REGISTRATION REPORT

# Part B
# Section 9
# Ecotoxicology

Detailed summary of the risk assessment

Product code: ADM.03500.F.2.B
(alternative codes: ADM.3500.F.2.B; MCW-2075)
Product name(s): see part A
Chemical active substance(s):
Prothioconazole, 250 g/L

Central zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: Country organisation/representative
as specified in Part A
Submission date: June 2021
MS Finalisation date: December 2022 (initial Core Assessment)
April 2023 (final Core Assessment)

# Version history

| When | What |
|---|---|
| 2021/06 | Version 1 Applicant |
| December 2022 | Initial zRMS assessment<br><br>The report in the dRR format has been prepared by the Applicant, therefore all comments, additional evaluations and conclusions of the zRMS are presented in grey commenting boxes. Minor changes are introduced directly in the text and highlighted in grey. Not agreed or not relevant information are ~~struck through and shaded for transparency~~. |
| April 2023 | Final report (Core Assessment updated following the commenting period)<br><br>Additional information/assessments included by the zRMS in the report in response to comments received from the cMS and the Applicant are highlighted in yellow. Information no longer relevant ~~is struck through and shaded~~. |

## DATA PROTECTION CLAIM

In order to present a dossier fully compliant with today's requirements (Reg. 284/2013), studies have been performed on ADM.03500.F.2.B. Under Article 59, Regulation 1107/2009/EC. On behalf of the Sponsor Company the applicant claims data protection for the studies conducted with ADM.03500.F.2.B. The data protection status and corresponding justification as valid for the respective country will be confirmed in the respective PART A.

## STATEMENT FOR OWNERSHIP

The summaries and evaluations contained in this document may be based on unpublished proprietary data submitted for the purpose of the assessment undertaken by the regulatory authority that prepared it.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authorities should not grant, amend, or renew a registration on the basis of the summaries and evaluation of unpublished proprietary data contained in this document unless they have received the data on which the summaries and evaluation are based, either –

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# 9 Ecotoxicology (KCP 10)

## 9.1 Critical GAP and overall conclusions

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

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Use-No. *** | **Member state(s)** | **Crop and/ or situation**<br><br>**(crop destination / purpose of crop)** | **F, Fn, Fpn G, Gn, Gpn or I **** | **Pests or Group of pests controlled**<br><br>(additionally: developmental stages of the pest or pest group) | **Application** | | | | **Application rate** | | | **PHI** (days) | **Remarks:**<br><br>e.g. g safener / synergist per ha | **Conclusion** | | | | | | |
| | | | | | Method / Kind | Timing / Growth stage of crop & season | Max. number a) per use b) per crop/ season | Min. interval between applications (days) | kg or L product / ha a) max. rate per appl. b) max. total rate per crop/season | g or kg as/ha<br><br>a) max. rate per appl. b) max. total rate per crop/season | Water L/ha<br><br>min / max | | | Birds | Mammals | Aquatic organisms | Bees | Non-target arthropods | Soil organisms | Non-target plants |
| **Zonal uses (field or outdoor uses, certain types of protected crops)** | | | | | | | | | | | | | | | | | | | | | |
| 1-4, 6-9, 11-15, 17-21, 23-26, 28-31, 33-36, 38-40, 42, 43, 45, 46 52-54, 169 | DE, AT, BE, NL, , IE, CZ, PL, SK, HU, SL | winter- & spring wheat, spring & winter barley triticale, rye, oats | F | *Septoria tritici, Drechslera tritici-repentis, Puccinia striiformis, Puccinia recondite, Fusarium + microdochium, Rhyncosporium secalis, Helmin-thosporium gra-mineum (Pyrenoph-ora teres), Ramu-laria collo-cygni, Puccinia hordei* | foliar, spraying, overall | BBCH 30-69 spring | a) 1 b) 1 | n.a. | a) 0.8 L/ha b) 0.8 L/ha | a) 200 b) 200 | 100 - 400 | n.r. | | A | A | R | A | A | A | A |

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Use-No. *** | **Member state(s)** | **Crop and/ or situation (crop destination / purpose of crop)** | **F, Fn, Fpn G, Gn, Gpn or I **** | **Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group) | **Application** | | | | **Application rate** | | | **PHI** (days) | **Remarks:** e.g. g safener / synergist per ha | **Conclusion** | | | | | | |
| | | | | | Method / Kind | Timing / Growth stage of crop & season | Max. number a) per use b) per crop/ season | Min. interval between applications (days) | kg or L product / ha a) max. rate per appl. b) max. total rate per crop/season | g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season | Water L/ha min / max | | | Birds | Mammals | Aquatic organisms | Bees | Non-target arthropods | Soil organisms | Non-target plants |
| 5, 10, 16, 22, 27, 32, 37, 41, 44, 47, 55 | DE, AT, BE, NL, , IE, CZ, PL, SK, HU, SL | winter- & spring oilseed rape | F | *Sclerotinia sclerotiorum Alternaria spp.* | foliar, spraying, overall | BBCH 50-73 spring | a) 1 b) 1 | n.a. | a) 0.7 L/ha b) 0.7 L/ha | a) 175 b) 175 | 100 - 400 | n.r. | | A | A | R | 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

** 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 “Conclusion”

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

**Remarks table:**

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

**zRMS comments:**

The report in the dRR format has been prepared by the Applicant, therefore all comments, additional evaluations and conclusions of the zRMS are presented in grey commenting boxes. Minor changes are introduced directly in the text and highlighted in grey. Not agreed or not relevant information ~~is struck~~ through and shaded for transparency.

## Overall conclusions

**zRMS comments:**

Conclusions presented in points 9.1.1.1 to 9.1.1.7 below were checked by the zRMS and amended where necessary.

### 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)

### 9.1.1.2 (KCP 10.1.3)

The risk assessment for terrestrial vertebrates was carried out according to the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438). No unacceptable risk for birds and mammals is expected for acute or long-term exposure to contaminated food indicated by $TER_A$ and $TER_{LT}$ values above the corresponding trigger values. Furthermore, no unacceptable risks are expected arising from other routes of direct exposure or secondary poisoning (residue uptake from drinking water or bioaccumulation in food chains). In conclusion, an acceptable overall risk for birds and mammals (and other terrestrial vertebrates) is indicated for the intended GAP uses of ADM.03500.F.2.B.

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

The risk assessment for aquatic organisms was carried out according to the Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (EFSA Journal 2013;11(7):3290). Based on Tier-1 (laboratory data) PEC/RAC calculations for the active substance prothioconazole and its metabolites potentially relevant in aquatic systems, no unacceptable risk for aquatic or sediment-dwelling organisms is indicated, if appropriate risk mitigation measures are applied (see table below). Further, the risk arising from bioaccumulation of the active substance and its metabolites is considered to be acceptable.

**Risk mitigation measures for:**

| Crop | Application rate [g a.s./ha] | BBCH | Risk mitigation measures |
|---|---|---|---|
| Spring cereals | 1× 200 | 30 | 10-m vegetated filter strip<br>(based on R1, R3 and R4, stream scenarios) |
| Spring cereals | 1× 200 | 69 | ~~No mitigation measures required~~<br>10-m vegetated filter strip<br>(based on R3 stream scenario~~)~~ |
| Winter cereals | 1× 200 | 30 | 10-m vegetated filter strip<br>(based on R1, stream, R3, stream and R4, stream scenarios) |
| Winter cereals | 1× 200 | 69 | 10-m vegetated filter strip<br>(based on R3, stream scenario) |
| Spring oilseed rape | 1× 175 | 50 | No mitigation measures required |
| Spring oilseed rape | 1× 175 | 73 | 10-m vegetated filter strip |

| | | | |
|---|---|---|---|
| | | | (based on R1 ~~R3,~~ stream scenario) |
| Winter oilseed rape | 1× 175 | 50 | 10-m vegetated filter strip<br>(based on R1, stream and R3, stream scenario) |
| Winter oilseed rape | 1× 175 | 73 | No mitigation measures required |

Concerned Member States must decide on applicability of indicated risk mitigation measures in their countries at the product authorisation.
Please note that additional aquatic risk assessment may be required by the concerned Member States that do not accept simulations performed according to FOCUS recommendations.

### 9.1.1.4 Effects on bees (KCP 10.3.1)

The evaluation of the acute risk for bees was performed in accordance with the recommendations of the Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002 rev.2 (final), October 17, 2002). Based on the Tier-1 risk assessment, it can be reasonably concluded that the intended GAP uses of ADM.03500.F.2.B in cereals and oilseed rape are of acceptable acute risk for bees under field conditions. Chronic and larval toxicity data for honeybees were submitted with the dossiers, since they are data requirements. ~~However, as for spray applications there is no noted Guidance on how to use this information in risk assessment, no deterministic chronic risk assessment for bees was provided by the applicant~~.

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

The risk assessment was conducted according to the ESCORT 2 Guidance Document (2000) and the Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002 rev 2 (final), October 17, 2002). Based on the results of worst-case laboratory tests with the standard test species *Aphidius rhopalosiphi* and *Typhlodromus pyri*, an overall acceptable risk for non-target arthropods colonised both in-field and off-field habitats can be concluded considering the intended GAP uses of ADM.03500.F.2.B in cereals and oilseed rape. Risk mitigation measures are not required.

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

The evaluation of the risk for soil organisms was performed in accordance with the recommendations of the Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002 rev 2 (final), October 17, 2002). Assessments were performed in consideration of the worst-case application scenario leading to maximum soil load, i.e. 1× 200 g a.s./ha (BBCH 30-69, 80 % crop interception) in cereals, covering the maximum application rates per crop and year.

*Soil macro- and mesofauna*

All $TER_{LT}$ values calculated for prothioconazole and its metabolites potentially relevant in soil are above the trigger values of 5, established for long-term exposure. Thus, an acceptable overall risk for earthworms and other soil organisms is indicated for the intended GAP uses of ADM.03500.F.2.B in cereals and oilseed rape.

*Soil microorganisms*

Effects within a range of ±25 % compared to the control were observed at exposure levels which exceed the maximum PEC values in soil calculated in consideration of the above-mentioned worst-case exposure scenario. Thus, an acceptable overall risk for soil microorganisms is indicated for the intended GAP uses of ADM.03500.F.2.B in cereals and oilseed rape.

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

The evaluation of the risk for non-target terrestrial plants 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). Based on the Tier 1 risk assessment ~~screening step recommended by the SANCO guideline for fungicides~~, a safe use (with respect to an acceptable risk for terrestrial non-target plants) can be concluded for the intended GAP uses of ADM.03500.F.2.B in cereals and oilseed rape. Risk mitigation measures are not required.

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

From the comprehensive set of ecotoxicity studies presented for ADM.03500.F.2.B (in addition to the toxicity data for the active substance and its metabolites), sufficient data are available for the assessment of the effects of ADM.03500.F.2.B to environmentally relevant species. Thus, further studies are not considered to be 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). The product ADM.03500.F.2.B is an emulsifiable concentrate (EC) containing 250 g/L of the active substance prothioconazole. It is a fungicide applied as spray to infested foliage of spring/winter cereals and oilseed rape. The timing of application is post-emergence (cereal BBCH 30-69 and oilseed BBCH 50-73). The worst-case application scenario leading to maximum soil load is a single post-emergence spray application at a rate of 200 g prothioconazole/ha to cereals (0.8 L prod./ha) and 175 g prothioconazole/ha to oilseed rape (0.7 L prod./ha). For a detailed summary of the GAP uses of ADM.03500.F.2.B, please refer to Table 9.1-1.

**Table 9.1-2: Critical use pattern of ADM.03500.F.2.B**

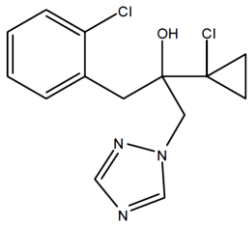
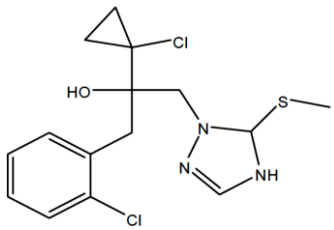
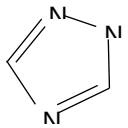
| **Grouping according to criterion** | | | |
|---|---|---|---|
| **Group** | **Intended uses** | **Relevant use parameters for grouping** | **Relevant exposure scenario** |
| **Effects on birds and mammals (*point CP 9.2 and CP 9.3*)** | | | |
| Field crops | Post-emergence application, (winter/spring) cereals, considering 1× 200 g a.s./ha, BBCH 30-69<br><br>Post-emergence application, (winter/spring) oilseed rape, considering 1× 175 g a.s./ha, BBCH 50-73 | Crop group according to EFSA Journal 2009; 7(12): 1438: Cereals & oilseed rape | Maximum application rates for cereals (1× 200 g/ha) and oilseed rape (1× 175 g/ha), considering indicator species (screening step) and generic focal species (Tier-1) relevant in treated fields according to EFSA exposure scenarios at time of application.<br>*Most critical routes of exposure:* Feeding on food items directly contaminated via spray application; bioaccumulation in food chains; residue uptake from drinking water. |
| **Effects on aquatic organisms (*point CP 9.5*)** | | | |
| Field crops | Post-emergence application, (winter/spring) cereals, considering 1× 200 g a.s./ha, BBCH 30-69<br><br>Post-emergence application, (winter/spring) oilseed rape, considering 1× 175 g a.s./ha, BBCH 50-73 | Crop group according to FOCUS (2001 & 2015): (spring/winter) cereals & oilseed rape | Maximum application rates for cereals (1× 200 g/ha) and oilseed rape (1× 175 g/ha), considering seasonal application rate scenarios as well as all relevant aquatic groups and calculated $PEC_{sw}$ values at FOCUS Step-1 to 4 (if required).<br>*Most critical routes of exposure:* Exposure in surfae water and sediment contaminated by spray drift, run-off and drainage |
| **Effects on bees (*point 9.6*)** | | | |
| Field crops | Post-emergence application, (winter/spring) cereals, considering 1× 200 g a.s./ha, BBCH 30-69<br><br>Post-emergence application, (winter/spring) oilseed rape, considering 1× 175 g a.s./ha, BBCH 50-73 | Crop group according to SANCO/10329/2002 rev.2 (final), October 17, 2002: Field crops | Maximum single application rate, i.e. 1× 0.8 L prod./ha [1× 200 g prothioconazole/ha]<br>*Most critical routes of exposure:* Acute contact and oral exposure from spray deposits (overspray, spray drift) and consumption of pollen and nectar from treated crops and weeds |

| Grouping according to criterion | | | |
|---|---|---|---|
| **Group** | **Intended uses** | **Relevant use parameters for grouping** | **Relevant exposure scenario** |
| **Effects on non-target arthropods (*point 9.7*)** | | | |
| Field crops | Post-emergence application, (winter/spring) cereals, considering 1× 200 g a.s./ha, BBCH 30-69<br><br>Post-emergence application, (winter/spring) oilseed rape, considering 1× 175 g a.s./ha, BBCH 50-73 | Crop group according to ESCORT 2 (2000): Field crops | Maximum application rate, i.e. 1× 0.8 L prod./ha [1× 200 g prothioconazole/ha] to field crops.<br><br>*Most critical routes of exposure:* Exposure via spray application in the in-field area and off-field area |
| **Effects on terrestrial soil meso-/macrofauna (*point CP 9.8*), soil microbial activity (*point CP 9.9*)** | | | |
| Field crops | Post-emergence application, (winter/spring) cereals, considering 1× 200 g a.s./ha, BBCH 30-69<br><br>Post-emergence application, (winter/spring) oilseed rape, considering 1× 175 g a.s./ha, BBCH 50-73 | Crop group according to SANCO/10329/2002 rev 2 (final), October 17, 2002: Cereals | 1× 200 g prothioconazole/ha, BBCH 30-69, considering 80 % crop interception<br>*Most critical routes of exposure:* Exposure in soil contaminated by spray application |
| **Effects on terrestrial non-target plants (*point 9.10*)** | | | |
| Field crops | Post-emergence application, (winter/spring) cereals, considering 1× 200 g a.s./ha, BBCH 30-69<br><br>Post-emergence application, (winter/spring) oilseed rape, considering 1× 175 g a.s./ha, BBCH 50-73 | Crop group according to SANCO/10329/2002 rev 2 (final), October 17, 2002: Field crops | Maximum single application rate, i.e. 1× 0.8 L prod./ha [1× 200 g prothioconazole/ha], as recommended by the guidance document for fungicides<br><br>*Most critical routes of exposure:* Exposure via spray application in the off-field area |

## 9.1.3 Consideration of metabolites

A list of metabolites found in environmental compartments in relevant amounts is provided below. The need for conducting a metabolite-specific risk assessment in the context of the evaluation of ADM.03500.F.2.B is indicated in the table.

**Table 9.1-3 Metabolites of prothioconazole potentially relevant in the environment**

| Metabolite | Molar mass | Chemical structure | Maximum observed occurrence in compartments | Risk assessment required? |
|---|---|---|---|---|
| Prothioconazole-desthio (M04) (JAU-desthio) | 312.2 g/mol |  | ~~soil: 57.1 %~~<br>~~water/sediment: 32.3 %~~ | **Yes**<br>Secondary poisoning terrestrial vertebrates;<br>Aquatic and sediment-dwelling organisms;<br>Soil macro- and mesofauna;<br>microorganisms |
| Prothioconazole-S-methyl (M01) (JAU-S-methyl) | 358.3 g/mol |  | ~~soil: 14.6 %~~<br>~~water/sediment: 77 %~~<br>~~(anaerobic)~~ | **Yes**<br>Secondary poisoning terrestrial vertebrates;<br>Aquatic and sediment-dwelling organisms;<br>Soil macro- and mesofauna;<br>microorganisms |
| 1,2,4-triazole (M13) | 69.065 g/mol |  | ~~water/sediment: 37.2 %~~ | **Yes**<br><br>aquatic and sediment-dwelling organisms; |

**zRMS comments:**

Metabolites relevant for soil and water compartment listed in Table 9.1-3 are the same as indicated in EFSA Scientific Report (2007) 106. It is noted that in the course of the EU review of prothioconazole metabolite JAU 6476-thiazocine was formed at >10% in photodegradation study in water, however according to EFSA Scientific Report (2007) 106, it was considered to be not relevant for evaluation in area of ecotoxicology.

The maximum occurrence is relevant for exposure evaluation, for information agreed in this area please refer to the Core Assessment, Part B, Section 8, where all respective data are provided and used in calculation of $PEC_{soil}$ and $PEC_{sw/sed}$ values, considered further in the risk assessment.
As the information on the maximum occurrence was not checked in detail, it was struck through in Table 9.1-3.

## 9.2 Effects on birds (KCP 10.1.1)

### 9.2.1 Toxicity data

Avian toxicity studies have been carried out with the active substance prothioconazole and its metabolite JAU-desthio. Full details of these studies are provided in the respective EU DAR and related documents. Effects on birds of ADM.03500.F.2.B were not evaluated as part of the EU assessments of prothioconazole.

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

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| Bobwhite quail (*Colinus virginianus*) | Prothioconazole technical | Acute toxicity | **$LD_{50}$ > 2000 mg a.s./kg bw** | EFSA Scientific Report (2007) 106, 1-98 |
| Bobwhite quail (*Colinus virginianus*) | Prothioconazole technical | Dietery 5 d Short-term | $LD_{50}$ > 1413 mg a.s/kg bw | EFSA Scientific Report (2007) 106, 1-98 |
| Mallard duck (*Anas platyrhynchos)* | Prothioconazole technical | Dietary 5 d Short-term | $LD_{50}$ > 2457 mg a.s./kg bw/day | EFSA Scientific Report (2007) 106, 1-98 |
| Bobwhite quail (*Colinus virginianus*) | JAU-desthio (M4) | Acute toxicity | $LD_{50}$ > 2000 mg met./kg bw | EFSA Scientific Report (2007) 106, 1-98 |
| Bobwhite quail (*Colinus virginianus*) | JAU-desthio (M4) | Dietary 5 d Short-term | **$LD_{50}$ > 297mg met./kg bw** | EFSA Scientific Report (2007) 106, 1-98 |
| Mallard duck (*Anas platyrhynchos*) | Prothioconazole technical | Reproductive toxicity | **NOEL = 78 mg a.s./kg bw/d** | EFSA Scientific Report (2007) 106, 1-98 |
| Bobwhite quail (*Colinus virginianus*) | JAU-desthio (M4) | Reproductive toxicity | **NOEL = 14.8 mg met./kg bw/d** | EFSA Scientific Report (2007) 106, 1-98 |

Birds are typically exposed to dry residues on their food items following the dilution and spraying of the formulated product. During these processes, much of the formulation constituents are likely to be lost by volatilisation. Since oral exposure is the main route of exposure, toxicity data for the active substance are therefore used in preference to data from tests with the formulated material. On this basis, the risk to birds and mammals from the proposed uses of ADM.03500.F.2.B will be assessed using data on the active substance prothioconazole. Exposure to ADM.03500.F.2.B via dermal and inhalation routes is considered unlikely, since at the time of application and for a short period thereafter, most wild birds and mammals will leave the immediate vicinity of spray operations in response to the human disturbance.

Furthermore, there is considered no increased risk from the formulated prothioconazole over that posed from the technical grade based on the mammalian toxicity data package and thus, the risk assessment for the formulation is covered by that for the active substance.

According to the current guidance document provided by EFSA, a separate short-term risk assessment is not intended and hence, it is recommended that the short-term dietary toxicity test is no longer part of the core data package. Instead, dietary effects are covered by the acute oral toxicity test resulting in a $LD_{50}$ as relevant endpoint that should be used for the $TER_A$ calculations.

**Metabolites**

JAU-desthio (M4) was considered to be the only major metabolite in foliage (EFSA Scientific Report (2007) 106) and acute and chronic toxicity studies were available to assess the risk. A total conversion of prothioconazole to the desthio metabolite was assumed at the screening level and in the Tier-1 assessment.

In conclusion, it is deemed acceptable to use a $LD_{50}$ of > 2000 mg/kg bw for the acute risk assessment for prothioconazole ~~as well as and~~ $LD_{50}$>297-mg a.s./kg bw for its metabolite JAU-desthio (M4). For the reproductive risk assessment, a NOEL of 78 mg/kg bw/d was considered for the parent compound and a NOEL of 14.8 mg/kg bw/d for the metabolite.

**zRMS comments:**

Avian toxicity data for prothioconazole and prothioconazole metabolite JAU 6476-desthio provided in Table 9.2-1 above were verified by zRMS and then confirmed that they are in line with EU agreed endpoints reported in EFSA EFSA Scientific Report (2007) 106.

It is noted that for the acute risk assessment the Applicant selected acute toxicity endpoints for both active compounds, which is considered acceptable by zRMS although that for the a.s.- prothioconazole lower endpoint from short-term study with $LD_{50}$ of 1413 mg p.m./kg is available.

In zRMS's opinion as no treatment related mortalities were observed in the short-term toxicity study for the a.s prothioconazole indicating that the dietary exposure have not resulted with increased mortality of tested birds and the acute $LD_{50}$>2000 kg a.s./kg bw is sufficiently protective to use in the risk assessment.

In case of the acute risk for metabolite JAU 6476-desthio acute $LD_{50}$ >2000 mg pm/kg bw is used by the Applicant, while short-term dietary studies with this compound with lower $LD_{50}$ of **297 mg pm/kg bw/d** should be considered as treatment related mortalities were observed in these short-term dietary studies.

