 10.2.3 Further testing on aquatic organisms

## A 2.3 KCP 10.3 Effects on arthropods

## A 2.3.1 KCP 10.3.1 Effects on bees

## A 2.3.1.1 KCP 10.3.1.1 Acute toxicity to bees

## A 2.3.1.1.1 KCP 10.3.1.1.1 Acute oral toxicity to bees

| | |
|---|---|
| Comments of zRMS: | The study was conducted to OECD guidance 213 and 214 and according to the principles of GLP. All validity criteria were met.<br>The study is considered to be reliable and suitable for the risk assessment. |

| | |
|---|---|
| Reference: | KCP 10.3.1.1.1 |
| Report | Franke, M., 2021, Oxathiapiprolin/Metalaxyl-M DC (A23109A) - Acute Toxicity to the Honeybee *Apis mellifera L*. under Laboratory Conditions, Report Number 21 48 BAA 0002. BioChem agrar, Labor für biologische und chemische, Analytik GmbH, Kupferstr. 6, 04827 Machern OT Gerichshain, Germany. (Syngenta File No. VV-892684) |
| Guideline(s): | OECD Guidelines for Testing of Chemicals, Method 213: Honeybees, acute oral toxicity test (1998)<br>OECD Guidelines for Testing of Chemicals, Method 214: Honeybees, acute contact toxicity test (1998) |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication (if vertebrate study) | N/A |

**Executive Summary**

In a 48-hr-acute oral toxicity study, honeybees (*Apis mellifera*) were exposed to A23109A *via* a feeding solution. The 48-hr $LD_{50}$ was > 959 µg A23109A/bee.

In a 48-hr-acute contact toxicity study, honeybees (*Apis mellifera*) were exposed to A23109A administered topically to adult bees. The 48-hr $LD_{50}$ was > 1000 µg A23109A/bee.

**Materials**

| | | |
|---|---|---|
| **Test Material** | A23109A | |
| **Lot/Batch #:** | JHU003-036-001 | |
| **Actual content of active ingredients:** | Metalaxyl-M: 17.2 % w/w corresponding to 185 g/L<br>CGA351920 (S-enantiomer): 0.57% w/w corresponding to 6.1 g/L<br>CGA329351 (R-enantiomer): 16.6 % w/w corresponding to 178 g/L<br>Oxathiapiprolin: 2.85 % w/w corresponding to 30.6 g/L | |
| **Description:** | DC (dispersible concentrate); brownish liquid | |
| **Stability of test compound:** | Stable | |
| **Reanalysis/expiry date:** | End of June 2022 | |
| **Treatments** | | |
| **Test rates:** | Contact test: | 1000, 500, 250, 125, 62.5 µg product/bee |

| | |
|---|---|
| | Oral test (offered): 1000, 500, 250, 125, 62.5 µg product/bee |
| | Oral test (consumed): 1000, 500, 250, 125, 62.5 µg product/bee |
| **Control:** | Contact test: water and wetting agent control (Tween solution at 1 % v/v)<br>Oral test: 50 % w/v sucrose solution |
| **Reference item:** | Dimethoate EC 400; 429.0 g/L; nominal dose levels of 0.250, 0.175, 0.123, 0.086 µg a.s./bee in contact test and 0.250, 0.150, 0.123 and 0.086 µg a.s./bee in oral test |
| **Administration:** | |
| **Test organisms** | |
| **Species:** | *Apis mellifera* L. Buckfast |
| **Source:** | BioChem agrar GmbH, Germany |
| **Food:** | 50 % w/v sucrose solution |
| **Test design** | |
| **Test cage description:** | Disposable test cages constructed of cardboard, with holes in the bottom for ventilation and a glass plate in front |
| **Replication:** | 3 |
| **No. of bees/rep:** | 10 |
| **Duration of test:** | 48 hours (contact test); 48 hours (oral test) |
| **Environmental test conditions** | |
| **Temperature:** | 24.1 – 25.2°C (contact and oral) |
| **Humidity:** | 50 - 66 % (contact and oral) |
| **Photoperiod:** | Darkness (except during observations) |

**Study Design and Methods**

Test facility: BioChem agrar GmbH, Kupferstr. 6, 04827 Machern OT Gerichshain, Germany
Experimental dates: Experimental start: 22 September 2020
Experimental completion: 24 September 2020

**Oral test procedures:** Bees were unfed from the time they were collected from the hives for 2 hours until treatment. Each group of bees was offered 200 µL (equivalent to 20 µL/bee) of the test material or toxic standard dispersed in aqueous sucrose solution. Treatments were calculated so that the target dose was contained in 200 µL. The doses were measured into the feeding tubes and the feeding tubes were weighed before the doses were made available to the bees. Once all test solutions were consumed, or after six hours (whichever was achieved first), the feeding tubes were replaced with similar tubes containing aqueous sucrose solution. All feeding tubes with test solutions were weighed to calculate actual mean consumption per bee for each treatment.

**Contact test procedures:** Bees were treated with 2 µL of droplets of the test solution, control or toxic standard applied to the dorsal surface of the thorax using topical application with a micro applicator. The bees were returned to the test unit, allowed to recover and fed with a continuous supply of 50 % w/v aqueous sucrose solution.

The mortality [%] per treatment was calculated from the number of dead bees and the total number of introduced bees per treatment group. Mortality in the test and reference item treatments was corrected with the corresponding control mortality according to the formula of SCHNEIDER-ORELLI (1947). The $LD_{50}$ values with 95 % confidence limits of the reference item treatment were calculated by means of a Probit analysis.

**Results**

Mortality data for the test material and toxic standard are summarised in the table below.

**Table A 20: Summary of acute oral toxicity of A23109A to the honeybee**

| Treatment (target dose) (µg product/ bee) | Treatment (based in consumption (µg product/ bee) | Mortality (%) | | Correct mortality # (%) | |
|---|---|---|---|---|---|
| | | 24 h | 48 h | 24 h | 48 h |
| 50 % w/v sucrose solution control | -- | 0.0 | 0.0 | - | - |
| 1000 | 959 | 33.3* | 33.3* | - | - |
| 500 | 461 | 3.3 | 6.7 | - | - |
| 250 | 245 | 0.0 | 0.0 | - | - |
| 125 | 125 | 0.0 | 0.0 | - | - |
| 62.5 | 62.5 | 0.0 | 0.0 | - | - |
| **48h $LD_{50}$ (µg product/bee)** | | **> 959** | | | |
| **95% C.I.** | | **--** | | | |

Mortality results are averages based on 3 replicates consisting of 10 bees each; corr.: corrected mortality (according to SCHNEIDER-ORELLI 1947); * Significant difference in pairwise comparison between treatment and sucrose solution control (Fisher`s Exact Binominal Test with Bonferroni Correction; α=0.05; one sided greater); Calculations are performed with non-rounded values

Sublethal effects were observed at the two highest dose rates of 959 and 461 µg product/bee at the 4-hr assessment. No behavioural abnormalities occurred in the further course up to 48 hours when treated with ≤ 959 µg A23109A/bee.

**Table A 21: Summary of acute contact toxicity of A23109A to the honeybee**

| Treatment (target dose) (µg product/bee) | Mortality (%) | | Correct mortality # (%) | |
|---|---|---|---|---|
| | 24 h | 48 h | 24 h | 48 h |
| Water control | 0.0 | 3.3 | - | - |
| 1 % v/v Tween®80 solution control | 0.0 | 0.0 | - | - |
| 1000 | 40.0* | 43.3* | - | - |
| 500 | 3.3 | 3.3 | - | - |
| 250 | 0.0 | 0.0 | - | - |
| 125 | 0.0 | 0.0 | - | - |
| 62.5 | 0.0 | 0.0 | - | - |
| **48h $LD_{50}$ (µg product/bee)** | **> 1000** | | | |
| **95% C.I.** | **--** | | | |

Mortality results are averages based on 3 replicates consisting of 10 bees each; corr.: corrected mortality (according to SCHNEIDER-ORELLI 1947); * Significant difference in pairwise comparison between treatment and tween solution control (Fisher`s Exact Binominal Test with Bonferroni Correction; α=0.05; one sided greater); Calculations are performed with non-rounded values

Sublethal effects were observed at the two highest dose rates of 1000 and 500 µg product/bee at the 4-h assessment. No behavioural abnormalities occurred in the further course up to 48 hours when treated with ≤ 1000 µg A23109A/bee.

**Validity criteria**

The test was considered to be valid as the mean mortality in the controls in the oral and contact toxicity test was ≤ 10 %, (actual 0%, only in Water control in contact test was 3.3%).

The 24-h $LD_{50}$ of the reference item in the oral toxicity test was ~~0.12~~ 0.115 µg a.s./bee (must be within the range of 0.10 to 0.35 µg a.s./bee).

The 24-h $LD_{50}$ of the reference item in the contact toxicity test was 0.143 µg a.s./bee (must be within the range of 0.10 to 0.30 µg a.s./bee).

**Conclusion**

In a 48-hr-acute oral toxicity study, honeybees (*Apis mellifera*) were exposed to A23109A *via* a feeding solution. The 48-hr $LD_{50}$ was > 959 µg product/bee.

In a 48-hr-acute contact toxicity study, honeybees (*Apis mellifera*) were exposed to A23109A administered topically to adult bees. The 48-hr $LD_{50}$ was > 1000 µg product/bee.

(Franke, M., 2021)

| | |
|---|---|
| Comments of zRMS: | The study was conducted to OECD guidance 246 and 247 and according to the principles of GLP. All validity criteria were met.<br>The study is considered to be reliable and suitable for the risk assessment. |

| | |
|---|---|
| Reference: | KCP 10.3.1.1.1 |
| Report | Amsel, K., 2022, Oxathiapiprolin/Metalaxyl-M DC (A23109A) - Acute toxicity to the bumblebee *Bombus terrestris* L. under laboratory conditions, Report Number 21 48 BBA 0033. BioChem agrar, Labor für biologische und chemische, Analytik GmbH, Kupferstr. 6, 04827 Machern OT Gerichshain, Germany. (Syngenta File No. VV-936483) |
| Guideline(s): | OECD (2017): Guideline for the testing of chemicals; Bumblebee; acute oral toxicity test, 247<br>OECD (2017): Guideline for the testing of chemicals; Bumblebees; acute contact toxicity test, 246 |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication<br>(if vertebrate study) | N/A |

**Executive Summary**

In a 48-hr acute oral toxicity study, bumble bees (*Bombus terrestris* L.) were exposed orally to A23109A *via* a feeding solution. The $LD_{50}$ was > 357.9 µg consumed product/bumble bee and the NOED was 357.9 µg consumed product/bumble bee after 48 hours.

In a 48-hr acute contact toxicity study, bumble bees (*Bombus terrestirs* L.) were exposed to A23109A administered topically to adult bees. The $LD_{50}$ was > 1000.0 µg product/bumble bee and the NOED was 1000.0 µg product/bumble bee after 48 hours.

## Materials

**Test Material**

| | |
|---|---|
| **Name:** | Metalaxyl-M/Oxathiapiprolin DC (A23109A) |
| **Other product code:** | CGA329351/SYN546539 SC (180/030) |
| **Product code:** | A23109A |
| **Lot/Batch #:** | JHU003-036-001 (other Batch ID: 1094822) |
| **Active substances content:** | Metalaxyl-M: 17.2 % w/w corresponding to 185 g/L<br>CGA351920 (S-enantiomer): 0.57% w/w corresponding to 6.1 g/L<br>CGA329351 (R-enantiomer): 16.6 % w/w corresponding to 178 g/L<br>Oxathiapiprolin: 2.85 % w/w corresponding to 30.6 g/L |
| **Description:** | Appearance: brownish liquid |
| **Density:** | 1074 kg/m³ |
| **Stability of test compound:** | Stable under recommended handling and storage conditions (< 30 °C) |
| **Reanalysis/expiry date:** | End of June 2022 |

**Treatments**

| | |
|---|---|
| **Test rates:** | Contact test: 1000.0, 500.0, 250.0, 125.0, 62.5 µg product/bumble bee<br>Oral test (offered): 1000.0, 500.0, 250.0, 125.0, 62.5 µg product/bumble bee<br>Oral test (consumed): 357.9, 266.6, 232.6, 122.5, 61.8 µg product/bumble bee |
| **Controls:** | Contact test: Deionised water, 0.5% TritonX solution<br>Oral test: Sucrose solution |
| **Reference item:** | Danadim® Progress (dimethoate EC 400; 401.7 g/L (analysed)) |
| **Administration:** | Contact test: 4 µL/bee<br>Oral test: 40 µL/bee |

**Test organisms**

| | |
|---|---|
| **Species:** | *Bombus terrestris* L. (bumble bee), worker bumble bees from queen right hives |
| **Source:** | Biobest Belgium N.V. Ilse Velden, 18, 2260 Westerlo, Belgium, supplied by: Katz Biotech AG, An der Birkenpfuhlheide 10, 15837 Baruth, Germany |
| **Food:** | 50% (w/v) sucrose solution |

**Test design**

| | |
|---|---|
| **Test cage description:** | Nicot cages (part of the Nicot queen bee rearing system) with a length of 7 cm and a diameter of 2 cm. |
| **Number of bees/ test unit (= replicate):** | 1 |
| **No. of bees/rep:** | 30 (+ 5 additionally replicates/treatment to account non-feeders in the oral test) |
| **Duration of test:** | 48 hours |

**Environmental test conditions**

| | |
|---|---|
| **Temperature:** | 23.9 – 24.9 °C |
| **Humidity:** | 51 – 80% |
| **Photoperiod:** | Constant darkness throughout the test (diffuse artificial light only during handling and assessments) |

## Study Design and Methods

Test facility: BioChem agrar Labor für biologische und chemische Analytik, GmbH Kupferstr. 6, 04827 Machern OT Gerichshain, Germany
Experimental dates: 22 September – 24 September 2021

**Oral test:** Plastic syringes (feeders) containing the corresponding application solution were used for application. The application volume was 40 µL/replicate (corresponding to 40 µL/bumble bee). The bumble bees were starved for approx. 4 hours prior to application start. Each unit was provided with the application solution for 4 hours, to ensure a sufficient uptake. The feeders were then removed, and the bumble bees were provided *ad libitum* with an untreated 50 % (w/v) aqueous sucrose solution. Treatments started with the control followed by the test item and finally the reference item. For dose verification the amount of application solution(s) consumed was determined by weighing the feeders before and after feeding using calibrated equipment.

**Contact test:** The application amount was 4 µL/bumble bee. After having been anaesthetised with $CO_2$ (the amount of anaesthetic used was minimised), the droplet of the application solution was applied individually to the dorsal side of the thorax of each bumble bee. Treatments started with the control followed by the test item and finally the reference item. For the toxic reference item group and the water treated control group, the water-wetting agent Triton X-100 was mixed into all application solutions. This reduced the surface tension of the applied solution and ensured that the drop of the application solution was spread out immediately after the application. After the application the bees were returned to the test units.

No statistical analysis was necessary since no control and test item mortality occurred during the contact and oral toxicity test.

**Results**

Analytical recoveries in dosing solutions are presented below.

**Table A 22: Analytical results - oral test**

| Target dosing solution (µg product/bee) | Measured concentration (mg a.i./kg) | % recovery |
|---|---|---|
| Sucrose | < LOD | - |
| 1000.0 | 586 | 98 |
| 500.0 | 285 | 95 |
| 250.0 | 143 | 95 |
| 125.0 | 72 | 96 |
| 62.5 | 36 | 97 |

LOD: 0.0026 mg/kg, corresponding to 0.062 µg/L

Mortality data for the test material and toxic standard are summarised in the table below.

**Table A 23: Summary of acute oral toxicity of A23109A to the bumble bee**

| Treatment (target dose) | Treatment (consumed) | Mortality (%) | | Corrected mortality (%) | |
|---|---|---|---|---|---|
| | | 24 h | 48 h | 24 h | 48 h |
| (µg product/bee) | | | | | |
| Sucrose | | 0.0 | 0.0 | - | - |
| 1000.0 | 357.9 (AT) | 0.0 | 0.0 | - | - |
| 500.0 | 266.6 (BT) | 0.0 | 0.0 | - | - |
| 250.0 | 232.6 (CT) | 0.0 | 0.0 | - | - |
| 125.0 | 122.5 (DT) | 0.0 | 0.0 | - | - |
| 62.5 | 61.8 (ET) | 0.0 | 0.0 | - | - |
| Toxic reference | 1.47 (AR) | 90.0 | 96.7 | - | - |
| $LD_{50}$ (µg consumed product/bee) | | > 357.9 | | | |
| 95% C.I. | | - | | | |
| NOED (µg consumed product/bee) | | 357.9 | | | |

Mortality results are averages based on 30 (control), 16 (AT), 18 (BT), 29 (CT), 30 (DT, ET, AR) replicates consisting of 1 bumblebee each

No sublethal effects were observed in the oral test.

**Table A 24: Analytical results - contact test**

| Target dosing solution (µg product/bee) | Measured concentration (mg a.i./L) | % recovery |
|---|---|---|
| 0.5% TritonX | < LOD | - |
| 1000.0 | 6677 | 94 |
| 500.0 | 3263.5 | 92 |
| 250.0 | 1622.1 | 91 |
| 125.0 | 782.0 | 88 |
| 62.5 | 401.8 | 90 |

LOD: 0.0026 mg/L, corresponding to 0.062 µg/L

**Table A 25: Summary of acute contact toxicity of A23109A to the bumble bee**

| Treatment (µg product/bee) | Mortality (%) | | Corrected mortality (%) | |
|---|---|---|---|---|
| | 24 h | 48 h | 24 h | 48 h |
| Water control | 0.0 | 0.0 | - | - |
| 0.5% TritonX | 0.0 | 0.0 | - | - |
| 1000.0 | 0.0 | 0.0 | - | - |
| 500.0 | 0.0 | 0.0 | - | - |
| 250.0 | 0.0 | 0.0 | - | - |
| 125.0 | 0.0 | 0.0 | - | - |
| 62.5 | 0.0 | 0.0 | - | - |
| Toxic reference | 100.0 | 100.0 | - | - |
| $LD_{50}$ (µg product/bee) | | > 1000.0 | | |
| 95% C.I. | | - | | |
| NOED (µg product/bee) | | 1000.0 | | |

Mortality results are averages based on 30 replicates consisting of 1 bumblebee each

No sublethal effects were observed in the contact test.

**Validity criteria**

The study is considered valid since the control and reference item validity criteria were met:

- the mean mortality in both control groups of the oral and contact test was ≤ 10 % at the end of the test (observed values 0%);
- the mean reference item mortality was ≥ 50 % at the end of the test (observed values of 100.0% and 96.7% for the contact and oral tests, respectively)

**Conclusion**

In a 48-hr acute oral toxicity study, bumble bees (*Bombus terrestris* L.) were exposed orally to A23109A *via* a feeding solution. The $LD_{50}$ was > 357.9 µg consumed product/bumble bee and the NOED was 357.9 µg consumed product/bumble bee after 48 hours.

In a 48-hr acute contact toxicity study, bumble bees (*Bombus terrestirs* L.) were exposed to A23109A administered topically to adult bees. The $LD_{50}$ was > 1000.0 µg product/bumble bee and the NOED was 1000.0 µg product/bumble bee after 48 hours.

(Amsel, K., 2022)

| Comments of zRMS: | Study not evaluated by zRMS. |
|---|---|

| | |
|---|---|
| Reference: | KCA1 8.3.1.1 |
| Report | Amsel, K., 2021, Metalaxyl-M - Acute toxicity to the bumblebee *Bombus terrestris* L. under laboratory conditions, Report Number 21 48 BBA 0011. BioChem agrar, Labor für biologische und chemische, Analytik GmbH, Kupferstr. 6, 04827 Machern OT Gerichshain, Germany. (Syngenta File No. VV-939735) |
| Guideline(s): | OECD (2017): Guideline for the testing of chemicals; Bumblebee; acute oral toxicity test, 247<br>OECD (2017): Guideline for the testing of chemicals; Bumblebees; acute contact toxicity test, 246 |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication (if vertebrate study) | N/A |

**Executive Summary**

In a 48-hr-acute oral toxicity study, bumble bees (*Bombus terrestris* L.) were exposed orally to metalaxyl-M *via* a feeding solution. The $LD_{50}$ was > 88.5 µg consumed a.s./bumble bee and the NOED was 88.5 µg consumed a.s./bumble bee after 48 hours.

In a 48-hr-acute contact toxicity study, bumble bees (*Bombus terrestirs* L.) were exposed to metalaxyl-M administered topically to adult bees. The $LD_{50}$ was > 200.0 µg a.s./bumble bee and the NOED was 200.0 µg a.s./bumble bee after 48 hours.

**Materials**

| | |
|---|---|
| **Test Material** | |
| **Name:** | Metalaxyl-M (CGA329351A) |
| **Lot/Batch #:** | 678767 |
| **Purity:** | 96.9 % w/w |
| **Description:** | Appearance: amber liquid |
| **Stability of test compound:** | Stable under recommended handling and storage conditions (< 30 °C) |
| **Reanalysis/expiry date:** | End of April 2023 |
| **Treatments** | |
| **Test rates:** | Contact test: 200.0, 100.0, 50.0, 25.0, 12.5 µg a.i./bumble bee<br>Oral test (offered): 200.0, 100.0, 50.0, 25.0, 12.5 µg a.i./bumble bee<br>Oral test (consumed): 88.5, 48.0, 42.3, 23.2, 11.9 µg |
| **Controls:** | Contact test: Deionised water, 0.5% TritonX solution, acetone<br>Oral test: Sucrose solution, sucrose solution + 5% (v/v) acetone |
| **Reference item:** | Danadim® Progress (dimethoate EC 400; 411.20 g/L (analysed)) |
| **Administration:** | Contact test: 2µL/bee<br>Oral test: 40 µL/bee |
| **Test organisms** | |
| **Species:** | *Bombus terrestris* L. (bumble bee), worker bumble bees from queen right hives |
| **Source:** | Biobest Belgium N.V. Ilse Velden, 18, 2260 Westerlo, Belgium, supplied by: Katz Biotech AG, An der Birkenpfuhlheide 10, 15837 Baruth, Germany |

| | |
|---|---|
| **Food:** | 50% (w/v) sucrose solution |
| **Test design** | |
| **Test cage description:** | Nicot cages (part of the Nicot queen bee rearing system) with a length of 7 cm and a diameter of 2 cm. |
| **Number of bees/ test unit (= replicate):** | 1 |
| **No. of bees/rep:** | 30 (+ 5 additionally replicates/treatment to account non-feeders in the oral test) |
| **Duration of test:** | 48 hours |
| **Environmental test conditions** | |
| **Temperature:** | 25.5 – 26.8 °C |
| **Humidity:** | 52 – 80% |
| **Photoperiod:** | Constant darkness throughout the test (diffuse artificial light only during handling and assessments) |

**Study Design and Methods**

Test facility: BioChem agrar
Labor für biologische und chemische Analytik GmbH
Kupferstr. 6, 04827 Machern OT Gerichshain, Germany
Experimental dates: 30 June – 02 July 2021

**Oral test:** Plastic syringes (feeders) containing the corresponding application solution were used for application. The application volume was 40 µL/replicate (corresponding to 40 µL/bumble bee). The bumble bees were starved for approx. 4 hours prior to application start. Each unit was provided with the application solution for 4 hours, to ensure a sufficient uptake. The feeders were then removed, and the bumble bees were provided *ad libitum* with an untreated 50 % (w/v) aqueous sucrose solution. Treatments started with the control followed by the test item and finally the reference item. For dose verification the amount of application solution(s) consumed was determined by weighing the feeders before and after feeding using calibrated equipment.

**Contact test:** A hand operated micro-applicator was used for application. The application amount was 2 µL/bumble bee. After having been anaesthetised with $CO_2$ (the amount of anaesthetic used was minimised), the droplet of the application solution was applied individually to the dorsal side of the thorax of each bumble bee. Treatments started with the control followed by the test item and finally the reference item. For the toxic reference item group and the water treated control group, the water-wetting agent Triton X-100 was mixed into all application solutions. This reduced the surface tension of the applied solution and ensured that the drop of the application solution was spread out immediately after the application. After the application the bees were returned to the test units.

No statistical analysis was necessary since no control and test item mortality occurred during the contact and oral toxicity test.

**Results**

Analytical recoveries in dosing solutions are presented below.

**Table A 26: Analytical results - oral test**

| Target dosing solution (µg a.i./bee) | Measured concentration (mg a.i./kg) | % recovery |
|---|---|---|
| Solvent | < LOD | - |
| 200.0 | 4282 | 101 |
| 100.0 | 2207 | 104 |
| 50.0 | 945 | 89 |
| 25.0 | 525.4 | 99 |
| 12.5 | 263.6 | 100 |

LOD: 0.0027 mg/kg, corresponding to 0.0647 µg/L

Mortality data for the test material and toxic standard are summarised in the table below.

**Table A 27: Summary of acute oral toxicity of metalaxyl-M to the bumble bee**

| Treatment (target dose) | Treatment (consumed) | Mortality (%) | | Corrected mortality (%) | |
|---|---|---|---|---|---|
| | | 24 h | 48 h | 24 h | 48 h |
| (µg a.i./bee) | | | | | |
| Water control | | 0.0 | 0.0 | - | - |
| Solvent control | | 0.0 | 0.0 | - | - |
| 200.0 | 88.5 (AT) | 0.0 | 0.0 | - | - |
| 100.0 | 48.0 (BT) | 0.0 | 0.0 | - | - |
| 50.0 | 42.3 (CT) | 0.0 | 0.0 | - | - |
| 25.0 | 23.2 (DT) | 0.0 | 0.0 | - | - |
| 12.5 | 11.9c(ET) | 0.0 | 0.0 | - | - |
| Toxic reference | 1.46 (AR) | 80.0 | 96.7 | - | - |
| $LD_{50}$ (µg consumed a.i./bee) | | > 88.5 | | | |
| 95% C.I. | | - | | | |
| NOED (µg consumed a.i./bee) | | 88.5 | | | |

Mortality results are averages based on 30 (AC, BC), 25 (AT), 20 (BT), 20 (CT), 23 (DT), 28 (ET), 30 (AR) replicates consisting of 1 bumblebee each

No sublethal effects were observed in the oral test.

**Table A 28: Analytical results - contact test**

| Target dosing solution (µg a.i./bee) | Measured concentration (mg a.i./L) | % recovery |
|---|---|---|
| Solvent | < LOD | - |
| 200.0 | 100878 | 101 |
| 100.0 | 46335 | 93 |
| 50.0 | 22113 | 88 |
| 25.0 | 11519 | 92 |
| 12.5 | 5712 | 91 |

LOD: 0.0027 mg/L, corresponding to 0.0647 µg/L

**Table A 29: Summary of acute contact toxicity of metalaxyl-M to the bumble bee**

| Treatment (µg a.i./bee) | Mortality (%) | | Corrected mortality (%) | |
|---|---|---|---|---|
| | 24 h | 48 h | 24 h | 48 h |
| Water control | 0.0 | 0.0 | - | - |
| 0.5% TritonX | 0.0 | 0.0 | - | - |
| Acetone | 0.0 | 0.0 | - | - |
| 200.0 | 0.0 | 0.0 | - | - |
| 100.0 | 0.0 | 0.0 | - | - |

| Treatment | Mortality (%) | | Corrected mortality (%) | |
|---|---|---|---|---|
| 50.0 | 0.0 | 0.0 | - | - |
| 25.0 | 0.0 | 0.0 | - | - |
| 12.5 | 0.0 | 0.0 | - | - |
| Toxic reference | 73.3 | 90.0 | - | - |
| $LD_{50}$ (µg a.i./bee) | | > 200.0 | | |
| 95% C.I. | | - | | |
| NOED (µg ai/bee) | | 200.0 | | |

Mortality results are averages based on 30 replicates consisting of 1 bumblebee each

No sublethal effects were observed in the contact test.

**Validity criteria**

The study is considered valid since the control and reference item validity criteria were met:

- the mean mortality in both control groups of the oral and contact test was ≤ 10 % at the end of the test (observed values 0%);
- the mean reference item mortality was ≥ 50 % at the end of the test (observed values of 90.0% and 96.7% for the contact and oral tests, respectively)

**Conclusion**

In a 48-hr-acute oral toxicity study, bumble bees (*Bombus terrestris* L.) were exposed orally to metalaxyl-M *via* a feeding solution. The $LD_{50}$ was > 88.5 µg consumed a.s./bumble bee and the NOED was 88.5 µg consumed a.s./bumble bee after 48 hours.

In a 48-hr-acute contact toxicity study, bumble bees (*Bombus terrestirs* L.) were exposed to metalaxyl-M administered topically to adult bees. The $LD_{50}$ was > 200.0 µg a.s./bumble bee and the NOED was 200.0 µg a.s./bumble bee after 48 hours.

(Amsel, K., 2021)

## A 2.3.1.1.2 KCP 10.3.1.1.2 Acute contact toxicity to bees

See Section KCP 10.3.1.1.1. above.

## A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

| | |
|---|---|
| Comments of zRMS: | The study was conducted to OECD guidance TG 245 and according to the principles of GLP. All validity criteria were met.<br>The study is considered to be reliable and suitable for the risk assessment. |

| | |
|---|---|
| Reference: | KCP 10.3.1.2 |
| Report | Dreßler K., (2020), Oxathiapiprolin/Metalaxyl-M DC (A23109A) – Chronic toxicity to the honeybee *Apis mellifera* L. in a 10-day continuous laboratory feeding study, Report Number 20 48 BAC 0044. BioChem agrar – Labor für biologische und chemische Analytik GmbH / Kupferstraße 6, 04827 Machern OT Gerichshain, Germany. (Syngenta File No. VV-896929) |

| | |
|---|---|
| Guideline(s): | OECD TG 245: Honeybee (*Apis mellifera* L.), chronic oral toxicity test (10-day feeding) (2017) |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication (if vertebrate study) | N/A |

**Executive Summary**

The toxicity of A23109A to the honeybee *Apis mellifera* was determined in a 10-day continuous oral exposure study.

The 10-day NOEC was determined to be 1.629 g A23109A/kg sucrose solution and the $LC_{50}$ is 6.464 g A23109A/kg sucrose solution. Based on actual consumption of the test solutions, the NOEDD was 57.4 µg consumed A23109A/bee/day and the $LDD_{50}$ was calculated to be 117 µg consumed A23109A/bee/day.

**Materials**

**Test Material**

| | |
|---|---|
| **Name:** | Oxathiapiprolin/Metalaxyl-M DC (A23109A) |
| **Product code:** | A23109A |
| **Lot/Batch #:** | JHU003-036-001 |
| **Purity:** | Metalaxyl-M: 17.2 % w/w corresponding to 185 g/L<br>CGA351920 (S-enantiomer): 0.57% w/w corresponding to 6.1 g/L<br>CGA329351 (R-enantiomer): 16.6 % w/w corresponding to 178 g/L<br>Oxathiapiprolin: 2.85 % w/w corresponding to 30.6 g/L |
| **Density:** | 1074 kg/$m^3$ |
| **Description:** | formulation type: DC (dispersible concentrate)<br>appearance: brownish liquid |
| **Stability of test compound:** | Stable under recommended handling and storage conditions (< 30 °C) |
| **Reanalysis/Expiry date:** | End of June 2022 |

**Treatments**

| | |
|---|---|
| **Treatment groups:** | 1 untreated control, 5 concentrations of A23109A, 1 concentration of the reference item |
| **Test rates/ concentrations:** | nominal dose rates of 1023, 512, 256, 128 and 64.0 µg A23109A/bee/day corresponding to concentrations of 26.050, 13.025, 6.513, 3.256 and 1.629 g A23109A/kg sucrose solution |
| **Controls:** | 1 untreated control fed with 50% (w/v) sucrose solution |
| **Reference item:** | Danadim® Progress (dimethoate) |
| **Application method:** | daily preparation of feeding solutions and provision *ad libitum* over a period of 10 days (according to OECD Guideline 245 (2017)) |
| **Analysis of test concentrations:** | Yes, all applied concentrations of the active ingredient oxathiapiprolin were analytically verified in samples taken on each day of application by RP-HPLC with MS-MS detection. Moreover, the storage stability of the active ingredient oxathiapiprolin in sample matrix (50% (w/v) sucrose solution) over the actual storage period was verified. |

**Test system**

| | |
|---|---|
| **Test organism:** | worker honeybee |
| **Species:** | *Apis mellifera* L. subspecies Buckfast |
| **Age:** | max. 2 days old |
| **Source:** | BioChem agrar GmbH, Kupferstraße 6, 04827 Machern OT Gerichshain, Germany |
| **Food:** | 50% (w/v) sucrose solution |

**Test Design**

| | |
|---|---|
| **Test cage description:** | aluminum cages;<br>dimensions: 95 mm (width) x 70 mm (height) x 60 mm (depth); with holes in the lateral walls for sufficient air supply and ventilation; two glass plates (one in front and one in the back) for observation of the bees |
| **Replication:** | 3 |
| **No. of bees/replicate:** | 10 |
| **Environmental test conditions** | |
| **Temperature:** | 32.3 – 33.3 °C |
| **Humidity:** | 56.7 – 63.9% |
| **Photoperiod:** | darkness (diffuse artificial light only during assessments) |
| **Duration of test:** | 10 days |

**Study Design and Methods**

Test facility: BioChem agrar GmbH, Kupferstraße 6, 04827 Machern OT Gerichshain, Germany
Experimental dates: 28 July to 07 August 2020

A23109A treatments were freshly prepared every day. The target amount of 1.550 g A23109A was weighted into a 50 mL volumetric flask and dissolved in 50% (w/v) sucrose solution to prepare a 31.0 g/L stock solution (highest concentrated feeding solution). The remaining feeding solutions were prepared by parallel dilution by adding 12.50, 6.250, 3.125 or 1.563 mL of the highest concentrated feeding solution to 50% (w/v) sucrose solution (total volume of each feeding solution: 25 mL). The control treatment was provided with 50% (w/v) sucrose solution.

To consider the evaporation from the feeders, three additional test units with untreated 50% (w/v) sucrose solution and no bees present were set up.

Bees were fed *ad libitum* with treated/untreated sugar solutions presented with syringe feeders, which were renewed every day. Feeders were weighed before and after they were offered, so that the food consumed could be determined by comparison of the weight of the remaining solution with the initial weight. The individual daily consumption was corrected each day by the number of surviving bees at each assessment date as well as by estimated sucrose evaporation.

Direct treatment effects (mortality and other observed biological effects) were assessed at daily intervals during the 10-day exposure period by visual counting of honeybees.

All applied concentrations of the active ingredient oxathiapiprolin were analytically verified in samples taken on each day of application by RP-HPLC with MS-MS detection. No residues of the active ingredient oxathiapiprolin were found in the control samples. Additionally, the storage stability of the active ingredient oxathiapiprolin in sample matrix (50% (w/v) sucrose solution) over the actual storage period was verified.

To determine the NOEC and NOEDD, survival data were analysed for statistically significant differences compared to the control group using Step-down Rao-Scott-Cochran-Armitage Test Procedure (one-sided greater, $\alpha = 0.05$). To determine the $LC_{50/20/10}$ and $LDD_{50/20/10}$ values along with their 95% confidence limits, Probit and Weibull analysis using linear max. likelihood regression were used, respectively.

**Results**

Analytical recoveries in the diets are presented below.

**Table A 30: Analytical results**

| Diet (mg oxathiapiprolin/kg)* | Analytical Recovery in Diet [mg oxathiapiprolin/kg] (% of nominal) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | D0 | D1 | D2 | D3 | D4 | D5 | D6 | D7 | D8 | D9 |
| Untreated control | < 30% of LOQ | < 30% of LOQ | < 30% of LOQ | < 30% of LOQ | < 30% of LOQ | < 30% of LOQ | < 30% of LOQ | < 30% of LOQ | < 30% of LOQ | < 30% of LOQ |
| 742 | 702 (94.6) | 711 (95.8) | 716 (96.5) | 727 (97.9) | 736 (99.1) | 722 (97.2) | 739 (99.6) | 733 (98.8) | 738 (99.4) | 748 (101) |
| 371 | 368 (99.1) | 356 (95.9) | 357 (96.3) | 363 (97.9) | 375 (101) | 380 (102) | 373 (100) | 380 (102) | 374 (101) | 373 (100) |
| 186 | 175 (94.4) | 170 (91.8) | 171 (92.3) | 173 (93.2) | 176 (94.7) | 178 (95.8) | 171 (91.9) | 174 (93.5) | 177 (95.2) | 173 (93.5) |
| 92.8 | 86.5 (93.3) | 87.7 (94.5) | 88.8 (95.7) | 87.2 (94.0) | 90.2 (97.2) | 92.2 (99.4) | 91.8 (98.9) | 88.3 (95.2) | 89.4 (96.4) | 90.7 (97.7) |
| 46.4 | 43.9 (94.7) | 43.2 (93.1) | 42.9 (92.5) | 43.7 (94.2) | 42.2 (90.9) | 44.6 (96.0) | 44.4 (95.6) | 43.4 (93.5) | 43.5 (93.6) | 44.0 (94.8) |

LOQ: 0.01 mg oxathiapiprolin/kg, corresponding to 0.25 µg oxathiapiprolin/L in diluted extracts
* based on the analysed content of oxathiapiprolin according to Certificate of Analysis of 22 July 2019 (2.85% w/w)

Mortality data for the test material and reference item are summarised in the table below.

**Table A 31: Summary of mortality of bees in the chronic toxicity test after 10 days**

| Nominal Concentration [g A23109A/kg sucrose solution] | Daily Consumed Dose based on bee consumption [µg A23109A/bee/day] | After 10 days | |
|---|---|---|---|
| | | Mean mortality | |
| | | absolute [%] | corrected [%] |
| Blank control | | 0.0 | -- |
| 26.050 | 148 | 100* | -- |
| 13.025 | 146 | 96.7* | -- |
| 6.513 | 117 | 46.7* | -- |
| 3.256 | 80.2 | 6.7* | -- |
| 1.629 | 57.4 | 0.0 | -- |
| Reference item | | 93.3 | -- |

Results are averages based on 3 replicates, containing 10 bees each; Calculations are performed with non-rounded values.
corrected: corrected mortality (according to SCHNEIDER-ORELLI 1947); Due to 0% mortality in the control group, no correction is needed.
* Statistically significant difference in pairwise comparison between treatment and untreated control group (Step-down Rao-Scott-Cochran-Armitage Test Procedure; $\alpha = 0.05$; one-sided greater)

During the course of the test, behavioural abnormalities were observed in the highest test item dose (148 µg consumed A23109A/bee/day). One bee out of nine remaining bees was observed as being affected (uncoordinated movements) on day 2. No other behavioural abnormalities were observed in any test item treatment group on any other assessment day.

Study endpoints are summarised in the table below.

**Table A 32: Study endpoints at 10 days**

| Treatment | Endpoints | After 10 d |
|---|---|---|
| **Test item doses** | $LDD_{10}$ [µg consumed A23109A/bee/day] | 90.6 (77.3 – 99.2) |
| | $LDD_{20}$ [µg consumed A23109A/bee/day] | 100 (88.9 – 108) |
| | $LDD_{50}$ [µg consumed A23109A/bee/day] | 117 (109 – 122) |
| | NOEDD [µg consumed A23109A/bee/day] | 57.4 |
| **Test item concentrations** | $LC_{10}$ [g A23109A/kg sucrose solution] | 3.786 (2.822 – 4.535) |
| | $LC_{20}$ [g A23109A/kg sucrose solution] | 4.549 (3.614 – 5.315) |
| | $LC_{50}$ [g A23109A/kg sucrose solution] | 6.464 (5.560 – 7.515) |
| | NOEC [g A23109A/kg sucrose solution] | 1.629 |

**Validity Criteria**

The test was considered valid;

- Average cumulative mortality was <15% in the sucrose solution control (actual 0%)
- Mortality was >50% in the toxic reference (actual 93.3%)

**Conclusion**

The toxicity of A23109A to the honeybee *Apis mellifera* was determined in a 10-day continuous oral exposure study.

The 10-day NOEC was determined to be 1.629 g A23109A/kg sucrose solution and the $LC_{50}$ is 6.464 g A23109A/kg sucrose solution. Based on actual consumption of the test solutions, the NOEDD was 57.4 µg consumed A23109A/bee/day and the $LDD_{50}$ was calculated to be 117 µg consumed A23109A/bee/day.

(Dreßler, K., 2020)

| Comments of zRMS: | Study not evaluated by zRMS. |
|---|---|

**Reference:** KCA1 8.3.1.2

**Report:** Ruhland S. (2021), Metalaxyl-M: Chronic toxicity to the honey bee *Apis mellifera* L. in a 10-day continuous laboratory feeding study. Report Number 21 48 BAC 0027, BioChem agrar Labor für biologische und chemische Analytik GmbH. (Syngenta File No. VV-932880).

**Guidelines:** OECD TG 245: Honeybee (*Apis mellifera* L.), chronic oral toxicity test (10-day feeding) (2017)

| | |
|---|---|
| **Deviations:** | None |
| **GLP:** | Yes |
| **Acceptability:** | Yes |
| **Duplication (if vertebrate study):** | N/A |

**Executive Summary**

The toxicity of metalaxyl-M to the honeybee *Apis mellifera* was determined in a 10-day continuous oral exposure study.

The 10-day NOEC was determined to be 0.328 g metalaxyl-M/kg sucrose solution and the $LC_{50}$ was 1.167 g metalaxyl-M/kg sucrose solution.

Based on actual consumption of the test solutions, the NOEDD was 8.55 µg metalaxyl-M/bee/day and the $LDD_{50}$ was calculated to be 20.3 µg metalaxyl-M/bee/day.

**Materials**

| | |
|---|---|
| **Test Material** | |
| **Name:** | Metalaxyl-M |
| **Lot/Batch #:** | 678767 (other batch ID: CAB2G030) |
| **Purity:** | Metalaxyl-M 96.9 % w/w |
| **Description:** | Substance type: TGAI (technical grade of active ingredient)<br>Appearance: amber liquid |
| **Stability of test compound:** | Stable under the given conditions |
| **Reanalysis/Expiry date:** | End of April 2023 |
| **Treatments** | |
| **Test rates:** | Nominal dose rates of 200, 79.8, 31.9, 12.8 and 5.11 µg metalaxyl-M/bee/day corresponding to concentrations of 5.124, 2.050, 0.820, 0.328 and 0.131 g Metalaxyl-M/kg sucrose solution |
| **Controls:** | 1 untreated control fed with 50% (w/v) sucrose solution<br>1 untreated control fed with 50% (w/v) sucrose solution + 5% (v/v) acetone |
| **Reference item:** | Danadim® Progress (Dimethoate) |
| **Application method:** | Daily preparation of feeding solutions and provision *ad libitum* over a period of 10 days via syringes (according to OECD TG 245 (2017)) |
| **Analysis of test concentrations:** | Yes, all applied concentrations of the active ingredient metalaxyl-M were analytically verified in samples taken on each day of application by RP-HPLC with MS/MS detection. Moreover, the storage stability of the active ingredient metalaxyl-M in sample matrix (50% (w/v) sucrose solution + 5% (v/v) acetone) over the actual storage period was verified. |
| **Test organisms** | |
| **Species:** | *Apis mellifera* L. subspecies Buckfast *(honeybee)* |
| **Age:** | Max. 2 days old |
| **Source:** | BioChem agrar GmbH, Kupferstraße 6, 04827 Machern OT Gerichshain, Germany |
| **Food:** | 50% (w/v) sucrose solution |
| **Test Design** | |
| **Test cage description:** | Aluminum cages;<br>dimensions: 95 mm (width) x 70 mm (height) x 60 mm (depth); with holes in the lateral walls for sufficient air supply and ventilation; two glass plates (one in front and one in the back) for observation of the bees |
| **Replication:** | 3 |
| **No. of bees/replicate:** | 10 |
| **Environmental test conditions** | |
| **Temperature:** | 32.9 – 33.4 °C |
| **Humidity:** | 57.3 – 63.3% |

| | |
|---|---|
| **Photoperiod:** | Darkness (diffuse artificial light only during feeding and assessments) |
| **Duration of test:** | 10 days |

**Study Design and Methods**

Test Facility: BioChem agrar GmbH, Kupferstraße 6, 04827 Machern OT Gerichshain, Germany

| | | |
|---|---|---|
| Experimental dates: | Experimental start date: | 20 July 2021 |
| | Experimental completion date: | 30 July 2021 |
| | Experimental start date (analytical phase): | 30 September 2021 |
| | Experimental completion date (analytical phase): | 12 October 2021 |

Metalaxyl-M treatments were freshly prepared every day. The target amount of 0.156 g metalaxyl-M was weighted into a 25 mL volumetric flask and dissolved in 50% (w/v) sucrose solution containing 5% (v/v) acetone to prepare a 6.047 g/L stock solution (highest concentrated feeding solution). The remaining feeding solutions were prepared by serial dilution by adding 10.0 mL of the previous feeding solution to 50% (w/v) sucrose solution + 5% (v/v) acetone (total volume of each feeding solution: 25 mL).

One control treatment was provided with 50% (w/v) sucrose solution. A second control treatment was provided with 50% (w/v) sucrose solution containing 5% (v/v) acetone.

To consider the evaporation from the feeders, three additional test units with untreated 50% (w/v) sucrose solution and untreated 50% (w/v) sucrose solution + 5% (v/v) acetone and no bees present were set up.

Bees were fed *ad libitum* with treated/untreated sugar solutions presented with syringe feeders, which were renewed every day. Feeders were weighed before and after they were offered, so that the food consumed could be determined by comparison of the weight of the remaining solution with the initial weight. The individual daily consumption was corrected each day by the number of surviving bees at each assessment date as well as by estimated sucrose evaporation.

Direct treatment effects (mortality and other observed biological effects) were assessed at daily intervals during the 10-day exposure period by visual counting of honeybees.

All applied concentrations of the active ingredient metalaxyl-M were analytically verified in samples taken on each day of application by RP-HPLC with MS/MS detection. The mean recovery rates of the active ingredient metalaxyl-M in samples of the test item feeding solutions were between ± 20% of the nominal concentrations. Therefore, the concentrations of active ingredients in the applied test item feeding solutions were verified and endpoints have been based on nominal concentrations. No residues of the active ingredient metalaxyl-M were found in the control samples. Moreover, the storage stability of metalaxyl-M in sample matrix (50% (w/v) aqueous sucrose solution + 5% (v/v) acetone) over the actual storage period was verified.

To determine the NOEC and NOEDD, mortality data were analysed for statistically significant differences compared to the solvent control (with acetone) using Step-down Cochran-Armitage Test Procedure (one-sided greater, $\alpha = 0.05$). To determine the $LC_{50/20/10}$ and $LDD_{50/20/10}$ values along with their 95% confidence limits, the survival data were arcsine-transformed and a probit analysis with linear max. likelihood regression and weighted linear regression, respectively, was performed.

**Results**

Analytical recoveries in the diets are presented below.

**Table A 33: Analytical results**

| Diet (mg metalaxyl-M/kg)* | Analytical Recovery in Diet [mg metalaxyl-M/kg] (% of nominal) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | D0 | D1 | D2 | D3 | D4 | D5 | D6 | D7 | D8 | D9 |
| Untreated acetone control | < 30% of LOQ | < 30% of LOQ | < 30% of LOQ | < 30% of LOQ | < 30% of LOQ | < 30% of LOQ | < 30% of LOQ | < 30% of LOQ | < 30% of LOQ | < 30% of LOQ |
| 5124 | 5591 (109) | 5351 (104) | 5406 (105) | 5457 (107) | 5777 (113) | 5663 (111) | 5729 (112) | 5542 (108) | 5628 (110) | 5704 (111) |
| 2050 | 2196 (107) | 2287 (112) | 2308 (113) | 2340 (114) | 2404 (117) | 2469 (120) | 2527 (123) | 2355 (115) | 2301 (112) | 2304 (112) |
| 820 | 957 (117) | 890 (109) | 911 (111) | 945 (115) | 927 (113) | 894 (109) | 980 (120) | 926 (113) | 929 (113) | 944 (115) |
| 328 | 356 (109) | 363 (111) | 363 (111) | 356 (109) | 395 (120) | 377 (115) | 367 (112) | 370 (113) | 379 (116) | 371 (113) |
| 131 | 145 (110) | 144 (110) | 145 (111) | 144 (110) | 152 (116) | 147 (112) | 147 (112) | 153 (116) | 151 (115) | 172 (131) |

LOQ: 0.010 mg/kg, corresponding to 0.24 µg/L in diluted extracts. Method limit of detection (LOD): 0.0027 mg/kg

Mortality data for the test material and reference item are summarised in the table below.

**Table A 34: Summary of mortality of bees in the chronic toxicity test after 10 days**

| Nominal Concentration [g Metalaxyl-M/kg sucrose solution] | Daily Consumed Dose based on bee consumption [µg Metalaxyl-M/bee/day] | After 10 days | |
|---|---|---|---|
| | | Mean mortality | |
| | | absolute [%] | corrected [%] |
| Blank control (50% (w/v) sucrose solution) | | 0.0 | -- |
| Solvent control (50% (w/v) sucrose solution containing 5% (v/v) acetone) | | 0.0 | -- |
| 5.124 | 17.6 | 100.0* | -- |
| 2.050 | 32.8 | 60.0* | -- |
| 0.820 | 25.9 | 46.7* | -- |
| 0.328 | 8.55 | 3.3 | -- |
| 0.131 | 4.39 | 3.3 | -- |
| Reference item | | 100.0 | -- |

Results are averages based on 3 replicates, containing 10 bees each; Calculations are performed with non-rounded values.
corrected: corrected mortality (according to SCHNEIDER-ORELLI 1947); Due to 0.0% mortality in both control groups, no correction is needed.

* Statistically significant difference in pairwise comparison between treatment and untreated control group BC (Step-down Cochran-Armitage Test Procedure; $\alpha = 0.05$; one-sided greater)

In the final assessment one bee was described as affected in the second highest test item dose (32.8 µg consumed a.i./bee/day).

Study endpoints are summarised in the table below.

**Table A 35: Study endpoints at 10 days**

| Treatment | Endpoints | After 10 d |
|---|---|---|
| **Test item doses** | $LDD_{50}$ [µg consumed Metalaxyl-M/bee/day] | 20.3 (12.0 – 75.5) |
| | $LDD_{20}$ [µg consumed Metalaxyl-M/bee/day] | 7.86 (1.91 – 13.3) |
| | $LDD_{10}$ [µg consumed Metalaxyl-M/bee/day] | 4.79 (0.45 – 8.66) |
| | NOEDD [µg consumed Metalaxyl-M/bee/day] | 8.55 |
| **Test item concentrations** | $LC_{50}$ [g Metalaxyl-M/kg food] | 1.167 (0.835 – 1.638) |
| | $LC_{20}$ [g Metalaxyl-M/kg food] | 0.444 (0.215 – 0.651) |
| | $LC_{10}$ [g Metalaxyl-M/kg food] | 0.268 (0.097 – 0.438) |
| | NOEC [g Metalaxyl-M/kg food] | 0.328 |

**Validity Criteria**

The test was considered valid;

- Average cumulative mortality was <15% in the sucrose solution control
- Average cumulative mortality was <15% in the acetone control
- Mortality was >50% in the toxic reference

**Conclusion**

The toxicity of metalaxyl-M to the honeybee *Apis mellifera* was determined in a 10-day continuous oral exposure study.

The 10-day NOEC was determined to be 0.328 g metalaxyl-M/kg sucrose solution and the $LC_{50}$ was 1.167 g metalaxyl-M/kg sucrose solution.

Based on actual consumption of the test solutions, the NOEDD was 8.55 µg metalaxyl-M/bee/day and the $LDD_{50}$ was calculated to be 20.3 µg metalaxyl-M/bee/day.

(Ruhland, S., 2021)

| Comments of zRMS: | Study not evaluated by zRMS. |
|---|---|

**Reference:** KCA 8.3.1.2

**Report:** Kling A. (2015), Metalaxyl-M SL (A13947A) - Assessment of Effects on the Adult Honey Bee, *Apis mellifera* L., in a 10 Day Chronic Feeding Test under Laboratory Conditions. Report Number S15-00380, Eurofins Agroscience Services EcoChem GmbH Eutinger Str. 24, 75223 Niefern-Öschelbronn, Germany (Syngenta file No. A13947A_11449/ VV-414721).