### 9.2.1.1 Justification for new endpoints

No new endpoints are proposed.

## 9.2.2 Risk assessment for spray applications

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

The product ADM.03500.F.2.B is an emulsifiable concentrate (EC) containing 250 g/L of the active substance prothioconazole. It is a fungicide applied as spray to infested foliage of (spring/winter) cereals and oilseed rape. The timing of application is post-emergence (cereal BBCH 30-69 and oilseed BBCH 50-73). The worst-case application scenario leading to maximum soil load is a single post-emergence spray application at a rate of 200 g prothioconazole/ha to cereals (0.8 L prod./ha) and 175 g prothioconazole/ha to oilseed rape (0.7 L prod./ha). For a detailed summary of the GAP uses of ADM.03500.F.2.B, please refer to Table 9.1-1.

Considering these GAP uses, the major potential routes of critical exposure were considered to be feeding on food items (e.g. vegetation and invertebrates) directly contaminated via spray application of the plant protection product.

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

**Screening assessment**

For the initial screening assessment, “indicator species” and exposure scenarios were selected as recommended in EFSA Journal 2009; 7(12): 1438. According to this guidance document, an “indicator species” is not a real species but by virtue of its size and feeding habits is considered to have higher exposure than other species that occur in a particular crop at a particular time.

In other words, if a low risk is estimated for the indicator species of concern, then an overall low risk can be concluded for all other (real) avian species exposed to ADM.03500.F.2.B. A summary of the intended uses and relevant avian indicator species is given in the table below.

**Table 9.2-2: Worst-case GAP use of ADM.03500.F.2.B and corresponding avian indicator species relevant for the screening assessments**

| Crop | Worst-case application scenario | Indicator species | Shortcut value for $TER_A/TER_{LT}$ |
|---|---|---|---|
| Cereals | Post-emergence,<br>BBCH 30-69,<br>1× 200 g/ha | Small omnivorous bird | 158.8 / 64.8 |
| Oilseed rape | Post-emergence,<br>BBCH 50-73,<br>1× 175 g/ha | | |

Exposure of terrestrial vertebrates to ADM.03500.F.2.B expressed as Daily Dietary Dose (DDD) was assessed separately for acute ($DDD_A$) and long-term exposure ($DDD_{LT}$). The DDD values were calculated according to the formula derived from the current EFSA guidance document. For the acute exposure assessment, shortcut values for 90$^{th}$ percentile RUDs ($SV_{90th}$) were taken into account as recommended in EFSA Journal 2009; 7(12): 1438.

For long-term exposure estimates, a time-frame of a few weeks after application is considered. Since the area of birds feeding on contaminated diet will be largely compared to the spatial scale of residue variation, shortcut values for mean percentile RUDs ($SV_m$) should be used. Furthermore, time-weighted average residues are considered to reflect long-term exposure in a more realistic manner in view of a residue decrease in relevant food over time.

According to the recommendations of current guidance, i.e. in consideration of a residue decline with a default first order $DT_{50}$ of 10 days and a time scale of 21 days, the time-weighted average factor is TWA = 0.53. Multiple Application Factors (MAF) were not taken into account with respect to the single application scenario of ADM.03500.F.2.B.

The risk for birds was assessed by calculating Toxicity Exposure Ratios (TER) considering the toxicity endpoints above and exposure expressed as Daily Dietary Dose (DDD). The results are presented in the table below.

## Prothioconazole

**Table 9.2-3: Screening assessment of the acute and long-term risk for birds due to the use of ADM.03500.F.2.B in cereals**

| | | | | | |
|---|---|---|---|---|---|
| **Intended use** | Cereals, 1× 200 g a.s./ha, BBCH 30 – 69 | | | | |
| **Active substance** | Prothioconazole | | | | |
| **Application rate (g/ha)** | 1× 200 | | | | |
| **MAF** | 1.0 | | | | |
| **Acute toxicity (mg/kg bw)** | > 2000 | | | | |
| **TER criterion** | 10 | | | | |
| **Crop scenario<br>Growth stage** | **Indicator species** | **$SV_{90}$** | **$MAF_{90}$** | **$DDD_{90}$<br>(mg/kg bw/d)** | **$TER_A$** |
| BBCH > 10 | Small omnivorous bird | 158.8 | 1.0 | 31.8 | > 63.0 |
| **Long-term toxicity<br>(mg/kg bw/d)** | 78 | | | | |
| **TER criterion** | 5 | | | | |
| **Crop scenario<br>Growth stage** | **Indicator species** | **$SV_m$** | **$MAF_m$ ×<br>TWA** | **$DDD_m$<br>(mg/kg bw/d)** | **$TER_{LT}$** |
| BBCH > 10 | Small omnivorous bird | 64.8 | 0.53 | 6.9 | 11.4 |

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

As outlined in the table above, the calculated $TER_A$ and $TER_{LT}$ values for the exposure to prothioconazole are above the trigger of 10 and 5, established for acute and long-term exposure, indicating an overall acceptable risk for birds in cereals already at screening level. Thus, no further refinements at Tier-1 level are required for prothioconazole.

**Table 9.2-4: Screening assessment of the acute and long-term risk for birds due to the use of ADM.03500.F.2.B in oilseed rape**

| | | | | | |
|---|---|---|---|---|---|
| **Intended use** | Oilseed rape, 1× 175 g a.s./ha, BBCH 50 - 73 | | | | |
| **Active substance** | Prothioconazole | | | | |
| **Application rate (g/ha)** | 1× 175 | | | | |
| **MAF** | 1.0 | | | | |
| **Acute toxicity (mg/kg bw)** | > 2000 | | | | |
| **TER criterion** | 10 | | | | |
| **Crop scenario<br>Growth stage** | **Indicator species** | **$SV_{90}$** | **$MAF_{90}$** | **$DDD_{90}$<br>(mg/kg bw/d)** | **$TER_A$** |
| BBCH > 10 | Small omnivorous bird | 158.8 | 1.0 | 27.8 | > 72.0 |
| **Long-term toxicity<br>(mg/kg bw/d)** | 78 | | | | |
| **TER criterion** | 5 | | | | |
| **Crop scenario<br>Growth stage** | **Indicator species** | **$SV_m$** | **$MAF_m$ × TWA** | **$DDD_m$<br>(mg/kg bw/d)** | **$TER_{LT}$** |
| BBCH > 10 | Small omnivorous bird | 64.8 | 0.53 | 6.0 | 13.0 |

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

As outlined in the table above, the calculated $TER_A$ and $TER_{LT}$ values for the exposure to prothioconazole are above the trigger of 10 and 5, established for acute and long-term exposure, indicating an overall acceptable risk for birds in oilseed rape already at screening level. Thus, no further refinements at Tier-1 level are required for prothioconazole.

## JAU-desthio (M4)

**Table 9.2-5: Screening assessment of the acute and long-term risk for birds due to the use of ADM.03500.F.2.B in cereals**

| | | | | | |
|---|---|---|---|---|---|
| **Intended use** | Cereals, 1× 200 g a.s./ha, BBCH 30 – 69 | | | | |
| **Active substance** | JAU-desthio (M4) | | | | |
| **Application rate (g/ha)** | 1× 200* | | | | |
| **MAF** | 1.0 | | | | |
| **Acute toxicity (mg/kg bw)** | > 297 ~~2000~~ | | | | |
| **TER criterion** | 10 | | | | |
| **Crop scenario<br>Growth stage** | **Indicator species** | **$SV_{90}$** | **$MAF_{90}$** | **$DDD_{90}$<br>(mg/kg bw/d)** | **$TER_A$** |
| BBCH > 10 | Small omnivorous bird | 158.8 | 1.0 | 31.8 | > 9.33 ~~63.0~~ |

| | | | | | |
|---|---|---|---|---|---|
| **Intended use** | Cereals, 1× 200 g a.s./ha, BBCH 30 – 69 | | | | |
| **Active substance** | JAU-desthio (M4) | | | | |
| **Application rate (g/ha)** | 1× 200* | | | | |
| **MAF** | 1.0 | | | | |
| **Long-term toxicity (mg/kg bw/d)** | 14.8 | | | | |
| **TER criterion** | 5 | | | | |
| **Crop scenario Growth stage** | **Indicator species** | **$SV_m$** | **$MAF_m$ × TWA** | **$DDD_m$ (mg/kg bw/d)** | **$TER_{LT}$** |
| BBCH > 10 | Small omnivorous bird | 64.8 | 0.53 | 6.9 | **2.2** |

Bold: below the relevant trigger, indicating an unacceptable risk at this assessment level
SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio
* TER calculation for the metabolite JAU-desthio was conducted with the application rate of the parent compound prothioconazole – representing an absolute worst-case approach

As outlined in the table above, the calculated $TER_A$ value for the exposure to JAU-desthio (M4) is below ~~above~~ the trigger of 10, indicating an unacceptable acute risk for birds in cereals ~~already~~ at screening level. ~~By contrast,~~ The $TER_{LT}$ value is also below the trigger of 5, and thus a Tier-1 acute and long-term risk assessment for the metabolite of concern is required.

**Table 9.2-6: Screening assessment of the acute and long-term risk for birds due to the use of ADM.03500.F.2.B in oilseed rape**

| | | | | | |
|---|---|---|---|---|---|
| **Intended use** | Oilseed rape, 1× 175 g a.s./ha, BBCH 50 – 73 | | | | |
| **Active substance** | JAU-desthio (M4) | | | | |
| **Application rate (g/ha)** | 1× 175* | | | | |
| **MAF** | 1.0 | | | | |
| **Acute toxicity (mg/kg bw)** | > 297 ~~2000~~ | | | | |
| **TER criterion** | 10 | | | | |
| **Crop scenario Growth stage** | **Indicator species** | **$SV_{90}$** | **$MAF_{90}$** | **$DDD_{90}$ (mg/kg bw/d)** | **$TER_A$** |
| BBCH > 10 | Small omnivorous bird | 158.8 | 1.0 | 27.8 ~~31.8~~ | > 10.7 ~~63.0~~ |
| **Long-term toxicity (mg/kg bw/d)** | 14.8 | | | | |
| **TER criterion** | 5 | | | | |
| **Crop scenario Growth stage** | **Indicator species** | **$SV_m$** | **$MAF_m$ × TWA** | **$DDD_m$ (mg/kg bw/d)** | **$TER_{LT}$** |
| BBCH > 10 | Small omnivorous bird | 64.8 | 0.53 | 6.0 | **2.5** |

Bold: below the relevant trigger, indicating an unacceptable risk at this assessment level
SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio
* TER calculation for the metabolite JAU-desthio was conducted with the application rate of the parent compound prothioconazole – representing an absolute worst-case approach

As outlined in the table above, the calculated $TER_A$ value for the exposure to JAU-desthio (M4) is above the trigger of 10, indicating an unacceptable acute risk for birds in oilseed rape already at screening level. By contrast, the $TER_{LT}$ value is below the trigger of 5, and thus a Tier-1 long-term risk assessment for the metabolite of concern is required.

**zRMS comments:**

Screening step in the risk assessment

The screening step risk assessment for prothioconazole is validated by zRMS.
$TER_A$ and $TER_{LT}$ values for the exposure to prothioconazole are above the trigger of 10 and 5 for acute and long-term exposure, indicating acceptable risk for birds.
It should be noted the acute risk for metabolite JAU 6476-desthio was performed by the Applicant with consideration of the acute $LD_{50}$ of >2000 mg pm/kg bw, while the toxicity endpoint from dietary study was more relevant for purposes of the acute risk assessment for this metabolite (please see in the commenting boxes under Table 9.2-1).
The evaluation presented in Table 9.2 - 5 and Table 9.2 - 6 above were amended accordingly with consideration of the $LD_{50}$ of 297 mg pm/kg bw/d.
In case of acute and long-term risk assessment by using the parent application for metabolite JAU 6476-desthio (assuming 100% conversion into JAU-desthio as a worst-case approach), the $TER_A$ and $TER_{LT}$ values are below the trigger of 10 and 5, respectively and Tier - 1 acute (for cereals only) and long-term risk assessment (for cereals and oilseed rape) for the metabolite of concern was required.

## Tier-1 risk assessment

For the Tier-1 risk assessment, "generic focal species" and exposure scenarios were selected as recommended in EFSA Journal 2009; 7(12): 1438. According to this current guidance document, a "generic focal species" *is not a real species, however it is considered to be representative of all those species potentially at risk*. In other words, if a low risk is estimated for the generic focal species of concern, then an overall low risk can be concluded for all other (real) avian species exposed to ADM.03500.F.2.B. A summary of the critical GAP uses and relevant avian indicator species is given in the table below.

**Table 9.2-7: Critical use pattern of ADM.03500.F.2.B and corresponding avian generic focal species relevant for Tier-1 acute and long – term assessments**

| Crop | Worst-case application scenario | EFSA crop group | EFSA Tier-1 scenario | Generic focal species (Representative) | Shortcut value for~~TER$_{LT}$~~ |
|---|---|---|---|---|---|
| **Tier 1 acute risk** | | | | | |
| Cereals | Post-emergence, BBCH 30-69, 1× 200 g/ha | Cereals | BBCH 30-39 | small omnivorous bird (lark) | 12.0 |
| | | | BBCH ≥ 40 | small omnivorous bird (lark) | 7.2 |
| **Tier 1 long-term risk** | | | | | |
| Cereals | Post-emergence, BBCH 30-69, 1× 200 g/ha | Cereals | BBCH 30 ~~10~~-39 | small omnivorous bird (lark) | 5.4 |
| | | | BBCH ≥ 40 | small omnivorous bird (lark) | 3.3 |
| **Tier 1 long-term risk** | | | | | |
| Oilseed rape | Post-emergence, BBCH 50-73, 1× 175 g/ha | Oilseed rape | BBCH 30-99 | Small insectivorous bird (dunnock) | 2.7 |
| | | | BBCH 30-39 | small omnivorous bird (lark) | 3.3 |
| | | | BBCH ≥ 40 | small omnivorous bird (lark) | 2.7 |
| | | | BBCH 30-39 | medium herbivorous/ granivorous bird (pigeon | 1.1 |
| | | | BBCH ≥ 40 | medium herbivorous/ granivorous bird (pigeon) | 0.9 |

The risk for birds was assessed by calculating Toxicity Exposure Ratios (TER) considering the toxicity

endpoints above and exposure expressed as Daily Dietary Dose (DDD). The results are presented in the table below.

### JAU-desthio (M4)

**Table 9.2-8: Tier-1 assessment of the long-term risk for birds due to the use of ADM.03500.F.2.B in cereals**

| | | | | | |
|---|---|---|---|---|---|
| **Intended use** | 1× 200 g a.s./ha, BBCH 30 – 69 | | | | |
| **Active substance** | JAU-desthio (M4) | | | | |
| **Application rate (g/ha)** | 1× 200* | | | | |
| **MAF** | 1.0 | | | | |
| **Long-term toxicity (mg/kg bw/d)** | 14.8 | | | | |
| **TER criterion** | 5 | | | | |
| **Crop scenario<br>Growth stage** | **Generic focal species** | **$SV_m$** | **$MAF_m$ × TWA** | **$DDD_m$ (mg/kg bw/d)** | **$TER_{LT}$** |
| Cereals, BBCH 30 ~~10~~-39 | Small omnivorous bird (lark) | 5.4 | 0.53 | 0.6 | 24.6 ~~25.9~~ |

| | | | | | |
|---|---|---|---|---|---|
| **Intended use** | 1× 200 g a.s./ha, BBCH 30 – 69 | | | | |
| **Active substance** | JAU-desthio (M4) | | | | |
| **Application rate (g/ha)** | 1× 200* | | | | |
| **MAF** | 1.0 | | | | |
| Cereals, BBCH ≥ 40 | Small omnivorous bird (lark) | 3.3 | 0.53 | 0.3 | 42.3 |

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio
* TER calculation for the metabolite JAU-desthio (M4) was conducted with the application rate of the parent compound prothioconazole – representing an absolute worst-case approach

As outlined in the table above, the calculated $TER_{LT}$ values for the exposure to JAU-desthio are above the trigger of 5, established for long-term exposure, indicating an acceptable long-term risk for birds in cereals at Tier-1 level (under still worst-case exposure assumptions). Thus, no further refinements are considered to be required for the metabolite.

**Table 9.2-9: Tier-1 assessment of the long-term risk for birds due to the use of ADM.03500.F.2.B in oilseed rape**

| | | | | | |
|---|---|---|---|---|---|
| **Intended use** | 1× 175 g a.s./ha, BBCH 50 – 73 | | | | |
| **Active substance** | JAU-desthio (M4) | | | | |
| **Application rate (g/ha)** | 1× 175* | | | | |
| **MAF** | 1.0 | | | | |
| **Long-term toxicity (mg/kg bw/d)** | 14.8 | | | | |
| **TER criterion** | 5 | | | | |
| **Crop scenario Growth stage** | **Generic focal species** | **$SV_m$** | **$MAF_m$ × TWA** | **$DDD_m$ (mg/kg bw/d)** | **$TER_{LT}$** |
| Oilseed rape, BBCH 30-99 | Small insectivorous bird (dunnock) | 2.7 | 0.53 | 0.25 ~~0.3~~ | 59.2 ~~44~~ |
| Oilseed rape, BBCH ≥ 40 | Small omnivorous bird (lark) | ~~2.7~~ | 0.53 | 0.25 ~~0.3~~ | 59.1 |
| BBCH 30-39 | small omnivorous bird (lark) | 3.3 | 0.53 | 0.31 | 47.7 |
| Oilseed rape, BBCH ≥ 40 | Medium herbivorous/ granivorous bird (pigeon) | 0.9 | 0.53 | 0.08 | 185 ~~177.3~~ |
| BBCH 30-39 | medium herbivorous/ granivorous bird (pigeon) | 1.1 | 0.53 | 0.1 | 148 |

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio
* TER calculation for the metabolite JAU-desthio (M4) was conducted with the application rate of the parent compound prothioconazole – representing an absolute worst-case approach

As outlined in the table above, the calculated $TER_{LT}$ values for the exposure to JAU-desthio are above the trigger of 5, established for long-term exposure, indicating an acceptable long-term risk for birds in oilseed rape at Tier-1 level (under still worst-case exposure assumptions). Thus, no further refinements are considered to be required for the metabolite.

**zRMS comments:**

**Tier 1 acute risk assessment**

**JAU-desthio (M4)**

Based on screening acute risk assessment performed at Table 9.2 - 5 and Tables 9.2 - 6 there is needs for further refinement. Therefore, Tier 1 acute risk assessment for cereals is performed by zRMS taking into account relevant species listed in the Table 9.2 - 7.

**Tier-1 assessment of acute risk for birds due to the use of ADM.03500.F.2.B in cereals.**

| | | | | | |
|---|---|---|---|---|---|
| **Intended use** | 1× 200 g a.s./ha, BBCH 30 – 69 | | | | |
| **Active substance** | JAU-desthio (M4) | | | | |
| **Application rate (g/ha)** | 1× 200* | | | | |
| **MAF** | 1.0 | | | | |
| **Acute toxicity (mg/kg bw/d)** | 297 | | | | |
| **TER criterion** | 10 | | | | |
| **Crop scenario Growth stage** | **Generic focal species** | **SVa** | **MAF** | **$DDD_m$ (mg/kg bw/d)** | **$TER_A$** |
| Cereals, BBCH 30 - 39 | small omnivorous bird (lark) | 12.0 | 1 | 2.40 | 123.8 |
| Cereals, BBCH ≥ 40 | small omnivorous bird (lark) | 7.2 | 1 | 1.44 | 206.3 |

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio
*TER calculation for the metabolite JAU-desthio (M4) was conducted with the application rate of the parent compound prothioconazole – representing an absolute worst-case approach

**Tier 1 long-term risk assessment**

The evaluation of the long-term risk for birds presented in Table 9.2-8 indicated acceptable risk in cereals.
The evaluation of the long-term risk for birds presented in Table 9.2-9 was amended with consideration of the relevant species and shortcut values for oilseed rape.

Overall, based on Applicants' and zRMS calculations, acceptable risk to birds from compounds active substance and metabolite JAU 6476-desthio may be concluded from the intended uses of ADM.03500.F.2.B.

### 9.2.2.2 Higher-tier risk assessment

Not considered to be 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 and a drinking water uptake rate of 0.46 L/kg bw/d (see Appendix K of EFSA Journal 2009; 7(12): 1438).

**Leaf scenario**

Since ADM.03500.F.2.B is not intended to be applied on leafy vegetables forming heads or crop plants with comparable water collecting structures at principal growth stage 4 or later, the leaf scenario does not have to be considered.

**Puddle scenario**

Due to the characteristics of the exposure scenario in connection with the standard assumptions for water

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

With K(f)oc > 500, the active substance prothioconazole ($K_{OC}$ = 1765) as well as the metabolite JAU-desthio (M4) ($K_{OC}$ = 523-625) belong to the group of more sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the worst-case application scenario (i.e. the maximum seasonal application rate of 1× 200 g/ha in cereals) covers the risk for water-drinking birds from all intended GAP uses of ADM.03500.F.2.B (for details, see point 9.1.2).

**Prothioconazole**

| | | | | |
|---|---|---|---|---|
| Effective application rate (g/ha) | = | 1× 200 | | |
| Acute toxicity (mg/kg bw) | = | > 2000 | quotient = | < 0.1 |
| Reprod. toxicity (mg/kg bw/d) | = | 78 | quotient = | 2.6 |

**JAU-desthio (M4)**

| | | | | |
|---|---|---|---|---|
| Effective application rate (g/ha) | = | 1× 200 | | |
| Acute toxicity (mg/kg bw) | = | > 297 ~~2000~~ | quotient = | < 0.7 ~~0.1~~ |
| Reprod. toxicity (mg/kg bw/d) | = | 14.8 | quotient = | 13.5 |

Since the ratio of effective application rate to relevant endpoint does not exceed the trigger of 3000 for more sorptive substances, no further considerations have to be taken into account.

**zRMS comment:**

The acceptable risk to birds via drinking water may be concluded for prothioconazole and its metabolite.

### 9.2.2.4 Effects of secondary poisoning

Based on a log $P_{OW}$ > 3 for the active substance prothioconazole (i.e. log Pow of 3.82 at pH 7; for details, see EFSA Scientific report (2007) 106, 1- 98), a potential for bioaccumulation has to be considered for this compound on a hypothetical basis. Thus, appropriate risk assessments were performed for exposure from accumulation in food chains in agreement with the current guidance document.
*Metabolites*

As outlined in the underlying residue definitions in the EFSA Scientific report (2007) 106, 1-98, the following metabolites in soil and surface water may have to be considered for the assessment:

| Compound | Major metabolite in | Log $P_{OW}$ |
|---|---|---|
| JAU-desthio (M4) | Soil, surface water | 3.04 |
| JAU-S-methyl (M1) | Soil, surface water | 4.19 |
| 1,2,4-triazole | Surface water | < 3 |

In conclusion, a potential for bioaccumulation may be expected for the active substance prothioconazole and its metabolites JAU-desthio (M4) and JAU-S-methyl (M1). Consequently, a deterministic risk assessment by calculating TER values was performed only for these compounds of concern.