**Guidelines:** None

**Deviations:** N/A

**GLP:** Yes

**Acceptability:** Yes

**Duplication (if vertebrate study):** N/A

**Executive Summary**

The effects of A13947A were assessed on young adult honey bees, *Apis mellifera*, in a 10 day chronic feeding test under laboratory conditions.

The $LC_{50}$ was calculated to be 5208 mg A13947A /kg food and the NOEC was determined to be 2500 mg A13947A /kg food. The $LDD_{50}$ was calculated to be 67.8 µg A13947A /bee/day and the NOEDD was determined to be 439 µg A13947A /bee/day.

## Materials

| | |
|---|---|
| **Test Material** | A13947A<br>metalaxyl-M SL |
| **Lot/Batch #:** | SMO3L0022 |
| **Actual content of active ingredients:** | Nominal: 45.2 % w/w; 480 g/L; analysed: 45.7 % w/w; 484 g/L |
| **Description:** | Orange liquid |
| **Stability of test compound:** | Stable under test conditions |
| **Reanalysis/Expiry date:** | 31 Jan 2017 |
| **Density:** | 1.058 g/cm³ |
| **Treatments** | |
| **Test concentrations:** | 625, 1250, 2500, 5000 and 10000 mg product/kg feeding solution (corresponding to 18.9, 31.3, 43.9, 65.6 and 166 µg A13947A/bee/day) |
| **Control:** | 50 % (w/v) aqueous sucrose solution |
| **Toxic standard:** | Dimethoate BAS 152 11 I (nominal: 400.0 g/L; measured 400.9 g/L) |
| **Administration:** | Test and reference item mixed with 50 % (w/v) aqueous sucrose solution. Control groups were treated with 50 % (w/v) aqueous sucrose solution only |
| **Test organisms** | |
| **Species:** | *Apis mellifera carnica* L. (Hymenoptera: Apidae) (young adult worker bees) |
| **Source:** | Beekeeper Klaus Hampel, Eutinger Straße 24, 75223 Niefern-Öschelbronn, Germany |
| **Food:** | 50 % (w/v) aqueous sucrose solution |
| **Test design** | |
| **Test cage description:** | Stainless steel (base: 8 cm x 4 cm; height: 6 cm). The front side of the cages was equipped with a transparent pane to enable observation. The bottom of the cages consisted of perforated steel, which guaranteed sufficient air supply. The cages were lined with filter paper. |
| **Replication:** | 4 replicates of 10 bees |
| **Duration of test:** | 10 days |
| **Environmental test conditions** | |
| **Temperature:** | 31.7 – 34.6 °C |
| **Humidity:** | 36.7 – 64.2 % |
| **Photoperiod:** | Constant darkness |

## Study Design and Methods

Experimental dates: 9th to 19th June 2015

Two days prior to test initiation, brood combs containing capped cells which were expected to hatch on the same day were transferred into a climatically controlled chamber from the honeybee colony. Brood combs from two hives were used to guarantee a sufficient number of bees for the test. One day prior to test start the 0 – 3 day old bees were transferred from combs to the test cages and kept under test conditions.

Feeding solutions were placed in syringes, the tips of which had been removed for access, and offered to the bees in each unit *ad libitum*. Bees in one replicate shared the feeding solution and thus received similar doses. Feeding solutions were replaced daily and the amount of feeding solution consumed was determined by weighing the syringe before and after feeding.

In order to determine the $LC_{50}$ and $LDD_{50}$ values a Weibull analysis was used. Only concentrations within a clear dose response were used for calculating $LC_{50}$ and $LDD_{50}$ values. Fisher's Exact Test with Bonferroni Correction (one-sided greater, $p \leq 0.05$) was used to evaluate whether there are significant differences between the mortality data of the control and the test item treatment group and to determine the NOEC and NOEDD based on mortality. Statistical calculations were made by using the statistical program ToxRat Professional 3.2.1.

**Results and Discussion**

Results are presented in the table below.

**Table A 36: Summary of semi-chronic toxicity of A13947A to honeybee adults**

| Treatment | 10 day cumulative mortality (corrected mortality) (%) | Overall mean consumption of feeding solution (mg/bee/day) | Dietary dose (based on actual measured consumption) (µg/bee/day) | Mean accumulated uptake of test item during the test period µg/bee) |
|---|---|---|---|---|
| **Control** | | | | |
| C (0) | 5.0 (-) | 30.3 | - | - |
| **A13947A (mg product/kg)** | | | | |
| 625 | 2.5 (-2.6) | 30.2 | 18.9 | 189 |
| 1250 | 0.0 (-5.3) | 25.1 | 31.3 | 313 |
| 2500 | 0.0 (-5.3) | 17.6 | 43.9 | 439 |
| 5000 | 42.5 (39.5) | 13.1 | 65.6 | 656 |
| 10000 | 100 (100) | 16.6 | 166 | 1796 |
| **Reference item (Perfekthion (mg a.s./kg)** | | | | |
| R (0.9) | 100 (100) | 16.9 | 0.02 | 0.17 |
| $LC_{50}$ | 5208 mg A13947A /kg food | | | |
| NOEC | 2500 mg A13947A /kg food | | | |
| $LDD_{50}$ | 67.8 µg A13947A /bee/day | | | |
| NOEDD | 439 µg A13947A /bee/day | | | |

**Conclusions**

The chronic toxicity of A13947A to honeybees was tested under laboratory conditions.

The 10 day $LDD_{50}$ was determined to be 67.8 µg A13947A /bee/day. The 10 day NOEDD was determined to be 439 µg A13947A /bee/day. The 10 day $LC_{50}$ was determined to be 5208 mg A13947A /kg food. The 10 day NOEC was determined to be 2500 mg A13947A /kg food.

(Kling A. 2015)

| Comments of zRMS: | Study not evaluated by zRMS. |
|---|---|

Reference: KCA2 8.3.1.2

Report: Tänzler, V. (2015), Oxathiapiprolin (DPX-QGU42) 100 g/L OD: Chronic oral toxicity to the honey bee, *Apis mellifera* L. (Hymenoptera, Apidae). Report Number 94441136. Institut für Biologische Analytik und Consulting IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany. DuPont Study No. DuPont-41989. (Study owner Corteva, Syngenta have access)

| | |
|---|---|
| Guideline(s): | OECD Guideline No. 213 (1998)<br>CEB No. 230 (2014) |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |

**Executive Summary**

This study was conducted in order to determine the chronic oral toxicity of Oxathiapiprolin 100 g/L OD on the honey bee (*Apis mellifera* L.) under laboratory conditions for a period of ten days. Mortality of the bees was used as the toxic endpoint. Sublethal effects such as changes in behaviour were also monitored and reported.

Under laboratory conditions 30 freshly emerged worker bees (*Apis mellifera* L.) per treatment level were exposed for 10 days to 5 concentrations (1500, 750, 375, 188 and 94 mg a.s./kg food corresponding to 14306, 7153, 3576, 1788 and 894 mg prod./kg food) of the test item-treated sugar solutions *ad libitum*. These concentrations led to actual mean dose levels of 43.6, 24.1, 12.1, 6.5 and 3.7 µg a.s./bee per day (corresponding to 415.8, 229.8, 115.4, 62.0 and 35.3 µg prod./bee per day). An untreated control and a reference item were included in this study.

At test end (10 days after start of exposure) there was 83.3, 13.3, 3.3, 3.3 and 0.0% mortality in the 1500, 750, 375, 188 and 94 ppm treatments, respectively (corresponding to 14306, 7153, 3576, 1788 and 894 ppm prod./kg food). A mortality of 6.7 % was observed in the water control group.

On day 7 two bees and day 9 to 10 three bees from the 1500 ppm treatment level were affected. On day 10 one bee from the 750 ppm treatment level was affected. No further test item-related behavioural effects were observed.

The reference item (dimethoate) at a concentration of 1 ppm (corresponding to a mean dose of 0.021 µg a.s./bee/day) led to 100% mortality on day 10.

**Materials**

| | |
|---|---|
| **Test material:** | Oxathiapiprolin 100 g/L OD |
| **Batch/Lot Number:** | QGU42-347 |
| **Purity:** | 100 g a.s./L (nominal); 103.7 g/L (measured) |
| **Description:** | OD (oil dispersion) |
| **CAS Registry Number:** | None for the formulation<br>1003318-67-9 for the active substance |
| **Test vehicle:** | 50 % w/v sucrose solution (500 g sucrose/L deionised water) |
| **Reference item:** | Perfekthion (BAS 152 11 I) |
| **Test organism:** | Worker honey bees (Insecta, Hymenoptera) |
| **Species:** | Adult *Apis mellifera carnica* L. |
| **Stage and Sex:** | Female worker bees |
| **Source:** | Honey bee colonies, disease-free and queen-right, bred by IBACON |
| **Collection:** | Two days before the start of the test, three brood combs with sealed brood and bees visibly starting to emerge were selected. The comb also contained pollen which served as a first feeding source for the freshly hatched bees. The combs were taken out from the hive and the adult bees were swept away. Then it was enclosed in an excluder box and stored in an incubator in the laboratory. The emerging bees remained in the excluder box for one further day.<br>The following day (test start), the freshly emerged worker bees were taken out from the excluder box with forceps and were transferred to the ready-prepared test units (cages) without the use of smoke and without anaesthetics. |

**Environmental conditions**

| | |
|---|---|
| **Temperature:** | 32 – 33 °C |
| **Relative Humidity:** | 58 – 59 % |
| **Application:** | The treated and untreated feeding solutions were offered *ad libitum* to each cage in syringes. The syringes were weighed daily before introduction into the cages and after the feeding interval (before daily replacement with new syringes containing fresh test solutions). |

**Study Design and Methods**

Experimental dates: 02 September 2014 to 12 September 2014

The study comprised 7 treatment groups (five dose rates of the test item, water control, one dose rate of the reference item) with 3 replicates each containing 10 bees. The final concentration of sugar in the test item feeding solutions offered to the bees was 50 % (w/v). The feeding solutions of the test item were prepared freshly every day.

The reference item was prepared with 50 % (w/v) sucrose solution. The stock solution of the reference item was prepared once at start of the test and stored at 4 °C ± 4 °C over a period of ten days. 50 % (w/v) sucrose solution was used for the untreated control. The feeding solutions of the reference item and untreated control were prepared freshly every 4 days and stored at 4 °C ± 4 °C.

The treated and untreated feeding solutions were offered *ad libitum* to each cage in syringes. The syringes were weighed daily before introduction into the cages and after the feeding interval (before daily replacement with new syringes containing fresh test solutions).

**Results and Discussion**

All study validity criteria were met.

Under laboratory conditions 30 freshly emerged worker bees (*Apis mellifera* L.) per treatment level were exposed for 10 days to 5 concentrations (1500, 750, 375, 188 and 94 mg a.s./kg food corresponding to 14306, 7153, 3576, 1788 and 894 mg prod./kg food) of the test item-treated sugar solutions *ad libitum*. These concentrations led to actual mean dose levels of 43.6, 24.1, 12.1, 6.5 and 3.7 µg a.s./bee per day (corresponding to 415.8, 229.8, 115.4, 62.0 and 35.3 µg prod./bee per day). An untreated control and a reference item were included in this study.

At test end (10 days after start of exposure) there was 83.3, 13.3, 3.3, 3.3 and 0.0 % mortality in the 1500, 750, 375, 188 and 94 ppm treatments, respectively (corresponding to 14306, 7153, 3576, 1788 and 894 mg prod./kg food). A mortality of 6.7 % was observed in the water control group.

On day 7 two bees and day 9 to 10 three bees from the 1500 ppm treatment level were affected. On day 10 one bee from the 750 ppm treatment level was affected. No further test item-related behavioural effects were observed.

The reference item (dimethoate) at a concentration of 1 ppm (corresponding to a dose of 0.021 µg a.s./bee/day) led to 100% mortality on day 10.

The results are summarised in the table below.

**Table A 37: Chronic toxicity of Oxathiapiprolin 100 g/L OD to honeybees**

| **Test Organism** | | | ***Apis mellifera* L.** | | | |
|---|---|---|---|---|---|---|
| **Treatment Group** | **Concentration [mg a.s./kg]** | **Concentration [mg prod./kg]** | **Dose Level[a] [µg a.s./bee]** | **Dose Level[a] [µg prod./bee]** | **Mortality at day 10[b] [% mean]** | **Corrected mortality [%]** |
| Oxathiapiprolin 100 g/L OD | 1500 | 14306 | 43.6 | 415.8 | 83.3 (*) | 82.1 |
| | 750 | 7153 | 24.1 | 229.8 | 13.3 (n.s.) | 7.1 |
| | 375 | 3576 | 12.1 | 115.4 | 3.3 (n.s.) | 0.0 |
| | 188 | 1788 | 6.5 | 62.0 | 3.3 (n.s.) | 0.0 |
| | 94 | 894 | 3.7 | 35.3 | 0.0 (n.s.) | 0.0 |
| Water Control | 0.0 | | 0.0 | | 6.7 | - |
| Reference Item | 1.0 | | 0.021 | | 100 | 100 |

[a] Mean dose per bee per day; dose measured based on consumed feeding solution
[b] Mortality at study termination 10 days after start of first feeding
n.s. = no statistical significant difference compared to the control
* = statistically significant difference compared to the control

**Table A 38: Chronic toxicity of Oxathiapiprolin 100 g/L OD to honeybees: Endpoints at test termination (day 10)**

| **$LC_{50}$** | **$LD_{50}$** | **NOEC** | **NOED** |
|---|---|---|---|
| 1148.1 mg a.s./kg food<br>10949.6 mg prod./kg food | 34.7 µg a.s./bee per day<br>330.9 µg prod./bee per day | 750 mg a.s./kg food<br>7153 mg prod./kg food | 24.1 µg a.s./bee per day<br>229.8 µg prod./bee per day |

Statistical analysis:
LC/LD: according to Probit Analysis (according to Finney 1971).
NOEC/NOED: Fisher`s Exact Test, pairwise comparison, one-sided greater, $\alpha = 0.05$

**Conclusions**

The chronic toxicity of Oxathiapiprolin 100 g/L OD was tested over 10 days.

The $LC_{50}$ value (10 days) was 1148.1 mg a.s./kg feeding solution (corresponding to 10949.6 mg prod./kg feeding solution). The $LD_{50}$ value (10 days) was 34.7 µg a.s./bee per day (corresponding to 330.9 µg prod./bee per day). The NOED and NOEC values (10 days) were 24.1 µg a.s./bee/day and 750 mg a.s./kg feeding solution, respectively (corresponding to 229.8 µg prod./bee per day and 7153 mg prod./kg feeding solution, respectively).

In addition, the $LC_{10}$ value (10 days) and the $LC_{20}$ value (10 days) were 791.4 mg a.s./kg feeding solution and 899.2 mg a.s./kg feeding solution, respectively (corresponding to 7547.7 mg prod./kg feeding solution and 8575.8 mg prod./kg feeding solution, respectively). Moreover the $LD_{10}$ value (10 days) and the $LD_{20}$ value (10 days) were 25.2 µg a.s./bee/day and 28.1 µg a.s./bee/day, respectively (corresponding to 240.3 µg prod./bee/day and 268.0 µg prod./bee/day).

The analytical recovery rate of the active ingredient oxathiapiprolin in the feeding solutions was between 103 and 128% of the nominal value.

(Tänzler V, 2015)

## A 2.3.1.3 KCP 10.3.1.3 Effects on honey bee development and other honey bee life stages

| | |
|---|---|
| Comments of zRMS: | The study was conducted to OECD guidance TG 239 and according to the principles of GLP. All validity criteria were met.<br>The study is considered to be reliable and suitable for the risk assessment. |

| | |
|---|---|
| Reference: | KCP 10.3.1.3 |
| Report | Schmidt K., (2021), Oxathiapiprolin/Metalaxyl-M DC (A23109A) – Repeated Exposure of the Honeybee Larvae (*Apis mellifera* L.) under Laboratory Conditions (until Adult Emergence up to Day 22), Report Number 20 48 BLC 0044. BioChem agrar Labor für biologische und chemische Analytik GmbH. (Syngenta File No. VV-895633). |
| Guideline(s): | OECD 239: Honeybee (*Apis mellifera* L.) larval toxicity test, repeated exposure |
| Deviations: | ~~None~~ One deviation dated 18 December 2020 regarding the relative humidity and temperature between D8 and D15 |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication (if vertebrate study) | N/A |

**Executive Summary**

The toxicity of Oxathiapiprolin/Metalaxyl-M DC (A23109A) to the larvae of the honeybee *Apis mellifera* was determined in a chronic exposure study over a 22-day period.

The 22-day NOEC was determined to be 155 mg product/kg diet and the LC50 is 784 mg product/kg diet.

**Materials**

| | |
|---|---|
| **Test Material** | Oxathiapiprolin/Metalaxyl-M DC (A23109A) |
| **Lot/Batch #:** | JHU003-036-001 |
| **Purity:** | Metalaxyl-M: 17.2 % w/w corresponding to 185 g/L<br>CGA351920 (S-enantiomer): 0.57% w/w corresponding to 6.1 g/L<br>CGA329351 (R-enantiomer): 16.6 % w/w corresponding to 178 g/L<br>Oxathiapiprolin: 2.85 % w/w corresponding to 30.6 g/L |
| **Description:** | Brownish liquid |
| **Stability of test compound:** | Stable under the given conditions |
| **Reanalysis/Expiry date:** | End of June 2022 |
| **Treatments** | |
| **Test rates:** | µg product/larva: 382, 153, 61, 24, 10<br>mg product/kg diet: 2417, 967, 387, 155, 62 |
| **Control:** | Untreated diet |
| **Toxic standard:** | Dimethoate tech., Purity: 98.8 ± 0.5%, 7.6 µg a.i./larva, 48 mg a.i./kg diet |
| **Analysis of test concentrations:** | The determination of oxathiapiprolin in final diet was conducted by an in-house developed method using reversed phase - high performance liquid chromatography (RP-HPLC) with mass-spectrometric (MS/MS) detection. |
| **Test organisms** | Worker honeybee larvae (Hymenoptera, Apoidea) |
| **Species:** | *Apis mellifera* L., *ssp: Buckfast* |
| **Age:** | The larvae were in first instar stage (L1) at grafting. |
| **Source:** | BioChem agrar GmbH |
| **Food:** | artificial diets containing glucose, fructose, yeast extract and water mixed with royal jelly at a weight ratio of 1 : 1 (D3-D6) |
| **Test Design** | |
| **Test cage description:** | Crystal polystyrene grafting cells (CNE Nicotplast, internal diameter 9 mm) were placed in 48 well plates |

| | | |
|---|---|---|
| **Replication:** | 3 replicates of the control, 3 replicates of each test and reference item dosage | |
| **No. of larvae/replicate:** | 12 | |
| **Environmental test conditions** | | |
| **Temperature:** | 25.8 – 35.0 °C | |
| **Humidity:** | D1-D8: | 92.5 – 99.9% |
| | D8-D15: | 29.5 – 99.9% |
| | D15-D22: | 63.4 – 67.2% |
| **Photoperiod:** | Illumination: constant darkness within the test chamber (diffuse artificial light only during handling and assessments) | |
| **Duration of test:** | 22 days | |

**Study Design and Methods**

Test facility: BioChem agrar GmbH, Kupferstr. 6, 04827 Machern OT Gerichshain, Germany

| | | |
|---|---|---|
| Experimental dates: | Experimental start date: | 31 August 2020 |
| | Experimental completion date: | 21 September 2020 |
| | Experimental start date (analytical phase): | 05 January 2021 |
| | Experimental completion date (analytical phase): | 06 January 2021 |

To obtain larvae of approximately the same age, the queen was confined for a maximum of 25 hours on a brood comb containing empty cells for egg laying. After removing the queen from the excluder cage, the comb in the colony for approximately 3 days for incubation after which time it was brought into the laboratory for grafting larvae into the individual rearing cells (test units). The diet was deposited at the bottom of each cell, then the young larvae were grafted into the cells using a grafting tool.

During development, larvae were fed with three diet compositions containing the test item on days 3, 4, 5 and 6 of the larval rearing period. The composition of the aqueous part (D-glucose, D-fructose, yeast extract) and the amount of diet per larva varied according to the larval age. The required amount of diet was prepared and stored at ≤ 5°C during the 6 days of feeding.

Mortality was observed and recorded throughout the study period to day 22 by visual assessment. At the end of the study, adult bees which left their cell and/or showed a normal development into an adult honeybee were counted as successfully emerged.

Statistical analysis of larval mortality at day 8, pupal mortality at day 22 and adult bee emergence at day 22 was conducted in order to determine the NOEC. To determine the NOEC and LOEC ($\alpha = 0.05$), survival data was analysed for statistically significant differences compared to the control group using Step-Down Cochran-Armitage Test followed by the Logit analysis using linear maximum likelihood regression and Weibull analysis using linear maximum likelihood regression. Mortality and emergence results were corrected for control mortality using an adaptation of Abbott's formula. Diet consumption and timing of pupal transfer were not evaluated for statistical significance due to the non-quantitative nature of the observations.

**Results**

Analytical recoveries in the diets are presented below.

**Table A 39: Analytical results**

| **Diet (mg a.i./kg)** | **Analytical Recovery in Diet** | |
|---|---|---|
| | **Mean measured concentration (mg a.i./kg)** | **% of nominal** |
| 68.9 | 63.0 | 91 |
| 27.6 | 24.8 | 90 |
| 11.02 | 10.26 | 93 |
| 4.41 | 3.89 | 88 |

| | | |
|---|---|---|
| 1.76 | 1.86 | 105 |
| 0.00 | n.d. | - |

LOQ: 0.010 mg/kg, corresponding to 0.24 µg/L in diluted extracts

Mortality and emergence data for the test material and reference item are summarised in the table below.

**Table A 40: Summary of mortality and emergence over 22 days**

| Nominal Concentration [mg product/kg diet] | Cumulative dose [µg product/larva] | Larval mortality day 8 | | Total mortality day 22 | | |
|---|---|---|---|---|---|---|
| | | Actual | Corrected | Actual | Corrected | Adult emergence |
| **Control** | | 0.0 | - | 22.2 | 0.0 | 77.8 |
| 2417 | 382 | 38.9* | - | 97.2 | 96.4 | 2.8* |
| 967 | 153 | 2.8 | - | 63.9 | 53.6 | 36.1* |
| 387 | 61 | 0.0 | - | 44.4 | 28.6 | 55.6* |
| 155 | 24 | 0.0 | - | 30.6 | 10.7 | 69.4 |
| 62 | 10 | 0.0 | - | 22.2 | 0.0 | 77.8 |
| **Reference item** | | 91.7 | - | 100.0 | 100.0 | 0.0 |

* Statistically significant difference between treatment and control

No sublethal effects were observed.

Study endpoints are summarised in the table below.

**Table A 41: Study endpoints**

| Treatment | Endpoints | After 22 d |
|---|---|---|
| **Test item doses** [µg product/larva per developmental period] | 22-day $ED_{10}$ [95% CL] | **30** (19 - 47) |
| | 22-day $ED_{20}$ [95% CL] | **52** (37 – 73) |
| | 22-day $ED_{50}$ [95% CL] | **124** (100 – 154) |
| | NOED | **24** |
| **Test item concentrations** [mg product/kg diet][3] | 22-day $EC_{10}$ [95% CL] | **187** (119 - 294) |
| | 22-day $EC_{20}$ [95% CL] | **331** (236 - 464) |
| | 22-day $EC_{50}$[95% CL] | **784** (630 - 976) |
| | NOEC | **155** |

**Validity Criteria**

The test was considered valid;

- Day 8 larval mortality was < 15 % in the control replicates (actual 0%)
- Day 22 adult bee emergence was >70% in the control replicates (actual 77.8%)
- Day 8 mortality in the dimethoate reference item was >50% (actual 91.7%)

**Conclusion**

The toxicity of Oxathiapiprolin/Metalaxyl-M DC (A23109A) to the larvae of the honeybee *Apis mellifera* was determined in a chronic exposure study over a 22-day period.

The 22-day NOEC was determined to be 155 mg product/kg diet and the $LC_{50}$ is 784 mg product/kg diet.

(Schmidt, K., 2021)

| Comments of zRMS: | Study not evaluated by zRMS. |
|---|---|

| | |
|---|---|
| Reference: | KCA1 8.3.1.3 |
| Report | Schmidt K., (2021), Metalaxyl-M – Repeated Exposure of the Honeybee (*Apis mellifera* L.) Larvae under Laboratory Conditions (until Adult Emergence up to Day 22), Report Number 21 48 BLC 0026. BioChem agrar Labor für biologische und chemische Analytik GmbH. (Syngenta File No. VV-930784). |
| Guideline(s): | OECD 239: Honeybee (*Apis mellifera* L.) larval toxicity test, repeated exposure |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication (if vertebrate study) | N/A |

**Executive Summary**

The toxicity of metalaxyl-M to the larvae of the honeybee *Apis mellifera* was determined in a chronic exposure study over a 22-day period.

The 22-day NOEC for adult emergence was determined to be 252.8 mg a.i./kg larval diet and the $EC_{50}$ is 483.1 mg a.i./kg larval diet. The 22-day NOED for adult emergence was 40.0 µg a.i./larva/development period and the $ED_{50}$ was calculated to be 76.4 µg a.i./larva/development period.

**Materials**

**Test Material:** Metalaxyl-M

| | | |
|---|---|---|
| **Lot/Batch #:** | 678767 | |
| **Purity:** | Metalaxyl-M: | 96.9 % w/w |
| **Description:** | Amber liquid | |
| **Stability of test compound:** | Stable under the given conditions | |
| **Recertification date:** | End of April 2023 | |

**Treatments**

| | | |
|---|---|---|
| **Test rates:** | Doses applied (based on non-GLP range-finding test): | |
| | µg a.i./larva: | 100.0, 40.0, 16.0, 6.4, 2.6 |
| | mg a.i./kg diet: | 632.0, 252.8, 101.1, 40.4, 16.2 |
| **Control:** | Untreated diet | |
| **Toxic standard:** | Dimethoate tech., Purity: 98.8 ± 0.5%, 7.6 µg a.i./larva, 48 mg a.i./kg diet | |
| **Analysis of test concentrations:** | The determination of metalaxyl-M in final diet was conducted by an in-house developed method using reversed phase - high performance liquid chromatography with mass-spectrometric (MS/MS) detection. | |

**Test organisms:** Worker honeybee larvae (Hymenoptera, Apoidea)

| | |
|---|---|
| **Species:** | *Apis mellifera* L., *ssp: Buckfast* |
| **Age:** | The larvae were in first instar stage (L1) at grafting. |
| **Source:** | BioChem agrar GmbH |
| **Food:** | artificial diets containing glucose, fructose, yeast extract and water mixed with royal jelly at a weight ratio of 1 : 1 (D3-D6) |

**Test Design**

| | |
|---|---|
| **Test cage description:** | Crystal polystyrene grafting cells (CNE Nicotplast, internal diameter 9 mm) were placed in 48 well plates |
| **Replication:** | 3 replicates of the control, 3 replicates of each test and reference item dose |

| | |
|---|---|
| **No. of larvae/replicate:** | 12 |
| **Environmental test conditions** | |
| **Temperature:** | 34.3 – 35.0°C (deviations ≤ 30 min are not reported) |
| **Humidity:** | D1-D8: 97.8%,<br>D8-D15: 80.2 – 83.4%,<br>D15-D22: 53.1 – 65.7%, deviations ≤ 30 min are not reported |
| **Photoperiod:** | Illumination: constant darkness within the test chamber (diffuse artificial light only during handling and assessments) |
| **Duration of test:** | 22 days |

**Study Design and Methods**

Test facility: BioChem agrar GmbH, Kupferstr. 6, 04827 Machern OT Gerichshain, Germany

| | | |
|---|---|---|
| Experimental dates: | Experimental start date: | 09 August 2021 |
| | Experimental completion date: | 30 August 2021 |
| | Experimental start date (analytical phase): | 23 September 2021 |
| | Experimental completion date (analytical phase): | 14 October 2021 |

To obtain larvae of approximately the same age, the queen was confined for a maximum of 25 hours on a brood comb containing empty cells for egg laying. After removing the queen from the excluder cage, the comb in the colony for approximately 3 days for incubation after which time it was brought into the laboratory for grafting larvae into the individual rearing cells (test units). The diet was deposited at the bottom of each cell, then the young larvae were grafted into the cells using a grafting tool.

During development, larvae were fed with three diet compositions containing the test item on days 3, 4, 5 and 6 of the larval rearing period. The composition of the aqueous part (D-glucose, D-fructose, yeast extract) and the amount of diet per larva varied according to the larval age. The required amount of diet was prepared and stored at ≤ 5°C during the 6 days of feeding.

Mortality was observed and recorded throughout the study period to day 22 by visual assessment. At the end of the study, adult bees which left their cell and/or showed a normal development into an adult honeybee were counted as successfully emerged.

Statistical analysis of larval mortality at day 8, pupal mortality at day 22 and adult bee emergence at day 22 was conducted in order to determine the NOEC. To determine the NOEC and LOEC ($p \leq 0.05$), survival data was analysed for statistically significant differences compared to the control group using Step-Down Cochran-Armitage Test followed by the Trimmed Spearman-Karber procedure. Mortality and emergence results were corrected for control mortality using an adaptation of Abbott's formula. Diet consumption and timing of pupal transfer were not evaluated for statistical significance due to the non-quantitative nature of the observations.

**Results**

Analytical recoveries in the diets are presented below.

**Table A** 42**: Analytical results**

| **Diet (mg metalaxyl-M/kg)** | **Analytical Recovery in Diet** | |
|---|---|---|
| | **Mean measured concentration (mg metalaxyl-M/kg)** | **% of nominal** |
| 632.0 | 682.2 | 108 |
| 252.8 | 281.8 | 111 |
| 101.1 | 110.6 | 109 |
| 40.45 | 42.9 | 106 |
| 16.18 | 17.3 | 107 |

| | | |
|---|---|---|
| 0.00 | - | - |

LOQ: 0.010 mg/kg, corresponding to 0.25 µg/L in diluted extracts. Method limit of detection (LOD): 0.0027 mg/kg

Mortality and emergence data for the test material and reference item are summarised in the table below.

**Table A 43: Summary of mortality and emergence over 22 days**

| Concentration | | Larval mortality day 8 | | Adult emergence day 22 | | |
|---|---|---|---|---|---|---|
| nominal | nominal | Actual | Corrected | Actual | Corrected | Adult emergence |
| [mg a.i./kg diet] | [µg a.i./larva] | | | | | |
| **Control** | | 0.0 | - | 19.4 | 0.0 | 80.6 |
| 100.0 | 632.0 | 33.3* | - | 58.3 | 48.3 | 41.7* |
| 40.0 | 252.8 | 19.4* | - | 25.0 | 6.9 | 75.0 |
| 16.0 | 101.1 | 11.1* | - | 33.3 | 17.2 | 66.7 |
| 6.4 | 40.4 | 8.3 | - | 25.0 | 6.9 | 75.0 |
| 2.6 | 16.2 | 8.3 | - | 16.7 | 0.0 | 83.3 |
| **Reference item** | | 100.0 | - | 100.0 | 100.0 | 0.0 |

Results are averages based on 3 replicates, containing 12 larvae each; abs.: absolute; corr.: corrected mortality (according to SCHNEIDER-ORELLI 1947): test and reference item treated group mortalities were corrected by AC; negative values were set to "0"; * Statistically significant difference between treatment and control (Step-Down Cochran-Armitage Test; α = 0.05; one-sided greater)

No sublethal effects were observed.

Study endpoints are summarised in the table below.

**Table A 44: Study endpoints**

| Treatment | Endpoints | After 22 d |
|---|---|---|
| **Test item doses [µg a.i./larva/development period]** | 22-day $ED_{10}$ | **n.d.** |
| | 22-day $ED_{20}$ | **n.d.** |
| | 22-day $ED_{50}$ [95% CL] [2] | **76.4** (61.3 – 95.3) |
| | NOED [1] | **40.0** |
| **Test item concentrations [mg a.i./kg diet]** | 22-day $EC_{10}$ | **n.d.** |
| | 22-day $EC_{20}$ | **n.d.** |
| | 22-day $EC_{50}$ [95% CL] [2] | **483.1** (387.1 – 602.9) |
| | NOEC [1] | **252.8** |

[1] Step-Down Cochran-Armitage Test; α = 0.05; one-sided greater; n.d.: not determined
[2] Trimmed Spearman-Karber procedure

### Validity Criteria

The test was considered valid;

- Day 8 larval mortality was < 15 % in the control replicates
- Day 22 adult bee emergence was >70% in the control replicates
- Day 8 mortality in the dimethoate reference item was >50%

### Conclusion

The toxicity of Metalaxyl-M to the larvae of the honeybee *Apis mellifera* was determined in a chronic exposure study over a 22-day period.

The 22-day NOEC for adult emergence was determined to be 252.8 mg a.i./kg larval diet and the $EC_{50}$ is 483.1 mg a.i./kg larval diet. The 22-day NOED for adult emergence was 40.0 µg a.i./larva/development period and the $ED_{50}$ was calculated to be 76.4 µg a.i./larva/development period.

(K. Schmidt, 2021)

| Comments of zRMS: | Study not evaluated by zRMS. |
|---|---|

**Reference:** KCA1 8.3.1.3

**Report:** Eckert J. (2016), Metalaxyl-M SL (A13947A) - Honey bee (*Apis mellifera* L.) Larval Toxicity Test (Repeated Exposure), Report Number S15-02457. Eurofins Agroscience Services EcoChem GmbH / Eurofins Agroscience Services Ecotox GmbH Eutinger Str. 24 75223 Niefern-Öschelbronn, Germany (Syngenta File No. VV-415529, A13947A_11455).

**Guidelines:** OECD DRAFT Guidance Document for testing chemicals: Honeybee (*Apis mellifera*) larval toxicity test, repeated exposure (2014)

OECD 237 Guidelines for testing chemicals: Honeybee (*Apis mellifera*) larval toxicity test, single exposure (2013)

**Deviations:** No

**GLP:** Yes

**Acceptability:** Yes

**Duplication (if vertebrate study):** N/A

**Executive Summary**

The purpose of this study was to determine the semi-chronic toxicity of A13947A to honeybee larvae *Apis mellifera* L. in an *in vitro* test after repeated oral application. The 8 day NOEC was determined to be a food concentration of 2250.0 mg product/kg diet. The 8 day $LC_{50}$ was determined to be 43136.4 mg product/kg diet. The 8 day $LD_{50}$ was determined to be 410.4 µg product/larva/development period. The 8 day NOED was determined to be 346.5 µg product/larva/development period.

**Materials**

| | |
|---|---|
| **Test Material** | A13947A<br>Metalaxyl-M SL |
| **Lot/Batch #:** | SMO3L0022 |
| **Actual content of active ingredients:** | 45.7 % w/w; 484 g/L |
| **Description:** | Orange liquid |
| **Stability of test compound:** | Stable under given conditions |
| **Reanalysis/Expiry date:** | 29 Jan 2014 |
| **Density:** | 1.058 g/cm³ |
| **Treatments** | |
| **Test concentrations:** | 562.5, 1125.0, 2250.0, 4500.0 and 9000.0 mg product/kg diet<br>86.6, 173.3, 346.5, 693.0 and 891.0 µg product/larva/development period |
| **Control:** | Untreated artificial diet |

| | |
|---|---|
| **Toxic standard:** | Dimethoate tech. (BAS 152 I), purity 98.8 % |
| **Application method:** | Oral application using a sterile pipette |
| **Test organisms** | |
| **Species:** | Worker honeybee larvae *Apis mellifera* L. subspecies *carnica* P. (Insecta, Hymenoptera, Apoidea) |
| **Age:** | First instar (L1) during grafting |
| **Source:** | Maintained at test facility |
| **Food:** | Artificial diet containing royal jelly, glucose, fructose and the test item |
| **Test Design** | |
| **Test cage description:** | Crystal polystyrene grafting cells (NICOTPLAST) having a diameter of 9 mm. Each cell was placed into a well of a sterile 48-well cellular culture plate, which was previously part-filled with a piece of dental roll |
| **Replication:** | 3 |
| **No. of larvae/replicate:** | 16 |
| **Environmental test conditions** | |
| **Temperature:** | 28.2 to 35.1 °C |
| **Humidity:** | 40.2 - 100.0 % |
| **Photoperiod:** | Constant darkness |
| **Duration of test:** | 8 days |

**Study Design and Methods**

Experimental dates: 19th June to 20th July 2015

The test/reference item was mixed into sterile filtered aqueous sugar solution. Several dilutions were prepared by adding further sugar solution. The royal jelly was added to each stock solution at a ratio of 1 : 1, based on (w/w), to reach the final test concentrations.

Honeybee larvae *Apis mellifera* L. were exposed to repeated oral application of 86.6, 173.3, 346.5, 693.0 and 891.0 µg product/larva/development period (equivalent to 562.5, 1125.0, 2250.0, 4500.0 and 9000.0 mg product/kg diet) in an *in vitro* test. One control group was included in the test. The larvae of the control treatment were fed with untreated artificial diet, which served as a vehicle for the test item and reference item.

The combs containing eggs were left in the excluder cages during the incubation stage until hatching on day 1 (D1). At D1, the combs were transferred to the laboratory using an insulated container in order to avoid temperature variation. In the laboratory three out of six combs were selected for grafting, containing the highest number of synchronized larvae. On D1 the test was initiated with larvae in excess. Therefore four reserve plates were prepared containing larvae of the same replicate hives. Before first application of the test item on day 3 (D3), it was assured that all larvae used were of similar size and alive. Therefore 9 to 14 non-suitable larvae per replicate were replaced across all treatment groups by individuals from the reserve plates, using larvae from the same replicate hive.

Cells were placed in 48 well plates filled up to 1/3 with a piece of dental roll. Each replicate unit consisted of 12 larvae, and there were 3 replicates per treatment and control. Each larva was fed daily between Day 3 and Day 6 using a sterile pipette.

The number of dead larvae was recorded on Day 4, Day 5, Day 6, Day 7 and Day 8. Any large amounts of unconsumed food or substantially undersized larvae were recorded on Day 8. After the last assessment (Day 8) the culture plates with all organisms were placed in a freezer.

For the statistical evaluation the statistics program ToxRat professional, Version 3.1.0 was used.

Fisher's Exact Test with Bonferroni Correction (one-sided greater, $\alpha = 0.05$) was used to evaluate whether there was a significant difference between the mortality data of the test item groups and the control group in order to determine the NOEC (No Observed Effect Concentration) on day 8 (D8). The corresponding NOED (No Observed Effect Dose) was calculated by taking into account the density of the larval diet (1.1 g/cm$^3$) and the cumulative feeding volume per larva (140 µL diet).

Fisher's Exact Test (one-sided greater, $\alpha = 0.05$) was used to evaluate whether there was a significant difference between the mortality data of the reference item group and the control group.

For day 8 (D8), the $LC_{50}$ with 95 % confidence limits was calculated by moving averages computation.

For day 8 (D8), the $LD_{50}$ with 95 % confidence limits was calculated by moving averages computation. The calculation of the $LD_{50}$ is based on the actual applied cumulative test item dose per larva and treatment group.

**Results and Discussion**

Mortality data and other observations for the test material and reference item are summarised in the table below.

**Table A 45: Summary of semi-chronic toxicity of A13947A to honeybee larvae**

| Item applied | Dosage [µg product/larva/ development period] | Concentration [mg product/kg diet] | Cumulative Mortality on Day 8 [%] |
|---|---|---|---|
| Control | - | - | 0.0 |
| A13947A | 86.6 | 562.5 | 2.1 |
| | 173.3 | 1125.0 | 0.0 |
| | 346.5 | 2250.0 | 8.3 |
| | 693.0 | 4500.0 | 93.8* |
| | 891.0 | 9000.0 | 100.0* |
| Reference item | 7.4 | 48.0 | 64.6 |
| Treatment | **Endpoints** | | **Day 8** |
| Test item doses | **NOED** | | 346.5 µg product/larva per development period |
| | **$LD_{50}$ (95 % confidence limits)** | | 410.4 µg product/larva/development period (376.3 – 447.5 µg product/larva/development period) |
| Test item concentrations | **NOEC** | | 2250.0 mg product/kg diet |
| | **$LC_{50}$ (95 % confidence limits)** | | 3136.4 mg product/kg diet (2829.0 – 3477.1 mg product/kg diet) |

* Significantly increased compared to control (Fisher's Exact Test with Bonferroni Correction, one sided greater, $\alpha = 0.05$)

**Analytical Verification**

An analytical method for the determination of metalaxyl-M was validated with regard to recovery, linearity of detector response, repeatability, specificity, limit of quantification, and limit of detection. The analytical method fulfils the requirements of guideline SANCO/3029/99 rev. 4, 11/07/2000.

**Validity Criteria**

Both of the validity criteria were met:

- Control mortality should be ≤ 15 % for larvae across all control replicates at day 8 (actual value 0.0 %)
- Reference item mortality should be ≥ 50 % for larvae across all reference replicates at day 8 (actual value 64.6 %)

**Conclusions**

The 8 day (D8) NOEC for A13947A was determined as 2250.0 mg product/kg diet.

The 8 day (D8) NOED for A13947A was calculated as 346.5 µg product/larva/development period.

The 8 day (D8) $LC_{50}$ for A13947A was determined as 3136.4 mg product/kg diet.

On day 8 (D8) the $LD_{50}$ for A13947A was determined as 410.4 µg product/larva/development period.

(Eckert J., 2016)

| Comments of zRMS: | Study not evaluated by zRMS. |
|---|---|

| | |
|---|---|
| Reference: | KCA2 8.3.1.3 |
| Report: | Oberrauch, S., (2017), Oxathiapiprolin (DPX-QGU42) technical: Honey bee (*Apis mellifera* L.) 22 day larval toxicity test (repeated exposure). Report Number S17-01639. Eurofins Agroscience Services EcoChem GmbH / Eurofins Agroscience Services Ecotox GmbH, Eutinger Str. 24, 75223 Niefern-Öschelbronn, Germany. DAS Study No. DuPont-48606. (Study owner Corteva, Syngenta have access) |
| Guideline(s): | OECD Guideline No. 239 (2016) |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |

**Executive Summary**

Effects of the test item oxathiapiprolin on the honey bee larvae, *Apis mellifera L.*, from repeated feeding exposure in a 22-day *in vitro* test were assessed.

On the first day (D1) of the dose response test synchronised honey bee larvae (*Apis mellifera* carnica Pollmann, first instar, L1) were transferred into 48-well plates where they were fed a standardised amount of artificial diet. On Days 3, 4, 5 and 6 of the test, five different concentrations (7.30, 18.2, 45.6, 114 and 285 mg oxathiapiprolin/kg diet) of the test item and one single concentration of the reference item (48.0 mg dimethoate/kg diet) were applied to the larvae with Diet B and Diet C. A control and solvent control group were included in the test and exposed for the same period of time under identical exposure conditions to the water and acetone treated artificial diet. Assessment of mortality was carried out during the larval phase on Days 4, 5, 6, 7 and 8. The presence of uneaten food was qualitatively recorded on Day 8. Assessment of mortality was carried out during the pupation phase on Days 15 and 22. Assessment of adult emergence was carried out on Day 22.

The No Observed Effect Concentration/Dose (NOEC/NOED) as well as the concentrations and doses causing 10, 20 and 50 % reduction of adult emergence ($EC_{10}$/$ED_{10}$, $EC_{20}$/$ED_{20}$ and the $EC_{50}$/$ED_{50}$) were determined for Day 22, where possible.

The measured concentrations of the test item stock solution and the test item solutions were within 20 % of nominal. The measured concentrations of the test item treated larval diet of the test item groups of 7.30, 18.2 and 45.6 mg oxathiapiprolin/kg diet were between 84 and 130 % of nominal, with mean recoveries across the application days of 104, 104 and 95% of nominal, per test item group. The nominal concentrations of the two highest test item groups of 114 and 285 mg oxathiapiprolin/kg diet were not confirmed. A possible reason for this could be the low water solubility of the test item. Therefore, they were excluded from the statistical evaluation.

On Day 8, the cumulative mortalities in the control and solvent control group were both 0.0 %. The

cumulative mortality in the reference item group was 97.9 %. On day 22 the adult emergence rate was 89.6 % in the control group and 85.4 % in the solvent control group.

There were no statistically significant differences in adult emergence compared to the solvent control group in any test item group that was statistically analysed.

On Day 22, the NOEC relating to adult emergence for oxathiapiprolin was determined as ≥ 45.6 mg oxathiapiprolin/kg diet, equivalent to a NOED of ≥ 7.02 µg oxathiapiprolin/larva per developmental period.

On Day 22, the $EC_{10}$ and the corresponding $ED_{10}$ relating to adult emergence for oxathiapiprolin could not be determined due to the lack of a clear concentration response relationship.

On Day 22, the $EC_{20}$ and $EC_{50}$ relating to adult emergence for oxathiapiprolin could not be determined since there was no reduction in emergence above 20 % in any of the concentrations that were statistically analysed. However, they can be regarded as > 45.6 mg oxathiapiprolin/kg diet, equivalent to an $ED_{20}$ and $ED_{50}$ of > 7.02µg oxathiapiprolin/larva per developmental period.

During the assessments of mortality and adult emergence no test item related other observations such as deviating sizes, appearances and malformations of the test organisms were made. On Day 8, uneaten food was observed in the solvent control group and in the test item groups T1, T4 and T5 with nominal concentrations of 7.30, 114 and 285 mg oxathiapiprolin/kg diet.

The study was considered valid since all validity criteria were met.

### Materials

| | |
|---|---|
| **Test material:** | Oxathiapiprolin technical |
| **Batch/Lot Number:** | QGU42-174 |
| **Purity:** | 95.8% by analysis |
| **Description:** | Solid, chrystalline, off-white |
| **CAS #** | 1003318-67-9 |
| **Stability in solution:** | Stable at normal temperatures and storage conditions |
| **Control:** | Water-treated Diet B and Diet C containing autoclaved, deionized water as solvent |
| **Solvent control:** | Acetone-treated Diet B and Diet C containing acetone as solvent |
| **Test vehicle:** | Test item-treated Diet B and Diet C containing aliquots of the test item solution prepared with acetone |
| **Test organism:** | Honey Bee larvae |
| **Species:** | *Apis mellifera carnica* Pollmann |
| **Age at grafting:** | First instar larvae, L1 |
| **Source:** | Test facility (Eurofins Agroscience Services EcoChem GmbH) |
| **Place of test:** | Eurofins Agroscience Services Ecotox GmbH Neulingen-Göbrichen Field Station, 75245 Neulingen-Göbrichen, Germany<br>All bee hives were located at the field station. |
| **Test chamber:** | Crystal polystyrene grafting cells (NICOTPLAST) having a diameter of 9 mm; each cell was placed into a well of a sterile 48-well cellular culture plate. The open plates were placed into a hermetically sealed desiccator, containing a dish filled with a saturated potassium sulphate ($K_2SO_4$) solution in order to keep a water saturated atmosphere from Day 1 until Day 8. On Day 8, the plates were transferred into a second desiccator containing a saturated sodium chloride (NaCl) solution. The desiccators were placed in an incubator with forced air circulation. On Day 15, each plate was covered by its lid and transferred from the desiccator into an incubator. The incubator contained a dish filled with deionised water in order to keep the adequate relative air humidity. |
| **Environmental conditions (biological phase)** | |
| **Temperature:** | 31.4 – 35.2 °C |
| **Relative Humidity:** | 39.1 – 100 % |
| **Exposure to light:** | None, except during grafting, feeding and assessments |

**Study Design and Methods**

Experimental dates: 30 May 2017 to 14 September 2017

*Experimental treatments*
Two controls, five test item concentrations of 7.30, 18.2, 45.6, 114 and 285 mg oxathiapiprolin/kg diet and one single concentration of the reference item (48.0 mg dimethoate/kg diet) were tested. For each treatment group, 48 larvae from three different hives were tested over 22 days. Each hive equates to one replicate, 16 larvae from each replicate were used. On Days 3, 4, 5 and 6 of the test diet B or diet C containing the test item solutions was applied to the larvae.

*Observations*
Assessments of mortality were carried out during the larval phase on Days 4, 5, 6, 7 and 8 and during the pupation phase on Days 15 and 22. The presence of uneaten food was qualitatively recorded on Day 8. Assessment of adult emergence was carried out on Day 22. Other observations (appearance, size, malformation of organisms) were recorded to aid in the interpretation of mortality in comparison to the solvent control group.

*Statistics*
Multiple Fisher's exact test with Bonferroni-Holm adjustment (one-sided greater, $\alpha = 0.05$) was used to evaluate whether there was a statistically significant difference between the mortality data of the test item groups and the solvent control group for larval mortality on Day 8. Multiple Chi²-test with Bonferroni-Holm adjustment (one-sided greater, $\alpha = 0.05$) was used to evaluate whether there was a statistically significant difference between the mortality data of the test item groups and the solvent control group for larval and pupal mortality on Day 15 and pupal mortality from Day 8 through 22 as well as for adult emergence on Day 22. Since the nominal concentrations of the highest two test item groups of 114 and 285 mg oxathiapiprolin/kg diet were not confirmed by the analytical verification, both test item groups were excluded from statistical analysis.

The NOEC was determined for adult emergence on Day 22. The corresponding NOED was calculated by taking into account the density of the larval diet (1.1g/cm$^3$) and the cumulative feeding volume per larva of 140 µL. The $EC_{10}$ with 95% confidence limits could not be determined due to the lack of a clear concentration response relationship. The $EC_{20}$ and $EC_{50}$ with 95% confidence limits could not be calculated since there was no reduction in emergence above 20 % in any of the concentrations that were statistically analysed, but can be regarded as above the highest concentration tested. The corresponding $ED_{20}$ and $ED_{50}$ values were calculated by taking into account the density of the larval diet (1.1 g/cm$^3$) and the cumulative feeding volume per larva of 140 µL.

**Results and Discussion**

Study results and endpoints are summarised in the table below.

**Table A 46: The effects on adult emergence of honey bees until Day 22 after repeated exposure of treated diet in the laboratory**

| Treatment Group | Concentration | | Cumulative Dose | | Adult Emergence on Day 22 |
|---|---|---|---|---|---|
| | | | | | (%) |
| Control | --- | --- | --- | --- | 89.6 |
| Solvent Control | --- | --- | --- | --- | 85.4 |
| Test Item (oxathiapiprolin) | 7.30 | [mg oxathiapiprolin/ kg diet][b] | 1.12 | [µg oxathiapiprolin/ larva per developmental period][b c] | 87.5 |
| | 18.2 | | 2.80 | | 89.6 |
| | 45.6 | | 7.02 | | 68.8 |
| | 114[a] | | 17.6[a] | | 83.3 |
| | 285[a] | | 43.9[a] | | 66.7 |
| **Endpoints for Adult Emergence on Day 22** | | | | | |

| Treatment Group | Concentration | | Cumulative Dose | | Adult Emergence on Day 22 |
|---|---|---|---|---|---|
| | | | | | (%) |
| **NOEC** | **$EC_{10}$** | | **$EC_{20}$** | | **$EC_{50}$** |
| [mg oxathiapiprolin/kg diet][b] | | | | | |
| ≥ 45.6 | n.d.[d] | | > 45.6[e] | | > 45.6[e] |
| **NOEC** | **$EC_{10}$** | **$EC_{20}$** | **$EC_{50}$** | **NOEC** | **$EC_{10}$** |
| [µg oxathiapiprolin/larva per developmental period][b,c] | | | | | |
| ≥ 7.02 | n.d.[d] | | ≥ 7.02[e] | | ≥ 7.02[e] |

[a] The test item groups of 114 and 285 mg oxathiapiprolin/kg diet (respectively 17.6 and 43.9 µg oxathiapiprolin/larva per developmental period) were excluded from statistical evaluations, since the nominal concentrations were not confirmed by the analytical dose verification

[b] Based on the analysed purity

[c] Based on the total feeding volume of 140 µL and the density of the diet of 1.1 $g/cm^3$

[d] The $EC_{10}/ED_{20}$ values for the adult emergence on Day 22 could not be determined due to the lack of a clear concentration/dose response relationship.