Food chain from earthworm to earthworm-eating birds
Residues in worms and the estimated theoretical exposure of earthworm eating birds were calculated with the following formulae:

**Equation 1: Calculation of Daily Dietary Dose for earthworm-eating birds and mammals**

$$\mathbf{DDD = PEC_{worm} \cdot f_{conv}} \quad \text{[mg/kg bw/d]}$$

where (1) $PEC_{worm} = PEC_{soil} \cdot BCF$

(2) $$BCF = \frac{(0.84 + 0.012 \cdot P_{ow})}{f_{oc} \cdot K_{oc}}$$

and
- $PEC_{worm}$ = predicted concentration in earthworms [mg/kg]
- $f_{conv}$ = factor in order to convert $PEC_{worm}$ to daily dose
- $PEC_{soil}$ = 3-week $PEC_{twa}$ in soil [mg/kg soil dry wt]
- BCF = bioconcentration factor in earthworms
- $P_{OW}$ = octanol/water partition coefficient
- $f_{OC}$ = organic carbon content of soil
- $K_{OC}$ = organic carbon adsorption coefficient

## Prothioconazole

A log $P_{OW}$ of 3.82 at pH 7 was determined for prothioconazole corresponding to a $P_{OW}$ of 6607. Using this $P_{OW}$, the $K_{OC}$ of 1765 (for details, see EFSA Scientific report (2007) 106, 1-98) and a default value of 0.02 for $f_{OC}$, the calculated bioconcentration factor in worms is 2.270. According to the recommendations of EFSA Journal 2009; 7(12): 1438, the $PEC_{twa(21\text{-}d)}$ in the upper 5 cm soil layer should be used for the $PEC_{worm}$ calculation.

As outlined under point 8.7 of Section 8 (*Environmental fate*), a maximum $PEC_{twa(21\text{-}d)}$ in soil of 0.010 mg a.s./kg was calculated for the intended GAP uses of ADM.03500.F.2.B in cereals. The relevant $TER_{LT}$ value for the generic standard bird (100-g bird eating 104.6 g per day) was based on the estimated residue in worms and long-term toxicity endpoints for birds already used in the risk assessment above.

**Table 9.2-10: Daily Dietary Dose (DDD) and Tier-1 $TER_{LT}$ for earthworm-eating birds**

| Species | $PEC_{twa(21-d)}$ [mg/kg] | | BCF | $PEC_{worm}$ [mg/kg] | $f_{conv}$ | DDD [mg/kg bw/d] | Endpoint [mg/kg bw/d] | | $TER_{LT}$ [1)] | TER trigger |
|---|---|---|---|---|---|---|---|---|---|---|
| **Cereals, 1× 0.200 kg a.s./ha, BBCH 30-69 (worst-case approach)** | | | | | | | | | | |
| Bird, 100 g | Prothiocon-azole | 0.010* | 2.270 | 0.023 | 1.05 | 0.026 | NOEL | 78 | 3273 | 5 |

1) According to EFSA Journal 2009; 7(12): 1438, only the long-term risk needs to be considered
*PECs 21TWA

As outlined in the table above, the $TER_{LT}$ value is above 5. Thus, an acceptable risk for earthworm-eating birds can be concluded. No further considerations have to be taken into account.

**JAU-desthio (M4)**

A $logP_{OW}$ of 3.04 was determined for the metabolite JAU-desthio (M4) corresponding to a $P_{OW}$ of 1096. Using this $P_{OW}$, the 575.4 ($K_{OC}$ = 523-625, n = 4; for details, see EFSA Scientific report (2007) 106, 1-98) and a default value of 0.02 for $f_{OC}$, the calculated bioconcentration factor in worms is 1.216. According to the recommendations of EFSA Journal 2009; 7(12): 1438, the $PEC_{twa(21-d)}$ in the upper 5 cm soil layer should be used for the $PEC_{worm}$ calculation.

As outlined under point 8.7 of Section 8 (*Environmental fate*), a maximum $PEC_{twa(21-d)}$ in soil of 0.021 mg met./kg was calculated for the intended GAP uses of ADM.03500.F.2.B in cereals. The relevant $TER_{LT}$ value for the generic standard bird (100-g bird eating 104.6 g per day) was based on the estimated residue in worms and long-term toxicity endpoints for birds already used in the risk assessment above.

**Table 9.2-11: Daily Dietary Dose (DDD) and Tier-1 $TER_{LT}$ for earthworm-eating birds**

| Species | $PEC_{twa(21-d)}$ [mg/kg] | | BCF | $PEC_{worm}$ [mg/kg] | $f_{conv}$ | DDD [mg/kg bw/d] | Endpoint [mg/kg bw/d] | | $TER_{LT}$ [1)] | TER trigger |
|---|---|---|---|---|---|---|---|---|---|---|
| **Cereals, 1× 0.200 kg a.s./ha, BBCH 30-69 (worst-case approach)** | | | | | | | | | | |
| Bird, 100 g | JAU-desthio (M4) | 0.025* ~~0.021~~ | 1.216 | 0.026 | 1.05 | 0.027 | NOEL | 14.8 | 551.8 | 5 |

1) According to EFSA Journal 2009; 7(12): 1438, only the long-term risk needs to be considered
*PECs21TWA

As outlined in the table above, the long-term TER value is above 5. Thus, an acceptable risk for earthworm-eating birds can be concluded. No further considerations have to be taken into account.

**JAU-S-methyl (M1)**

A log $P_{OW}$ of 4.19 was determined for the metabolite JAU-S-methyl (M1) corresponding to a $P_{OW}$ of 15488. Using this $P_{OW}$, the mean $K_{OC}$ of 2556.3 ($K_{OC}$ = 1974-2995, n=4; for details, see EFSA Scientific report (2007) 106, 1-98) and a default value of 0.02 for $f_{OC}$, the calculated bioconcentration factor in worms is 3.652. According to the recommendations of EFSA Journal 2009; 7(12): 1438, the $PEC_{twa(21-d)}$ in the upper 5 cm soil layer should be used for the $PEC_{worm}$ calculation.

As outlined under point 8.7 of Section 8 (*Environmental fate*), a maximum $PEC_{twa(21-d)}$ in soil of 0.007 mg met./kg was calculated for the intended GAP uses of ADM.03500.F.2.B in cereals. The relevant $TER_{LT}$ value for the generic standard bird (100-g bird eating 104.6 g per day) was based on the estimated residue in worms and long-term toxicity endpoints for birds already used in the risk assessment above.

**Table 9.2-12: Daily Dietary Dose (DDD) and Tier-1 $TER_{LT}$ for earthworm-eating birds**

| Species | $PEC_{twa(21-d)}$ [mg/kg] | | BCF | $PEC_{worm}$ [mg/kg] | $f_{conv}$ | DDD [mg/kg bw/d] | Endpoint [mg/kg bw/d] | | $TER_{LT}$ [1)] | TER trigger |
|---|---|---|---|---|---|---|---|---|---|---|
| **Cereals, 1× 0.200 kg a.s./ha, BBCH 30-69 (worst-case approach)** | | | | | | | | | | |
| Bird, 100 g | JAU-S-methyl (M1) | 0.007 | 3.652 | 0.026 | 1.05 | 0.027 | NOEL | 7.8 [2)] | 290.6 | 5 |

1) According to EFSA Journal 2009; 7(12): 1438, only the long-term risk needs to be considered

2) As no toxicity data are available for the metabolite of concern, it was assumed that the metabolite is 10× toxic than the parent compound (*absolute worst-case approach*)

As outlined in the table above, the long-term TER value is above 5. Thus, an acceptable risk for earthworm-eating birds can be concluded. No further considerations have to be taken into account.

Food chain from fish to fish-eating birds

Data on bioconcentration of the active substance prothioconazole in fish are available in the context of the EU evaluation process. Explicit reference is made to the underlying results summarised and evaluated in the DAR Prothioconazole July 2005 – Volume 3, B.9 and stated as agreed endpoint in the EFSA Scientific report (2007) 106, 1-98.

**Equation 2: Calculation of Daily Dietary Dose for fish-eating birds and mammals**

$$\mathbf{DDD = PEC_{fish} \cdot f_{conv}} \quad \text{[mg/kg bw/d]}$$

where $PEC_{fish} = PEC_{sw} \cdot BCF$

and
- $PEC_{fish}$ = predicted concentration in fish [mg/kg]
- $f_{conv}$ = factor in order to convert $PEC_{fish}$ to daily dose
- $PEC_{sw}$ = 3-week $PEC_{twa}$ in surface water [mg/L]
- BCF = bioconcentration factor in fish

The maximum FOCUS Step-2 and Step-3 $PEC_{twa,21d}$ for prothioconazole and the metabolites JAU-desthio (M4) and JAU-S-methyl (M1) as well as the BCF value of 19.7 (whole fish) for the parent compound, the BCF value of 65 for JAU-desthio (M4) (experimentally determined) as well as the BCF value of 319.3 for JAU-S-methyl (M1) (estimated using the calculation model BCFBAF™ (formerly called BCFWIN™, see below) as part of EPISUITE 4.1) were considered for the calculation of the corresponding $PEC_{fish}$ values.

For the JAU-S-methyl (M1) risk assessment, it was conservatively assumed that the metabolite is 10× more toxic to terrestrial vertebrates than the parent compound, since no experimentally determined NOEL is available. The relevant $TER_{LT}$ value for the generic standard bird (1000-g bird eating 159 g fish per day) was based on the estimated residue in fish and ecologically relevant long-term endpoints already justified in the risk assessment above.

## Prothioconazole

**Table 9.2-13: Daily Dietary Dose (DDD) and Tier-1 $TER_{LT}$ for fish-eating birds**

| Species | Max. FOCUS Step-3 $PEC_{twa, 21d}$ value [µg/L] | | BCF | $PEC_{fish}$ [mg/kg] | $f_{conv}$ | DDD [mg/kg bw/d] | Endpoint [mg/kg bw/d] | | $TER_{LT}$ [1)] | TER trigger |
|---|---|---|---|---|---|---|---|---|---|---|
| **Cereals, 1× 0.200 kg a.s./ha, BBCH 30-69 (worst-case approach)** | | | | | | | | | | |
| Bird, 1000 g | Prothiocona-zole | 0.299 [2)] | 19.7 | 0.006 | 0.159 | 0.001 | NOEL | 78 | 83284 | 5 |

1) According to EFSA Journal 2009; 7(12): 1438, only the long-term risk needs to be considered

2) Maximum FOCUS Step-3 $PEC_{twa,21d}$ for the parent compound in spring cereals at BBCH 30 (D1, ditch), covering also the GAP uses in oilseed rape

As outlined in the table above, the $TER_{LT}$ value for prothioconazole is above the relevant trigger value of 5, indicating an acceptable risk for fish-eating birds.

## JAU-desthio (M4)

**Table 9.2-14: Daily Dietary Dose (DDD) and Tier-1 $TER_{LT}$ for fish-eating birds**

| Species | Max. FOCUS Step-2 $PEC_{twa, 21d}$ value [µg/L] | | BCF | $PEC_{fish}$ [mg/kg] | $f_{conv}$ | DDD [mg/kg bw/d] | Endpoint [mg/kg bw/d] | | $TER_{LT}$ [1)] | TER trigger |
|---|---|---|---|---|---|---|---|---|---|---|
| **Cereals, 1× 0.200 kg a.s./ha, BBCH 30-69 (worst-case approach)** | | | | | | | | | | |
| Bird, 1000 g | JAU-desthio (M4) | 3.08[2)] ~~2.803~~ [2)] | 65 | 0.200 ~~0.182~~ | 0.159 | 0.031 ~~0.029~~ | NOEL | 14.8 | 477.5 ~~510.9~~ | 5 |

1) According to EFSA Journal 2009; 7(12): 1438, only the long-term risk needs to be considered

2) Maximum FOCUS Step-2 $PEC_{twa,21d}$ for the metabolite in spring / winter cereals at BBCH 30 (March-May; June-Sept.), covering also the GAP uses in oilseed rape

As outlined in the table above, the $TER_{LT}$ value for JAU-desthio (M4) is above the relevant trigger value of 5, indicating an acceptable risk for fish-eating birds.

## JAU-S-methyl (M1)

The metabolite JAU-S-methyl (M1) has a logPow of 4.3 (at pH 4-9), therefore this is over the threshold for needing to consider bioconcentration in the aquatic environment. Thus, the BCF of JAU-S-methyl (M1) was modelled using QSAR data. BCFBAF™ (formerly called BCFWIN™) as part of EPISUITE 4.1 was used to model the BCFs of JAU-S-methyl (M1). The input parameters used are summarised in the table below.

**Table 9.2-15: BCF model input parameters**

| Compound | LogPow | SMILES |
|---|---|---|
| JAU-S-methyl (M1) | 4.19 | n1(CC(O)(C3(CL)CC3)Cc2ccccc2CL)ncnc1SC |

The 'middle trophic level' was considered in the report to be most representative of fish weight likely to be consumed by an avian or terrestrial piscivore; therefore, only the mid trophic level BCF was reported. The model outputs are summarised in the table below.

**Table 9.2-16: BCF model outputs**

| Compound | Estimated BCF (EPISUITE/BCFBAF v3.01) (L/kg wet wt) | Reference |
|---|---|---|
| JAU-S-methyl (M1) | Regression based: BCF = 319.3<br>Arnot-Grobas, mid-trophic: BCF = 800.1 | EPISUITE |

It is assumed that for JAU-S-methyl (M1) the regression based estimate can be relied upon most heavily, but for maximum conservatism also the Arnot-Grobas BCF values that include and exclude biotransformation rate estimates was taken into consideration (see table below).

**Table 9.2-17: Daily Dietary Dose (DDD) and Tier-1 $TER_{LT}$ for fish-eating birds**

| Species | Max. FOCUS Step-2 $PEC_{twa,\ 21d}$ value [µg/L] | | BCF | $PEC_{fish}$ [mg/kg] | $f_{conv}$ | DDD [mg/kg bw/d] | Endpoint [mg/kg bw/d] | | $TER_{LT}$ [1)] | TER trigger |
|---|---|---|---|---|---|---|---|---|---|---|
| **Cereals, 1× 0.200 kg a.s./ha, BBCH 30-69 (worst-case approach)** | | | | | | | | | | |
| Bird, 1000 g | JAU-S-methyl (M1) | 0.709 [2)] | 319.3 | 0.226 | 0.159 | 0.036 | NOEL | 7.8 [3)] | 216.7 | 5 |
| | | | 800.1 | 0.567 | | 0.090 | | | 86.5 | |

1) According to EFSA Journal 2009; 7(12): 1438, only the long-term risk needs to be considered

2) Maximum FOCUS Step-2 $PEC_{twa,21d}$ for the metabolite in spring / winter cereals (March-May; June-Sept.) at BBCH 30, covering also the GAP uses in oilseed rape

3) As no toxicity data are available for the metabolite of concern, it was assumed that the metabolite is 10× toxic than the parent compound (*absolute worst-case approach*)

As outlined in the table above, the $TER_{LT}$ value for JAU-S-methyl (M1) is above the relevant trigger value of 5, indicating an acceptable risk for fish-eating birds.

**zRMS comments:**

The Applicants' approach in evaluation of the risk of secondary poisoning is in line with EFSA (2009). Compounds selected for this assessment are agreed by the zRMS. Evaluation was not triggered for remaining metabolites of active substance due to their log Pow <3.

Some additional corrections were added in tables above in case PECs 21 dTWA values according to evaluation in area of Section 8.

Despite all corrections of the zRMS, acceptable risk of secondary exposure from all relevant compounds could be concluded for birds.

### 9.2.2.5 Biomagnification in terrestrial food chains

Biomagnification is considered to be low. Thus, no further considerations have to be taken into account.

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

Not considered to be relevant.

## 9.2.4 Overall conclusions

Based on the GAP uses intended for ADM.03500.F.2.B in cereals and oilseed rape, no unacceptable risk for birds is expected for acute or long-term exposure to contaminated food indicated by Tier-1 TER values above the corresponding trigger values. Furthermore, no unacceptable risks are expected arising from other routes of direct exposure or secondary poisoning (residue uptake from drinking water or bioaccumulation in food chains). In conclusion, an acceptable overall risk for birds is indicated for the intended GAP uses of ADM.03500.F.2.B.

## 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 the active substance prothioconazole and its metabolite JAU-desthio (M4). Full details of these studies are provided in the respective EU DAR and related documents. Effects on mammals of ADM.03500.F.2.B were not evaluated as part of the EU assessment of the active substance.

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

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| Rat | Prothioconazole technical | Acute toxicity | $LD_{50}$ > 6200 mg a.s./kg bw | EFSA Scientific Report (2007) 106, 1-98 |
| Mouse | JAU-desthio (M4) | Acute toxicity | $LD_{50}$ = 2235 mg met./kg bw | EFSA Scientific Report (2007) 106, 1-98 |
| Rat | Prothioconazole technical | Reproductive toxicity | NOAEL = 95.6 mg a.s./kg bw/d | EFSA Scientific Report (2007) 106, 1-98 |
| Rat | JAU-desthio (M4) | Reproductive toxicity | NOAEL = 10 mg met./kg bw/d | EFSA Scientific Report (2007) 106, 1-98 |

**zRMS comments:**

Mammalian toxicity data for prothioconazole and JAU-desthio provided in Tables 9.3-1 above are in line with EU agreed endpoints reported in EFSA Scientific Report (2007) 106.

Mammals are typically exposed to dry residues on their food items following the dilution and spraying of the formulated product. During these processes, much of the formulation constituents are likely to be lost by volatilisation. Therefore, where oral exposure is the main route of exposure, toxicity data for the active substance are used in preference to data from tests with the formulated material. Exposure to ADM.03500.F.2.B via dermal and inhalation routes is considered unlikely, since at the time of application and for a short period thereafter, most wild mammals will leave the immediate vicinity of spray operations in response to the human disturbance.

In this context, it should be noted that the acute toxicity study with the product is designed as fixed dose procedure according to OECD 420 or as acute toxic class test according to OECD 423 in consideration of only few test organisms exposed to only one or two test dose rates. Those study systems are performed for purposes of classification and labelling of the product and are not suitable for the derivation of a precise $LD_{50}$ used for the ecotoxicological risk assessment. Therefore, and for the reason given in the paragraph above, the EU agreed endpoints determined for the active substance should preferably be used as key endpoints for the risk assessment.

As already justified in the avian risk assessment above, the metabolite JAU-desthio (M4), which occurs in plant material in amounts of > 10% of the total radioactive residue (TRR), is more toxic than its parent prothioconazole (up to a factor of 9.5 for the long-term toxicity) and thus is also considered in the risk assessment for mammals.

In conclusion, it is deemed acceptable to use a $LD_{50}$ of > 6200 mg/kg bw for the acute risk assessment for prothioconazole as well as a $LD_{50}$ of 2235 mg/kg bw for its metabolite JAU-desthio (M4). For the reproductive risk assessment, a NOEL of 95.6 mg/kg bw/d was considered for the parent compound and a NOEL of 10.0 mg/kg bw/d for the metabolite.

### 9.3.1.1 Justification for new endpoints

No new endpoints are proposed.

## 9.3.2 Risk assessment for spray applications

The evaluation of the risk for mammals was performed in accordance with the recommendations of the current "Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA" (EFSA Journal 2009; 7(12): 1438), hereafter referred to as EFSA Journal 2009; 7(12): 1438.

The product ADM.03500.F.2.B is an emulsifiable concentrate (EC) containing 250 g/L of the active substance prothioconazole. It is a fungicide applied as spray to infested foliage of spring/winter cereals and oilseed rape. The timing of application is post-emergence (cereal BBCH 30-69 and oilseed BBCH 50-73). The worst-case application scenario leading to maximum soil load is a single post-emergence spray application at a rate of 200 g prothioconazole/ha to cereals (0.8 L prod./ha) and 175 g prothioconazole/ha to oilseed rape (0.7 L prod./ha). For a detailed summary of the GAP uses of ADM.03500.F.2.B, please refer to Table 9.1-1.

Considering these GAP uses, the major potential routes of critical exposure were considered to be feeding on food items (e.g. vegetation and invertebrates) directly contaminated via spray application of the plant protection product.

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

**Screening assessment**

For the initial screening assessment, “indicator species” and exposure scenarios were selected as recommended in EFSA Journal 2009; 7(12): 1438. According to this guidance document, an “indicator species” is not a real species but by virtue of its size and feeding habits is considered to have higher exposure than other species that occur in a particular crop at a particular time.

In other words, if a low risk is estimated for the indicator species of concern, then an overall low risk can be concluded for all other (real) mammalian species exposed to ADM.03500.F.2.B. A summary of the intended uses and relevant mammalian indicator species is given in the table below.

**Table 9.3-2: Worst-case GAP use of ADM.03500.F.2.B and corresponding mammalian indicator species relevant for the screening assessments**

| Crop | Worst-case application scenario | Indicator species | Shortcut value for $TER_A/TER_{LT}$ |
|---|---|---|---|
| Cereals | Post-emergence,<br>BBCH 30-69,<br>1× 200 g/ha | Small herbivorous mammal | 118.4 / 48.3 |
| Oilseed rape | Post-emergence,<br>BBCH 50-73,<br>1× 175 g/ha | | |

Exposure of terrestrial vertebrates to ADM.03500.F.2.B expressed as Daily Dietary Dose (DDD) was assessed separately for acute ($DDD_A$) and long-term exposure ($DDD_{LT}$). The DDD values were calculated according to the formula derived from the current EFSA guidance document. For the acute exposure assessment, shortcut values for 90$^{th}$ percentile RUDs ($SV_{90th}$) were taken into account as recommended in EFSA Journal 2009; 7(12): 1438.

For long-term exposure estimates, a time-frame of a few weeks after application is considered. Since the area of mammals feeding on contaminated diet will be largely compared to the spatial scale of residue variation, shortcut values for mean percentile RUDs ($SV_m$) should be used. Furthermore, time-weighted average residues are considered to reflect long-term exposure in a more realistic manner in view of a residue decrease in relevant food over time.

According to the recommendations of current guidance, i.e. in consideration of a residue decline with a default first order $DT_{50}$ of 10 days and a time scale of 21 days, the time-weighted average factor is TWA = 0.53. Multiple Application Factors (MAF) were not taken into account with respect to the single application scenario of ADM.03500.F.2.B.

The risk for mammals was assessed by calculating Toxicity Exposure Ratios (TER) considering the toxicity endpoints above and exposure expressed as Daily Dietary Dose (DDD). The results are presented in the table below.

## Prothioconazole

**Table 9.3-3: Screening assessment of the acute and long-term risk for mammals due to the use of ADM.03500.F.2.B in cereals**

| | | | | | |
|---|---|---|---|---|---|
| **Intended use** | Cereals, 1× 200 g a.s./ha, BBCH 30 – 69 | | | | |
| **Active substance** | Prothioconazole | | | | |
| **Application rate (g/ha)** | 1× 200 | | | | |
| **MAF** | 1.0 | | | | |
| **Acute toxicity (mg/kg bw)** | > 6200 | | | | |
| **TER criterion** | 10 | | | | |
| **Crop scenario<br>Growth stage** | **Indicator species** | **$SV_{90}$** | **$MAF_{90}$** | **$DDD_{90}$<br>(mg/kg bw/d)** | **$TER_A$** |
| BBCH > 10 | Small herbivorous mammal | 118.4 | 1.0 | 23.7 ~~1~~ | > 261.6 ~~3~~ |
| **Long-term toxicity<br>(mg/kg bw/d)** | 95.6 | | | | |
| **TER criterion** | 5 | | | | |
| **Crop scenario<br>Growth stage** | **Indicator species** | **$SV_m$** | **$MAF_m$ × TWA** | **$DDD_m$<br>(mg/kg bw/d)** | **$TER_{LT}$** |
| BBCH > 10 | Small herbivorous mammal | 48.3 | 0.53 | 5.1 | 18.7 |

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

As outlined in the table above, the calculated $TER_A$ and $TER_{LT}$ values for the exposure to prothioconazole are above the trigger of 10 and 5, established for acute and long-term exposure, indicating an overall acceptable risk for mammals in cereals already at screening level. Thus, no further refinements at Tier-1 level are required for prothioconazole.