[e] The $EC_{20}/ED_{20}$ and $EC_{50}/ED_{50}$ for the adult emergence on Day 22 could not be determined, since there was no reduction in emergence above 20 % in any of the concentrations that were statistically analysed. Therefore, the $EC_{20}/ED_{20}$ and $EC_{50}/ED_{50}$ values can be regarded as above the highest concentration/dose statistically tested.

**Conclusions**

The measured concentrations of the test item stock solution and the test item solutions were within ± 20 % of nominal. The measured concentrations of the test item treated larval diet of the test item groups of 7.30, 18.2, and 45.6 mg oxathiapiprolin/kg diet were between 84 and 130 % of nominal, with mean recoveries across the application days of 104, 104 and 95 % of nominal, per test item group.

The nominal concentrations of the two highest test item groups of 114 and 285 mg oxathiapiprolin/kg diet were not confirmed. A possible reason for this could be the low water solubility of the test item. Therefore, they were excluded from the statistical evaluation.

On Day 8 the cumulative mortalities in the control and solvent control group were both 0.0 %. The cumulative mortality in the reference item group was 97.9%. On Day 22, the adult emergence rate was 89.6 % in the control group and 85.4 % in the solvent control group. Thus, the validity criteria were met and the study was deemed valid.

On Day 22, the NOEC relating to adult emergence for oxathiapiprolin was determined as ≥ 45.6 mg oxathiapiprolin/kg diet, equivalent to a NOED of ≥ 7.02 µg oxathiapiprolin/larva per developmental period.
On Day 22, the $EC_{10}$ and the corresponding $ED_{10}$ relating to adult emergence for oxathiapiprolin could not be determined due to the lack of a clear concentration response relationship.

On Day 22, the $EC_{20}$ and $EC_{50}$ relating to adult emergence for oxathiapiprolin could not be determined since there was no reduction in emergence above 20 % in any of the concentrations that were statistically analysed. However, they can be regarded as > 45.6 mg oxathiapiprolin/kg diet, equivalent to an $ED_{20}$ and $ED_{50}$ of > 7.02 µg oxathiapiprolin/larva per developmental period.

(Oberrauch S, 2017)

## A 2.3.1.4 KCP 10.3.1.4 Sub-lethal effects

## A 2.3.1.5 KCP 10.3.1.5 Cage and tunnel tests

## A 2.3.1.6 KCP 10.3.1.6 Field tests with honeybees

## A 2.3.2 KCP 10.3.2 Effects on non-target arthropods other than bees

### A 2.3.2.1 KCP 10.3.2.1 Standard laboratory testing for non-target arthropods

| | |
|---|---|
| Comments of zRMS: | The study follows the guideline specified by Blümel *et al.* (2000) and according to the principles of GLP.<br>The study is considered to be valid and suitable for the risk assessment. |

| | |
|---|---|
| Reference: | KCP 10.3.2.1 |
| Report | Fallowfield L. (2020). Oxathiapiprolin/metalaxyl-M DC (A23109A) – A Rate-Response Laboratory Study to Determine the Effects of Fresh Residues on the Predatory Mite *Typhlodromus pyri* (Acari: Phytoseiidae). Report Number SYN-20-71. Mambo-Tox, A Division of Cawood Scientific Ltd, University Science Park, Southampton SO16 7NP, United Kingdom (Syngenta file no VV-887718) |
| Guideline(s): | Blümel *et al*. (2000). Laboratory residual contact test with the predatory mite *Typhlodromus pyri* (Acari: Phytoseiidae) for regulatory testing of plant protection products |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication (if vertebrate study) | N/A |

**Executive Summary**

The effects of A23109A on the predatory mite *Typhlodromus pyri* were assessed in a laboratory test. Mites were exposed to rates equivalent to 2000, 1000, 500, 250 and 125 mL A23109A/ha. The 7-day $LR_{50}$ value was 252.4 mL A23109A/ha with 95% confidence interval values of 135.5 and 384.0 mL A23109A/ha. The NOER value for mite mortality was < 125 mL A23109A/ha, the lowest rate tested. The $ER_{50}$ value was found to lie between 125 and 250 mL A23109A/ha. The NOER value for reproduction was 125 mL A23109A/ha.

**Materials**

| | |
|---|---|
| **Test Material** | Metalaxyl-M/Oxathiapiprolin DC (180/030) |
| **Product Code** | A23109A |
| **Lot/Batch #:** | JHU003-036-001 |
| **Actual content of active ingredient:** | Metalaxyl-M: 17.2% w/w (corresponding to 185 g/L)<br>(incl. its s-enantiomer)<br>CGA351920 (S-enantiomer) = 0.57% w/w corresponding to 6.1 g/L)<br>CGA329351 (R-enantiomer) = 16.6% w/w corresponding to 178 g/L)<br>Oxathiapiprolin: 2.85% w/w (corresponding to 30.6 g/L) |
| **Description:** | Brownish liquid |
| **Stability of test compound:** | Stable under standard conditions |
| **Reanalysis/Expiry date:** | End June 2022 |
| **Density** | 1074 kg/m$^3$ |
| **Treatments** | |
| **Test rates:** | 2000, 1000, 500, 250 and 125 mL test item/ha |
| **Control:** | Purified water |
| **Toxic standard:** | Dimethoate (an EC formulation containing 417.0 g a.s./L), applied at a rate of 15 mL product/ha |

| | |
|---|---|
| **Spray volume rate:** | 200 L/ha |
| **Application method:** | Calibrated laboratory track-sprayer |
| **Test organisms** | |
| **Species:** | *Typhlodromus pyri* |
| **Age:** | < 24-hr-old protonymphs |
| **Source:** | In-house culture, originally obtained in April 1995 from P.K. Nützlingszuchten, Welzheim, Germany and supplemented with further mites from the same source in 1996 and 1997. |
| **Feeding:** | 1:1 v/v almond (Prunus sp. var. a mix of Aldrich, Nonpareil and Wood Colony) and apple (Malus sp. var. Red Delicious) pollen. |
| **Test design** | |
| **Arenas:** | Glass plates formed from two microscope slide cover slips (each 2.2 cm x 4.0 cm in area) joined together with two additional cover slips glued to the top and bottom ends of the main cover slips. 12 cm2 arena created on slides by non-drying sticky insect gel barrier. |
| **Replication:** | 3 per treatment |
| **No. of mites/arena :** | 20 |
| **Duration of test:** | 14 days |
| **Environmental test conditions** | |
| **Temperature:** | 24.0-25.3ºC |
| **Humidity:** | 72-84 % |
| **Photoperiod:** | 16 h (400-1300 lux) |

**Study Design and Methods**

Experimental dates: 12 October 2020 to 26 October 2020
Dilutions were prepared in purified water, shortly before applications were made and the solutions were thoroughly agitated to ensure their homogeneity. Treatments were applied to glass plates which were left to dry and then used to construct the test arenas. Mites were then introduced to the arenas and their survival assessed over a 7-day period, by which time the mites in the control were adult. The sex of the mites was determined and they were then left in situ so that their reproduction could be assessed over a further 7 days. The mean number of eggs produced per female between 7 and 14 days after treatment (DAT) was calculated.

The mean percentage mortality after 7 days was calculated for the individual treatments and corrected for any losses in the control treatment using Abbott's formula. In order to determine the no observed effect-rate (NOER) in terms of mite survival, the percentage mortality in each treatment was compared to the control using step-down Cochran-Armitage test procedure ($\alpha = 0.05$, one sided, > control). The median lethal rate ($LR_{50}$) with respect to mortality was determined using Probit analysis.

In order to determine the NOER for reproduction, the results for eggs per female in each replicate were compared statistically. The data were checked for normality (Shapiro-Wilk) and for homogeneity of variance (Levene's test) prior to analysis by Williams' multiple sequential t-test procedure ($\alpha = 0.05$, one sided, < control). The median effect rate ($ER_{50}$) with respect to reproduction was visually extrapolated from the data.

**Results**

Mortality and fecundity are summarised in the table below.

**Table A 47: Effects of A22422Aon mortality and fecundity of *Typhlodromus pyri*, when exposed in a laboratory test.**

| Treatment | Mean % mortality at 7 DAT [a)] | Mean corrected % mortality at 7 DAT [b)] | Mean eggs/female from 7 to 14 DAT [c)] | % Effect on reproduction compared to control [d)] |
|---|---|---|---|---|
| **Control** | 1.7 | - | 4.97 | - |

| | | | | |
|---|---|---|---|---|
| 2000 mL A23109A/ha | 96.7 * | 96.6 | ~ | - |
| 1000 mL A23109A/ha | 91.7 * | 91.5 | ~ | - |
| 500 mL A23109A/ha | 85.0 * | 84.7 | ~ | - |
| 250 mL A23109A/ha | 55.0 * | 54.2 | 2.07 * | 58.4 |
| 125 mL A23109A/ha | 15.0 * | 13.6 | 5.05 | -1.6 |
| **Toxic reference** | 96.7 * | 96.6 | ~ | - |

a) Individual test item treatments were compared to the control using step-down Cochran-Armitage test procedure and for the toxic reference treatment Fisher's exact binomial test was used ($\alpha = 0.05$, one-sided, > control). Treatments that differed significantly from the control are indicated with an asterisk (*).
b) Calculated using Abbott's formula. A positive value indicates an increase in mortality, relative to the control.
c) Individual treatments were compared to the control by Williams' multiple sequential t-test procedure ($\alpha = 0.05$, one-sided, < control). The treatment rate that differed significantly from the control is indicated with an asterisk (*).
d) A negative value indicates an increase and a positive value indicates a decrease in egg production.
~ Treatment not assessed.

**Validity criteria**

The test was considered valid;

- Mortality in the control treatment was 1.7% (must not be > 20 %.)
- Corrected mortality in the toxic reference treatment was 96.6% (must be over 50 %)
- The mean cumulative number of eggs produced from 7 to 14 days was 4.97 per female in the control (should be equal to or greater than 4.0 per female).

**Conclusion**

The effects of A23109A on the predatory mite *Typhlodromus pyri* were assessed in a laboratory test. Mites were exposed to rates equivalent to 2000, 1000, 500, 250 and 125 mL test item/ha. The 7-day $LR_{50}$ value was 252.4 mL test item/ha, with 95% confidence interval values of 135.5 and 384.0 mL A23109A/ha. Based on statistical comparison with the control, the NOER value with respect to mite survival was < 125 mL A23109A/ha, the lowest rate tested.

The $ER_{50}$ value was found to lie between 125 and 250 mL A23109A/ha. The NOER value with respect to mite reproduction was 125 mL A23109A/ha.

(Fallowfield, L., 2020)

| | |
|---|---|
| Comments of zRMS: | The study follows the guideline specified by Mead Briggs *et al.* and according to the principles of GLP. The study is considered to be valid and suitable for the risk assessment. |

Reference: KCP 10.3.2.1

Report: Stevens, J. (2020). Oxathiapiprolin/Metalaxyl-M DC (A23109A) – A Rate-Response Laboratory Study to Determine the Effects of Fresh Residues on the Parasitic Wasp *Aphidius rhopalosiphi* (Hymenoptera, Braconidae). Report Number SYN-20-72. Mambo-Tox, A Division of Cawood Scientific Ltd., 2 Venture Road, University Science Park, Southampton SO16 7NP, United Kingdom (Syngenta file No. VV-887720)

Guideline(s): Mead-Briggs *et al.* (2000). A laboratory test for evaluating the effects of plant protection products on the parasitic wasp, *Aphidius rhopalosiphi* (DeStephani-Perez) (Hymenoptera, Braconidae)

Deviations: None

| | |
|---|---|
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication (if vertebrate study) | N/A |

**Executive Summary**

The effects of A23109A on the parasitic wasp *Aphidius rhopalosiphi* were assessed in a laboratory test. Wasps were exposed to rates equivalent to 2000, 1000, 500, 250 and 125 mL product/ha. The 48-h $LR_{50}$ value for A23109A was 257.73 mL/ha, with 95% confidence limits of 139.51 and 419.81 mL A23109A/ha. The NOER value with respect to wasp survival was 125 mL test item/ha. In terms of effects on the reproductive performance of surviving wasps, the $ER_{50}$ value was > 125 mL test item/ha and the NOER value was 125 mL test item/ha. The overall NOER value was 125 mL test item/ha.

## Materials

| | |
|---|---|
| **Test Material** | Metalaxyl-M/oxathiapiprolin DC (180/030) |
| **Product Code:** | A23109A |
| **Lot/Batch #:** | 1094822/ JHU003-036-001 |
| **Actual content of active ingredient:** | Metalaxyl-M: 17.2% w/w (corresponding to 185 g/L)<br>(incl. its s-enantiomer)<br>CGA351920 (S-enantiomer) = 0.57% w/w corresponding to 6.1 g/L)<br>CGA329351 (R-enantiomer) = 16.6% w/w corresponding to 178 g/L)<br>Oxathiapiprolin: 2.85% w/w (corresponding to 30.6 g/L) |
| **Density:** | 1.074 kg/$m^3$ |
| **Description:** | Brownish liquid |
| **Stability of test compound:** | Stable under standard conditions |
| **Reanalysis/Expiry date:** | End June 2022 |
| **Treatments** | |
| **Test rates:** | 2000, 1000, 500, 250 and 125 mL product/ha |
| **Control:** | Purified water |
| **Toxic standard:** | Dimethoate (an EC formulation containing nominally 400 g a.s./L), applied at a rate of 0.05 mL product/ha |
| **Spray volume rate:** | 200 L/ha |
| **Application method:** | Calibrated laboratory track-sprayer |
| **Test organisms** | |
| **Species:** | *Aphidius rhopalosiphi* (Hymenoptera, Braconidae) |
| **Age:** | < 48 h |
| **Source:** | In-house culture, originally established using wasps from a commercial supplier (Katz Biotech AG, Baruth, Germany) |
| **Feeding:** | 1:3 v/v solution of honey in water on cotton wool |
| **Test design - Mortality phase** | |
| **Arenas:** | Treated surfaces of glass plates (10 cm x 10 cm) used to form floors and ceilings of shallow test arenas, the walls of which comprised a square frame made from metal casing, with mesh-covered ventilation holes present in the side walls of the casing. |
| **Replication:** | 4 arenas per treatment |
| **No. of wasps/arena :** | 10 ( > 5 ♀) |
| **Test design - Fecundity phase** | |
| **Arenas:** | Pots containing approximately 15 barley seedlings (*Hordeum vulgare* var. Laureate) and previously infested per pot with approximately > 100 adults and nymphs of a mixed cereal aphid culture (*Metopolophium dirhodum* and *Rhopalosiphum padi*), were enclosed within clear acrylic cylinders (9 cm in diameter, 20 cm high), the tops of which were covered with nylon mesh netting. |
| **Replication:** | 15 pots per treatment being assessed |

**No. of wasps/arena :** 1 ♀
**Duration of test:** 13 days

**Environmental test conditions**
**Temperature:** Mortality phase: 20.3-20.8ºC. Fecundity phase: 20.3-20.9ºC
**Humidity:** Mortality phase: 73-76%.
**Photoperiod:** Mortality phase: 16 h (922-944 lux). Fecundity phase: 16 h (1007-1061 lux for aphid parasitisation phase; 4771-4800 lux for pupal wasp development).

**Study Design and Methods**

Experimental dates: 20 October 2020 to 02 November 2020
Dilutions of test item prepared in purified water shortly before use and the solutions were thoroughly agitated to ensure their homogeneity. Treatments were applied to glass plates which were left to dry and then used to construct the test arenas. The wasps were introduced into these arenas and their mortality was assessed after 2, 24 and 48 h.

To assess any sub-lethal effects, reproduction assessments were then carried out for the control and for the only treatment rate of the test item resulting in ≤ 60% corrected mortality with sufficient 'live' wasps available. Female wasps were confined individually over untreated aphid-infested barley plants for 24 h, before being removed. The plants were left for a further 10 days before recording the number of aphid 'mummies' (pupal wasps) that had developed on plants where wasps had been found alive after the 24-h oviposition period.

The percentage mortality of the wasps in the bioassay over 48 h was calculated. Mortality was defined as the numbers of *moribund* and *dead* wasps combined. The corrected percentage mortality (taking into account any control treatment losses) was derived using Abbott's formula. The *median lethal rate* ($LR_{50}$) value for the test item was estimated by Probit regression analysis. Where there was treatment mortality at 48 h, this was also compared to mortality in the control using step-down Cochran-Armitage test procedure (one-sided, > control, $\alpha = 0.05$).

For the data from the reproduction assessments, the data sets from each treatment were checked for normality (Shapiro-Wilk test, $p > 0.01$) and homogeneity of variance (Levene's test, $p > 0.01$), prior to comparison by two-sample t-test procedure (one-sided, < control, $\alpha = 0.05$).

**Results and Discussion**

The results of mortality and reproduction are summarised in the table below.

**Table A 48: Effects of fresh residues of A23109A on mortality and reproduction of *Aphidius rhopalosiphi*, when exposed under laboratory test conditions**

| Treatment | % mortality at 48 h [a] | % corrected mortality at 48 h (M-value) [b] | Number females successfully assessed for reproduction | Mean number mummies per surviving female [c] | % change in reproduction compared to control (R-value) [d] |
|---|---|---|---|---|---|
| **Control** | 0.0 | - | 15 | 63.9 | - |
| **2000 mL A23109A/ha** | 100 * | 100 | ~ | ~ | ~ |
| **1000 mL A23109A/ha** | 95.0 * | 95.0 | ~ | ~ | ~ |
| **500 mL A23109A/ha** | 87.5 * | 87.5 | ~ | ~ | ~ |
| **250 mL A23109A/ha** | 60.0 * | 60.0 | ~ | ~ | ~ |
| **125 mL A23109A/ha** | 5.0 | 5.0 | 12 | 69.3 | -8.4 |
| **Toxic reference** | 97.5 * | 97.5 | ~ | ~ | ~ |

a) The individual test item treatments were compared to the control using step-down Cochran-Armitage test procedure and the toxic reference treatment was compared to the control using Fisher's exact binomial test (one-sided, > control, $\alpha = 0.05$); an asterisk (*) indicates where there were significant differences.

b) Derived using Abbott's formula.

c) The results were compared to the control using two-sample t-test procedure (one-sided, < control, $\alpha = 0.05$), an asterisk (*) indicates where there were significant differences.

d) Percentage change in reproduction, relative to the control. A negative value indicates an increase.

~ Treatment not assessed.

From the mortality data, the 48-h $LR_{50}$ value was 257.73 mL test item/ha, with 95% confidence limits of 139.51 and 419.81 mL test item/ha). Based on the statistical outcome, the NOER value with respect to survival was 125 mL product/ha.

From the fecundity data, the $ER_{50}$ value was > 125 mL test item/ha. Based on the statistical outcome, the NOER value with respect to reproduction was 125 mL test item/ha.

**Validity criteria**

The test was considered valid;

- Mortality within the control treatment at 48 hours was 0.0% (should not exceed 13%, i.e. 5 wasps from 40)
- Corrected mortality within the toxic-reference treatment at 48 hours was 97.5% (should exceed 50%)
- In the reproduction assessment, the mean number of mummies/females in the control treatment was 63.9, with no zero values (should be > 5.0 mummies/female and no more than two zero values)

**Conclusions**

In a laboratory test to determine the effects of fresh residues of A23109A on the parasitic wasp *Aphidius rhopalosiphi*, the 48-h $LR_{50}$ value was 257.73 mL test item/ha, with 95% confidence limits of 139.51 and 419.81 mL test item/ha). Based on statistical comparison with the control, the NOER value for wasp survival was 125 mL test item/ha.

In terms of effects on the reproductive performance of surviving wasps, the $ER_{50}$ value for A23109A was > 125 mL test item/ha and the NOER value for reproduction was 125 mL test item/ha.

The overall NOER value was 125 mL test item/ha.

(Stevens, J., 2020)

## A 2.3.2.2 KCP 10.3.2.2 Extended laboratory testing, aged residue studies with non-target arthropods

| | |
|---|---|
| Comments of zRMS: | The study follows the guideline specified by Blümel *et al.* (2000) and according to the principles of GLP.<br>The study is considered to be valid and suitable for the risk assessment. |

| | |
|---|---|
| Reference: | KCP 10.3.2.2 |
| Report | Fallowfield L. (2021). Oxathiapiprolin/metalaxyl-M DC (A23109A) – A Rate-Response Extended Laboratory Study to Determine the Effects of Fresh Residues on the Predatory Mite *Typhlodromus pyri* (Acari: Phytoseiidae). Report Number SYN-20-77. Mambo-Tox, A Division of Cawood Scientific Ltd, University Science Park, Southampton SO16 7NP, United Kingdom (Syngenta file no VV-895497) |

| | |
|---|---|
| Guideline(s): | Blümel *et al*. (2000). Laboratory residual contact test with the predatory mite *Typhlodromus pyri* (Acari: Phytoseiidae) for regulatory testing of plant protection products |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication (if vertebrate study) | N/A |

**Executive Summary**

The effects of A23109A on the predatory mite *Typhlodromus pyri* were assessed in an extended laboratory test. Mites were exposed to rates equivalent to 2000, 1000, 500, 250 and 125 mL A23109A/ha. The 7-day $LR_{50}$ value was > 2000 mL A23109A/ha, the highest rate tested. The NOER value for mite mortality was 2000 mL A23109A/ha. The $ER_{50}$ value was > 2000 mL A23109A/ha and the NOER value for reproduction was 2000 mL A23109A/ha.

**Materials**

| | |
|---|---|
| **Test Material** | Metalaxyl-M/oxathiapiprolin DC (180/030) |
| **Product Code** | A23109A |
| **Lot/Batch #:** | JHU003-036-001 |
| **Actual content of active ingredient:** | Metalaxyl-M: 17.2% w/w (corresponding to 185 g/L) (incl. its s-enantiomer)<br>CGA351920 (S-enantiomer) = 0.57% w/w corresponding to 6.1 g/L<br>CGA329351 (R-enantiomer) = 16.6% w/w corresponding to 178 g/L<br>Oxathiapiprolin: 2.85% w/w (corresponding to 30.6 g/L) |
| **Description:** | Brownish liquid |
| **Stability of test compound:** | Stable under standard conditions |
| **Reanalysis/Expiry date:** | End June 2022 |
| **Density** | 1074 kg/$m^3$ |
| **Treatments** | |
| **Test rates:** | 2000, 1000, 500, 250 and 125 mL test item/ha |
| **Control:** | Purified water |
| **Toxic standard:** | Dimethoate (an EC formulation containing 417.0 g a.s./L), applied at a rate of 37.5 mL product/ha |
| **Spray volume rate:** | 200 L/ha |
| **Application method:** | Calibrated laboratory track-sprayer |
| **Test organisms** | |
| **Species:** | *Typhlodromus pyri* |
| **Age:** | < 24-hr-old protonymphs |
| **Source:** | In-house culture, originally obtained in April 1995 from P.K. Nützlingszuchten, Welzheim, Germany and supplemented with further mites from the same source in 1996 and 1997. |
| **Feeding:** | 1:1 v/v almond (*Prunus* sp. var. a mix of Aldrich, Nonpareil and Wood Colony) and apple (*Malus* sp. var. Red Delicious) pollen. |
| **Test design** | |
| **Arenas:** | Leaf discs cut from the first true leaves taken from dwarf French bean plants. 12.5 $cm^2$ arena created on leaf discs by a non-drying sticky insect gel barrier. |
| **Replication:** | 3 per treatment |
| **No. of mites/arena :** | 20 |
| **Duration of test:** | 14 days |
| **Environmental test conditions** | |
| **Temperature:** | 24.5-25.1°C |
| **Humidity:** | 63-75% |
| **Photoperiod:** | 16 h (800-1450 lux) |

**Study Design and Methods**

Experimental dates: 26 January 2021 to 09 February 2021
Dilutions were prepared in purified water, shortly before applications were made and the solutions were thoroughly agitated to ensure their homogeneity. Treatments were applied to leaf discs which were left to dry and then used to construct the test arenas. Mites were then introduced to the arenas and their survival assessed over a 7-day period, by which time the mites in the control were adult. The sex of the mites was determined and they were then left in situ so that their reproduction could be assessed over a further 7 days. The mean number of eggs produced per female between 7 and 14 days after treatment (DAT) was calculated.

The mean percentage mortality after 7 days was calculated for the individual treatments and corrected for any losses in the control treatment using Abbott's formula. In order to determine the *no-observed-effect rate* (NOER) in terms of mite survival, the percentage mortality in each treatment was compared to the control firstly by using qualitative trend analysis by contrasts (monotonicity of concentration/response) and then by $chi^2$ 2x2 table test with Bonferroni correction ($\alpha = 0.05$, one sided, > control). The *median lethal rate* ($LR_{50}$) with respect to mortality was visually extrapolated from the data.

In order to determine the NOER for reproduction, the results for eggs per female in each replicate were compared statistically. The data were checked for normality (Shapiro-Wilk) and for homogeneity of variance (Levene's test) prior to analysis by multiple sequentially-rejective t-test after Bonferroni-Holm ($\alpha = 0.05$, one sided, < control). The *median effect rate* ($ER_{50}$) with respect to reproduction was visually extrapolated from the data.

**Results**

Mortality and fecundity are summarised in the table below.

**Table A 49: Effects of A23109A on mortality and fecundity of *Typhlodromus pyri*, when exposed in an extended laboratory test**

| Treatment | Mean % mortality at 7 DAT [a)] | Mean corrected % mortality at 7 DAT [b)] | Mean eggs/female from 7 to 14 DAT [c)] | % Effect on reproduction compared to control [d)] |
|---|---|---|---|---|
| **Control** | 13.3 | - | 7.7 | - |
| 2000 mL A23109A/ha | 13.3 | 0.0 | 7.8 | -1.9 |
| 1000 mL A23109A/ha | 23.3 | 11.5 | 6.6 | 14.5 |
| 500 mL A23109A/ha | 13.3 | 0.0 | 7.1 | 8.4 |
| 250 mL A23109A/ha | 23.3 | 11.5 | ~ | - |
| 125 mL A23109A/ha | 15.0 | 1.9 | ~ | - |
| **Toxic reference** | 93.3 * | 92.3 | ~ | - |

a) Individual test item treatments were compared to the control using $chi^2$ 2x2 table test with Bonferroni correction and for the toxic reference treatment Fisher's exact binomial test ($\alpha = 0.05$, one-sided, > control). Treatments that differed significantly from the control are indicated with an asterisk (*)

b) Calculated using Abbott's formula. A positive value indicates an increase in mortality, relative to the control

c) Individual treatments were compared to the control by multiple sequentially-rejective t-test after Bonferroni-Holm ($\alpha = 0.05$, one-sided, < control). No treatment rate differed significantly from the control

d) A positive value indicates a decrease and a negative value indicates an increase in egg production

~ Treatment not assessed

**Validity criteria**

The test was considered valid;

- Mortality in the control treatment was 13.3% (must not be > 20 %.)

- Corrected mortality in the toxic reference treatment was 92.3% (must be over 50%)
- The mean cumulative number of eggs produced from 7 to 14 days was 7.7 per female in the control (should be equal to or greater than 4.0 per female)

**Conclusion**

In an extended laboratory test to determine the effects of fresh residues of A23109A on the predatory mite *Typhlodromus pyri*, the 7 day $LR_{50}$ value was > 2000 mL test item/ha, the highest rate tested. Based on statistical comparison with the control, the NOER value with respect to mite survival was 2000 mL A23109A/ha.

The $ER_{50}$ value was > 2000 mL A23109A/ha. The NOER value with respect to mite reproduction was 2000 mL A23109A/ha.

(Fallowfield, L., 2021)

| | |
|---|---|
| Comments of zRMS: | The study follows the guideline specified by Mead Briggs *et al.* and according to the principles of GLP. The study is considered to be valid and suitable for the risk assessment. |

| | |
|---|---|
| Reference: | KCP 10.3.2.2 |
| Report | Stevens, J, (2021), Oxathiapiprolin/metalaxyl-M DC (A23109A) – A Rate-Response Extended Laboratory Study of the Effects of Fresh Residues on the Parasitic Wasp *Aphidius rhopalosiphi* (Hymenoptera, Braconidae). Report Number SYN-20-78. Mambo-Tox, A Division of Cawood Scientific Ltd., 2 Venture Road, University Science Park, Southampton, SO16 7NP (Syngenta file no VV-895493) |
| Guideline(s): | Mead-Briggs *et al.* (2009). An extended laboratory test for evaluating the effects of plant protection products on the parasitic wasp, *Aphidius rhopalosiphi* |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication (if vertebrate study) | N/A |

**Executive Summary**

The effects of A23109A, on the parasitic wasp *Aphidius rhopalosiphi* were assessed in an extended laboratory test. Wasps were exposed to rates equivalent to 2000, 1000, 500, 250 and 125 mL A23109A/ha. The 48-h $LR_{50}$ value was > 2000 mL A23109A/ha, the highest rate tested. The $ER_{50}$ value for reproduction was > 2000 mL A23109A/ha. The overall NOER was 2000 mL A23109A/ha.

**Materials**

| | |
|---|---|
| **Test Material** | Metalaxyl-M/oxathiapiprolin DC (180/030) |
| **Product Code** | A23109A |
| **Lot/Batch #:** | JHU003-036-001 |
| **Actual content of active ingredients:** | Metalaxyl-M: 17.2% w/w (corresponding to 185 g/L) (incl. its s-enantiomer)<br>CGA351920 (S-enantiomer) = 0.57% w/w corresponding to 6.1 g/L<br>CGA329351 (R-enantiomer) = 16.6% w/w corresponding to 178 g/L |

| | |
|---|---|
| | Oxathiapiprolin: 2.85% w/w (corresponding to 30.6 g/L) |
| **Description:** | Brownish liquid |
| **Stability of test compound:** | Stable under standard conditions |
| **Reanalysis/Expiry date:** | End June 2022 |
| **Density:** | 1074 kg/$m^3$ |
| **Treatments** | |
| **Test rates:** | 2000, 1000, 500, 250 and 125 mL test item/ha |
| **Control:** | Purified water |
| **Toxic standard:** | Dimethoate (an EC formulation containing nominally 400 g a.s./L), applied at a rate of 10 mL product/ha |
| **Spray volume rate:** | 400 L/ha |
| **Application method:** | Calibrated laboratory track-sprayer |
| **Test organisms** | |
| **Species:** | *Aphidius rhopalosiphi* |
| **Age:** | < 48 h |
| **Source:** | In-house culture, originally established using wasps from a commercial supplier (Katz Biotech AG, Baruth, Germany) |
| **Feeding:** | 10% w/v fructose solution sprayed onto test plants |
| **Test design - Mortality phase** | |
| **Arenas:** | Pots of approximately 10 treated barley (*Hordeum vulgare*) seedlings, approximately 10 cm tall, enclosed within clear acrylic cylinders (8 cm in diameter, 20 cm high), the tops of which were covered with nylon mesh netting |
| **Replication:** | 6 per treatment rate |
| **No. of wasps/arena:** | 5 ♀ |
| **Test design - Fecundity phase** | |
| **Arenas:** | Pots containing approximately 15 untreated barley seedlings. Infested with > 100 adults and nymphs of a mixed cereal aphid culture containing *M. dirhodum* and *R. padi*) three days previously. The wasps were confined over the pots of plants using clear acrylic cylinders (9 cm in diameter, 20 cm high), the tops of which were covered with nylon mesh netting. |
| **Replication:** | 15 per treatment analysed |
| **No. of wasps/arena:** | 1 ♀ |
| **Duration of test:** | 13 days |
| **Environmental test conditions** | |
| **Temperature:** | Mortality phase: 19.8-21.2ºC. Fecundity phase: 19.8-21.0ºC |
| **Humidity:** | Mortality phase: 66-79%. |
| **Photoperiod:** | Mortality phase: 16 h (approx. 1099-1122 lux). Fecundity phase: 16 h (approx. 4591-4880 lux). |

## Study Design and Methods

Experimental dates: 16 February 2021 – 01 March 2021

Dilutions of the test item were prepared in purified water shortly before use and the solutions were thoroughly agitated to ensure their homogeneity. Treatments were sprayed onto separate batches of pots of seedling barley (*Hordeum vulgare*) using a laboratory track-sprayer. Once dry, the barley plants in each pot were enclosed within cylindrical, ventilated collars. Five wasps were confined in each arena, with six replicates (i.e. a total of 30 wasps) prepared for each treatment. The behaviour of the wasps was assessed during the first 3 h after treatment and also at 24 and 48 h, to determine whether there was any apparent repellence from the treated plants, and wasp survival was assessed over a period of 48 h. To assess sub-lethal effects on reproduction, assessments were then carried out for the control and for the treatment rates of 2000, 1000 and 500 mL A23109A/ha. Female wasps were confined individually over untreated aphid-infested barley plants for 24 h, before being removed. The plants were left for a further 10 days before the number of aphid mummies that had developed on plants where wasps had been found alive after the 24-h oviposition period was recorded.

The percentage mortality of the insects in the bioassay over the initial 48 h was calculated. Mortality was

defined as the numbers of *moribund* and *dead* insects combined. The corrected percentage mortality (taking into account any control treatment losses) was derived using Abbott's formula. Regression analysis of the results proved to be unsuitable. Where there was test item treatment mortality at 48 h, this was first compared to that in the control using qualitative trend analysis by contrasts (monotonicity of rate/response), followed by a chi² 2x2 table test with Bonferroni correction (one-sided, > control, $\alpha = 0.05$); the toxic reference treatment was compared by Fisher's exact binomial test (one-sided, > control, $\alpha = 0.05$). As a measurement of repellence of insects from the treated foliage during the initial 3 h of the bioassay, the percentages of wasps settled on the plants in each replicate was calculated for each of the five assessment occasions. A mean value was then obtained for each replicate. These values were angularly transformed (square root arcsine). The test item and control treatment data were checked for normality (Shapiro-Wilk test, $p > 0.01$) and homogeneity of variance (Levene's test, $p > 0.01$). As equality of variance could not be assumed, the individual test item treatments were compared to the control using multiple sequentially-rejective Welch's t-test after Bonferroni-Holm (one-sided, < control, $\alpha = 0.05$). The toxic reference treatment was compared to the control using Student's t-test for homogenous variances (one-sided, < control, $\alpha = 0.05$). For the percentages of wasps settled on the plants at 24 h and 48 h after treatment, a mean value was obtained for each replicate across the two assessment occasions. These values were angularly transformed (square root arcsine). The data for the control and test item treatment were checked for normality (Shapiro-Wilk test, $p > 0.01$) and for equality of variance (Levene's test, $p > 0.01$) and for trend analysis by contrasts (monotonicity of concentration/response), before being compared by Williams' multiple sequential t-test procedure (one-sided, < control, $\alpha = 0.05$).

For the reproduction assessments, the data sets from each treatment were checked for normality (Shapiro-Wilk test, $p > 0.01$) and homogeneity of variance (Levene's test, $p > 0.01$) and for trend analysis by contrasts (monotonicity of concentration/response), before being compared by Dunnett's multiple t-test procedure (one-sided, < control, $\alpha = 0.05$).

**Results and Discussion**

Mortality, wasp behaviour and reproduction are summarised in the table below.

**Table A 50: Effects of fresh residues of A23109A on mortality, behaviour and reproduction of *Aphidius rhopalosiphi*, when exposed under extended laboratory test conditions**

| Treatment | Mean % mortality at 48 h [a] | Mean % corrected mortality at 48 h (M-value) [b] | % wasps settled on treated plants [c] | | Mean number mummies per surviving female [d] | % change in reproduction compared to control (R-value) [e] |
|---|---|---|---|---|---|---|
| | | | Initial 3 h | 24 & 48 h | | |
| **Control** | 3.3 | - | 42.7 | 44.0 | 69.8 | - |
| **2000 mL A23109A/ha** | 6.7 | 3.4 | 32.0 | 28.6 | 70.7 | -1.3 |
| **1000 mL A23109A/ha** | 3.3 | 0.0 | 26.0 | 29.3 | 71.6 | -2.6 |
| **500 mL A23109A/ha** | 6.7 | 3.4 | 30.7 | 36.8 | 71.9 | -3.0 |
| **250 mL A23109A/ha** | 0.0 | -3.4 | 38.7 | 45.0 | - | - |
| **125 mL A23109A/ha** | 0.0 | -3.4 | 42.0 | 36.7 | - | - |
| **Toxic reference** | 76.7 * | 75.9 | 27.3 * | - | - | - |

a) The results for individual test item treatments were compared to the control using chi² 2x2 table test with Bonferroni correction, and the toxic reference treatment was compared to the control using Fisher's exact binomial test (one-sided, > control, $\alpha = 0.05$). Treatment rates that differed significantly are marked with an asterisk (*).

b) Derived using Abbott's formula.

c) For the initial 3 h assessments, the test item treatments were compared to the control using multiple sequentially-rejective Welch's t-test after Bonferroni-Holm (one-sided, < control, $\alpha = 0.05$). The toxic reference treatment was compared to the control using Student's t-test for homogenous variances (one-sided, < control, $\alpha = 0.05$). For the combined 24 and 48 h

assessments, the test item treatments were compared to the control using Williams' multiple sequential t-test procedure (one-sided, < control, α = 0.05). Treatments that differed significantly are indicated with an asterisk (*).

d) The results were compared using Dunnett's multiple t-test (one-sided, < control, α = 0.05). No treatments differed significantly.

e) A negative value indicates an increase.

For the mortality data, the $LR_{50}$ value was > 2000 mL A23109A/ha. Based on the statistical outcome, the NOER value with respect to survival was 2000 mL A23109A/ha.

For the fecundity data, the $ER_{50}$ value was > 2000 mL A23109A/ha. Based on the statistical outcome, the NOER value with respect to reproduction was 2000 mL A23109A/ha.

**Validity criteria**

The test was considered valid;

- Mortality within the control treatment at 48 hours was 3.3% (should not exceed 10%, i.e. 3 wasps from 30)
- Corrected mortality within the toxic-reference treatment at 2 hours was 0.0% (should be less than 25%) and at 48 hours was 75.9% (should be 50-100%)
- The mean number of mummies in the control treatment per surviving female was 69.8 (must be > 5.0 per female)
- In the reproduction assessment, there should be no more than two zero values in the control treatment. There were no zero values
- Although not a criterion stated in the guideline, for the purpose of this study a validity criterion was set such that there should be a minimum of 30% of observations of wasps settled on the treated plants in the control treatment during the initial 3 h of the behavioural assessments. Control settling was 42.7% during the initial 3 h

**Conclusions**

In an extended laboratory test to determine the effects of A23109A on the parasitic wasp *Aphidius rhopalosiphi*, the 48-h $LR_{50}$ value was > 2000 mL A23109A/ha, the highest rate tested. Based on statistical comparison with the control, the NOER value with respect to wasp survival was 2000 mL A23109A/ha.

In assessments of the reproductive performance of surviving wasps, the $ER_{50}$ value was > 2000 mL A23109A/ha. Based on statistical comparison with the control, the NOER value for reproduction was 2000 mL A23109A/ha.

The overall NOER value for survival and reproduction was, therefore, 2000 mL A23109A/ha.

(Stevens, J., 2021)

| | |
|---|---|
| Comments of zRMS: | The study follows the guideline specified by Grimm *et al.* and according to the principles of GLP. The study is considered to be valid and suitable for the risk assessment. |

Reference: KCP 10.3.2.2

Report: Tew, G, (2021). Oxathiapiprolin/metalaxyl-M DC (A23109A) – A Rate-Response Extended Laboratory Study of the Effects of Freshly Treated Substrate on the Rove Beetle, *Aleochara bilineata* (Coleoptera, Staphylinidae). Report Number SYN-20-79. Mambo-Tox, A Division of Cawood Scientific Ltd., 2 Venture Road, University Science Park, Southampton SO16 7NP, United Kingdom. (Syngenta File No. VV-905353)

| | |
|---|---|
| Guideline(s): | Grimm *et al.* A test for evaluating the chronic effects of plant protection products on the rove beetle *Aleochara bilineata* Gyll. under laboratory and extended laboratory conditions. (2000) |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication (if vertebrate study) | N/A |

**Executive Summary**

The effects of A23109A on adults of the parasitic beetle *Aleochara bilineata* and their larval offspring were assessed in an extended laboratory test. Adult beetles were exposed to soil treated with concentrations of A23109A equivalent to 2000, 1000, 500, 250 and 125 mL/ha. The median effect rate ($ER_{50}$) value was > 2000 mL A23109A/ha and NOER value for reproduction was 2000 mL A23109A/ha.

**Materials**

| | |
|---|---|
| **Test Material** | Metalaxyl-M/Oxathiapiprolin DC |
| **Lot/Batch #:** | JHU003-036-001 |
| **Actual content of active ingredients:** | Metalaxyl-M: 17.2 % w/w corresponding to 185 g/L<br>CGA351920 (S-enantiomer): 0.57% w/w corresponding to 6.1 g/L<br>CGA329351 (R-enantiomer): 16.6 % w/w corresponding to 178 g/L<br>Oxathiapiprolin: 2.85 % w/w corresponding to 30.6 g/L |
| **Description:** | Brownish liquid |
| **Stability of test compound:** | Stable under standard conditions |
| **Reanalysis/Expiry date:** | End June 2022 |
| **Density:** | 1074 kg/m3 |
| **Treatments** | |
| **Test rates:** | 2000, 1000, 500, 250 and 125 mL A23109A/ha |
| **Control:** | Purified water |
| **Toxic standard:** | Dimethoate, EC formulation of nominally 400 g a.s./L, applied at 3.8 L product/ha |
| **Application method:** | Laboratory track-sprayer |
| **Test organisms** | |
| **Species:** | *Aleochara bilineata* |
| **Age:** | Adult beetles ≈ 3 days old at the start of the test (physiologically) |
| **Source:** | Commercial supplier (De Groene Vlieg, Nieuwe Tonge, the Netherlands) |
| **Food:** | raw minced beef |
| **Host pupae for larvae to parasitize:** | Delia antiqua Meigen. (Diptera, Anthomyiidae), |
| **Test design - Mortality phase** | |
| **Arenas:** | Clear polystyrene boxes filled to a depth of 4 cm with loamy sand (LUFA 2.1). Beetles placed in the boxes once treatments have been applied. Onion fly pupae (500 per replicate) placed in the soil at 7, 14 and 21 days after treatment (DAT). |
| **Substrate:** | Lufa 2.1 soil. |
| **Replication:** | 4 arenas per treatment, |
| **No. of beetles/arena:** | 10 male and 10 female beetles |
| **Test design – Fecundity phase** | |
| **Arenas:** | Plastic pots, 9 cm in diameter and 5 cm deep. The lid and base of each pot had a central area removed and covered with nylon netting (ca. 0.5 mm by 0.5 mm mesh size for lid, ca. 2 mm by 3 mm mesh size for base). The netting at the base acted as a sieve, so that when the adult beetles emerged, they fell through into collecting chambers below (i.e. pots 9 cm diameter by 9 cm deep). |
| **Duration of test:** | 81 days. |

**Environmental test conditions**

| | |
|---|---|
| **Temperature:** | 19.1-20.3°C |
| **Humidity:** | 75-84% RH. |
| **Photoperiod:** | 16 hours (800-900 lux) |

### Study Design and Methods

Test facility: Mambo-Tox, A Division of Cawood Scientific Ltd.
Experimental dates: 25 January 2021 – 19 April 2021
Treatments were applied to the test arenas and the adult beetles were introduced. At days 7, 14 and 21 during exposure, 500 *D. antiqua* pupae were carefully buried in the soil (depth *ca.* 2-3 cm) and homogeneously distributed within the test unit and completely covered with the substrate. At 28 DAT days all surviving adult beetles were removed from the substrate and the number was recorded. The substrate and the parasitized onion fly pupae were returned to the climatic room in the original test units with gentle aeration. 28 days after application the pupae were sieved out of the soil and the pupae of each replicate were transferred to a separate emergence container. Emerging beetles were counted and removed from the emergence containers at least 3 times per week; emergence of the F1-generation was monitored until the control treatment fell below a rate of two beetles per replicate per day.
All values presented throughout this report were calculated using the original raw data and were not based on rounded values, as presented in summary tables. Statistical analyses were performed using validated computer software (ToxRat Solutions GmbH, 2018).

The aim of the study was to determine whether the individual test-item treatments led to a reduction in the parasitic success of the treated beetles, relative to the water control. The mean number of offspring produced per replicate and a measure of the standard deviation were calculated for each treatment. The percentage of the fly pupae provided (nominally 6000 per treatment) that gave rise to F1 beetles was determined for each treatment.

Individual test-item treatments were compared to the control by Dunnett's multiple t-test procedure (one sided, < control, $\alpha = 0.05$). The toxic reference was compared to the control by Student's t-test for homogeneous variances (one-sided, < control, $\alpha = 0.05$). An asterisk (*) indicates where there was a statistically significant reduction in numbers of progeny.

### Results

Mortality and reproduction are summarised in the table below.

**Table A 51: Effects of A23109A on reproduction of *Aleochara bilineata***

| Rate (mL product/ha) | % mortality | corrected % mortality [a)] | Mean number of $F_1$ progeny [b)] | % effect on reproduction [c)] |
|---|---|---|---|---|
| **Control** | 10.0 | - | 590.3 | - |
| 2000 | 16.3 | 6.9 | 552.0 | 6.5 |
| 1000 | 16.3 | 6.9 | 552.0 | 6.5 |
| 500 | 16.3 | 6.9 | 551.8 | 6.5 |
| 250 | 17.5 | 8.3 | 580.5 | 1.7 |
| 125 | 17.5 | 8.3 | 520.8 | 11.8 |
| **Toxic ref** | 100 | 100 | 0.3* | 100.0 |

a) Values corrected using Abbott's formula. Negative values indicate a decrease and positive values an increase in mortality with respect to the control
b) Individual test-item treatments were compared to the control by Dunnett's multiple t-test procedure (one sided, < control, $\alpha = 0.05$). The toxic reference was compared to the control by Student's t-test for homogeneous variances (one-sided, < control, $\alpha = 0.05$). An asterisk (*) indicates where there was a statistically significant reduction in numbers of progeny
c) The percentage change in numbers of F1 progeny, relative to the control. Positive values indicate a decrease in numbers, relative to the control

### Validity criteria

The validity criteria are listed below

- The average number of hatched beetles per replicate of the F1-generation in the control was 590.3 (must be > 400)
- The reduction of the reproductive capacity in the reference item treatment relative to control was 100.0% (must be ≥ 50 %)

All of the validity criteria were met.

**Conclusion**

The effects of A23109A on adults of the parasitic beetle *Aleochara bilineata* and their larval offspring were assessed in an extended laboratory test. For reproduction, the $ER_{50}$ value was > 2000 mL A23109A/ha and the NOER value was 2000 mL A23109A/ha.

(Tew, G., 2021)

| | |
|---|---|
| Comments of zRMS: | The study follows the guideline specified by Vogt *et al.* and according to the principles of GLP. The study is considered to be valid and suitable for the risk assessment. |

| | |
|---|---|
| Reference: | KCP 10.3.2.2 |
| Report | Vaughan R. (2021). Oxathiapiprolin/Metalaxyl-M DC (A23109A) – A Rate-Response Extended Laboratory Study to Evaluate the Effects of Fresh Residues on the Green Lacewing, *Chrysoperla carnea* (Neuroptera, Chrysopidae). Report Number SYN-20-80. Mambo-Tox, A Division of Cawood Scientific Ltd, University Science Park, Southampton SO16 7NP, United Kingdom. (Syngenta File No. VV-902193) |
| Guideline(s): | Vogt, H. *et al*. (2000). Laboratory method to test effects of plant protection products on larvae of *Chrysoperla carnea* (Neuroptera: Chrysopidae) |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication (if vertebrate study) | N/A |

**Executive Summary**

The effects of A23109A on the green lacewing *Chrysoperla carnea* were assessed in an extended laboratory test. The $LR_{50}$ was estimated to be > 2000 mL product/ha. The NOER with respect to lacewing survival was 2000 mL product/ha. With respect to lacewing reproduction, the $ER_{50}$ was estimated to be > 2000 mL product/ha and the NOER was 2000 mL product/ha.

**Materials**

| | |
|---|---|
| **Test Material** | Metalaxyl-M/oxathiapiprolin DC (180/030) |
| **Lot/Batch #:** | JHU003-036-001/1094822 |
| **Actual content of active ingredients:** | Metalaxyl-M 17.2% w/w (185 g/L)<br>CGA351920 (S-enantiomer) 0.57% w/w (6.1 g/L)<br>CGA329351 (R-enantiomer) 16.6% w/w (178 g/L)<br>Oxathiapiprolin 2.85% w/w (30.6 g/L) |
| **Description:** | Brownish liquid |

| | |
|---|---|
| **Stability of test compound:** | Stable under standard conditions |
| **Reanalysis/Expiry date:** | End of June 2022 |
| **Density:** | 1074 kg/$m^3$ |
| **Treatments** | |
| **Test rates:** | 2000, 1000, 500, 250 and 125 mL test item/ha |
| **Control:** | Purified water |
| **Toxic standard:** | Dimethoate (an EC formulation containing nominally 400 g a.s./L), applied at a rate of 40 mL product/ha |
| **Spray volume rate:** | 200 L/ha |
| **Application method:** | Calibrated laboratory track-sprayer |
| **Test organisms** | |
| **Species:** | *Chrysoperla carnea* (Neuroptera, Chrysopidae) |
| **Age:** | 2-3 days at start of test |
| **Source:** | Lacewing eggs obtained from culture maintained at Test Facility |
| **Feeding:** | Larvae: eggs of *Sitotroga cerealella.*<br>Adults: artificial diet, honey water and purified water. |
| **Test design - Exposure phase** | |
| **Arenas:** | Excised dwarf French bean leaf sandwiched between 7.5 cm x 7.5 cm glass plate and Perspex sheet, with 5-cm-diameter plastic collar treated with Fluon to confine larva. Ventilated lid placed on top. |
| **Replication:** | 40 per treatment rate |
| **No. of larvae/arena:** | 1 |
| **Test design – Reproductive phase** | |
| **Arenas:** | Clear polystyrene box (15 cm x 27 cm x 10 cm) with close fitting lid. Fibrous tissue placed under each lid as oviposition site. |
| **Replication:** | 1 or 2 boxes per treatment analysed (not considered as replication for statistical purposes). |
| **Duration of test:** | 37 days |
| **Environmental test conditions** | |
| **Temperature:** | 23.4-25.3ºC |
| **Humidity:** | 60-78% RH |
| **Photoperiod:** | 2100-3900 lux |

### Study Design and Methods

Test facility: Mambo-Tox, A Division of Cawood Scientific Ltd.
Experimental dates: 12 February 2021 to 26 March 2021
Dilutions of the test item were prepared shortly before treatments were applied, and the solutions were thoroughly agitated to ensure their homogeneity. Treatments were sprayed onto the target leaves (first true leaves of *Phaseolus vulgaris*) using a laboratory track-sprayer. Once dry, the leaves were used to line the floor of test arenas. A single larva was confined in each arena, with 40 replicates prepared for each treatment. Assessments of treatment effects were made every 1-3 days until the larvae pupated. The number of successfully emerging adult lacewings was then recorded. To assess sub-lethal effects on reproduction, assessments were then carried out for the control and for the test item treatment rates of 2000, 1000 and 500 mL/ha. The sex of the adult lacewings was determined, and they were transferred to oviposition boxes. Eggs were sampled and counted over two 24-h periods. The eggs were then monitored in order to assess the number that successfully hatched.

The percentage pre-imaginal mortality of the insects in the bioassay was calculated. Pre-imaginal mortality was defined as the numbers of insects that did not successfully reach adulthood. The corrected percentage mortality was derived using Abbott's formula. The $LR_{50}$ was estimated by extrapolation from the data, since corrected mortality did not exceed 50% in any of the test item treatments. Where there was treatment mortality, this was also compared to mortality in the control using the $chi^2$ 2x2 table test with Bonferroni correction (one-sided, > control, $\alpha = 0.05$). For the reproduction assessments, effects on lacewing reproduction in the individual test item treatments are normally assessed on the basis of 'triggers', as specified in the guideline of Vogt *et al.* (2000). Namely, if treatment effects are to be deemed harmless, there should be a mean of ≥ 15 eggs produced per female per day (n = 2) and the mean egg-hatching rate should be ≥ 70%.