**Table 9.3-4: Screening assessment of the acute and long-term risk for mammals due to the use of ADM.03500.F.2.B in oilseed rape**

| | | | | | |
|---|---|---|---|---|---|
| **Intended use** | Oilseed rape, 1× 175 g a.s./ha, BBCH 50 – 73 | | | | |
| **Active substance** | Prothioconazole | | | | |
| **Application rate (g/ha)** | 1× 175 | | | | |
| **MAF** | 1.0 | | | | |
| **Acute toxicity (mg/kg bw)** | > 6200 | | | | |
| **TER criterion** | 10 | | | | |
| **Crop scenario<br>Growth stage** | **Indicator species** | **$SV_{90}$** | **$MAF_{90}$** | **$DDD_{90}$<br>(mg/kg bw/d)** | **$TER_A$** |
| BBCH > 10 | Small herbivorous mammal | 118.4 | 1.0 | 20.7 | > 299.5.2 |
| **Long-term toxicity<br>(mg/kg bw/d)** | 95.6 | | | | |
| **TER criterion** | 5 | | | | |
| **Crop scenario<br>Growth stage** | **Indicator species** | **$SV_m$** | **$MAF_m$ ×<br>TWA** | **$DDD_m$<br>(mg/kg bw/d)** | **$TER_{LT}$** |
| BBCH > 10 | Small herbivorous mammal | 48.3 | 0.53 | 4.5 | 21.3 |

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

As outlined in the table above, the calculated $TER_A$ and $TER_{LT}$ values for the exposure to prothioconazole are above the trigger of 10 and 5, established for acute and long-term exposure, indicating an overall acceptable risk for mammals in oilseed rape already at screening level. Thus, no further refinements at Tier-1 level are required for prothioconazole.

**zRMS comments:**

The screening step risk assessment for prothioconazole is agreed by the zRMS.

Acceptable acute and long-term risk may be concluded for mammals exposed to prothioconazole in ADM.03500.F.2.B.

## JAU-desthio (M4)

**Table 9.3-5: Screening assessment of the acute and long-term risk for mammals due to the use of ADM.03500.F.2.B in cereals**

| | | | | | |
|---|---|---|---|---|---|
| **Intended use** | Cereals, 1× 200 g a.s./ha, BBCH 30 – 69 | | | | |
| **Active substance** | JAU-desthio (M4) | | | | |
| **Application rate (g/ha)** | 1× 200* | | | | |
| **MAF** | 1.0 | | | | |
| **Acute toxicity (mg/kg bw)** | 2235 | | | | |
| **TER criterion** | 10 | | | | |
| **Crop scenario<br>Growth stage** | **Indicator species** | **$SV_{90}$** | **$MAF_{90}$** | **$DDD_{90}$<br>(mg/kg bw/d)** | **$TER_A$** |
| BBCH > 10 | Small herbivorous mammal | 118.4 | 1.0 | 23.7 | 94.4 |

| | | | | | |
|---|---|---|---|---|---|
| **Intended use** | Cereals, 1× 200 g a.s./ha, BBCH 30 – 69 | | | | |
| **Active substance** | JAU-desthio (M4) | | | | |
| **Application rate (g/ha)** | 1× 200* | | | | |
| **MAF** | 1.0 | | | | |
| **Long-term toxicity (mg/kg bw/d)** | 10.0 | | | | |
| **TER criterion** | 5 | | | | |
| **Crop scenario Growth stage** | **Indicator species** | **$SV_m$** | **$MAF_m$ × TWA** | **$DDD_m$ (mg/kg bw/d)** | **$TER_{LT}$** |
| BBCH > 10 | Small herbivorous mammal | 48.3 | 0.53 | 5.1 | **2.0** |

Bold: below the relevant trigger, indicating an unacceptable risk at this assessment level
SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio
* TER calculation for the metabolite JAU-desthio was conducted with the application rate of the parent compound prothioconazole – representing an absolute worst-case approach

As outlined in the table above, the calculated $TER_A$ value for the exposure to JAU-desthio (M4) is above the trigger of 10, established for acute exposure, indicating an acceptable acute risk for mammals in cereals already at screening level. By contrast, the $TER_{LT}$ value is below the trigger of 5, and thus a Tier-1 long-term risk assessment for the metabolite of concern is required.

**Table 9.3-6: Screening assessment of the acute and long-term risk for mammals due to the use of ADM.03500.F.2.B in oilseed rape**

| | | | | | |
|---|---|---|---|---|---|
| **Intended use** | Oilseed rape, 1× 175 g a.s./ha, BBCH 50 – 73 | | | | |
| **Active substance** | JAU-desthio (M4) | | | | |
| **Application rate (g/ha)** | 1× 175* | | | | |
| **MAF** | 1.0 | | | | |
| **Acute toxicity (mg/kg bw)** | 2235 | | | | |
| **TER criterion** | 10 | | | | |
| **Crop scenario Growth stage** | **Indicator species** | **$SV_{90}$** | **$MAF_{90}$** | **$DDD_{90}$ (mg/kg bw/d)** | **$TER_A$** |
| BBCH > 10 | Small herbivorous mammal | 118.4 | 1.0 | 20.7 | 107.9 |
| **Long-term toxicity (mg/kg bw/d)** | 10.0 | | | | |
| **TER criterion** | 5 | | | | |
| **Crop scenario Growth stage** | **Indicator species** | **$SV_m$** | **$MAF_m$ × TWA** | **$DDD_m$ (mg/kg bw/d)** | **$TER_{LT}$** |
| BBCH > 10 | Small herbivorous mammal | 48.3 | 0.53 | 4.5 | **2.2** |

Bold: below the relevant trigger, indicating an unacceptable risk at this assessment level
SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio
* TER calculation for the metabolite JAU-desthio was conducted with the application rate of the parent compound prothioconazole – representing an absolute worst-case approach

As outlined in the table above, the calculated $TER_A$ value for the exposure to JAU-desthio (M4) is above the trigger of 10, established for acute exposure, indicating an acceptable acute risk for mammals in oilseed rape already at screening level. By contrast, the $TER_{LT}$ value is below the trigger of 5, and thus a Tier-1 long-term risk assessment for the metabolite of concern is required.

**Tier-1 risk assessment**

For the Tier-1 risk assessment, "generic focal species" and exposure scenarios were selected as recommended in EFSA Journal 2009; 7(12): 1438. According to this current guidance document, a "generic focal species" *is not a real species, however it is considered to be representative of all those species potentially at risk*. In other words, if a low risk is estimated for the generic focal species of concern, then an overall low risk can be concluded for all other (real) mammalian species exposed to ADM.03500.F.2.B. A summary of the critical GAP uses and relevant mammalian indicator species is given in the table below.

**Table 9.3-7: Critical use pattern of ADM.03500.F.2.B and corresponding mammalian generic focal species relevant for Tier-1 assessments**

| Crop | Worst-case application scenario | EFSA crop group | EFSA Tier-1 scenario | Generic focal species (Representative) | Shortcut value for $TER_{LT}$ |
|---|---|---|---|---|---|
| Cereals | Post-emergence, BBCH 30-69, 1× 200 g/ha | Cereals | BBCH ≥ 20 | Small insectivorous mammal (shrew) | 1.9 |
| | | | BBCH ≥ 40 | Small herbivorous mammal (vole) | 21.7 |
| | | | BBCH 30-39 | Small omnivorous mammal (mouse) | 3.9 |
| | | | BBCH ≥ 40 | Small omnivorous mammal (mouse) | 2.3 |
| Oilseed rape | Post-emergence, BBCH 50-73, 1× 175 g/ha | Oilseed rape | BBCH ≥ 20 | Small insectivore (shrew) | 1.9 |
| | | | BBCH ≥ 40 | Small herbivore (vole) | 18.1 |
| | | | All season | Large herbivore (lagomorph) | 14.3 |
| | | | BBCH ≥ 40 | Small omnivore (mouse) | 1.9 |

Exposure parameters like MAF and TWA were implemented as already justified in the corresponding risk assessment for birds. For the Tier-1 standard risk assessment, PT, PD and AV were set to 1, and thus not considered for exposure mitigation; i.e. animals satisfy their entire food demand in the exposed area, feed on a single food type and contaminated diet will not be avoided. Based on these worst-case assumptions, the Daily Dietary Dose (DDD) was calculated according to the standard formula of the EFSA guideline.

The risk for mammals was assessed by calculating Toxicity Exposure Ratios (TER) considering the toxicity endpoints presented above and exposure expressed as Daily Dietary Dose (DDD). The results are presented in the table below.

## JAU-desthio (M4)

**Table 9.3-8: Tier-1 assessment of the long-term risk for mammals due to the use of ADM.03500.F.2.B in cereals**

| | | | | | |
|---|---|---|---|---|---|
| **Intended use** | 1× 200 g a.s./ha, BBCH 30 – 69 | | | | |
| **Active substance** | JAU-desthio (M4) | | | | |
| **Application rate (g/ha)** | 1× 200* | | | | |
| **MAF** | 1.0 | | | | |
| **Long-term toxicity (mg/kg bw/d)** | 10.0 | | | | |
| **TER criterion** | 5 | | | | |
| **Crop scenario Growth stage** | **Generic focal species** | **$SV_m$** | **$MAF_m$ × TWA** | **$DDD_m$ (mg/kg bw/d)** | **$TER_{LT}$** |
| Cereals, BBCH ≥ 20 | Small insectivorous mammal (shrew) | 1.9 | 0.53 | 0.2 | 49.7 |
| Cereals, BBCH ≥ 40 | Small herbivorous mammal (vole) | 21.7 | 0.53 | 2.3 | **4.3** |
| Cereals, BBCH 30-39 | Small omnivorous mammal (mouse) | 3.9 | 0.53 | 0.4 | 24.2 |
| Cereals, BBCH ≥ 40 | Small omnivorous mammal (mouse) | 2.3 | 0.53 | 0.2 | 41.0 |

Bold: below the relevant trigger, indicating an unacceptable risk at this assessment level
SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio
* TER calculation for the metabolite JAU-desthio (M4) was conducted with the application rate of the parent compound prothioconazole – representing an absolute worst-case approach

As outlined in the table above, the calculated $TER_{LT}$ value for the exposure to JAU-desthio (M4) are above the trigger of 5, established for long-term exposure, except for the generic focal species "vole". Thus, further refinements might be considered to be required for JAU-desthio (M4). However, it should be noted that TER calculations above for the metabolite JAU-desthio (M4) were conducted with the application rate of the parent compound prothioconazole which represents an absolute worst-case approach.

According to the DAR (2005) for prothioconazole, the real percentage of JAU-desthio (M4) in cereals is 35 % of the total radioactive residue (TRR). Hence, the exposure is about 3 times lower than the parent. As wheat can be considered as surrogate for monocotyledonous plants, and the diet of the common vole consist of grass and cereals for the exposure scenario in cereals according to the EFSA Journal 2009; 7(12): 1438, it is deemed acceptable to refine the exposure rate for the metabolite of concern.

**Table 9.3-9: Tier-1 assessment of the long-term risk for mammals due to the use of ADM.03500.F.2.B based on actual percentage of JAU-desthio (M4) in cereals**

| | | | | | |
|---|---|---|---|---|---|
| **Intended use** | 1× 200 g a.s./ha, BBCH 30 – 69 | | | | |
| **Active substance** | JAU-desthio (M4) | | | | |
| **Application rate (g/ha)** | 1× 70* | | | | |
| **MAF** | 1.0 | | | | |
| **Long-term toxicity (mg/kg bw/d)** | 10.0 | | | | |
| **TER criterion** | 5 | | | | |
| **Crop scenario Growth stage** | **Generic focal species** | **$SV_m$** | **$MAF_m$ × TWA** | **$DDD_m$ (mg/kg bw/d)** | **$TER_{LT}$** |
| Cereals, BBCH ≥ 40 | Small herbivorous mammal (vole) | 21.7 | 0.53 | 0.8 | 12.4 |

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

* According to the DAR (2005) for prothioconazole, the real percentage of JAU-desthio (M4) in cereals is 35 % of the total radioactive residue (TRR). Thus, in the risk assessment for the vole, an exposure rate of 200*0.35 = 70 g/ha was considered

Taking this into account, the TER for JAU-desthio (M4) is 12.4 and thus clearly above the trigger of 5 (see table below). In conclusion, an acceptable risk can be concluded also for JAU-desthio (M4). Additionally, further supportive refinement options were provided for the TER calculation conducted with the application rate of the parent compound prothioconazole under point 9.3.2.2 below.

**Table 9.3-10: Tier-1 assessment of the long-term risk for mammals due to the use of ADM.03500.F.2.B in oilseed rape**

| | | | | | |
|---|---|---|---|---|---|
| **Intended use** | 1× 175 g a.s./ha, BBCH 50 – 73 | | | | |
| **Active substance** | JAU-desthio (M4) | | | | |
| **Application rate (g/ha)** | 1× 175* | | | | |
| **MAF** | 1.0 | | | | |
| **Long-term toxicity (mg/kg bw/d)** | 10.0 | | | | |
| **TER criterion** | 5 | | | | |
| **Crop scenario Growth stage** | **Generic focal species** | **$SV_m$** | **$MAF_m$ × TWA** | **$DDD_m$ (mg/kg bw/d)** | **$TER_{LT}$** |
| Oilsee rape, BBCH ≥ 20 | Small insectivore (shrew) | 1.9 | 0.53 | 0.2 | 56.7 |
| Oilsee rape, BBCH ≥ 40 | Small herbivore (vole) | 18.1 | 0.53 | 1.7 | 6.0 |
| Oilsee rape, all season | Large herbivore (lagomorph) | 14.3 | 0.53 | 1.3 | 7.5 |
| Oilsee rape, BBCH ≥ 40 | Small omnivore (mouse) | 1.9 | 0.53 | 0.2 | 56.7 |

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

* TER calculation for the metabolite JAU-desthio (M4) was conducted with the application rate of the parent compound prothioconazole – representing an absolute worst-case approach

As outlined in the table above, the calculated $TER_A$ and $TER_{LT}$ values for the exposure to JAU-desthio (M4) are above the trigger of 10 and 5, established for acute and long-term exposure, indicating an overall acceptable risk for mammals in oilseed rape at Tier-1. Thus, no further refinements are required for the metabolite.

**zRMS comments:**

The screening step and Tier 1 risk assessment for metabolite JAU 6476-desthio are agreed by the zRMS.

Based on performed calculations acceptable acute risk may be concluded for mammals exposed to the metabolite.
The chronic risk is acceptable for most of the species, with exception of small herbivorous mammal - vole.
The applicant's refinement was provided with consideration of the real percentage of JAU-desthio (M4) in cereals which is 35 % of the total radioactive residue (TRR) from information reported in the DAR.
zRMS is in the opinion that refinement based on max. application rate of 200 g pm/ha should be taken into account as a worst case scenario.
Other option of refinement of the risk from this metabolite is presented in point 9.3.2.2 below.

### 9.3.2.2 Higher-tier risk assessment

The risk assessments for mammals performed so far (Tier-1) were based on worst-case exposure assumptions. In the following Tier-2 approach, exposure parameters were refined to assess the risk of the species potentially of concern in a more realistic way.

**Deposition Factor:** In the EFSA guidance document (Appendix E – impact of crop interception on residues on plant food items), it is stated that the deposition factors provided for the different crops and growth stages in the guidance document are likely to reflect conservative estimates. In the context of a higher-tier assessment, the more detailed values of the FOCUS groundwater report can therefore be used for refinement. Thus, the deposition values reported in the latest 'Generic Guidance for Tier-1 FOCUS Ground Water Assessment' (vers. 2.2; May 2014) could be considered for refinement purposes. These values are in line with the updated interception values recommended by the EFSA (EFSA Journal 2014;12(5):3662, Appendix C, pages 27/28) which are considered as "*well-documented data and thus act as robust and representative values*" for regulatory risk assessments and which are also included now in the "Guidance document on work sharing in the Northern Zone in the authorisation of plant protection products" (May 2018, version 7). Based on the updated crop interception values, it is deemed acceptable to consider a $f_{dep}$ of 0.1 instead of 0.3 (as considered by EFSA guidance document at Tier-1 level) for cereal crop stages at BBCH 40-69 (growth stages relevant for the risk assessment of the common vole).

**Table 9.3-11: Tier-1 assessment of the long-term risk for mammals due to the use of ADM.03500.F.2.B in cereals**

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Intended use** | 1× 200 g a.s./ha, BBCH 30 – 69 | | | | | | | | |
| **Active substance** | JAU-desthio (M4) | | | | | | | | |
| **Application rate (g/ha)** | 1× 200* | | | | | | | | |
| **MAF** | 1.0 | | | | | | | | |
| **Long-term toxicity (mg/kg bw/d)** | 10.0 | | | | | | | | |
| **TER criterion** | 5 | | | | | | | | |
| **Generic focal species** | **Food item** | **FIR/bw** | **RUD** | **$A_{MAF}$** | **TWA** | **fdep** | **PT** | **$DDD_m$ (mg/kg bw/d)** | **$TER_{LT}$** |
| Small herbivore (vole), BBCH ≥ 40 | 100 % grass | 1.33 | 54.2 | 0.2 | 0.53 [2)] | 0.1 | 1 | 0.8 | 12.5 ~~13.1~~ |

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

* TER calculation for the metabolite JAU-desthio (M4) was conducted with the application rate of the parent compound prothioconazole – representing an absolute worst-case approach

As outlined in the table above, the calculated $TER_{LT}$ value for the exposure to JAU-desthio (M4) are above the trigger of 5, established for long-term exposure. Thus, no further refinements are considered to be required for JAU-desthio (M4).

**zRMS comments:**

Based on performed calculations with consideration a $f_{dep}$ of 0.1 instead of 0.3 for cereal crop stages at BBCH 40-69, acceptable long-term risk may be concluded for small mammals – vole exposed to the metabolite.

Overall, based on Applicants' calculations, acceptable risk to mammals from metabolite JAU 6476-desthio may be concluded from the intended uses of ADM.03500.F.2.B.

### *Supportive "weight-of-evidence" approach*

Nevertheless, it should be mentioned that the refined TER calculations are still based on overly conservative exposure assumptions from several points of view, e.g., it was considered that the generic focal species exclusively feed on contaminated food items from the in-field area contaminated at standard residue levels. Additional refinements decreasing the realistic exposure estimates were not taken into account. However, these refinement options would provide further confidence in the conclusion that mammals are not at risk from exposure to ADM.03500.F.2.B.

**Table 9.3-12: Supportive "weight-of-evidence" approach for mammals with respect to the intended GAP uses of ADM.03500.F.2.B at higher tier level**

| Point | Argumentation |
|---|---|
| Residues in foliage<br><br>*Relevant for herbivorous and omnivorous species* | As outlined in the DAR (2005) and the EFSA Scientific Report (2007) 106 for prothioconazole, a fast foliar residue decline (significantly below the Tier-1 $DT_{50}$ of 10 d) was determined for JAU-desthio (M4) indicated by a mean foliar $DT_{50}$ of 3.2 days (n = 8 trials). It would be deemed acceptable to use this refined $DT_{50}$ for the recalculation of the Time-Weighted Average Factor ($f_{twa}$). If considering a $f_{twa}$ of 0.22 instead of the default value of 0.53, an acceptable risk can be concluded for small herbivorous mammals, indicated by a $TER_{LT}$ of 10.5 under still worst-case exposure assumptions (considering the application rate of the parent compound prothioconazole and the Tier-1 $f_{dep}$ of 0.3 as recommended by the EFSA guidance document at Tier-1 level). |
| Proportion of diet obtained in the treated area (PT)<br><br>*Relevant for all generic focal species* | For a maximum conservative risk assessment, it was assumed that the PT for mammals is 1, although it is very likely that mammals do not obtain their food exclusively from the treated area. For a refined but still conservative risk assessment for mammals, it seems reasonable to assume that the proportion of diet obtained in the treated area will be lower than 100 %. Thus, it should be recognized that using a PT of 1 (as done in the Tier-1 risk assessment above) is an overly conservative approach and should be taken into account when assessing the acceptability of the determined refined long-term TER values. |
| Relevance of voles in agricultural landscapes and for the environmental risk assessment | Under the current scheme of mammalian risk assessment, the common vole (*Microtus arvalis*) is the representative herbivorous mammal species. Common vole population dynamics, habitat and food preferences, their potential as agricultural pests and the use of the common vole in mammalian environmental risk assessment processes were reviewed by Jacob *et al*. (2014). The common vole is primarily a grassland species that is well adapted to steppe habitats. Primary habitats are meadows, set-a-side land, flower strips, grassy field verges, alfalfa and clover fields. They prefer to inhabit undisturbed short vegetation and can be found in grass leys in forests after clear cuts and other grassy habitats. This habitat preference is of advantage for voles as survival of voles is greater in primary habitats where refuges are more abundant than in secondary habitats (agricultural areas).<br>Furthermore, it must also be considered that secondary habitats cannot maintain common vole populations for long periods given the seasonal nature of farming. Populations in secondary / agricultural habitats will be regularly disrupted by harvest and tilling. Agricultural areas (secondary habitats) are colonised when the carrying capacity of primary grassland habitats are exceeded. |

| | |
|---|---|
| Relevance of voles in agricultural landscapes and for the environmental risk assessment (*continued*) | This occurs during multi-annual outbreaks (every 2-5 years), when population sizes can exceed 1000 individuals per hectare. When colonizing secondary habitats, rodents damage crops directly by feeding on shoots and leaves, which results in yield losses or to decreased crop quality. In addition, secondary damage results in plants becoming more susceptible to attack by viral, bacterial and fungal diseases. Indirect costs associated with rodent management can be substantial.<br>During vole population outbreaks to agricultural grassland grazing livestock may have to be transferred early from pastures to stables, resulting in additional husbandry costs for fodder. In such cases, in-crop common vole population control management is commonly practised to avoid significant crop damage. However, even with extensive direct action during outbreaks, *Microtus populations* are seen to recover relatively quickly although data to confirm recovery are scarce.<br>These findings, along with the exceptional reproductive potential of common voles, indicate that common voles will overcome adverse effects of in-crop following application of plant protection products at the landscape level. When considering benefits and damage caused by the common vole during periodic outbreaks, the associated crop losses and management cost suggest that this species is the most serious vertebrate pest in European agriculture. The species' status as a crop pest, its high fecundity, resilience to disturbance and intermittent colonisation of crop habitats are important characteristics that should be reflected in risk assessment.<br>Based on the information provided in the scientific literature, it seems justified to modify elements of the current risk assessment scheme for plant protection products including the use of **(1)** realistic food intake rates, **(2)** reduced assessment trigger values (already applied in certain EU member states e.g. Germany, or to consider **(3)** the use of alternate focal rodent species in European risk assessment:<br>**(1)** EFSA/2009/1438 indicates that a 25 g vole must consume 1.33 times its own body weight (the default food intake rate / bodyweight (FIR/bw)) to satisfy the theoretical daily energy expenditure (DEE). However, in laboratory studies, common voles have been found to only consume about a third of their body weight per day and values as low as 10% based on the uptake of dry matter have been reported. As shown in laboratory studies, even at low temperatures when food uptake is highest, an amount of food equivalent to about 50% of the body weight is eaten, although this was not verified under field conditions.<br>**(2)** The common vole is a model species that exists in cropped areas and, given body weight and food intake rates, does represent a worst-case exposure model. It seems therefore reasonable to consider an adjustment in the trigger values to account for reduced uncertainty, associated with the evaluation of derived TER values from acute and reproduction dietary risk assessments (e.g. Germany already accepts trigger values of $\geq 5$ in the acute and $\geq 2$ in the long-term risk assessment).<br>**(3)** The use of alternate focal species that exist alongside the common vole in the field such as the wood mouse or small mammals within the same feeding guild (e.g. field vole) is a pragmatic approach to risk assessment where common voles are not widely distributed (e.g. proposed for the Northern Zone). However, this position, although pragmatic, cannot be consistently applied across member states as often sufficient field data is not available. In these cases, the pragmatic use of the wood mouse as the representative small mammal species is considered a viable alternate species, considered protective of small mammals in agricultural landscapes. |

**zRMS comments:**

The risk assessment for vole is considered acceptable based on refinement risk assessment provided in Table 9.3-11.
Supportive "weight-of-evidence" approach presented above 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 Journal 2009; 7(12): 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 K(f)oc > 500, the active substance prothioconazole ($K_{OC}$ = 1765) as well as the metabolite JAU-desthio (M4) ($K_{OC}$ = 523-625) belong to the group of more sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the worst-case application scenario (i.e. the maximum seasonal application rate of 1× 200 g/ha in cereals) covers the risk for water-drinking mammals from all intended GAP uses of ADM.03500.F.2.B (for details, see point 9.1.2).