**Results**

Mortality and fecundity are summarised in the tables below.

**Table A 52: Effect of A23109A on mortality, hatching and pupation of *Chrysoperla carnea***

| Treatment (mL product/ha) | % mortality [a] | % corrected mortality (M-value) [b] | Mean number eggs/female/day [c] | Mean % egg viability [d] | Mean viable eggs/female/ day |
|---|---|---|---|---|---|
| **Control** | 15.4 | - | 30.4 | 89.9 | 27.3 |
| **2000** | 12.5 | -3.4 | 35.9 | 91.5 | 32.8 |
| **1000** | 22.5 | 8.4 | 37.1 | 90.4 | 33.5 |
| **500** | 20.0 | 5.5 | 34.3 | 91.3 | 31.3 |
| **250** | 20.0 | 5.5 | - | - | - |
| **125** | 17.5 | 2.5 | - | - | - |
| **Toxic reference** | 85.0 * | 82.3 | - | - | - |

a) The results for individual treatments were compared to the control using the $chi^2$ 2x2 table test with Bonferroni correction (one-sided, > control, $\alpha = 0.05$). The result for the toxic reference treatment was compared to the control using Fisher's exact binomial test (one-sided, > control, $\alpha = 0.05$). Significant differences are indicated by an asterisk (*).

b) Derived using Abbott's formula.

c) Based on two 24-h long assessments made for each oviposition box in each treatment.

d) Based on all eggs laid on the fibrous tissue sheet lining the lid of each oviposition box.

- Treatment not assessed.

**Validity Criteria**

The test was considered valid;

- Pre-imaginal mortality within the control treatment was 15.4% (should not exceed 20%, *i.e*. 8 lacewings from 40)
- Corrected pre-imaginal mortality within the toxic-reference treatment was 82.3% (should exceed 50%)
- Mean egg production per female per day in the control treatment was 30.4 (must be ≥ 15.0 per female per day)
- Mean egg viability in the control treatment was 89.9 (must be ≥ 70%)

**Conclusions**

The effects of A23109A on the green lacewing *Chrysoperla carnea* were assessed in an extended laboratory test. The $LR_{50}$ was estimated to be > 2000 mL product/ha. The NOER with respect to lacewing survival was 2000 mL product/ha. With respect to lacewing reproduction, the $ER_{50}$ was estimated to be > 2000 mL product/ha and the NOER was 2000 mL product/ha.

(Vaughan, R., 2021)

## A 2.3.2.3 KCP 10.3.2.3 Semi-field studies with non-target arthropods

## A 2.3.2.4 KCP 10.3.2.4 Field studies with non-target arthropods

## A 2.3.2.5 KCP 10.3.2.5 Other routes of exposure for non-target arthropods

## A 2.4 KCP 10.4 Effects on non-target soil meso- and macrofauna

## A 2.4.1 KCP 10.4.1 Earthworms

| Comments of zRMS: | Study not evaluated by zRMS. |
|---|---|

| | |
|---|---|
| Reference: | KCP 10.4.1 |
| Report | Friedrich, S. (2021) Oxathiapiprolin/metalaxyl-M DC (A23109A) - Acute Toxicity to the Earthworm *Eisenia andrei* in Artificial Soil, Report Number 21 48 TEA 0001. BioChem agrar GmbH, Kupferstraße 6, 04827 Machern OT Gerichshain, Germany. (Syngenta File No. VV-892721) |
| Guideline(s): | OECD Guidelines for Testing of Chemicals, Method 207: Earthworm, Acute Toxicity Tests (1984) |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication (if vertebrate study) | N/A |

### Executive Summary

In an acute toxicity test, in which earthworms (*Eisenia andrei*) were exposed to A23109A, the overall NOEC was determined to be 1000 mg test item/kg soil d.w. The 14-d $LC_{50}$ could not be calculated, but it can be concluded that the $LC_{50}$ is greater than 1000 mg test item/kg soil d.w., the highest concentration tested.

### Materials

| | | |
|---|---|---|
| **Test Material** | A23109A | |
| **Lot/Batch #:** | JHU003-036-001 | |
| **Actual content of active ingredients:** | Metalaxyl-M (incl. its S-enantiomer) | 17.2 % w/w corresponding to 185 g/L |
| | CGA351920 (S-enantiomer) | 0.57 % w/w corresponding to 6.1 g/L |
| | CGA329351 (R-enantiomer) | 16.6 % w/w corresponding to 178 g/L |
| | Oxathiapiprolin | 2.85 % w/w corresponding to 30.6 g/L |
| **Description:** | brownish liquid | |
| **Stability of test compound:** | stable under recommended handling and storage conditions (< 30 °C) | |
| **Reanalysis/Expiry date:** | end of June 2022 | |
| **Density:** | 1074 kg/m³ | |
| **Treatments** | | |

| | |
|---|---|
| **Test concentrations:** | 62.5, 125, 250, 500, 1000 mg test item/kg soil dry weight |
| **Control:** | Untreated (quartz sand only) |
| **Toxic standard:** | 2-chloroacetamide in deionised water at concentrations of 14.1, 18.3, 23.8, 31.0 and 40.3 mg/kg soil d.w. (separate GLP study). |
| **Test organisms** | |
| **Species:** | *Eisenia andrei* (BOUCHÉ, 1972) |
| **Age and weight range at test start:** | adult worms, 4 months old with clitellum<br>354 – 537 mg/worm |
| **Source:** | W. Neudorff GmbH KG, An der Mühle 3, 31860 Emmerthal, Germany; reared under ambient laboratory conditions in the test facility |
| **Feeding:** | none |
| **Test design** | |
| **Vessels:** | 1 L glass jars with clear lids |
| **Substrate:** | artificial soil comprising 10 % sphagnum peat, 20 % kaolin clay (kaolinite content > 30 %), 69.5 % industrial quartz sand (> 50 % of the particles between 50 and 200 µm) and 0.5 % calcium carbonate. 751 g soil wet weight, corresponding to 556 g dry weight of artificial soil was added to each test vessel. |
| **Replication:** | 4 |
| **No. of worms/vessel:** | 10 |
| **Duration of test:** | 14 days (14 days adult mortality) |
| **Environmental test conditions** | |
| **Temperature:** | 18.7 – 20.5 °C |
| **pH of soil:** | test start: 6.01 – 6.06<br>test end: 5.75 – 5.84 |
| **Water content of soil:** | test start: 55.6 – 55.7 % of max. WHC<br>test end: 54.5 – 54.9 % of max. WHC |
| **Photoperiod:** | continuous light (approximately 570 lux) |

## Study Design and Methods

BioChem agrar GmbH, Kupferstraße 6, 04827 Machern OT Gerichshain, Germany.
Experimental dates: 09 December 2020 to 23 December 2020
Approximately 24 hours prior to 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 untreated artificial soil for approximately 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 only. The test vessels were then filled with the treated soil. The acclimatised test animals were washed, gently dried on a paper towel, weighed and randomly placed onto the test substrate.

Assessments were performed after 7 and 14 days. The final number of surviving adult earthworms, the behaviour and pathological symptoms as well as their biomass change was recorded on day 14.

Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm test and the Williams-t-test were used to compare the control mortality and biomass with the independent test item groups. The $LC_{50}$ at could not be quantified due to the absence of a toxic effect of the test item at the tested concentrations.

## Results

Mortality and fecundity are summarised in the table below.

**Table A 53: Effects of A23109A on mortality of earthworms**

| Endpoint | Treatment group [mg test item/kg soil d.w.] | | | | | |
|---|---|---|---|---|---|---|
| | **Control** | **62.5** | **125** | **250** | **500** | **1000** |
| **Mortality of adult worms after 14 days (%)** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 2.5 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Mean biomass change at 14 days (mg/worm)** | -44.4 | -43.3 | -41.6 | -47.2 | -45.5 | -47.4 |
| **Mean biomass change (0-14 d) (%)** | -10.2 | -9.9 | -9.5 | -10.9 | -10.5 | -11.0 |
| **$LC_{50}$** | > 1000 | | | | | |
| **NOEC (mortality)** | 1000 | | | | | |
| **NOEC (biomass)** | 1000 | | | | | |

No statistically significant differences between control and test item were calculated for mortality (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, $\alpha = 0.05$, one-sided greater) and for biomass (Williams-t-test, $\alpha = 0.05$, one-sided greater)
d.w.: dry weight (of artificial soil)

**Validity Criteria**

The test was considered valid;

- There was 0.0% mortality in the control (must be ≤ 10%)

**Conclusion**

In an acute toxicity test, in which earthworms (*Eisenia andrei*) were exposed to A23109A, the overall NOEC was determined to be 1000 mg test item/kg soil d.w. The 14-d $LC_{50}$ could not be calculated, but it can be concluded that the $LC_{50}$ is greater than 1000 mg test item/kg soil d.w., the highest concentration tested.

(Friedrich, S., 2021)

## A 2.4.1.1 KCP 10.4.1.1 Earthworms - sub-lethal effects

| | |
|---|---|
| Comments of zRMS: | The study was conducted to OECD guidance 222 and according to the principles of GLP. All validity criteria were met.<br>The study is considered to be reliable. |

| | |
|---|---|
| Reference: | KCP 10.4.1.1 |
| Report | Friedrich, S. (2021) Oxathiapiprolin/metalaxyl-M DC (A23109A) – Sublethal Effects on the Reproduction of the Earthworm *Eisenia andrei* in Artificial Soil, Report Number 21 48 TEC 0005. BioChem agrar GmbH, Kupferstraße 6, 04827 Machern OT Gerichshain, Germany. (Syngenta File No. VV-892682) |
| Guideline(s): | OECD Guidelines for Testing of Chemicals, Method 222: Earthworm Reproduction Test (2016) |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |

Duplication N/A
(if vertebrate study)

### Executive Summary

In an earthworm reproduction study, in which earthworms (*Eisenia andrei*) were exposed to A23109A, the NOEC for mortality was determined to be 1000 mg test item/kg soil d.w. The NOEC for biomass change and reproduction was determined to be 556 and 309 mg test item/kg soil d.w., respectively. The $EC_{10}$, $EC_{20}$ and $EC_{50}$ values for reproduction were calculated to be 302, 413 and 752 mg test item/kg soil d.w., respectively.

### Materials

| | |
|---|---|
| **Test Material** | Oxathiapiprolin/metalaxyl-M DC (A23109A) |
| **Lot/Batch #:** | JHU003-036-001 |
| **Actual content of active ingredients:** | Metalaxyl-M<br>(incl. its S-enantiomer) 17.2 % w/w corresponding to 185 g/L<br>CGA351920 (S-enantiomer) 0.57 % w/w corresponding to 6.1 g/L<br>CGA329351 (R-enantiomer) 16.6 % w/w corresponding to 178 g/L<br>Oxathiapiprolin 2.85 % w/w corresponding to 30.6 g/L |
| **Description:** | brownish liquid |
| **Stability of test compound:** | stable under recommended handling and storage conditions (< 30 °C) |
| **Reanalysis/Expiry date:** | end of June 2022 |
| **Density:** | 1074 kg/m³ |
| **Treatments** | |
| **Test rates:** | 16.3, 29.4, 52.9, 95.3, 171, 309, 556, 1000 mg test item/kg soil dry weight (spacing factor: 1.8) |
| **Control:** | untreated (deionised water) |
| **Toxic standard:** | Maypon Flow (carbendazim, SC 500) was tested at concentrations of 2.2 and 4.3 mg a.i./kg soil dry weight (separate GLP study BioChem project No.: 21 48 TEC 0011). |
| **Test organisms** | |
| **Species:** | *Eisenia andrei* (BOUCHÉ, 1972) |
| **Age and weight range at test start:** | adult worms, 4 months old with clitellum<br>368 – 540 mg/worm |
| **Source:** | reared under ambient laboratory conditions in the test facility (originally purchased from W. Neudorff GmbH KG, An der Mühle 3, 31860 Emmerthal, Germany) |
| **Feeding:** | air-dried and finely ground horse manure |
| **Test design** | |
| **Vessels:** | plastic vessel (16.5 cm x 12 cm x 6 cm) with a lid pervious to air and light. |
| **Substrate:** | artificial soil comprising 10 % sphagnum peat, 20 % kaolin clay (kaolinite content > 30 %), 69.5 % industrial quartz sand (> 50 % of the particles between 50 and 200 µm) and 0.5 % calcium carbonate. 810 g soil wet weight, corresponding to 600 g dry weight of artificial soil was added to each test vessel. |
| **Replication:** | 8 replicates for the control group and 4 replicates for the treated groups |
| **No. of worms/vessel :** | 10 |
| **Duration of test:** | 8 weeks (4 weeks adult mortality and biomass change; 4 weeks juvenile development) |
| **Environmental test conditions** | |
| **Temperature:** | 18.1 – 20.2 °C |
| **pH of soil:** | test start: 5.95 – 6.01<br>test end: 5.65 – 5.76 |
| **Water content of soil:** | test start: 57.2 – 57.5 % of max. WHC<br>test end: 55.6 – 56.8 % of max. WHC |
| **Photoperiod:** | 16 hours light : 8 hours dark (approximately 570 lux) |

**Study Design and Methods**

BioChem agrar GmbH, Kupferstraße 6, 04827 Machern OT Gerichshain, Germany.
Experimental dates: 02 December 2020 to 27 January 2021
The test concentrations were prepared by dispersing an exactly weighed amount of the test item in deionised water to make a stock solution. This stock solution was diluted with deionised water for each test concentration and was thoroughly mixed with the artificial soil using a laboratory mixer, achieving a final nominal water content of 40-60 % of WHC. The control was treated with deionised water only. After a randomising procedure according to the worm fresh weight, selected groups of 10 worms were then randomly assigned to each treatment group. Four replicates were used per test item concentration and eight replicates were used for the control. One day after application, 5 g air-dried and finely ground horse manure was scattered on the soil surface of each test vessel. The feeding interval was weekly during the first four weeks of the test.

After four weeks, the adult worms were removed from the test vessels, and mortality and the body weight of the surviving worms were determined. After all of the adult worms had been removed, the soil in each vessel was mixed with 5 g horse manure. Four weeks later, the number of surviving juveniles and any morphological alterations were recorded. Observations of behavioural and pathological symptoms were observed weekly.

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 $EC_x$ values (number of juveniles) were calculated using the Probit analysis. Confidence limits (95 %) of the $EC_x$ values were computed by normal approximation. For identifying the NOEC values the Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm test and the Williams-t-test were used to compare the control with the independent test item groups.

**Results**

Mortality and reproduction are summarised in the table below.

**Table A 54: Effect of A23109A on mortality, growth and reproduction of *Eisenia andrei***

| Endpoint | Treatment group (mg test item/kg soil d.w.) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Control** | **16.3** | **29.4** | **52.9** | **95.3** | **171** | **309** | **556** | **1000** |
| Mortality of adult worms after 4 weeks (%) | 1.3 | 0.0 | 2.5 | 0.0 | 0.0 | 0.0 | 0.0 | 2.5 | 10.0 |
| Mean biomass change after 4 weeks (%) | 28.8 | 26.7 | 29.3 | 27.2 | 26.4 | 28.9 | 27.0 | 24.6 | 21.7* |
| Mean number of juveniles after 8 weeks | 225.0 | 229.8 | 220.5 | 221.3 | 228.8 | 228.3 | 199.8 | 149.3* | 77.8* |
| Change of reproduction compared to control (%) | - | -2.1 | 2.0 | 1.7 | -1.7 | -1.4 | 11.2 | 33.7 | 65.4 |
| | Endpoint (mg test item/kg soil d.w.) | | | | | | | | |
| NOEC (mortality) | 1000 | | | | | | | | |
| NOEC (biomass change) | 556 | | | | | | | | |
| NOEC (reproduction) | 309 | | | | | | | | |
| $LC_{50}$ (mortality) [1] | > 1000 | | | | | | | | |
| $EC_{10}$ (reproduction) [2] | 302 (95 % confidence limits 275 – 331) | | | | | | | | |
| $EC_{20}$ (reproduction) [2] | 413 (95 % confidence limits 387 – 440) | | | | | | | | |
| $EC_{50}$ (reproduction) [2] | 752 (95 % confidence limits 720 – 785) | | | | | | | | |

Not statistically significant compared to control regarding mortality (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm for mortality, $\alpha = 0.05$, one-sided greater)

* statistically significantly different compared to control (Williams-t-test for biomass change and reproduction, $\alpha = 0.05$, one-sided smaller)
Negative % values for change of reproduction = increase, relative to control
[1] based on estimation of the data, [2] Probit analysis using linear max. likelihood regression

**Validity criteria**

The test is considered valid:

- Adult mortality was 1.3 % in the control treatment (must be ≤ 10%)
- The mean number of juveniles per control replicate was 169 to 284 (must be ≥30)
- The coefficient of variation for reproduction in the control treatment was 18.0 % (must be ≤ 30%)

**Conclusion**

In an earthworm reproduction study, in which earthworms (*Eisenia andrei*) were exposed to A23109A, the NOEC for mortality was determined to be 1000 mg test item/kg soil d.w. The NOEC for biomass change and reproduction was determined to be 556 and 309 mg test item/kg soil d.w., respectively. The $EC_{10}$, $EC_{20}$ and $EC_{50}$ values for reproduction were calculated to be 302, 413 and 752 mg test item/kg soil d.w., respectively.

(Friedrich, S., 2021)

## A 2.4.1.2 KCP 10.4.1.2 Earthworms - field studies

## A 2.4.2 KCP 10.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

## A 2.4.2.1 KCP 10.4.2.1 Species level testing

| | |
|---|---|
| Comments of zRMS: | The study was conducted to OECD guidance 232 and according to the principles of GLP. All validity criteria were met.<br>The study is considered to be reliable. |

| | |
|---|---|
| Reference: | KCP 10.4.2.1 |
| Report | Friedrich, S. (2021) Oxathiapiprolin/metalaxyl-M DC (A23109A) - Effects on the Reproduction of the Collembolan *Folsomia candida*. Report Number 21 48 TCC 0003. BioChem agrar GmbH, Kupferstraße 6, 04827 Machern OT Gerichshain, Germany (Syngenta File No. VV-891167) |
| Guideline(s): | OECD Guidelines for Testing of Chemicals, Method 232: Collembolan Reproduction Test in Soil (2016) |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication (if vertebrate study) | N/A |

**Executive Summary**

In a Collembola reproduction study with A23109A, the NOEC for mortality of the parental collembolans was determined to be 1000 mg test item/kg soil dry weight. The $LC_{50}$ could not be calculated, but it can be concluded that the $LC_{50}$ is higher than 1000 mg test item/kg soil d.w., the highest concentration tested. The NOEC for reproduction was determined to be 556 mg test item/kg soil dry weight. The $EC_{10}$, $EC_{20}$ and $EC_{50}$ values for reproduction were calculated to be 675, 811 and > 1000 mg test item/kg soil dry weight, respectively.

## Materials

| | |
|---|---|
| **Test Material** | Oxathiapiprolin/metalaxyl-M DC (A23109A) |
| **Lot/Batch #:** | JHU003-036-001 |
| **Actual content of active ingredients:** | Metalaxyl-M<br>(incl. its S-enantiomer) 17.2 % w/w corresponding to 185 g/L<br>CGA351920 (S-enantiomer) 0.57 % w/w corresponding to 6.1 g/L<br>CGA329351 (R-enantiomer) 16.6 % w/w corresponding to 178 g/L<br>Oxathiapiprolin 2.85 % w/w corresponding to 30.6 g/L |
| **Description:** | brownish liquid |
| **Stability of test compound:** | stable under recommended handling and storage conditions (< 30 °C) |
| **Reanalysis/Expiry date:** | end of June 2022 |
| **Density:** | 1074 kg/m³ |
| **Treatments** | |
| **Test rates:** | 16.3, 29.4, 52.9, 95.3, 171, 309, 556, 1000 mg test item/kg soil dry weight (spacing factor: 1.8) |
| **Control:** | untreated (deionised water only) |
| **Toxic standard:** | boric acid (separate GLP study BioChem project No.: 20 48 TCC 0064) |
| **Application method** | soil incorporation |
| **Test organisms** | |
| **Species:** | *Folsomia candida* |
| **Age:** | juvenile collembolans, 9-12 days old |
| **Source:** | originally purchased from "Biologische Bundesanstalt (BBA)", Berlin-Dahlem. reared under ambient laboratory conditions in the test facility |
| **Feeding:** | 2 mg granulated dry yeast at the start of the test and after 14 days |
| **Test design** | |
| **Vessels:** | glass container (approximately 150 mL) covered with a lid |
| **Substrate:** | artificial soil comprising 5 % sphagnum peat, 20 % kaolin clay (kaolinite content > 30 %), 74.7 % industrial quartz sand (> 50 % of the particles between 50 and 200 µm) and 0.3 % calcium carbonate. 37.5 g wet weight soil, corresponding to 30 g dry weight of artificial soil was added to each test vessel. |
| **Replication:** | 8 replicates for the control group and 4 replicates for the treated groups |
| **No. of Collembola/vessel :** | 10 |
| **Duration of test:** | 28 days |
| **Environmental test conditions** | |
| **Temperature:** | 18.2 – 20.0 ºC |
| **pH of soil:** | test start: 5.97 – 6.06<br>test end: 5.68 – 5.80 |
| **Water content of soil:** | test start: 57.2 – 57.5 % of max. WHC<br>test end: 55.9 – 56.6 % of max. WHC |
| **Photoperiod:** | 16 hours light : 8 hours dark photoperiod, approximately 560 lux |

## Study Design and Methods

Test facility: BioChem agrar GmbH, Kupferstraße 6, 04827 Machern OT Gerichshain, Germany
Experimental dates: 08 December 2020 to 05 January 2021
The test concentrations were prepared by dispersing an exactly weighed amount of the test item in deionised water to make a stock solution. This stock solution was diluted with deionised water for each test concentration and was thoroughly mixed with the artificial soil using a laboratory mixer, achieving a final nominal water content of 40-60 % of WHC. The control was treated with deionised water only.

After the application ten juvenile collembolans were transferred to the substrate surface of each test vessel using an aspirator. Four replicates were used per test item concentration and eight replicates were used for the control. The test organisms were fed twice during the test (at the start of the test and after 14 days) with approximately 2 mg of granulated dry yeast per test vessel. Four weeks after introducing the test organisms, the surviving parental collembolans and offspring (juveniles) were counted.

All values presented throughout this report were calculated using the original raw data and were not based on rounded values.

The percentage mortality of the springtails was calculated for each treatment, both before and after correction for any control treatment losses using Abbott's formula. Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm and Williams-t-test were used to compare the control with the independent test item groups. The ECx values were calculated by using Probit analysis using the maximum likelihood regression.

**Results**

Mortality and fecundity are summarised in the table below.

**Table A 55: Effects of residues of A23109A on mortality and reproduction of *Folsomia candida***

| Endpoint | Treatment group (mg test item/kg soil dry weight) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Control** | **16.3** | **29.4** | **52.9** | **95.3** | **171** | **309** | **556** | **1000** |
| % Mortality of parental collembolans after 4 weeks | 1.3 | 0.0 | 0.0 | 0.0 | 0.0 | 2.5 | 0.0 | 0.0 | 7.5 |
| % Corrected mortality (Abbott) | - | -1.3 | -1.3 | -1.3 | -1.3 | 1.3 | -1.3 | -1.3 | 6.3 |
| Mean number of juveniles after 4 weeks | 1323 | 1328 | 1332 | 1365 | 1286 | 1358 | 1372 | 1332 | 837* |
| % Reduction of reproduction compared to control | - | -0.4 | -0.7 | -3.2 | 2.7 | -2.6 | -3.7 | -0.7 | 36.7 |
| | **Endpoint (mg test item/kg soil dry weight)** | | | | | | | | |
| NOEC (mortality) | 1000 | | | | | | | | |
| NOEC (reproduction) | 556 | | | | | | | | |
| $LC_{50}$ (mortality) [1] | > 1000 | | | | | | | | |
| $EC_{10}$ (reproduction) [2] | 675 (95 % confidence limits 563 – 808) | | | | | | | | |
| $EC_{20}$ (reproduction) [2] | 811 (95 % confidence limits 731 – 900) | | | | | | | | |
| $EC_{50}$ (reproduction) [2] | > 1000 | | | | | | | | |

Not statistically significantly different compared to control regarding mortality (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, $\alpha = 0.05$, one-sided greater)
* statistically significant different compared to the control Williams-t-test for reproduction, $\alpha = 0.05$, one-sided smaller)
[1] based on estimation of the data, [2] Probit analysis
Negative % values for change of reproduction = increase, relative to control

**Validity criteria**

The validity criteria are as follows:

- Control treatment mortality was 1.3 % (must be ≤ 20%)

- The mean number of juveniles recorded in the control treatment was 1323 (must be ≥ 100 per replicate)
- The coefficient of variation of reproduction in the control was 6.6 % (must be < 30%)

**Conclusion**

In a Collembola reproduction study with A23109A, the NOEC for mortality of the parental collembolans was determined to be 1000 mg test item/kg soil dry weight. The $LC_{50}$ could not be calculated, but it can be concluded that the $LC_{50}$ is higher than 1000 mg test item/kg soil d.w., the highest concentration tested. The NOEC for reproduction was determined to be 556 mg test item/kg soil dry weight. The $EC_{10}$, $EC_{20}$ and $EC_{50}$ values for reproduction were calculated to be 675, 811 and > 1000 mg test item/kg soil dry weight, respectively.

(Friedrich, S., 2021)

| | |
|---|---|
| Comments of zRMS: | The study was conducted to OECD guidance 226 and according to the principles of GLP. All validity criteria were met.<br>The study is considered to be reliable. |

| | |
|---|---|
| Reference: | KCP 10.4.2.1 |
| Report | Schulz, L. (2021), Oxathiapiprolin/metalaxyl-M DC (A23109A) - Effects on the Reproduction of the Predatory Mite *Hypoaspis aculeifer*, Report Number 21 48 THC 0003. BioChem agrar Labor für biologische und chemische Analytik GmbH, Kupferstraße 6, 04827 Machern OT Gerichshain, Germany (Syngenta File No. VV-890311) |
| Guideline(s): | OECD Guideline 226: Predatory mite (*Hypoaspis* (*Geolaelaps*) *aculeifer*) reproduction test in soil (2016) |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication<br>(if vertebrate study) | N/A |

**Executive Summary**

In a 14-day *Hypoaspis aculeifer* reproduction study with A23109A, the $LC_{50}$ for mortality and the $EC_{10}$, $EC_{20}$ and $EC_{50}$ values for reproduction could not be calculated, but it can be concluded that these values are higher than 1000 mg test item/kg soil dry weight, the highest concentration tested. The NOEC for mortality and the NOEC for reproduction were determined to be equal or higher than 1000 mg test item/kg soil dry weight.

**Materials**

| | |
|---|---|
| **Test Material** | Oxathiapiprolin/metalaxyl-M DC (A23109A) |
| **Lot/Batch #:** | JHU003-036-001 |
| **Actual content of active ingredients:** | Metalaxyl-M (incl. its S-enantiomer) 17.2 % w/w corresponding to 185 g/L<br>CGA351920 (S-enantiomer) 0.57 % w/w corresponding to 6.1 g/L<br>CGA329351 (R-enantiomer) 16.6 % w/w corresponding to 178 g/L<br>Oxathiapiprolin 2.85 % w/w corresponding to 30.6 g/L |
| **Description:** | brownish liquid |
| **Stability of test compound:** | Stable under the given conditions |

| | |
|---|---|
| **Reanalysis/Expiry date:** | End of June 2022 |
| **Treatments** | |
| **Test rates:** | 16, 29, 53, 95, 171, 309, 556, 1000 mg test item/kg soil dry weight (spacing factor: 1.8) |
| **Control:** | prepared with deionised water |
| **Toxic standard:** | Dimethoate 400 EC (400g/L, nominal) (separate GLP study) |
| **Test organisms** | |
| **Species** | *Hypoaspis aculeifer* (CANESTRINI) |
| **Source:** | Obtained synchronised from "Katz Biotech AG", Baruth, Germany, on 12 November 2020 and kept in the test facility under ambient laboratory conditions until test start |
| **Food:** | *Tyrophagus putrescentiae* (SCHRANK) |
| **Age at test start:** | Adult (31-33 days old) |
| **Test design** | |
| **Vessels:** | 160 mL WECK-jar with glass lid |
| **Substrate:** | - 5 % sphagnum peat; origin: Torfwerk Moorkultur Ramsloh, 26683 Saterland, Germany, classified according to DIN 11540 (as close to pH 5.5-6.0 as possible, no visible plant remains, finely ground, dried to measured moisture content)<br>- 20 % kaolin clay (kaolinite content > 30 %); type: Kaolin W, origin: ERBSLÖH Lohrheim GmbH, 65558 Lohrheim, Germany<br>- 0.25 % calcium carbonate; origin: MERCK KGaA, 64271 Darmstadt, Germany<br>- 74.75 % industrial quartz sand; type: Millisil W3, origin: Quarzwerke GmbH, 50207 Frechen, Germany (predominantly fine sand with more than 50 % of the particles between 50 and 200 µm)<br>- deionised water |
| **Replication:** | control: 8 (+ 2 replicates for determination of water content and pH-value; without predatory mites)<br>treated group: 4 (+ 2 replicates for determination of water content and pH-value; without predatory mites) |
| **No. of mites/arena:** | 10 adult females |
| **Duration of test:** | 14 days |
| **Environmental test conditions** | |
| **Temperature:** | 19.4 -21.4 °C |
| **pH:** | test start: 6.4 - 6.5<br>test end: 6.4 - 6.5 |
| **Water content of soil:** | test start: 16.63 - 17.22 (equivalent to 47.65 - 49.31 % of maximum WHC)<br>test end: 16.91 - 17.25 (equivalent to 48.43 - 49.41 % of maximum WHC) |
| **Photoperiod:** | duration: light : dark = 16 h : 8 h<br>intensity: 488 lux |

**Study Design and Methods**

Test facility: BioChem agrar GmbH, Kupferstraße 6, 04827 Machern OT Gerichshain, Germany
Experimental dates: 16 November 2020 - 03 December 2020
Adult females of the soil mite *Hypoaspis aculeifer* were exposed to different concentrations of Oxathiapiprolin/metalaxyl-M DC (A23109A) incorporated into the test soil. An exactly weighed amount of the test item was mixed with purified water to make a stock solution, and appropriate volumes of this stock solution were further diluted with deionised water to obtain the test concentrations such that, when added to pre-moistened artificial soil, a final moisture content value of approximately 50% WHC was achieved. Adult females were transferred to the test vessels which contained untreated (control) or test item treated artificial soil. Ten adult females were introduced to each test vessel. As a source of food, cheese mites (*Tyrophagus putrescentiae*) were added to the soil surface every 2-3 days. The test was carried out under controlled light-dark cycle. Fourteen days after introducing the test organisms, the surviving mites and the juveniles of *Hypoaspis aculeifer* were extracted by heat/light extraction and counted. From these data the mortality of the adult females and the reproductive output were calculated.

The statistical analysis was performed with the software ToxRat Professional 3.3.0 (RATTE, 2018). The Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm and Dunnett's Multiple t-test Procedure were used to compare the control with the independent test item groups.

**Results**

Mortality and fecundity are summarised in the table below.

**Table A 56: Effects of residues of A23109A on mortality and reproduction of *Hypoaspis aculeifer***

| Endpoint | Treatment group (mg test item/kg soil d.w.) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Control** | **16** | **29** | **53** | **95** | **171** | **309** | **556** | **1000** |
| | **Mortality of adult mites after 14 days** | | | | | | | | |
| **% mortality** | 1.3 | 2.5 | 2.5 | 5.0 | 0.0 | 5.0 | 2.5 | 2.5 | 2.5 |
| **% corrected mortality** | - | 1.3 | 1.3 | 3.8 | -1.3 | 3.8 | 1.3 | 1.3 | 1.3 |
| | **Number of juveniles after 14 days** | | | | | | | | |
| **Mean no. progeny per replicate** | 220.5 | 214.5 | 220.5 | 212.8 | 230.5 | 211.5 | 221.3 | 211.3 | 223.5 |
| **standard deviation** | 16.6 | 8.2 | 13.8 | 13.9 | 13.4 | 14.9 | 18.0 | 9.2 | 20.4 |
| **coefficient of variation (%)** | 7.5 | 3.8 | 6.2 | 6.5 | 5.8 | 7.0 | 8.1 | 4.4 | 9.1 |
| **% reduction compared to control** | - | 2.7 | 0.0 | 3.5 | -4.5 | 4.1 | -0.3 | 4.2 | -1.4 |
| **NOEC (mortality)** | **≥ 1000** | | | | | | | | |
| **$LC_{50}$ (mortality)** | **> 1000** | | | | | | | | |
| **NOEC (reproduction)** | **≥ 1000** | | | | | | | | |
| **$EC_{10}$ (reproduction)** | **> 1000** | | | | | | | | |
| **$EC_{20}$ (reproduction)** | **> 1000** | | | | | | | | |
| **$EC_{50}$ (reproduction)** | **> 1000** | | | | | | | | |

Not statistically significantly different compared to the control (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm for mortality, $\alpha = 0.05$, one-sided greater and Dunnett's Multiple t-test Procedure for reproduction, $\alpha = 0.05$, one-sided smaller)
Negative % values for reduction of reproduction = increase, relative to control

## Validity Criteria

The validity criteria for the control group were met:

- Mean mortality of adult females: ≤ 20% (observed: 1.3 %)
- Mean number of juveniles per replicate: ≥ 50 (calculated: 220.5)
- Coefficient of variation (mean number of juveniles per replicate): ≤ 30 % (calculated: 7.5 %)

**Conclusion**

In a 14-day *Hypoaspis aculeifer* reproduction study with A23109A, the $LC_{50}$ for mortality and the $EC_{10}$, $EC_{20}$ and $EC_{50}$ values for reproduction could not be calculated, but it can be concluded that these values are higher than 1000 mg test item/kg soil dry weight, the highest concentration tested. The NOEC for mortality and the NOEC for reproduction were determined to be equal or higher than 1000 mg test item/kg soil dry weight.

(Schulz, L., 2021)

## A 2.4.2.2 KCP 10.4.2.2 Higher tier testing

## A 2.5 KCP 10.5 Effects on soil nitrogen transformation

| | |
|---|---|
| Comments of zRMS: | The study was conducted to OECD guidance and according to the principles of GLP. All validity criteria were met.<br>The study is considered to be reliable and suitable for the risk assessment. |

| | |
|---|---|
| Reference: | KCP 10.5 |
| Report | Schulz, L. (2021), Oxathiapiprolin/metalaxyl-M DC (A23109A) - Oxathiapiprolin/Metalaxyl-M DC (A23109A) – Effects on the Activity of Soil Microflora (Nitrogen and Carbon Transformation Tests), Report Number 21 48 SMO 0002. BioChem agrar Labor für biologische und chemische Analytik GmbH, Kupferstraße 6, 04827 Machern OT Gerichshain, Germany (Syngenta File No. VV-894279) |
| Guideline(s): | OECD guideline 216, Soil Microorganisms: Nitrogen Transformation Test (2000)<br>OECD guideline 217, Soil Microorganisms: Carbon Transformation Test (2000) |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |
| Duplication (if vertebrate study) | N/A |

**Executive Summary**

The test item A23109A (tested at 2.88 mg/kg soil dry weight corresponding to 2.01 L test item/ha and 9.59 mg/kg soil dry weight corresponding to 6.70 L test item/ha) caused no adverse effects (deviation from control < 25 %, OECD 216/217) on soil nitrogen transformation (measured as $NO_3$-N-production) and on soil carbon transformation (measured as $O_2$-consumption) at the end of the 28-day incubation period.

**Materials**

| | |
|---|---|
| **Test Material** | Oxathiapiprolin/metalaxyl-M DC (A23109A) |
| **Lot/Batch #:** | JHU003-036-001 |
| **Actual content of active ingredients:** | Metalaxyl-M (incl. its S-enantiomer) 17.2 % w/w corresponding to 185 g/L<br>CGA351920 (S-enantiomer) 0.57 % w/w corresponding to 6.1 g/L<br>CGA329351 (R-enantiomer) 16.6 % w/w corresponding to 178 g/L<br>Oxathiapiprolin 2.85 % w/w corresponding to 30.6 g/L |

| | |
|---|---|
| **Description:** | brownish liquid |
| **Stability of test compound:** | Stable under the given conditions |
| **Reanalysis/Expiry date:** | End of June 2022 |
| **Density:** | 1074 kg/m³ |
| **Treatments** | |
| **Test rates:** | 2.88 mg test item/kg soil dry weight (2.01 L test item/ha)<br>9.59 mg test item/kg soil dry weight (6.70 L test item/ha) |
| **Control:** | Deionised water |
| **Toxic standard:** | Dinoterb (tested in separate GLP study) |
| **Test design** | |
| **Soil type:** | loamy sand (DIN ISO 11277; DIN 4220) |
| **Test units:** | Nitrogen transformation test: wide-mouth glass flasks (500 mL)<br>Carbon transformation test: stainless steel vessels (4 L) |
| **Replication:** | 3 |
| **Sampling intervals :** | 0 (3 hours after application), 7, 14 and 28 days |
| **Duration of test:** | 28 days |
| **Environmental test conditions** | |
| **Temperature:** | 19.0 - 21.4 °C |
| **pH of soil:** | Nitrogen transformation test: 5.9 - 6.0, Carbon transformation test: 5.8 - 6.0 |
| **Soil moisture content:** | Approximately 45 % of maximum water holding capacity |
| **Photoperiod:** | Continuous darkness |

## Study Design and Methods

Test facility: BioChem agrar GmbH, Kupferstraße 6, 04827 Machern OT Gerichshain, Germany
Experimental dates: 06 January 2021 to 03 February 2021
Soil samples were treated with A23109A at two doses, 2.88 and 9.59 mg A23109A/kg dry soil (corresponding to 2.01 L test item/ha and 6.70 L test item/ha, respectively). Test concentrations related to a soil depth of 5 cm and a soil density of 1.5 g/cm$^3$.

The test item was mixed with deionised water and the test solution was subsequently mixed with the soil (carbon transformation test: laboratory mixer, nitrogen transformation test: hand stirrer). Water was added to the soil to achieve a water content of approximately 45 % of WHC. 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.

Three replicate soil samples were prepared for each treatment rate and the control for the nitrogen transformation test and carbon transformation test.

Mean nitrogen content (mg $NO_3$/kg soil d.w.), standard deviation and coefficient of variation as well as the mean nitrogen content/day (mg $NO_3$/kg soil d.w./day) were calculated for each treatment group and sampling date.

For the evaluation of the results the relative deviations (%) of the test item treatment groups from the control were calculated (based on the mean nitrogen content/day) for each sampling date.

The cumulative $O_2$ consumption after 12 hours was calculated (using regression analysis; the goodness of fit ($R^2$) was > 0.99 in all replicates and on all days). Furthermore, standard deviation and coefficient of variation were calculated for each treatment group and sampling dates.

For evaluation of the results the relative deviations (%) of the test item treatment groups from the control were calculated for each sampling date. A 2-sided Student-t-test at 5 % significance level was performed for statistical evaluation

## Results

Results from the Nitrogen transformation test and the Carbon transformation test are summarised in the tables below.

**Table A 57: Effects on Nitrogen Transformation in Soil after Treatment with the Test Item**

| Time Interval (days) | Control | | 2.88 mg test item/kg soil dry weight | | | 9.59 mg test item/kg soil dry weight | | |
|---|---|---|---|---|---|---|---|---|
| | $NO_3$-N [mg/kg soil d.w.] | $NO_3$-N [mg/kg soil d.w./day] | $NO_3$-N [mg/kg soil d.w.] | $NO_3$-N [mg/kg soil d.w./day] | Deviation from control [%] [1)] | $NO_3$-N [mg/kg soil d.w.] | $NO_3$-N [mg/kg soil d.w./day] | Deviation from control [%] [1)] |
| **0 - 7** | 52.6 | 2.76 | 50.8 | 2.57 | -6.7 | 50.3 | 2.61 | -5.4 |
| **0 - 14** | 64.4 | 2.22 | 66.3 | 2.39 | +7.7 | 63.7 | 2.26 | +1.8 |
| **0 - 28** | 86.9 | 1.91 | 87.9 | 1.97 | +2.7 | 86.6 | 1.95 | +1.7 |

1) based on NO3-nitrogen-production; - = inhibition; + = stimulation

Not statistically significant difference to the control (Student-t-test for homogeneous variances, 2-sided, $\alpha = 0.05$).

The calculations were performed with non-rounded values.

**Table A 58: Effects on Carbon Transformation in Soil after Treatment with the Test Item**

| Days after application | Control | 2.88 mg test item/kg soil dry weight | | 9.59 mg test item/kg soil dry weight | |
|---|---|---|---|---|---|
| | $O_2$-consumption [mg/kg soil d.w./h] | $O_2$-consumption [mg/kg soil d.w./h] | Deviation from control [%] [1)] | $O_2$-consumption [mg/kg soil d.w./h] | Deviation from control [%] [1)] |
| **0** | 12.81 | 123.38 | +4.5 | 12.26 | -4.3 |
| **7** | 11.99 | 11.95 | -0.3 | 11.23* | -6.3 |
| **14** | 11.11 | 11.07 | -0.4 | 10.61* | -4.5 |
| **28** | 11.32 | 11.52 | +1.8 | 11.00 | -2.8 |

1) based on O2-consumption; - = inhibition; + = stimulation

* = statistically significant differences between the control and the test item treatments were calculated (Student-t-test for homogeneous variances, 2-sided, $\alpha = 0.05$).

The calculations were performed with non-rounded values.

**Validity criteria**

The validity criteria are listed below:

- The coefficient of variation in the control group of the nitrogen and carbon transformation tests (for the whole test duration) were at maximum 6.6 and 2.6 % respectively (must be ≤ 15 %)
- The toxic standard caused effects of +26.9 %, +43.2 % and +27.2 % at concentrations 6.80, 13.60 and 27.20 mg/kg soil d.w. in the Nitrogen transformation test after a 28 day exposure, demonstrating the sensitivity of the test system (must be ≥ 25 %)
- The toxic standard caused effects of -25.4 %, -42.4 % and -48.3 % at concentrations 6.80, 13.60 and 27.20 mg/kg soil d.w., respectively in the Carbon transformation test after a 28 day exposure, demonstrating the sensitivity of the test system (must be ≥ 25 %)

**Conclusion**

The test item A23109A tested at 2.88 mg/kg soil dry weight and 9.59 mg/kg soil dry (corresponding to 2.01 L test item/ha and 6.70 L test item/ha, respectively) caused no adverse effects (deviation from control < 25 %, OECD 216/217) on soil nitrogen transformation (measured as $NO_3$-N-production) and on soil carbon transformation (measured as $O_2$-consumption) at the end of the 28-day incubation period.

(Schulz, L. 2021)

## 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

| Comments of zRMS: | The study is considered to be reliable and suitable for the risk assessment. |
|---|---|

| | |
|---|---|
| Reference: | KCP 10.6.1 |
| Report | Jones, K (2020), Oxathiapiprolin/metalaxyl-M DC (A23109A) - Phytotoxicity to Non-Target Plants Screening Test, ACE-20-109, AgroChemex Ltd., Aldhams Farm Research Station, Dead Lane, Lawford, Manningtree, Essex, CO11 2NF, United Kingdom. (Syngenta File No. VV-890173) |
| Guideline(s): | Study was carried out following a standardised study protocol based on Syngenta herbicide profiling test |
| Deviations: | None that had an impact on the study results |
| GLP: | Yes. No claim of GLP was made for the soil analysis |
| Acceptability: | Yes |
| Duplication (if vertebrate study) | N/A |

**Executive Summary**

The effects of A23109A on the seedling emergence and vegetative vigour of six non-target plant species (onion, wheat, sugar beet, oilseed rape, cucumber and soybean) were assessed following a range of applications, (0 (deionised water only), 62.5, 125, 250, 500 and 1000 mL A23109A/ha. In both seedling emergence and vegetative vigour test none of the tested species showed any phytotoxic effects up to and including the top does of 1000 mL A23109A /ha.

**Materials**

| | |
|---|---|
| **Test material** | A23109A |
| **Lot/Batch #:** | JHU003-036-001 |
| **Actual content of active ingredients:** | metalaxyl-M (incl. its s-enantiomer): 17.2 % w/w corresponding to 185 g/L<br>CGA351920 (S-enantiomer): 0.57 % w/w corresponding to 6.1 g/L<br>CGA329351 (R-enantiomer): 16.6 % w/w corresponding to 178 g/L<br>oxathiapiprolin: 2.85% w/w corresponding to 30.6 g/L |
| **Description:** | brownish liquid |
| **Stability of test compound:** | Stable under normal conditions |
| **Reanalysis/expiry date:** | End of June 2022 |
| **Density** | 1074 Kg/$m^3$ (1.074 g/mL) |
| **Treatments** | |
| **Test concentrations:** | 62.5, 125, 250, 500 and 1000 mL A23109A/ha |
| **Control:** | Deionised water |
| **Spray volume:** | 200 L/ha ± 10% |
| **Application method:** | Mardrive cabinet sprayer |
| **Test organisms** | |
| **Species:** | Onion (*Allium cepa*), Wheat (*Triticum aestivum*), Sugar beet (*Beta vulgaris*), Oilseed rape (*Brassica napus*), Cucumber (*Cucumis sativus*), Soybean (*Glycine max*). |
| **Test soil:** | Sandy loam mixed in a ratio of approximately 2 parts sterile loam and 1 part |

| | |
|---|---|
| | sand. 125 g slow release eionized (Osmocote® Pro) incorporated into 30 litres of soil mix prior to study start. Organic carbon content 0.9 %. |
| **Test design** | |
| **Test vessels:** | Non-porous plastic pots were used (9 x 9 x10 cm; W x D x H). |
| **Sampling interval:** | Seedling emergence: Visual phytotoxicity assessment undertaken 28 days after the application of the test item.<br>Vegetative vigour: Visual phytotoxicity assessment undertaken 21 days after the application of the test item. |
| **Replication:** | Three pots per treatment with four seeds/plants per pot (six seeds/plants for onion). |
| **Duration:** | 28 days for seedling emergence test; 21 days for vegetative vigour test. |
| **Environmental conditions** | |
| **Test temperature:** | Min: 18.5°C – Max: 25.6°C (Mean: 21.5°C) |
| **Humidity:** | Min: 44.6% – Max: 72.9% (Mean: 55.4%) |
| **Soil pH:** | 7.4 |
| **Lighting:** | Min: 0.4 kilolux – Max: 27.8 kilolux (Mean: 4.1 kilolux) |

**Study Design and Methods**

Test facility: AgroChemex Ltd., Aldhams Farm Research Station, Dead Lane, Lawford, Manningtree, Essex, CO11 2NF, United Kingdom.
Experimental dates: 21 October 2020 – 18 November 2020
For the vegetative vigour, seeds were germinated in seed trays of Levington F1 compost and four seedlings (six for onion) were transplanted shortly after emergence at BBCH Growth Stage 10 into plastic pots. After planting, the pots were placed in the glasshouse where the seedlings were allowed to develop into plants with two to four true leaves (BBCH growth 12 -14) before being used in the study.

For the seedling emergence, four seeds (six for onion) were sown in plastic pot with a depth of 1-2 cm prior to the application.

Foliage of plants of two monocot species (*Allium cepa,* and *Triticum aestivum*) and four dicot species (*Beta vulgaris, Brassica napus, Cucumis sativus* and *Glycine max*) were sprayed with A23109A to assess the vegetative vigour. For the seedling emergence test, A23109A was applied directly to the soil.

On the day of application, a primary stock solution was prepared by diluting 1.6153 g of A23109A into 300 mL eionized water. The solution was mixed by swirling and inversion and it served as the spray mixture for the 1000 mL A23109A/ha application rate. Lower dose rates (500, 250, 125 and 62.5 mL A23109A/ha) were prepared by serial dilution.

The spray solutions were not analysed to determine the active ingredient concentration and no statistical analysis was required for this study.

At the final assessments for seedling emergence and vegetative vigour the level of phytotoxicity was recorded using a visual scale of 0 to 10:

**Table A 59: Assessment of Injury Scale**

| Rating | Nominal % effect | Description of effects |
|---|---|---|
| 0 | 0 | Vigorous healthy plants, emergence of normal amount of seeds, indistinguishable from control |
| 1 | 10 | Vigorous, but with slight discoloration, malformation or stunting – slightly impaired emergence, growth or development |
| 2 | 20 | Less vigorous, with discoloration, malformation or stunting – slightly impaired growth and development, recovery likely, rate of emergence slightly reduced |
| 3 | 30 | Less vigorous, with obvious discoloration, malformation or stunting – impaired growth and development, recovery likely, rate of emergence reduced |
| 4 | 40 | Less vigorous, with more pronounced discoloration, malformation or stunting – recovery possible, clear reduction of rate of emergence |

| | | |
|---|---|---|
| 5 | 50 | Poor vigour due to discoloration, malformation or stunting – recovery possible, emergence rate only about half of the control |
| 6 | 60 | Poor vigour due to discoloration, malformation or stunting and senescence – recovery doubtful, emergence of only a minor part of the seeds |
| 7 | 70 | Very poor vigour due to discoloration, malformation, stunting or senescence – still growing but recovery unlikely, emergence of few seeds only |
| 8 | 80 | Very poor vigour due to severe discoloration, malformation, stunting or senescence – recovery unlikely, emergence of very few seeds only |
| 9 | 90 | Very poor vigour – not all tissue dead but further growth unlikely, only some germination |
| 10 | 100 | Complete destruction of plant parts above ground, complete inhibition of germination |

**Results**

The results of the visual observation of phytotoxicity in the vegetative vigour and seedling emergence tests are given in the tables below.

**Table A 60: Visual Observation of Phytotoxicity in the Vegetative Vigour Test**

| Application rate (mL A23109A/ha) | 0 (deionised water only) | 62.5 | 125 | 250 | 500 | 1000 |
|---|---|---|---|---|---|---|
| **Onion** | 0 | 0 | 0 | 0 | 0 | 0 |
| **Wheat** | 0 | 0 | 0 | 0 | 0 | 0 |
| **Sugar beet** | 0 | 0 | 0 | 0 | 0 | 0 |
| **Oilseed rape** | 0 | 0 | 0 | 0 | 0 | 0 |
| **Cucumber** | 0 | 0 | 0 | 0 | 0 | 0 |
| **Soybean** | 0 | 0 | 0 | 0 | 0 | 0 |

**Table A 61 Visual Observation of Phytotoxicity in the Seedling Emergence Test**

| Application rate (mL A23109A/ha) | 0 (deionised water only) | 62.5 | 125 | 250 | 500 | 1000 |
|---|---|---|---|---|---|---|
| **Onion** | 0 | 0 | 0 | 0 | 0 | 0 |
| **Wheat** | 0 | 0 | 0 | 0 | 0 | 0 |
| **Sugar beet** | 0 | 0 | 0 | 0 | 0 | 0 |
| **Oilseed rape** | 0 | 0 | 0 | 0 | 0 | 0 |
| **Cucumber** | 0 | 0 | 0 | 0 | 0 | 0 |
| **Soybean** | 0 | 0 | 0 | 0 | 0 | 0 |

**Validity criteria**

The test was considered valid;

- The control plants did not exhibit visible phytotoxic effects (*e.g.* chlorosis, necrosis, and wilting, leaf and stem deformation) and the plants exhibited only normal variation in growth and morphology for that particular species
- For the seedling emergence there was at least 70% mean emergence in the untreated control seedlings
- Environmental conditions for a particular species were identical and growing media contained the same amount of soil matrix, support media or substrate from the same source

**Conclusions**

**Seedling emergence:**

None of the tested species showed any phytotoxic effects up to and including the top does of 1000 mL A23109A/ha.

**Vegetative vigour:**

None of the tested species showed any phytotoxic effects up to and including the top does of 1000 mL A23109A/ha.

(Jones, K., 2020)

## A 2.6.2 KCP 10.6.2 Testing on non-target plants

## A 2.6.3 KCP 10.6.3 Extended laboratory studies on non-target plants

## A 2.6.4 KCP 10.6.4 Semi-field and field tests 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