**Prothioconazole**

| | | | | |
|---|---|---|---|---|
| Effective application rate (g/ha) | = | 1× 200 | | |
| Acute toxicity (mg/kg bw) | = | > 6200 | quotient = | < 0.03 |
| Reprod. toxicity (mg/kg bw/d) | = | 95.6 | quotient = | 2.1 |

**JAU-desthio (M4)**

| | | | | |
|---|---|---|---|---|
| Effective application rate (g/ha) | = | 1× 200 | | |
| Acute toxicity (mg/kg bw) | = | > 2235 | quotient = | < 0.09 |
| Reprod. toxicity (mg/kg bw/d) | = | 10 | quotient = | 20 |

Since the ratio of effective application rate to relevant endpoint does not exceed the trigger of 3000 for more sorptive substances, no further considerations have to be taken into account.

**zRMS comments:**

The acceptable risk to mammals via drinking water may be concluded for prothioconazole and its metabolite.

### 9.3.2.4 Effects of secondary poisoning

As already justified in the corresponding risk assessment for birds (for details, see point 9.2.2.4), a potential for bioaccumulation is expected for the active substance prothioconazole and its metabolites JAU-desthio (M4) and JAU-S-methyl (M1) (log Pow > 3). Consequently, deterministic risk assessments by calculating TER values were performed only for these compounds of concern.

Food chain from earthworm to earthworm-eating mammals

Estimated theoretical exposure of earthworm-eating mammals was calculated with Equation 1 (p. 27), based on the same exposure input parameters considered in the respective risk assessment for birds. The relevant $TER_{LT}$ value for the generic standard mammals (10-g mammal eating 12.8 g worms per day) was based on the estimated residue in worms and the ecologically relevant long-term endpoint already justified in the risk assessment above:

## Prothioconazole

**Table 9.3-13: Daily Dietary Dose (DDD) and Tier-1 $TER_{LT}$ for earthworm-eating mammals**

| Species | $PEC_{twa(21-d)}$ [mg/kg] | | BCF | $PEC_{worm}$ [mg/kg] | $f_{conv}$ | DDD [mg/kg bw/d] | Endpoint [mg/kg bw/d] | | $TER_{LT}$ [1)] | TER trigger |
|---|---|---|---|---|---|---|---|---|---|---|
| **Cereals, 1× 0.200 kg a.s./ha, BBCH 30-69 (worst-case approach)** | | | | | | | | | | |
| Mammal, 10 g | Prothiocon-azole | 0.010 | 2.270 | 0.023 | 1.28 | 0.029 | NOAEL | 96.5 | 3321 | 5 |

1) According to EFSA Journal 2009; 7(12): 1438, only the long-term risk needs to be considered

As outlined in the table above, the $TER_{LT}$ value is above 5. Thus, an acceptable risk for earthworm-eating mammals can be concluded. No further considerations have to be taken into account.

## JAU-desthio (M4)

**Table 9.3-14: Daily Dietary Dose (DDD) and Tier-1 $TER_{LT}$ for earthworm-eating mammals**

| Species | $PEC_{twa(21-d)}$ [mg/kg] | | BCF | $PEC_{worm}$ [mg/kg] | $f_{conv}$ | DDD [mg/kg bw/d] | Endpoint [mg/kg bw/d] | | $TER_{LT}$ [1)] | TER trigger |
|---|---|---|---|---|---|---|---|---|---|---|
| **Cereals, 1× 0.200 kg a.s./ha, BBCH 30-69 (worst-case approach)** | | | | | | | | | | |
| Mammal, 10 g | JAU-desthio (M4) | 0.025* ~~0.021~~ | 1.216 | 0.026 | 1.28 | 0.033 | NOAEL | 10.0 | 305.9 | 5 |

1) According to EFSA Journal 2009; 7(12): 1438, only the long-term risk needs to be considered
*PECs 21 TWA

As outlined in the table above, the long-term TER value is above 5. Thus, an acceptable risk for earthworm-eating mammals can be concluded. No further considerations have to be taken into account.

## JAU-S-methyl (M1)

**Table 9.3-15: Daily Dietary Dose (DDD) and Tier-1 $TER_{LT}$ for earthworm-eating mammals**

| Species | $PEC_{twa(21-d)}$ [mg/kg] | | BCF | $PEC_{worm}$ [mg/kg] | $f_{conv}$ | DDD [mg/kg bw/d] | Endpoint [mg/kg bw/d] | | $TER_{LT}$ [1)] | TER trigger |
|---|---|---|---|---|---|---|---|---|---|---|
| **Cereals, 1× 0.200 kg a.s./ha, BBCH 30-69 (worst-case approach)** | | | | | | | | | | |
| Mammal, 10 g | JAU-S-methyl (M1) | 0.007 | 3.652 | 0.026 | 1.28 | 0.033 | NOAEL | 9.65 [2)] | 294.9 | 5 |

1) According to EFSA Journal 2009; 7(12): 1438, only the long-term risk needs to be considered
2) As no toxicity data are available for the metabolite of concern, it was assumed that the metabolite is 10× toxic than the parent compound (*absolute worst-case approach*)

As outlined in the table above, the long-term TER value is above 5. Thus, an acceptable risk for earthworm-eating mammals can be concluded. No further considerations have to be taken into account.

Food chain from fish to fish-eating mammals

Estimated theoretical exposure of fish-eating mammals was calculated with Equation 2 (p. 29), based on the same exposure input parameters considered in the respective risk assessment for birds. The relevant $TER_{LT}$ values for the generic standard mammals (3000-g mammal eating 425 g fish per day) was based on the estimated residue in fish and the ecologically relevant long-term endpoint already justified in the risk assessment above:

## Prothioconazole

**Table 9.3-16: Daily Dietary Dose (DDD) and Tier-1 $TER_{LT}$ for fish-eating mammals**

| Species | Max. FOCUS Step-3 $PEC_{twa,\ 21d}$ value [µg/L] | | BCF | $PEC_{fish}$ [mg/kg] | $f_{conv}$ | DDD [mg/kg bw/d] | Endpoint [mg/kg bw/d] | | $TER_{LT}$ [1)] | TER trigger |
|---|---|---|---|---|---|---|---|---|---|---|
| **Cereals, 1× 0.200 kg a.s./ha, BBCH 30-69 (worst-case approach)** | | | | | | | | | | |
| Mammals, 3000 g | Prothiocona-zole | 0.299 [2)] | 19.7 | 0.006 | 0.142 | 0.001 | NOEL | 96.5 | 115372 | 5 |

1) According to EFSA Journal 2009; 7(12): 1438, only the long-term risk needs to be considered

2) Maximum FOCUS Step-3 $PEC_{twa,21d}$ for the parent compound in spring cereals at BBCH 30 (D1, ditch), covering also the GAP uses in oilseed rape

As outlined in the table above, the $TER_{LT}$ value for prothioconazole is above the relevant trigger value of 5, indicating an acceptable risk for fish-eating mammals.

## JAU-desthio (M4)

**Table 9.3-17: Daily Dietary Dose (DDD) and Tier-1 $TER_{LT}$ for fish-eating mammals**

| Species | Max. FOCUS Step-2 $PEC_{twa,\ 21d}$ value [µg/L] | BCF | $PEC_{fish}$ [mg/kg] | $f_{conv}$ | DDD [mg/kg bw/d] | Endpoint [mg/kg bw/d] | | $TER_{LT}$ [1)] | TER trigger |
|---|---|---|---|---|---|---|---|---|---|
| **Cereals, 1× 0.200 kg a.s./ha, BBCH 30-69 (worst-case approach)** | | | | | | | | | |
| Mammals, 3000 g | JAU-desthio (M4) | 3.08 [2)] ~~2.803~~ [2] | 65 | 0.200 ~~0.182~~ | 0.142 | 0.028 ~~0.026~~ | NOEL | 10 | 357.1 ~~386.5~~ | 5 |

1) According to EFSA Journal 2009; 7(12): 1438, only the long-term risk needs to be considered

2) Maximum FOCUS Step-2 $PEC_{twa,21d}$ for the metabolite in spring / winter cereals at BBCH 30 (March-May; June-Sept.), covering also the GAP uses in oilseed rape

As outlined in the table above, the $TER_{LT}$ value for JAU-desthio (M4) is above the relevant trigger value of 5, indicating an acceptable risk for fish-eating mammals.

## JAU-S-methyl (M1)

**Table 9.3-18: Daily Dietary Dose (DDD) and Tier-1 $TER_{LT}$ for fish-eating mammals**

| Species | Max. FOCUS Step-2 $PEC_{twa,\ 21d}$ value [µg/L] | BCF | $PEC_{fish}$ [mg/kg] | $f_{conv}$ | DDD [mg/kg bw/d] | Endpoint [mg/kg bw/d] | | $TER_{LT}$ [1)] | TER trigger |
|---|---|---|---|---|---|---|---|---|---|
| **Cereals, 1× 0.200 kg a.s./ha, BBCH 30-69 (worst-case approach)** | | | | | | | | | |
| Mammals, 3000 g | JAU-S-methyl (M1) | 0.709 [2] | 319.3 | 0.226 | 0.142 | 0.032 | NOEL | 9.65 [3)] | 300.2 | 5 |
| | | | 800.1 | 0.567 | | 0.081 | | | 119.8 | |

1) According to EFSA Journal 2009; 7(12): 1438, only the long-term risk needs to be considered

2) Maximum FOCUS Step-2 $PEC_{twa,21d}$ for the metabolite in spring / winter cereals (March-May; June-Sept.) at BBCH 30, covering also the GAP uses in oilseed rape

3) As no toxicity data are available for the metabolite of concern, it was assumed that the metabolite is 10× toxic than the parent compound (*absolute worst-case approach*)

As outlined in the table above, the $TER_{LT}$ value for JAU-S-methyl (M1) is above the relevant trigger value of 5, indicating an acceptable risk for fish-eating mammals.

**zRMS comments:**

The Applicants' approach in evaluation of the risk of secondary poisoning is in line with EFSA (2009). Compounds selected for this assessment are agreed by the zRMS. Evaluation was not triggered for remaining metabolites of active substance due to their log Pow <3.
Some additional corrections were added in tables above in case PECs 21dTWA values according to evaluation in area of Section 8.

Despite all corrections of the zRMS, acceptable risk of secondary exposure from all relevant compounds could be concluded for mammals

#### 9.3.2.5 Biomagnification in terrestrial food chains

Not considered to be relevant.

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

Not considered to be relevant.

### 9.3.4 Overall conclusions

Based on the GAP uses intended for ADM.03500.F.2.B, no unacceptable risk for mammals is expected for acute or long-term exposure to contaminated food indicated by Tier-1/Tier-2 TER values above the corresponding trigger values. Furthermore, no unacceptable risks are expected arising from other routes of direct exposure or secondary poisoning (residue uptake from drinking water or bioaccumulation in food chains). In conclusion, an acceptable overall risk for mammals is indicated for the intended GAP uses of ADM.03500.F.2.B.

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

According to the new data requirements set forth in the Annex to Reg. (EU) no 283/2013 and 284/2013, at present toxicity tests might be requested for birds and mammals but not for amphibians and reptiles. Nevertheless, it is stated that relevant data, including data from the open literature for the active substance of concern, regarding the potential effects to amphibians and reptiles shall be presented and taken into account in the risk assessment, if available.

However, it should be noted that no official risk assessment guideline has been developed so far that could be used to estimate the extent of different exposure routes for amphibians and reptiles under natural conditions. Further, almost no validated standard protocols are yet available for amphibian and reptile testing. The only official test guidelines are the amphibian metamorphosis assay (AMA; not developed to generate endpoints for risk assessment other than endocrine disruption) (OECD 231, September 2009) and the larval amphibian growth and development assay (LAGDA) (OECD 241, July 2015).

In the absence of appropriate test and risk assessment guidelines, only information from the open literature on potential side effects on reptiles and amphibians could be taken into account to estimate a theoretical risk to amphibians and reptiles following the intended uses of ADM.03500.F.2.B. This approach is in line with the recommendations of the guidance document SANCO/10181/2013, Section 4, where it is stated that waivers are acceptable for data requirements for which no agreed test methods or guidance documents are available.

*Aquatic life stages of amphibians*
According to the new 'Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters' (EFSA Journal 2013; 11 (7): 3290), aquatic life stages of amphibians should be included in the risk assessment for aquatic organisms. In the review article from Weltje *et al.* (2013)[1] pairwise comparisons of acute and chronic toxicity data obtained from laboratory tests with different fish and amphibian species were done to determine whether sensitivity systematically differs between these two groups of organisms. As a result, the authors could demonstrate that fish and amphibian toxicity data are highly correlated and fish are more sensitive than amphibians in almost all cases. They concluded that acute and chronic risk to the aquatic life stages of amphibians could be considered as covered by the currently requested risk assessment for aquatic organisms (in particular fish). Similar conclusions can be found also from other authors (e.g. Fryday & Thompson, 2012)[2] and are in line with the EFSA Journal 2013; 11 (7): 3290.

In summary, no adverse effects on aquatic life stages of amphibians need to be expected for the intended uses of ADM.03500.F.2.B, since acceptable effects on fish and other aquatic organisms were identified in the corresponding risk assessment (for details please refer to point 9.5 (*Effects on aquatic organisms*) of this section).

*Reptiles and terrestrial life stages of amphibians*
Reptiles and terrestrial life stages of amphibians will be addressed in future in a revised guidance document on terrestrial ecotoxicology. At present, a separate risk assessment for reptiles and terrestrial life stages of amphibians is not possible.

While a relatively large number of toxicity data were found for aquatic life stages of amphibians suitable for comparisons with fish data, a far smaller number of studies of variable quality are available on effects of pesticides on terrestrial stages of amphibians or reptiles. This makes a comparison with other terrestrial vertebrate data, i.e. for birds and mammals, more difficult.

[1] Weltje L, Simpson P, Gross M, Crane M & Wheeler J, 2013. Environmental Toxicology and Chemistry, 32, 984–994
[2] Fryday S & Thompson H, 2012. Supporting Publications 2012: EN-343, 348 pp.

However, for reptiles the risk from dietary exposure can be assumed much lower than for birds and mammals, since reptiles are poikilothermic and thus unlike birds and mammals they do not have to feed regularly (e.g. to maintain body temperature). As a result, feeding activity may be restricted to warm days and will be negligible during hibernation or at cold days (Fryday & Thompson, 2009[3]).

In addition, Fryday & Thompson (2012) found several examples where adult amphibians were tested in the same study under the same conditions as birds and mammals. In almost all cases, amphibians were less sensitive than birds and/or mammals, indicating that the currently requested and conducted risk assessments for terrestrial vertebrates exposed to prothioconazole are sufficiently conservative for the terrestrial phase of amphibians and reptiles.

In conclusion, based on the uses intended for ADM.03500.F.2.B, an acceptable risk for terrestrial vertebrates (including amphibians and reptiles) can be reasonably expected for acute or long-term exposure to food burdened with residues of prothioconazole (and metabolites), as indicated by TER values that are above the corresponding trigger values. For details, please refer to data points 9.2 (*Effects on birds*) and 9.3 (*Effects on terrestrial vertebrates other than birds*) of this section.

**zRMS comments:**

As currently there are no agreed rules or criteria for evaluation of the risk to other terrestrial vertebrates like reptiles and amphibians, this issue should be addressed once respective guidance is available and EU agreed endpoints concluded.

[3] Fryday S and Thompson H, 2009. Literature reviews on ecotoxicology of chemicals with a special focus on plant protection products. Lot 1. Exposure of reptiles to plant protection products. EFSA (CFT/EFSA/PPR/2008/01).

## 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 the active substance prothioconazole and its relevant metabolites in aquatic systems. Full details of these studies are provided in the respective EU DAR and related documents.

Two major metabolites were detected in the water/sediment study, JAU-desthio (M4) and 1,2,4-triazole. The metabolite JAU-S-methyl (M1) was considered to be a major metabolite in soil and thus might contaminate surface water via drainage and/or run-off.

Toxicity studies show that JAU-desthio (M4) is of less or similar acute toxicity to fish and daphnids. It is of higher toxicity towards algae as well as fish in early life stage toxicity studies. 1,2,4-triazole is of lower toxicity than prothioconazole and can be regarded as not ecotoxicologically relevant. The acute toxicity to fish and daphnids from JAU-S-methyl (M1) and the toxicity to algae are similar to prothioconazole and this metabolite can be considered as not ecotoxicologically relevant according to the EFSA Scientific Report (2007) 106. Nevertheless, since data for the metabolites JAU-S-methyl (M1) and 1,2,4-triazole are available, for maximum conservatism also risk assessments for these metabolites were provided below.

Effects on aquatic organisms of ADM.03500.F.2.B were not evaluated as part of the EU assessment of the active substance. For the purpose of a comparison between the toxicity of technical prothioconazole and the toxicity of formulated prothioconazole, studies on fish, *Daphnia* and algae conducted with ADM.03500.F.2.B were taken into account. As there is no evidence that the active substance or the formulated product is significantly more toxic, the overall lowest endpoints of the respective aquatic groups were used for the risk assessments.

**Table 9.5-1: Endpoints and effect values relevant for the risk assessment for aquatic organisms – active substance and its relevant metabolite(s) in aquatic systems**

| Species | Substance | Time scale | Results | Reference |
|---|---|---|---|---|
| **Toxicity to fish** | | | | |
| *Oncorhynchus mykiss* | Prothioconazole technical | acute | **$LC_{50}$ = 1.83 mg a.s./$L_{mm}$** | EFSA Scientific Report (2007) 106, 1- 98 |
| *Lepomis macrochirus* | Prothioconazole | 96 h, s | $LC_{50}$ = 4.59 mg a.s./$L_{mm}$ | EFSA Scientific Report (2007) 106, 1- 98 |
| *Cyprinus carpio* | Prothioconazole | 96 h, s | $LC_{50}$ = 6.91 mg a.s./$L_{mm}$ | EFSA Scientific Report (2007) 106, 1- 98 |
| *Oncorhynchus mykiss* | JAU-desthio (M4) (*metabolite of prothioconazole*) | acute | **$LC_{50}$ = 6.63 mg met./$L_{mm}$** | EFSA Scientific Report (2007) 106, 1- 98 |
| *Leuciscus idus melanotus* | JAU 6476-desthio | 96 h, s | $LC_{50}$ = 13.2 mg met./$L_{mm}$ | EFSA Scientific Report (2007) 106, 1- 98 |
| *Oncorhynchus mykiss* | JAU-S-methyl (M1) (*metabolite of prothioconazole*) | acute | **$LC_{50}$ = 1.8 mg met./$L_{nom}$** | EFSA Scientific Report (2007) 106, 1- 98 |

| Species | Substance | Time scale | Results | Reference |
|---|---|---|---|---|
| *Oncorhynchus mykiss* | 1,2,4-Triazole (*metabolite of prothioconazole*) | acute | **$LC_{50}$ = 498 mg met./$L_{mm}$** | EFSA Scientific Report (2007) 106, 1- 98 |
| *Oncorhynchus mykiss* | ADM.03500.F.2.B (Prothioconazole EC 250) | acute | **$LC_{50}$ = 0.57 mg a.s./$L_{gm}$** | KCP 10.2.1/01 xxxxxxx 2020, report no.: S19-03475 |
| *Oncorhynchus mykiss* | Prothioconazole technical | chronic, ELS | **NOEC = 0.308 mg a.s./L** | EFSA Scientific Report (2007) 106, 1- 98 |
| *Oncorhynchus mykiss* | JAU-desthio (M4) (*metabolite of prothioconazole*) | chronic, ELS | **NOEC = 0.00334 mg met./L** | EFSA Scientific Report (2007) 106, 1- 98 |
| *Oncorhynchus mykiss* | 1,2,4-Triazole (*metabolite of prothioconazole*) | chronic | NOEC = 3.2 mg met./L | EFSA Scientific Report (2007) 106, 1- 98 |
| **Toxicity to aquatic invertebrates** | | | | |
| *Daphnia magna* | Prothioconazole technical | acute | **$EC_{50}$ = 1.3 mg a.s./$L_{nom}$** | EFSA Scientific Report (2007) 106, 1- 98 |
| *Daphnia magna* | JAU-desthio (M4) (*metabolite of prothioconazole*) | acute | **$EC_{50}$ > 10 mg met./$L_{nom}$** | EFSA Scientific Report (2007) 106, 1- 98 |
| *Daphnia magna* | JAU-S-methyl (M1) (*metabolite of prothioconazole*) | acute | **$EC_{50}$ = 2.8 mg met./$L_{nom}$** | EFSA Scientific Report (2007) 106, 1- 98 |
| *Daphnia magna* | 1,2,4-Triazole (*metabolite of prothioconazole*) | acute | **$EC_{50}$ = 900 mg met./$L_{nom}$** | EFSA Scientific Report (2007) 106, 1- 98 |
| *Daphnia magna* | ADM.03500.F.2.B (Prothioconazole EC 250) | acute | $EC_{50}$ = 1.96 mg a.s./$L_{nom}$ | KCP 10.2.1/02 Zetzmann, M., 2020, report no.: S19-03474 |
| *Daphnia magna* | Prothioconazole technical | chronic | **NOEC = 0.56 mg a.s./$L_{nom}$** | EFSA Scientific Report (2007) 106, 1- 98 |
| *Daphnia magna* | JAU-desthio (M4) (*metabolite of prothioconazole*) | chronic | **NOEC = 0.10 mg met./$L_{nom}$** | EFSA Scientific Report (2007) 106, 1- 98 |

| Species | Substance | Time scale | Results | Reference |
|---|---|---|---|---|
| **Toxicity to sediment-dwelling organisms** | | | | |
| *Chironomus riparius* | Prothioconazole technical | chronic | **NOEC = 9.14 mg a.s../$L_{nom}$** | EFSA Scientific Report (2007) 106, 1- 98 |
| *Chironomus riparius* | JAU-desthio (M4) (*metabolite of prothioconazole*) | chronic | **NOEC = 2.0 mg met./$L_{nom}$** | EFSA Scientific Report (2007) 106, 1- 98 |
| **Toxicity to algae** | | | | |
| *Pseudokirchneriella subcapitata* | Prothioconazole technical | Sub-chronic | $E_bC_{50}$ = 1.1 mg a.s./L $_{im}$<br>**$E_rC_{50}$ = 2.18 mg a.s./L $_{im}$** | EFSA Scientific Report (2007) 106, 1- 98 |
| *Scenedesmus subspicatus* | JAU-desthio (M4) (*metabolite of prothioconazole*) | Sub-chronic | $E_bC_{50}$ = 0.073 mg met./L<br>**$E_rC_{50}$ = 0.55 mg met./L** | EFSA Scientific Report (2007) 106, 1- 98 |
| *Pseudokirchneriella subcapitata* | JAU-S-methyl (M1) (*metabolite of prothioconazole*) | Sub-chronic | $E_bC_{50}$ = 3.77 mg met./$L_{im}$<br>**$E_rC_{50}$ = 47.4 mg met./$L_{im}$** | EFSA Scientific Report (2007) 106, 1- 98 |
| *Pseudokirchneriella subcapitata* | 1,2,4-Triazole (*metabolite of prothioconazole*) | Sub-chronic | $E_bC_{50}$ = 8.2 mg met./L<br>**$E_rC_{50}$ = 22.5 mg met./L** | EFSA Scientific Report (2007) 106, 1- 98 |
| *Pseudokirchneriella subcapitata* | ADM.03500.F.2.B (Prothioconazole EC 250) | Sub-chronic | $E_yC_{50}$ = 0.484 mg a.s./L<br>**$E_rC_{50}$ = 0.752 mg a.s./L** | KCP 10.2.1/03<br>Schuler, L., 2020; report no. S19-03473 |
| **Toxicity to aquatic macrophytes** | | | | |
| *Lemna gibba* | ADM.03500.F.2.B (Prothioconazole EC 250) | Sub-chronic | $E_yC_{50}$ = 0.0183 mg a.s./L<br>**$E_rC_{50}$ = 0.264 mg a.s./L** | KCP 10.2.1/04<br>Weber, K., 2020; report no. S19-03476 |
| **Fish bioconcentration** | | | | |
| *Lepomis macrochirus* | Prothioconazole | Bioconcentration | BCF 19.7 (whole fish wet weight)<br>Clearance time ($CT_{50}$ days):0.8<br>Level of residues (%) after 14 days depuration phase: 9% | EFSA Scientific Report (2007) 106, 1- 98 |
| *Lepomis macrochirus* | JAU 6476-desthio | Bioconcentration | BCF 65 (whole fish wet weight)<br>Clearance time ($CT_{50}$ days):0.4-0.5<br>Level of residues (%) after 14 days depuration phase: 4% | EFSA Scientific Report (2007) 106, 1- 98 |

**in bold** values used in the risk assessment
s: static; ss: semi-static; f: flow-through; nom: based on nominal concentrations; mm: based on mean measured concentrations; im: based on initial measured concentrations
*Endpoint value according to agreement in PRAPeR expert meeting on triazole metabolites (PRAPeR 13, January 2007).

**zRMS comment:**

Aquatic toxicity data for prothioconazole and PTZ-desthio provided in Tables 9.5-1 above are in line with EU agreed endpoints reported in EFSA Scientific Report (2007) 106.

No studies on effects of prothioconazole and metabolite JAU 6476-desthio to *Lemna gibba* were available during the first EU review. It is noted that testing of aquatic macrophytes was not required for prothioconazole being a fungicide. Studies on effects of the formulated product on aquatic organisms listed in Table 9.5-3 were evaluated by the zRMS and considered acceptable. Summaries of the performed studies together with zRMS evaluation may be found in Appendix 2.

#### 9.5.1.1 Justification for new endpoints

In addition to the active substance and metabolite toxicity data, new endpoints are provided for acute toxicity of the formulated product ADM.03500.F.2.B. These studies are considered to be required according to Regulation (EC) No. 284/2013.

### 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", (EFSA Journal 2013; 11(7):3290).

*Regulatory Acceptable Concentrations (RAC)*
Accordingly, the Regulatory Acceptable Concentrations (RAC) relevant for the Tier-1 risk assessment were determined in consideration of the above-justified endpoints. The RAC is defined as concentration at which no adverse effects are expected for the respective aquatic representatives. It was calculated by dividing the endpoints ($LC_{50}$, $EC_{50}$, or NOEC) by the corresponding assessment factor (100/10).

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

The following text is added due to agreements during the Central Zone harmonisation meetings. It should be noted that this text has no impact on the outcome of zonal evaluation of formulation ADM.03500.F.2.B which was performed in line with the EU agreed methodology.

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

Prothioconazole

**Table 9.5-2:** **Prothioconazole: Acceptability of risk (PEC/RAC < 1) for each organism group based on FOCUS Step 1 and 2 calculations for the maximum application rate of ADM.03500.F.2.B: 1× 200 g a.s./ha post-emergence to spring / winter cereals at BBCH 30- 69**

| **Group** | | | | **Tier-1 assessment (based on laboratory data)** | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | **Fish acute** | **Fish acute** | **Fish chronic** | **Invertebrates acute** | **Invertebrates chronic** | **Sediment-dweller chronic** | **Algae sub-chronic** | **Algae sub-chronic** | **Macrophytes sub-chronic** |
| **Test species** | | ***O. mykiss*** | ***O. mykiss*** | ***O. mykiss*** | ***D. magna*** | ***D. magna*** | ***C. riparius*** | ***P. subcapitata*** | ***P. subcapitata*** | ***L. gibba*** |
| **Endpoint** | | $LC_{50}$ | $LC_{50}$ | NOEC | $EC_{50}$ | NOEC | NOEC | $E_rC_{50}$ | $E_rC_{50}$ | $E_rC_{50}$ |
| **(µg/L)** | | 1830 | 570* | 308 | 1300 | 560 | 9140 | 2180 | 752* | 264***** |
| **AF** | | 100 | 100 | 10 | 100 | 10 | 10 | 10 | 10 | 10 |
| **RAC (µg/L)** | | 18.3 | 5.7 | 30.8 | 13 | 56.0 | 914 | 218 | 75.2 | 26.4 |
| **FOCUS Scenario** | **$PEC_{SW}$ max (µg/L)** | | | | | | | | | |
| **Step 1** | | | | | | | | | | |
| | 21.72 | **1.16** | **3.8** | 0.7 | **1.7** | 0.4 | < 0.1 | 0.1 | 0.3 | 0.8 |
| **Step 2** | | | | | | | | | | |
| March-May / June-Sept. | 1.84 | 0.10 | 0.3 | --- | 0.1 | --- | --- | | --- | |

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**
*Endpoints based on formulation study( expressed as a.s.)

As outlined in the table above, for the maximum application to spring / winter cereals at BBCH 30-69, all PEC/RAC ratios for the active substance prothioconazole are below the relevant trigger of 1 at FOCUS Step 2 at the latest. In conclusion, no mitigation measures are required.

**Table 9.5-3:** **<u>Prothioconazole</u>: Acceptability of risk (PEC/RAC < 1) for each organism group based on FOCUS Step 1 and 2 calculations for the maximum application rate of ADM.03500.F.2.B: 1× 175 g a.s./ha post-emergence to spring / winter oilseed rape at BBCH 50-73**

| **Group** | | | | **Tier-1 assessment (based on laboratory data)** | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | **Fish acute** | **Fish acute** | **Fish chronic** | **Invertebrates acute** | **Invertebrates chronic** | **Sediment-dweller chronic** | **Algae sub-chronic** | **Algae sub-chronic** | **Macrophytes sub-chronic** |
| **Test species** | | ***O. mykiss*** | ***O. mykiss*** | ***O. mykiss*** | ***D. magna*** | ***D. magna*** | ***C. riparius*** | ***P. subcapitata*** | ***P. subcapitata*** | ***L. gibba*** |
| **Endpoint** | | $LC_{50}$ | $LC_{50}$ | NOEC | $EC_{50}$ | NOEC | NOEC | $E_rC_{50}$ | $E_rC_{50}$ | $E_rC_{50}$ |
| **(µg/L)** | | 1830 | 570* | 308 | 1300 | 560 | 9140 | 2180 | 752* | 264* |
| **AF** | | 100 | 100 | 10 | 100 | 10 | 10 | 10 | 10 | 10 |
| **RAC (µg/L)** | | 18.3 | 5.7 | 30.8 | 13 | 56.0 | 914 | 218 | 75.2 | 26.4 |
| **FOCUS Scenario** | **$PEC_{sw\ max}$ (µg/L)** | | | | | | | | | |
| **Step 1** | | | | | | | | | | |
| | 19.01 | **1.03** | **3.8** | 0.6 | **1.7** | 0.4 | < 0.1 | 0.09 | 0.3 | 0.8 |
| **Step 2** | | | | | | | | | | |
| March-May / June-Sept. | 1.61 | 0.08 | 0.3 | --- | 0.1 | --- | --- | --- | --- | --- |

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

As outlined in the table above, for the maximum application to spring / winter oilseed rape at BBCH 50-73, all PEC/RAC ratios for the active substance prothioconazole are below the relevant trigger of 1 at FOCUS Step 2 at the latest. In conclusion, no mitigation measures are required.

JAU-S-methyl (M1):

**Table 9.5-4: JAU-S-methyl (M1): Acceptability of risk (PEC/RAC < 1) for each organism group based on FOCUS Step 1 and 2 calculations for the maximum application rate of ADM.03500.F.2.B: 1× 200 g a.s./ha post-emergence to spring / winter cereals at BBCH 30-69**

| **Group** | | **Tier-1 assessment (based on laboratory data)** | | |
|---|---|---|---|---|
| | | **Fish acute** | **Invertebrates acute** | **Algae sub-chronic** |
| **Test species** | | ***O. mykiss*** | ***D. magna*** | ***P. subcapitata*** |
| **Endpoint** | | $LC_{50}$ | $EC_{50}$ | $E_rC_{50}$ |
| **(µg/L)** | | 1800 | 2800 | 47400 |
| **AF** | | 100 | 100 | 10 |
| **RAC (µg/L)** | | 18.0 | 28 | 4740 |
| **FOCUS Scenario** | **$PEC_{SW\ max}$ (µg/L)** | | | |
| **Step 1** | | | | |
| | 15.891 | 0.9 | 0.6 | << 0.1 |
| **Step 2** | | | | |
| March-May / June-Sept. | 1.474 | --- | --- | --- |

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration

As outlined in the table above, for post-emergence applications to spring / winter cereals at BBCH 30-69, all PEC/RAC ratios for the metabolite JAU-S-methyl (M1) are below the relevant trigger of 1 at FOCUS Step 1. In conclusion, no mitigation measures are required.

**Table 9.5-5:** **<u>JAU-S-methyl (M1):</u> Acceptability of risk (PEC/RAC < 1) for each organism group based on FOCUS Step 1 and 2 calculations for the maximum application rate of ADM.03500.F.2.B: 1× 175 g a.s./ha post-emergence to spring / winter oilseed rape at BBCH 50-73**

| **Group** | | **Tier-1 assessment (based on laboratory data)** | | |
|---|---|---|---|---|
| | | **Fish acute** | **Invertebrates acute** | **Algae sub-chronic** |
| **Test species** | | ***O. mykiss*** | ***D. magna*** | ***P. subcapitata*** |
| **Endpoint** | | $LC_{50}$ | $EC_{50}$ | $E_rC_{50}$ |
| **(µg/L)** | | 1800 | 2800 | 47400 |
| **AF** | | 100 | 100 | 10 |
| **RAC (µg/L)** | | 18.0 | 28 | 4740 |
| **FOCUS Scenario** | **$PEC_{SW\ max}$ (µg/L)** | | | |
| **Step 1** | | | | |
| | 13.905 | 0.8 | 0.5 | << 0.1 |
| **Step 2** | | | | |
| March-May / June-Sept. | 1.290 | --- | --- | --- |

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration

As outlined in the table above, for post-emergence applications to spring / winter oilseed rape at BBCH 50-73, all PEC/RAC ratios for the metabolite JAU-S-methyl (M1) are below the relevant trigger of 1 at FOCUS Step 1. In conclusion, no mitigation measures are required.

1,2,4-triazole:

**Table 9.5-6:** **1,2,4-triazole: Acceptability of risk (PEC/RAC < 1) for each organism group based on FOCUS Step 1 and 2 calculations for the maximum application rate of ADM.03500.F.2.B: 1× 200 g a.s./ha post-emergence to spring / winter cereals at BBCH 30-69**

| **Group** | | **Tier-1 assessment (based on laboratory data)** | | | |
|---|---|---|---|---|---|
| | | **Fish acute** | **Fish chronic** | **Invertebrates acute** | **Algae sub-chronic** |
| **Test species** | | ***O. mykiss*** | ***O. mykiss*** | ***D. magna*** | ***P. subcapitata*** |
| **Endpoint** | | $LC_{50}$ | $LC_{50}$ | $EC_{50}$ | $E_rC_{50}$ |
| **(µg/L)** | | 498000 | 3200 | 900000 | 22500 |
| **AF** | | 100 | 10 | 100 | 10 |
| **RAC (µg/L)** | | 4980 | 320 | 9000 | 2250 |
| **FOCUS Scenario** | **$PEC_{sw}$ max (µg/L)** | | | | |
| **Step 1** | | | | | |
| | 5.15 ~~4.587~~ | 0.001 ~~<< 0.1~~ | 0.016 ~~<< 0.1~~ | 0.000572 ~~<< 0.1~~ | 0.0022 ~~<< 0.1~~ |
| **Step 2** | | | | | |
| March-May / June-Sept. | 0.22 ~~0.198~~ | --- | --- | --- | --- |

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration

As outlined in the table above, for post-emergence applications to spring / winter cereals at BBCH 30-69, all PEC/RAC ratios for the metabolite JAU-S-methyl (M1) are below the relevant trigger of 1 at FOCUS Step 1. In conclusion, no mitigation measures are required.

**Table 9.5-7:** **<u>1,2,4-triazole:</u> Acceptability of risk (PEC/RAC < 1) for each organism group based on FOCUS Step 1 and 2 calculations for the maximum application rate of ADM.03500.F.2.B: 1× 175 g a.s./ha post-emergence to spring / winter oilseed rape at BBCH 50-73**

| **Group** | | **Tier-1 assessment (based on laboratory data)** | | | |
|---|---|---|---|---|---|
| | | **Fish acute** | **Fish chronic** | **Invertebrates acute** | **Algae sub-chronic** |
| **Test species** | | ***O. mykiss*** | ***O. mykiss*** | ***D. magna*** | ***P. subcapitata*** |
| **Endpoint** | | $LC_{50}$ | $LC_{50}$ | $EC_{50}$ | $E_rC_{50}$ |
| **(µg/L)** | | 498000 | 3200 | 900000 | 22500 |
| **AF** | | 100 | 10 | 100 | 10 |
| **RAC (µg/L)** | | 4980 | 320 | 9000 | 2250 |
| **FOCUS Scenario** | **$PEC_{sw\ max}$ (µg/L)** | | | | |
| **Step 1** | | | | | |
| | 4.51 ~~4.014~~ | 0.001 ~~<< 0.1~~ | 0.016 ~~<< 0.1~~ | 0.000572 ~~<< 0.1~~ | 0.0022 ~~<< 0.1~~ |
| **Step 2** | | | | | |
| March-May / June-Sept. | 0.15 ~~0.130~~ | --- | --- | --- | --- |

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration

As outlined in the table above, for post-emergence applications to spring / winter oilseed rape at BBCH 50-73, all PEC/RAC ratios for the metabolite JAU-S-methyl (M1) are below the relevant trigger of 1 at FOCUS Step 1. In conclusion, no mitigation measures are required.

JAU-desthio (M4):

**Table 9.5-8:** **JAU-desthio (M4): Acceptability of risk (PEC/RAC < 1) for each organism group based on FOCUS Step 1, 2, 3 and 4 calculations for the maximum application rate of ADM.03500.F.2.B: 1× 200 g a.s./ha post-emergence to spring cereals at BBCH 30**

| **Group** | | **Tier-1 assessment (based on laboratory data)** | | | | | |
|---|---|---|---|---|---|---|---|
| | | **Fish acute** | **Fish chronic** | **Invertebrates acute** | **Invertebrates chronic** | **Sediment-dweller chronic** | **Algae sub-chronic** |
| **Test species** | | ***O. mykiss*** | ***O. mykiss*** | ***D. magna*** | ***D. magna*** | ***C. riparius*** | ***S. subspicatus*** |
| **Endpoint** | | $LC_{50}$ | NOEC | $EC_{50}$ | NOEC | NOEC | $E_rC_{50}$ |
| **(µg/L)** | | 6630 | 3.34 | (>) 10000 | 100 | 2000 | 550 |
| **AF** | | 100 | 10 | 100 | 10 | 10 | 10 |
| **RAC (µg/L)** | | 66.3 | 0.334 | (>) 100 | 10 | 200 | 55 |
| **FOCUS Scenario** | **$PEC_{SW\ max}$ (µg/L)** | | | | | | |
| **Step 1** | | | | | | | |
| | 39.12<br>~~31.124~~ | 0.6<br>~~0.5~~ | **117.1**<br>~~**93.2**~~ | 0.4<br>~~0.3~~ | **3.9**<br>~~**3.1**~~ | 0.2 | 0.7<br>~~0.6~~ |
| **Step 2** | | | | | | | |
| March-May / June – Sept. | 3.63<br>~~3.279~~ | --- | **10.9**<br>~~**9.8**~~ | --- | 0.37<br>~~0.3~~ | --- | --- |
| **Step 3** | | | | | | | |
| D1, ditch | 0.165 | --- | 0.5 | --- | --- | --- | --- |
| D1, stream | 0.063 | --- | 0.2 | --- | --- | --- | --- |
| D3, ditch | 0.041 | --- | 0.1 | --- | --- | --- | --- |
| D4, pond | 0.008 | --- | < 0.1 | --- | --- | --- | --- |
| D4, stream | 0.027 | --- | 0.1 | --- | --- | --- | --- |
| D5, pond | 0.008 | --- | < 0.1 | --- | --- | --- | --- |

| Group | | Tier-1 assessment (based on laboratory data) | | | | | |
|---|---|---|---|---|---|---|---|
| | | **Fish acute** | **Fish chronic** | **Invertebrates acute** | **Invertebrates chronic** | **Sediment-dweller chronic** | **Algae sub-chronic** |
| **Test species** | | ***O. mykiss*** | ***O. mykiss*** | ***D. magna*** | ***D. magna*** | ***C. riparius*** | ***S. subspicatus*** |
| **Endpoint** | | $LC_{50}$ | NOEC | $EC_{50}$ | NOEC | NOEC | $E_rC_{50}$ |
| **(µg/L)** | | 6630 | 3.34 | (>) 10000 | 100 | 2000 | 550 |
| **AF** | | 100 | 10 | 100 | 10 | 10 | 10 |
| **RAC (µg/L)** | | 66.3 | 0.334 | (>) 100 | 10 | 200 | 55 |
| **FOCUS Scenario** | **$PEC_{SW\ max}$ (µg/L)** | | | | | | |
| D5, stream | 0.037 | --- | 0.1 | --- | --- | --- | --- |
| R4, stream | 0.563 | --- | **1.7** | --- | --- | --- | --- |
| **Step 4, 10-m VS** | | | | | | | |
| R4, stream | 0.256 | --- | 0.8 | --- | --- | --- | --- |

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; VS = Vegetative strip

As outlined in the table above, for the maximum application to spring cereals at BBCH 30, all PEC/RAC ratios for the metabolite JAU-desthio (M4) are below the relevant trigger of 1 at FOCUS Step 4, 10-m VS at the latest. Thus, no further considerations have to be taken into account.

**Table 9.5-9:** **JAU-desthio (M4): Acceptability of risk (PEC/RAC < 1) for each organism group based on FOCUS Step 1, 2 and 3 calculations for the maximum application rate of ADM.03500.F.2.B: 1× 200 g a.s./ha post-emergence to spring cereals at BBCH 69**

| Group | | Tier-1 assessment (based on laboratory data) | | | | | |
|---|---|---|---|---|---|---|---|
| | | **Fish acute** | **Fish chronic** | **Invertebrates acute** | **Invertebrates chronic** | **Sediment-dweller chronic** | **Algae sub-chronic** |
| **Test species** | | ***O. mykiss*** | ***O. mykiss*** | ***D. magna*** | ***D. magna*** | ***C. riparius*** | ***S. subspicatus*** |
| **Endpoint** | | $LC_{50}$ | NOEC | $EC_{50}$ | NOEC | NOEC | $E_rC_{50}$ |
| **(µg/L)** | | 6630 | 3.34 | (>) 10000 | 100 | 2000 | 550 |
| **AF** | | 100 | 10 | 100 | 10 | 10 | 10 |
| **RAC (µg/L)** | | 66.3 | 0.334 | (>) 100 | 10 | 200 | 55 |
| **FOCUS Scenario** | **$PEC_{SW\ max}$ (µg/L)** | | | | | | |
| **Step 1** | | | | | | | |
| | 39.12<br>~~31.124~~ | 0.6<br>~~0.5~~ | **117.1**<br>~~**93.2**~~ | 0.4<br>~~0.3~~ | **3.9**<br>~~**3.1**~~ | 0.2 | 0.7<br>~~0.6~~ |
| **Step 2** | | | | | | | |
| March-May / June – Sept. | 1.72<br>~~1.440~~ | --- | **5.2**<br>~~**4.3**~~ | --- | 0.2<br>~~0.1~~ | --- | --- |
| **Step 3** | | | | | | | |
| D1, ditch | 0.191 | --- | 0.6 | --- | --- | --- | --- |
| D1, stream | 0.074 | --- | 0.2 | --- | --- | --- | --- |
| D3, ditch | 0.056 | --- | 0.2 | --- | --- | --- | --- |
| D4, pond | 0.008 | --- | < 0.1 | --- | --- | --- | --- |
| D4, stream | 0.035 | --- | 0.1 | --- | --- | --- | --- |
| D5, pond | 0.008 | --- | < 0.1 | --- | --- | --- | --- |

| Group | | Tier-1 assessment (based on laboratory data) | | | | | |
|---|---|---|---|---|---|---|---|
| | | **Fish acute** | **Fish chronic** | **Invertebrates acute** | **Invertebrates chronic** | **Sediment-dweller chronic** | **Algae sub-chronic** |
| **Test species** | | ***O. mykiss*** | ***O. mykiss*** | ***D. magna*** | ***D. magna*** | ***C. riparius*** | ***S. subspicatus*** |
| **Endpoint** | | $LC_{50}$ | NOEC | $EC_{50}$ | NOEC | NOEC | $E_rC_{50}$ |
| **(µg/L)** | | 6630 | 3.34 | (>) 10000 | 100 | 2000 | 550 |
| **AF** | | 100 | 10 | 100 | 10 | 10 | 10 |
| **RAC (µg/L)** | | 66.3 | 0.334 | (>) 100 | 10 | 200 | 55 |
| **FOCUS Scenario** | **$PEC_{SW\ max}$ (µg/L)** | | | | | | |
| D5, stream | 0.049 | --- | 0.1 | --- | --- | --- | --- |
| R4, stream | 0.027 | --- | 0.1 | --- | --- | --- | --- |

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration

As outlined in the table above, for the maximum application to spring cereals at BBCH 30, all PEC/RAC ratios for the metabolite JAU-desthio (M4) are below the relevant trigger of 1 at FOCUS Step 3 at the latest. Thus, no further considerations have to be taken into account.

**Table 9.5-10:** **JAU-desthio (M4): Acceptability of risk (PEC/RAC < 1) for each organism group based on FOCUS Step 1, 2, 3 and 4 calculations for the maximum application rate of ADM.03500.F.2.B: 1× 200 g a.s./ha post-emergence to winter cereals at BBCH 30**

| **Group** | | **Tier-1 assessment (based on laboratory data)** | | | | | |
|---|---|---|---|---|---|---|---|
| | | **Fish acute** | **Fish chronic** | **Invertebrates acute** | **Invertebrates chronic** | **Sediment-dweller chronic** | **Algae sub-chronic** |
| **Test species** | | ***O. mykiss*** | ***O. mykiss*** | ***D. magna*** | ***D. magna*** | ***C. riparius*** | ***S. subspicatus*** |
| **Endpoint** | | $LC_{50}$ | NOEC | $EC_{50}$ | NOEC | NOEC | $E_rC_{50}$ |
| **(µg/L)** | | 6630 | 3.34 | (>) 10000 | 100 | 2000 | 550 |
| **AF** | | 100 | 10 | 100 | 10 | 10 | 10 |
| **RAC (µg/L)** | | 66.3 | 0.334 | (>) 100 | 10 | 200 | 55 |
| **FOCUS Scenario** | **$PEC_{SW\ max}$ (µg/L)** | | | | | | |
| **Step 1** | | | | | | | |
| | 39.12<br>~~31.124~~ | 0.6<br>~~0.5~~ | **117.1**<br>~~93.2~~ | 0.4<br>~~0.3~~ | **3.9**<br>~~3.1~~ | 0.2 | 0.7<br>~~0.6~~ |
| **Step 2** | | | | | | | |
| March-May / June – Sept. | 3.63<br>~~3.279~~ | --- | **10.9**<br>~~9.8~~ | --- | 0.37<br>~~0.3~~ | --- | --- |
| **Step 3** | | | | | | | |
| D1, ditch | 0.021 | --- | 0.1 | --- | --- | --- | --- |
| D1, stream | 0.042 | --- | 0.1 | --- | --- | --- | --- |
| D2, ditch | 0.143 | --- | 0.4 | --- | --- | --- | --- |
| D2, stream | 0.150 | --- | 0.4 | --- | --- | --- | --- |
| D3, ditch | 0.020 | --- | 0.1 | --- | --- | --- | --- |
| D4, pond | 0.006 | --- | < 0.1 | --- | --- | --- | --- |
| D4, stream | 0.024 | --- | 0.1 | --- | --- | --- | --- |
| D5, pond | 0.007 | --- | < 0.1 | --- | --- | --- | --- |
| D5, stream | 0.036 | --- | 0.1 | --- | --- | --- | --- |

| **Group** | | **Tier-1 assessment (based on laboratory data)** | | | | | |
|---|---|---|---|---|---|---|---|
| | | **Fish acute** | **Fish chronic** | **Invertebrates acute** | **Invertebrates chronic** | **Sediment-dweller chronic** | **Algae sub-chronic** |
| **Test species** | | ***O. mykiss*** | ***O. mykiss*** | ***D. magna*** | ***D. magna*** | ***C. riparius*** | ***S. subspicatus*** |
| **Endpoint** | | $LC_{50}$ | NOEC | $EC_{50}$ | NOEC | NOEC | $E_rC_{50}$ |
| **(µg/L)** | | 6630 | 3.34 | (>) 10000 | 100 | 2000 | 550 |
| **AF** | | 100 | 10 | 100 | 10 | 10 | 10 |
| **RAC (µg/L)** | | 66.3 | 0.334 | (>) 100 | 10 | 200 | 55 |
| **FOCUS Scenario** | **$PEC_{SW\ max}$ (µg/L)** | | | | | | |
| D6, ditch | 0.011 | --- | < 0.1 | --- | --- | --- | --- |
| R1, pond | 0.058 | --- | 0.2 | --- | --- | --- | --- |
| R1, stream | 0.506 | --- | **1.5** | --- | --- | --- | --- |
| R3, stream | 0.441 | --- | **1.3** | --- | --- | --- | --- |
| R4, stream | 0.651 | --- | **1.9** | --- | --- | --- | --- |
| **Step 4, 10-m VS** | | | | | | | |
| R1, stream | 0.230 | --- | 0.7 | --- | --- | --- | --- |
| R3, stream | 0.201 | --- | 0.6 | --- | --- | --- | --- |
| R4, stream | 0.296 | --- | 0.9 | --- | --- | --- | --- |

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; VS = Vegetative strip

As outlined in the table above, for the maximum application to winter cereals at BBCH 30, all PEC/RAC ratios for the metabolite JAU-desthio (M4) are below the relevant trigger of 1 at FOCUS Step 4, 10-m VS at the latest. Thus, no further considerations have to be taken into account.

**Table 9.5-11:** **JAU-desthio (M4): Acceptability of risk (PEC/RAC < 1) for each organism group based on FOCUS Step 1, 2, 3 and 4 calculations for the maximum application rate of ADM.03500.F.2.B: 1× 200 g a.s./ha post-emergence to winter cereals at BBCH 69**

| **Group** | | **Tier-1 assessment (based on laboratory data)** | | | | | |
|---|---|---|---|---|---|---|---|
| | | **Fish acute** | **Fish chronic** | **Invertebrates acute** | **Invertebrates chronic** | **Sediment-dweller chronic** | **Algae sub-chronic** |
| **Test species** | | ***O. mykiss*** | ***O. mykiss*** | ***D. magna*** | ***D. magna*** | ***C. riparius*** | ***S. subspicatus*** |
| **Endpoint** | | $LC_{50}$ | NOEC | $EC_{50}$ | NOEC | NOEC | $E_rC_{50}$ |
| **(µg/L)** | | 6630 | 3.34 | (>) 10000 | 100 | 2000 | 550 |
| **AF** | | 100 | 10 | 100 | 10 | 10 | 10 |
| **RAC (µg/L)** | | 66.3 | 0.334 | (>) 100 | 10 | 200 | 55 |
| **FOCUS Scenario** | **$PEC_{SW\ max}$ (µg/L)** | | | | | | |
| **Step 1** | | | | | | | |
| | 39.12<br>~~31.124~~ | 0.6<br>~~0.5~~ | **117.1**<br>~~93.2~~ | ~~0.4~~<br>~~0.3~~ | **3.9**<br>~~3.1~~ | 0.2 | 0.7<br>~~0.6~~ |
| **Step 2** | | | | | | | |
| March-May /<br>June – Sept. | 1.72<br>~~1.440~~ | --- | **5.2**<br>~~4.3~~ | --- | 0.2<br>~~0.1~~ | --- | --- |
| **Step 3** | | | | | | | |
| D1, ditch | 0.176 | --- | 0.5 | --- | --- | --- | --- |
| D1, stream | 0.063 | --- | 0.2 | --- | --- | --- | --- |
| D2, ditch | 0.186 | --- | 0.6 | --- | --- | --- | --- |
| D2, stream | 0.194 | --- | 0.6 | --- | --- | --- | --- |
| D3, ditch | 0.071 | --- | 0.2 | --- | --- | --- | --- |
| D4, pond | 0.009 | --- | 0.0 | --- | --- | --- | --- |
| D4, stream | 0.036 | --- | 0.1 | --- | --- | --- | --- |
| D5, pond | 0.008 | --- | 0.0 | --- | --- | --- | --- |
| D5, stream | 0.050 | --- | 0.1 | --- | --- | --- | --- |
| D6, ditch | 0.073 | --- | 0.2 | --- | --- | --- | --- |

| **Group** | | **Tier-1 assessment (based on laboratory data)** | | | | | |
|---|---|---|---|---|---|---|---|
| | | **Fish acute** | **Fish chronic** | **Invertebrates acute** | **Invertebrates chronic** | **Sediment-dweller chronic** | **Algae sub-chronic** |
| **Test species** | | ***O. mykiss*** | ***O. mykiss*** | ***D. magna*** | ***D. magna*** | ***C. riparius*** | ***S. subspicatus*** |
| **Endpoint** | | $LC_{50}$ | NOEC | $EC_{50}$ | NOEC | NOEC | $E_rC_{50}$ |
| **(µg/L)** | | 6630 | 3.34 | (>) 10000 | 100 | 2000 | 550 |
| **AF** | | 100 | 10 | 100 | 10 | 10 | 10 |
| **RAC (µg/L)** | | 66.3 | 0.334 | (>) 100 | 10 | 200 | 55 |
| **FOCUS Scenario** | **$PEC_{SW\ max}$ (µg/L)** | | | | | | |
| R1, pond | 0.074 | --- | 0.2 | --- | --- | --- | --- |
| R1, stream | 0.307 | --- | 0.9 | --- | --- | --- | --- |
| R3, stream | 0.456 | --- | **1.4** | --- | --- | --- | --- |
| R4, stream | 0.027 | --- | 0.1 | --- | --- | --- | --- |
| **Step 4, 10-m VS** | | | | | | | |
| R3, stream | 0.162 | --- | 0.5 | --- | --- | --- | --- |

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; VS = Vegetative strip

As outlined in the table above, for the maximum application to winter cereals at BBCH 69, all PEC/RAC ratios for the metabolite JAU-desthio (M4) are below the relevant trigger of 1 at FOCUS Step 4, 10-m VS at the latest. Thus, no further considerations have to be taken into account.

**Table 9.5-12:** **<u>JAU-desthio (M4):</u> Acceptability of risk (PEC/RAC < 1) for each organism group based on FOCUS Step 1, 2, 3 and 4 calculations for the maximum application rate of ADM.03500.F.2.B: 1× 175 g a.s./ha post-emergence to spring oilseed rape at BBCH 50**

| **Group** | | **Tier-1 assessment (based on laboratory data)** | | | | | |
|---|---|---|---|---|---|---|---|
| | | **Fish acute** | **Fish chronic** | **Invertebrates acute** | **Invertebrates chronic** | **Sediment-dweller chronic** | **Algae sub-chronic** |
| **Test species** | | ***O. mykiss*** | ***O. mykiss*** | ***D. magna*** | ***D. magna*** | ***C. riparius*** | ***S. subspicatus*** |
| **Endpoint** | | $LC_{50}$ | NOEC | $EC_{50}$ | NOEC | NOEC | $E_rC_{50}$ |
| **(µg/L)** | | 6630 | 3.34 | (>) 10000 | 100 | 2000 | 550 |
| **AF** | | 100 | 10 | 100 | 10 | 10 | 10 |
| **RAC (µg/L)** | | 66.3 | 0.334 | (>) 100 | 10 | 200 | 55 |
| **FOCUS Scenario** | **$PEC_{SW\ max}$ (µg/L)** | | | | | | |
| **Step 1** | | | | | | | |
| | 34.23<br>~~27.233~~ | 0.5<br>~~0.4~~ | **102.5**<br>~~**81.5**~~ | 0.3<br>~~0.3~~ | **3.2**<br>~~2.7~~ | 0.2<br>~~0.1~~ | 0.6<br>~~0.5~~ |
| **Step 2** | | | | | | | |
| March-May / June – Sept. | 1.34<br>~~1.010~~ | --- | **4.0**<br>~~**3.0**~~ | --- | 0.1 | --- | --- |
| **Step 3** | | | | | | | |
| D1, ditch | 0.154 | --- | 0.5 | --- | --- | --- | --- |
| D1, stream | 0.055 | --- | 0.2 | --- | --- | --- | --- |
| D3, ditch | 0.040 | --- | 0.1 | --- | --- | --- | --- |
| D4, pond | 0.008 | --- | 0.0 | --- | --- | --- | --- |
| D4, stream | 0.031 | --- | 0.1 | --- | --- | --- | --- |
| D5, pond | 0.007 | --- | 0.0 | --- | --- | --- | --- |
| D5, stream | 0.034 | --- | 0.1 | --- | --- | --- | --- |
| R1, pond | 0.021 | --- | 0.1 | --- | --- | --- | --- |
| R1, stream | 0.187 | --- | 0.6 | --- | --- | --- | --- |

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; VS = Vegetative strip

As outlined in the table above, for the maximum application to spring oilseed rape at BBCH 50, all PEC/RAC ratios for the metabolite JAU-desthio (M4) are below the relevant trigger of 1 at FOCUS Step 3 at the latest. Thus, no further considerations have to be taken into account.

**Table 9.5-13:** **JAU-desthio (M4): Acceptability of risk (PEC/RAC < 1) for each organism group based on FOCUS Step 1, 2, 3 and 4 calculations for the maximum application rate of ADM.03500.F.2.B: 1× 175 g a.s./ha post-emergence to spring oilseed rape at BBCH 73**

| **Group** | | **Tier-1 assessment (based on laboratory data)** | | | | | |
|---|---|---|---|---|---|---|---|
| | | **Fish acute** | **Fish chronic** | **Invertebrates acute** | **Invertebrates chronic** | **Sediment-dweller chronic** | **Algae sub-chronic** |
| **Test species** | | ***O. mykiss*** | ***O. mykiss*** | ***D. magna*** | ***D. magna*** | ***C. riparius*** | ***S. subspicatus*** |
| **Endpoint** | | $LC_{50}$ | NOEC | $EC_{50}$ | NOEC | NOEC | $E_rC_{50}$ |
| **(µg/L)** | | 6630 | 3.34 | (>) 10000 | 100 | 2000 | 550 |
| **AF** | | 100 | 10 | 100 | 10 | 10 | 10 |
| **RAC (µg/L)** | | 66.3 | 0.334 | (>) 100 | 10 | 200 | 55 |
| **FOCUS Scenario** | **$PEC_{SW\ max}$ (µg/L)** | | | | | | |
| **Step 1** | | | | | | | |
| | 34.23<br>~~27.233~~ | 0.5<br>~~0.4~~ | **102.5**<br>~~81.5~~ | 0.3 | **3.2**<br>~~2.7~~ | 0.2<br>~~0.1~~ | 0.6<br>~~0.5~~ |
| **Step 2** | | | | | | | |
| March-May / June – Sept. | 1.34<br>~~1.010~~ | --- | **4.0**<br>~~3.0~~ | --- | 0.1 | --- | --- |
| **Step 3** | | | | | | | |
| D1, ditch | 0.161 | --- | 0.5 | --- | --- | --- | --- |
| D1, stream | 0.062 | --- | 0.2 | --- | --- | --- | --- |
| D3, ditch | 0.053 | --- | 0.2 | --- | --- | --- | --- |
| D4, pond | 0.008 | --- | 0.0 | --- | --- | --- | --- |
| D4, stream | 0.032 | --- | 0.1 | --- | --- | --- | --- |
| D5, pond | 0.007 | --- | 0.0 | --- | --- | --- | --- |
| D5, stream | 0.045 | --- | 0.1 | --- | --- | --- | --- |
| R1, pond | 0.083 | --- | 0.2 | --- | --- | --- | --- |
| R1, stream | 0.404 | --- | **1.2** | --- | --- | --- | --- |
| **Step 4, 10-m VS** | | | | | | | |

| Group | | Tier-1 assessment (based on laboratory data) | | | | | |
|---|---|---|---|---|---|---|---|
| | | **Fish acute** | **Fish chronic** | **Invertebrates acute** | **Invertebrates chronic** | **Sediment-dweller chronic** | **Algae sub-chronic** |
| **Test species** | | ***O. mykiss*** | ***O. mykiss*** | ***D. magna*** | ***D. magna*** | ***C. riparius*** | ***S. subspicatus*** |
| **Endpoint** | | $LC_{50}$ | NOEC | $EC_{50}$ | NOEC | NOEC | $E_rC_{50}$ |
| **(µg/L)** | | 6630 | 3.34 | (>) 10000 | 100 | 2000 | 550 |
| **AF** | | 100 | 10 | 100 | 10 | 10 | 10 |
| **RAC (µg/L)** | | 66.3 | 0.334 | (>) 100 | 10 | 200 | 55 |
| **FOCUS Scenario** | **$PEC_{SW\ max}$ (µg/L)** | | | | | | |
| R1 ~~R3~~, stream | 0.184 | --- | 0.6 | --- | --- | --- | --- |

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; VS = Vegetative strip

As outlined in the table above, for the maximum application to spring oilseed rape at BBCH 73, all PEC/RAC ratios for the metabolite JAU-desthio (M4) are below the relevant trigger of 1 at FOCUS Step 4, 10-m VS at the latest. Thus, no further considerations have to be taken into account.

**Table 9.5-14:** **<u>JAU-desthio (M4):</u> Acceptability of risk (PEC/RAC < 1) for each organism group based on FOCUS Step 1, 2, 3 and 4 calculations for the maximum application rate of ADM.03500.F.2.B: 1× 175 g a.s./ha post-emergence to winter oilseed rape at BBCH 50**

| **Group** | | **Tier-1 assessment (based on laboratory data)** | | | | | |
|---|---|---|---|---|---|---|---|
| | | **Fish acute** | **Fish chronic** | **Invertebrates acute** | **Invertebrates chronic** | **Sediment-dweller chronic** | **Algae sub-chronic** |
| **Test species** | | ***O. mykiss*** | ***O. mykiss*** | ***D. magna*** | ***D. magna*** | ***C. riparius*** | ***S. subspicatus*** |
| **Endpoint** | | $LC_{50}$ | NOEC | $EC_{50}$ | NOEC | NOEC | $E_rC_{50}$ |
| **(µg/L)** | | 6630 | 3.34 | (>) 10000 | 100 | 2000 | 550 |
| **AF** | | 100 | 10 | 100 | 10 | 10 | 10 |
| **RAC (µg/L)** | | 66.3 | 0.334 | (>) 100 | 10 | 200 | 55 |
| **FOCUS Scenario** | **$PEC_{SW\ max}$ (µg/L)** | | | | | | |
| **Step 1** | | | | | | | |
| | 34.23<br>~~27.233~~ | 0.4<br>~~0.4~~ | **102.5**<br>~~**81.5**~~ | 0.3<br>~~0.3~~ | **3.2**<br>~~**2.7**~~ | 0.2<br>~~0.1~~ | 0.6<br>~~0.5~~ |
| **Step 2** | | | | | | | |
| March-May / June – Sept. | 1.34<br>~~1.010~~ | --- | **4.0**<br>~~**3.0**~~ | --- | 0.1 | --- | --- |
| **Step 3** | | | | | | | |
| D2, ditch | 0.124 | --- | 0.4 | --- | --- | --- | --- |
| D2, stream | 0.138 | --- | 0.4 | --- | --- | --- | --- |
| D3, ditch | 0.018 | --- | 0.1 | --- | --- | --- | --- |
| D4, pond | 0.006 | --- | 0.0 | --- | --- | --- | --- |
| D4, stream | 0.022 | --- | 0.1 | --- | --- | --- | --- |
| D5, pond | 0.006 | --- | 0.0 | --- | --- | --- | --- |
| D5, stream | 0.031 | --- | 0.1 | --- | --- | --- | --- |
| R1, pond | 0.055<br>~~0.049~~ | --- | 0.1 | --- | --- | --- | --- |
| R1, stream | 0.402 | --- | **1.2** | --- | --- | --- | --- |

| Group | | Tier-1 assessment (based on laboratory data) | | | | | |
|---|---|---|---|---|---|---|---|
| | | **Fish acute** | **Fish chronic** | **Invertebrates acute** | **Invertebrates chronic** | **Sediment-dweller chronic** | **Algae sub-chronic** |
| **Test species** | | ***O. mykiss*** | ***O. mykiss*** | ***D. magna*** | ***D. magna*** | ***C. riparius*** | ***S. subspicatus*** |
| **Endpoint** | | $LC_{50}$ | NOEC | $EC_{50}$ | NOEC | NOEC | $E_rC_{50}$ |
| **(µg/L)** | | 6630 | 3.34 | (>) 10000 | 100 | 2000 | 550 |
| **AF** | | 100 | 10 | 100 | 10 | 10 | 10 |
| **RAC (µg/L)** | | 66.3 | 0.334 | (>) 100 | 10 | 200 | 55 |
| **FOCUS Scenario** | **$PEC_{SW\ max}$ (µg/L)** | | | | | | |
| R3, stream | 0.373 | --- | **1.1** | --- | --- | --- | --- |
| **Step 4, 10-m VFS** | | | | | | | |
| R1, stream | 0.182 | --- | 0.5 | --- | --- | --- | --- |
| R3, stream | 0.170 | --- | 0.5 | --- | --- | --- | --- |

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; VS = Vegetative strip

As outlined in the table above, for the maximum application to winter oilseed rape at BBCH 50, all PEC/RAC ratios for the metabolite JAU-desthio (M4) are below the relevant trigger of 1 at FOCUS Step 4, 10-m VFS at the latest. Thus, no further considerations have to be taken into account.

**Table 9.5-15:** **<u>JAU-desthio (M4):</u> Acceptability of risk (PEC/RAC < 1) for each organism group based on FOCUS Step 1, 2, 3 and 4 calculations for the maximum application rate of ADM.03500.F.2.B: 1× 175 g a.s./ha post-emergence to winter oilseed rape at BBCH 73**

| **Group** | | **Tier-1 assessment (based on laboratory data)** | | | | | |
|---|---|---|---|---|---|---|---|
| | | **Fish acute** | **Fish chronic** | **Invertebrates acute** | **Invertebrates chronic** | **Sediment-dweller chronic** | **Algae sub-chronic** |
| **Test species** | | ***O. mykiss*** | ***O. mykiss*** | ***D. magna*** | ***D. magna*** | ***C. riparius*** | ***S. subspicatus*** |
| **Endpoint** | | $LC_{50}$ | NOEC | $EC_{50}$ | NOEC | NOEC | $E_rC_{50}$ |
| **(µg/L)** | | 6630 | 3.34 | (>) 10000 | 100 | 2000 | 550 |
| **AF** | | 100 | 10 | 100 | 10 | 10 | 10 |
| **RAC (µg/L)** | | 66.3 | 0.334 | (>) 100 | 10 | 200 | 55 |
| **FOCUS Scenario** | **$PEC_{SW\ max}$ (µg/L)** | | | | | | |
| **Step 1** | | | | | | | |
| | 34.23<br>~~27.233~~ | 0.5<br>~~0.4~~ | **102.5**<br>~~81.5~~ | 0.3 | **3.2**<br>~~2.7~~ | 0.2<br>~~0.1~~ | 0.6<br>~~0.5~~ |
| **Step 2** | | | | | | | |
| March-May / June – Sept. | 1.34<br>~~1.010~~ | --- | **3.0** | --- | 0.1 | --- | --- |
| **Step 3** | | | | | | | |
| D2, ditch | 0.157 | --- | 0.5 | --- | --- | --- | --- |
| D2, stream | 0.164 | --- | 0.5 | --- | --- | --- | --- |
| D3, ditch | 0.045 | --- | 0.1 | --- | --- | --- | --- |
| D4, pond | 0.008 | --- | 0.0 | --- | --- | --- | --- |
| D4, stream | 0.032 | --- | 0.1 | --- | --- | --- | --- |
| D5, pond | 0.007 | --- | 0.0 | --- | --- | --- | --- |
| D5, stream | 0.043 | --- | 0.1 | --- | --- | --- | --- |
| R1, pond | 0.025 | --- | 0.1 | --- | --- | --- | --- |
| R1, stream | 0.226 | --- | 0.7 | --- | --- | --- | --- |

| Group | | Tier-1 assessment (based on laboratory data) | | | | | |
|---|---|---|---|---|---|---|---|
| | | **Fish acute** | **Fish chronic** | **Invertebrates acute** | **Invertebrates chronic** | **Sediment-dweller chronic** | **Algae sub-chronic** |
| **Test species** | | ***O. mykiss*** | ***O. mykiss*** | ***D. magna*** | ***D. magna*** | ***C. riparius*** | ***S. subspicatus*** |
| **Endpoint** | | $LC_{50}$ | NOEC | $EC_{50}$ | NOEC | NOEC | $E_rC_{50}$ |
| **(µg/L)** | | 6630 | 3.34 | (>) 10000 | 100 | 2000 | 550 |
| **AF** | | 100 | 10 | 100 | 10 | 10 | 10 |
| **RAC (µg/L)** | | 66.3 | 0.334 | (>) 100 | 10 | 200 | 55 |
| **FOCUS Scenario** | **$PEC_{SW\ max}$ (µg/L)** | | | | | | |
| R3, stream | 0.283 | --- | 0.8 | --- | --- | --- | --- |

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; VS = Vegetative strip

As outlined in the table above, for the maximum application to winter oilseed rape at BBCH 73, all PEC/RAC ratios for the metabolite JAU-desthio (M4) are below the relevant trigger of 1 at FOCUS Step 3 at the latest. Thus, no further considerations have to be taken into account.

**zRMS comments:**

The aquatic risk assessment presented above has been amended accordingly with consideration of the surface water exposure agreed in the course of evaluation in area of Section 8.

Based on the performed calculations following conclusions may be derived:

1. **Spring cereals BBCH 30, 1 x 200 g a.s./ha:**

- Prothioconazole: acceptable risk with no need for risk mitigation measures
- JAU 6476-desthio:
    - D scenarios: risk acceptable with no need for risk mitigation measures
    - scenario R4: risk acceptable with 10 m VFS
    - scenario R1, R3 (surrogate scenarios for spring cereals comes from scenarios for winter cereals) with 10 m VFS.

2. **Spring cereals BBCH 69, 1 x 200 g a.s./ha:**

- Prothioconazole: acceptable risk with no need for risk mitigation measures
- JAU 6476-desthio:
    - D scenarios: risk acceptable with no need for risk mitigation measures
    - scenario R4: risk acceptable with no need for risk mitigation measures
    - scenario R3 (surrogate scenario for spring cereals comes from scenarios of winter cereals) with 10 m VFS.
    - scenario R1 (surrogate scenario for spring cereals comes from scenarios for winter cereals) risk acceptable with no need for risk mitigation measures

The risk from R scenarios not defined for spring cereals is covered by the risk assessment performed for these scenarios available for winter cereals.

3. **Winter cereals BBCH 30, 1 x 200 g a.s./ha :**

- Prothioconazole: acceptable risk with no need for risk mitigation measures
- JAU 6476-desthio:
    - D scenarios: risk acceptable with no need for risk mitigation measures
    - scenarios R1, R3 and R4 risk acceptable with 10 m VFS

4. **Winter cereals BBCH 69, 1 x 200 g a.s./ha:**

- Prothioconazole: acceptable risk with no need for risk mitigation measures
- JAU 6476-desthio:
    - D scenarios: risk acceptable with no need for risk mitigation measures
    - scenario R3: risk acceptable with 10 m VFS.

5. **Spring oilseed rape BBCH 50, 1 x175 g a.s./ha:**

- Prothioconazole: acceptable risk with no need for risk mitigation measures
- JAU 6476-desthio:

- D, R1 scenarios: risk acceptable with no need for risk mitigation measures

**6. Spring oilseed rape BBCH 73, 1 x 175 g a.s./ha:**

- Prothioconazole: acceptable risk with no need for risk mitigation measures
- JAU 6476-desthio:
  - D scenario risk acceptable with no need for risk mitigation measures
  - scenario R1: risk acceptable with 10 m VFS.

**7. Winter oilseed rape BBCH 50, 1 x 175 g a.s./ha:**

- Prothioconazole: acceptable risk with no need for risk mitigation measures
- JAU 6476-desthio:
  - D scenarios: risk acceptable with no need for risk mitigation measures
  - scenario R1 and R3: risk acceptable with 10 m VFS.

**8. Winter oilseed rape BBCH 73, 1 x 175 g a.s./ha:**

- Prothioconazole: acceptable risk with no need for risk mitigation measures
- JAU 6476-desthio:
  - D and R scenarios: risk acceptable with no need for risk mitigation measures

For remaining metabolites of active substance, the risk is acceptable in both crops with no need for risk mitigation measures.

Based on the performed calculations acceptable risk following application of ADM.03500.F.2.B according to the Central Zone GAP may be concluded provided that:

- 10 m vegetated filter strip to surface water bodies are respected for spring cereals at BBCH 30 (based on R1, R3 and R4 scenarios)
- 10 m vegetated filter strip to surface water bodies are respected for spring cereals at BBCH 69 (based on R3 scenario)

- 10 m vegetated filter strip to surface water bodies are respected for winter cereals at BBCH 30 (based on R1, R3 and R4 scenarios)
- 10 m vegetated filter strip to surface water bodies are respected for winter cereals at BBCH 69 (based on R3 scenario)
-
- 10 m vegetated filter strip to surface water bodies are respected for spring oilseed rape at BBCH 73 (based on R3 scenario)
- No risk mitigation for spring oilseed rape at BBCH 50

- 10 m vegetated filter strip to surface water bodies are respected for winter oilseed rape at BBCH 50 (based on R1, R3 scenarios)
- No risk mitigation for winter oilseed rape at BBCH 73

Concerned Member States must decide on applicability of indicated risk mitigation measures in their countries at the product authorisation.

Please note that additional aquatic risk assessment may be required by the concerned Member States that do not accept simulations performed according to FOCUS recommendations.

**Bioaccumulation**

The BCF in whole fish for prothioconazole was determined as 19.7 and as 65 for the metabolite JAU-desthio. Both the parent and the metabolite were rapidly depurated. The log Pow for the 1,2,5-triazole was stated to be < 3 and bioconcentration would therefore be of no concern. The metabolite JAU-S-methyl has a predicted log Pow of 4.19. No bioconcentration study is available, since the concentration in surface water was predicted to be low. Nevertheless, BCFBAF™ (formerly called BCFWIN™) as part of EPISUITE 4.1 was used to model the BCFs of JAU-S-methyl (M1). The regression-based BCF was 310.3.

### 9.5.3 Overall conclusions

Based on PEC/RAC calculations for the active substance prothioconazole and its metabolites, no unacceptable risk for aquatic organisms is indicated. Appropriate risk mitigation measures might be required. However, it should be noted that the recommendation of precautions for the protection of aquatic life depends on the critical GAP uses which may vary in the respective EU Member States (MS) as well as on $PEC_{sw}$ modelling and risk mitigation measures individually approved by each competent national authority. On this account, risk mitigation measures are identified at Member State level and therefore addressed in Part A as well as in the National Addenda to Part B (MS level) submitted along with this core assessment. Further, the risk arising from bioaccumulation of the active substance prothioconazole and metabolites is considered to be low.

**zRMS comments:**

Conclusions above were amended accordingly with consideration of the outcome of the performed risk assessment.

Please note that Additional calculations may be required by cMS that do not accept surface water exposure derived using FOCUS models.

The acceptability and applicability of the indicated risk mitigation measures has to be confirmed at the cMS level.

The following text is added due to agreements during the Central Zone harmonisation meetings. It should be noted that this text has no impact on the outcome of zonal evaluation of formulation ADM.03500.F.2.B, which was performed in line with the EU agreed methodology.

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

## 9.6 Effects on bees (KCP 10.3.1)

### 9.6.1 Toxicity data

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

In addition, a new acute and chronic toxicity study on adult honey bees as well as a honey bee larval toxicity test following repeated exposure have been performed with ADM.03500.F.2.B, the formulation for which authorisation is sought, to meet the data requirements set in the Annex to Reg. (EU) 284/2013. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

Endpoints relevant for the risk assessment of bees are listed in the table below.

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

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| **Acute toxicity** | | | | |
| *Apis mellifera* | Prothioconazole technical | contact | 48-h $LD_{50}$ > 200 µg a.s./bee | EFSA Scientific Report (2007) 106, 1-98 |
| | | oral | 48-h $LD_{50}$ > 71 µg a.s./bee | |
| *Apis mellifera* | ADM.03500.F.2.B (Prothioconazole EC 250) | contact | 96-h $LD_{50}$ = 125.3 µg a.s./bee | KCP 10.3.1.1/01 Sekine, T., 2020a, report no.: 137191035 |
| | | oral | 48-h $LD_{50}$ > 106.6 µg a.s./bee | |
| **Chronic toxicity** | | | | |
| *Apis mellifera* | ADM.03500.F.2.B (Prothioconazole EC 250) | oral, adults | 10-d $LDD_{50}$ = 14.4 µg a.s./bee/d | KCP 10.3.1.2/01: Sekine, T., 2020b, report no.: 137191136 |
| *Apis mellifera* | ADM.03500.F.2.B (Prothioconazole EC 250) | oral, larvae | 8-d NOED = 9.2 µg a.s./larva<br>22-NOED = 9.2 µg a.s./larva | KCP 10.3.1.3/01: Colli, M., 2020, report no.: BT109/19 |

**Justification for new endpoints**

In addition to the active substance data for acute toxicity, new endpoints are provided for acute and chronic toxicity of the formulated product ADM.03500.F.2.B to adult honeybees as well as for honeybee larval toxicity. These studies are considered to be required according to Regulation (EC) No. 284/2013.

**zRMS comments:**

Acute bee toxicity endpoints prothioconazole provided in Tables 9.6 - 1 above are in line with EU agreed endpoints reported in EFSA Scientific Report (2007) 106.

Studies on effects of the formulated product to bees listed in Table 9.6 - 1 were evaluated by the zRMS and considered acceptable. The reported endpoints are confirmed. Summary of the performed studies together with zRMS evaluation may be found in Appendix 2.

It is noted that in order to fulfil the data requirements as set by Commission Regulation (EU) No 284/2013, studies on chronic and larvae toxicity were performed with the formulated product.

## 9.6.2 Risk assessment

The evaluation of the risk for bees was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev 2 (final), October 17, 2002). The recently developed "EFSA Guidance Document on the risk of plant protection products on bees (*Apis mellifera, Bombus* spp. and solitary bees)" (EFSA Journal 2013; 11(7): 3295; updated version published on 4 July 2014) is not yet voted and therefore not taken into account.

The product ADM.03500.F.2.B is an emulsifiable concentrate (EC) containing 250 g/L of the active substance prothioconazole. It is a fungicide applied as spray to infested foliage of spring/winter cereals and oilseed rape. The timing of application is post-emergence (cereal BBCH 30-69 and oilseed rape BBCH 50-73). The worst-case application scenario leading to maximum soil load is a single post-emergence spray application at a rate of 200 g prothioconazole/ha to cereals (0.8 L prod./ha) and 175 g prothioconazole/ha to oilseed rape (0.7 L prod./ha). For a detailed summary of the GAP uses of ADM.03500.F.2.B, please refer to Table 9.1-1.

### 9.6.2.1 Hazard quotients (HQ) for bees

The exposure assessment was conducted using the critical GAP use approach with a single application rate of 200 g prothioconazole/ha covering all other application rates per crop and year. If an acceptable risk can be concluded for this worst-case application scenario, then an acceptable risk can also be concluded for all other intended application scenarios.

**Acute contact exposure**

Hazard Quotients [expressed as application rate (in g/ha) / $LD_{50}$ (in µg/bee)] confirming an acceptable acute contact risk for bees were calculated considering the lowest contact $LD_{50}$ values and the maximum single application rate of 0.8 L prod./ha [equivalent to 200 g a.s./ha]. Accordingly, contact HQ values were calculated as follows:

**Table 9.6-2: Acute contact exposure - Assessment of the risk for honeybees due to the use of ADM.03500.F.2.B**

| **Intended use** | Cereals, 1 × 200 g a.s./ha at BBCH 30-69 (worst-case approach) | | |
|---|---|---|---|
| **Active substance**<br>**Application rate (g/ha)** | Prothioconazole<br>1 × 200 g a.s./ha | | |
| **Test design** | **$LD_{50}$ (lab.) (µg a.s./bee)** | **Single application rate (g a.s./ha)** | **$HQ_{contact}$ criterion: HQ ≤ 50** |
| Contact toxicity | > 200 | 200 | < 1 |
| **Product**<br>**Application rate (g/ha)** | ADM.03500.F.2.B<br>1 × 200 g a.s./ha | | |
| **Test design** | **$LD_{50}$ (lab.) (µg a.s./bee)** | **Single application rate (g a.s./ha)** | **$HQ_{contact}$ criterion: HQ ≤ 50** |
| Contact toxicity | 125.3 | 200 | 1.6 |

HQ: Hazard quotients for contact exposure.

As outlined in the table above, $HQ_{contact}$ values for the active substance prothioconazole and the formulated product are clearly below the corresponding trigger, indicating a low acute contact risk for bees.

### Acute oral exposure

Hazard Quotients [expressed as application rate (in g/ha) / $LD_{50}$ (in µg/bee)] confirming an acceptable acute oral risk for bees were calculated considering the lowest oral $LD_{50}$ values and the maximum single application rate of 0.8 L prod./ha [equivalent to 200 g a.s./ha]. Accordingly, oral HQ values were calculated as follows:

**Table 9.6-3: Acute oral exposure – Assessment of the risk for honeybees due to the use of ADM.03500.F.2.B**

| **Intended use** | Cereals, 1 × 200 g a.s./ha at BBCH 30-69 (worst-case approach) | | |
|---|---|---|---|
| **Active substance**<br>**Application rate (g/ha)** | Prothioconazole<br>1 × 200 g a.s./ha | | |
| **Test design** | **$LD_{50}$ (lab.) (µg a.s./bee)** | **Single application rate (g a.s./ha)** | **$HQ_{contact}$ criterion: HQ ≤ 50** |
| Oral toxicity | > 71 | 200 | < 2.8 |
| **Product**<br>**Application rate (g/ha)** | ADM.03500.F.2.B<br>1 × 200 g a.s./ha | | |
| **Test design** | **$LD_{50}$ (lab.) (µg a.s./bee)** | **Single application rate (g a.s./ha)** | **$HQ_{contact}$ criterion: HQ ≤ 50** |
| Oral toxicity | > 106.6 | 200 | < 1.9 |

HQ: Hazard quotients for coral exposure.

As outlined in the table above, $HQ_{oral}$ values for the active substance and the formulated product are clearly below the corresponding trigger, indicating a low acute oral risk for bees.

**zRMS comments:**

The acute risk assessment for bees presented in Table 9.6 - 2 and Table 9.6 - 3 is agreed by the zRMS.

Overall, acceptable risk to bees may be concluded from the intended uses of ADM.03500.F.2.B.

Please note that the evaluation has been performed in line with SANCO/10329/2002 rev 2 final, as according to conclusions of the Central Zone Steering Committee (CZSC), recommendations of EFSA (2013) should not be considered for the zonal evaluations until the guidance is noted at the EU level.

### Chronic oral exposure

Chronic oral toxicity data on adult honeybees and honeybee larvae were generated to address the new data requirements set in the Annex to Reg. (EU) 283 and 284/2013. For the details of the studies, please refer to KCP 10.3.1.2/01 and KCP 10.3.1.3/01 in Appendix 2. Overall, prothioconazole is of low toxicity to bees with respect to the intended GAP uses of ADM.03500.F.2.B. However, no deterministic risk assessment was conducted for chronic exposure, as there is currently no approved assessment scheme.

**zRMS comments:**

The chronic and larvae risk assessment is not required according to SANCO/10329/2002 rev 2 final.
Due to the fact that the chronic tests are available for adult bee and larvae, the screening step and Tier 1 risk assessment in line with EFSA (2013) for request of some cMS, in Central Zone has been performed by the zRMS below, using endpoints from submitted studies.

Chronic risk assessment to bees:

All steps for the chronic risk assessment, i.e. the screening step, 1st and 2nd oral tier calculations were performed using the corresponding EFSA Bee calculator Tool (Bee-Tool v.3) provided by EFSA.

Screening step risk assessment

The chronic risk to adult honey bees and honey bee larvae bees from the use of product ADM.03500.F.2.B were assessed using the maximum single application rates and the 'exposure toxicity ratios' (ETRs).

| Test | Endpoint µg prod./bee | Calculation factor [a)] | ETR [a)] | Trigger [a)] | Risk acceptable? |
|---|---|---|---|---|---|
| **Cereals, BBCH 30-69 Maximum application dose 200 g a.s./ha** | | | | | |
| Honey bee, chronic | 14.4 | 7.6 / 10.6 | **0.106** | 0.03 | **No** |
| Honey bee, Larvae chronic | 9.2 | 4.4 / 6.1 | 0.10 | 0.2 | Yes |
| **Oilseed rape BBCH 50-73 Maximum application dose 175 g a.s/ha** | | | | | |
| Honey bee, chronic | 14.4 | 7.6 / 10.6 | **0.092** | 0.03 | **No** |
| Honey bee, larvae | 9.2 | 4.4 / 6.1 | 0.08 | 0.2 | Yes |

ETR values in bold are above the trigger value
[a)]Application scenario used for calculations: downward spraying / up- and sideward spraying

Considering the proposed uses of ADM.03500.F.2.B at a maximum application rate of 200 g a.s./ha, in cereals and 175 g a.s./ha in oilseed rape an unacceptable effects are expected for adult honey bees following chronic exposure. A potential risk of formulation is still needed. Therefore, 1st tier oral risk assessments for adult bees were carried out (see Table below).

1st tier, oral chronic risk assessment

In the screening step, potential risk was indicated for adult honey bees following the chronic exposure as well as for honey bee larvae. In the following, a crop and life stage-specific (adult/larvae) risk assessment is carried out, which is a first step of refinement. On the one hand, this takes into account crop dependent exposure factors (Ef), and on the other hand it considers SV values, which depend on default values for pollen and nectar consumption, sugar content in nectar, residues (RUDs) in pollen and nectar as well as crop attractiveness (see table below).
It is noted that 1st tier risk assessment scheme in EFSA (2013) allows for distinguishing between particular BBCH stages of the crop in question. Therefore it was decided by the zRMS to perform separate risk assessment for particular stages at which will be applied to cereals and oilseed rape .

**1st tier oral risk assessment for honey bees (chronic)**

| Crop (Crop group according to EFSA tool) | Endpoint | ETR (oral exposure scenario) chronic | | | | | Trigger |
|---|---|---|---|---|---|---|---|
| | | Treated crop | Weeds | Field margin | Adjacent crop | Next crop | |
| **Maximum single application rate: 0.200 kg a.s./ha BBCH 30-69 cereals** | | | | | | | |
| Cereals | adult, chronic | 0.009 | 0.015 | 0.000 | 0.000 | 0.005 | 0.03 |
| **Maximum single application rate: 0.175 kg a.s./ha, BBCH 50-73 oilseed rape** | | | | | | | |
| Oilseed rape | adult, chronic | 0.000 | 0.000 | 0.000 | 0.000 | 0.005 | 0.03 |

Based on calculations provided above for application to cereals and oilseed rape acceptable chronic risk to adult bees can be concluded.

Overall conclusion:

Acceptable acute oral and contact risk to adult bees from the intended uses of ADM.03500.F.2.B. could be concluded.

Based on calculation provided according to EFSA GD, 2013 the chronic risk to larvae bee at the screening step is considered to be acceptable in contrast to the chronic risk for adult bees where an unacceptable risk is noted.

However, at Tier 1 calculation ETRs values for chronic risk to adult bees were below respective triggers values, indicating an acceptable from the intended uses of ADM.03500.F.2.B.

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

Not relevant.

### 9.6.3 Effects on bumble bees

In the absence of official test guidelines for Non-Apis bees regarding acute (solitary bees) and/or chronic toxicity (solitary bees and bumblebees), no toxicity tests with bumblebees and solitary bees were provided and are not considered to be required according to the EU data requirements. This is in line with the recommendations of the guidance document SANCO/10181/2013, Section 4, where it is stated that waivers are acceptable for data requirements for which no agreed test methods or guidance documents are available.

### 9.6.4 Effects on solitary bees

No data are currently available for solitary bees. For justification, please refer to point 9.6.3.

### 9.6.5 Overall conclusions

Based on the risk assessment for bees according to SANCO/10329/2002 rev 2 (final), October 17, 2002, it can be reasonably concluded that all intended GAP uses of ADM.03500.F.2.B are of low risk to bees under field conditions.

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

## 9.7.1 Toxicity data

Effects on non-target arthropods of the formulation ADM.03500.F.2.B were not evaluated as part of the EU assessment of the active substance prothioconazole. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

The effects of ADM.03500.F.2.B on non-target arthropods were evaluated within the framework of standard laboratory tests using artificial substrate. Tests with the standard species *Aphidius rhopalosiphi* and *Typhlodromus pyri* were conducted. Endpoints relevant for the risk assessment of non-target arthropods are listed in the table below.

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

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| *Aphidius rhopalosiphi* | ADM.03500.F.2.B (Prothioconazole EC 250) | Standard lab test (2-D), glass plates, test rates: 25 – 400 g a.s./ha | $LR_{50}/ER_{50}$ > 400 g a.s./ha | KCP 10.3.2/01 Röhlig, U., 2020a, report no.: 19 48 NAL 0006 |
| *Typhlodromus pyri* | ADM.03500.F.2.B (Prothioconazole EC 250) | Standard lab test (2-D), glass plates, test rates: 12.5– 200 g a.s./ha | $LR_{50}/ER_{50}$ > 200 g a.s./ha | KCP 10.3.2/02 Röhlig, U., 2020b, report no.: 19 48 NTL 0006A |

2 D: 2-dimensional application test system (e.g. glass plates or leaf discs); 3-D: 3-dimensional application test system

### 9.7.1.1 Justification for new endpoints

New endpoints are provided for the formulated product ADM.03500.F.2.B. Those endpoints are considered to be more relevant in terms of non-target arthropod exposure under field conditions than effects of the active substance applied as technical grade.

## 9.7.2 Risk assessment

The evaluation of the risk for non-target arthropods was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002), and in consideration of the recommendations of the guidance document ESCORT 2 (Candolfi, 2001).

The product ADM.03500.F.2.B is an emulsifiable concentrate (EC) containing 250 g/L of the active substance prothioconazole. It is a fungicide applied as spray to infested foliage of spring/winter cereals and oilseed rape. The timing of application is post-emergence (cereal BBCH 30-69 and oilseed rape BBCH 50-73). The worst-case application scenario leading to maximum soil load is a single post-emergence spray application at a rate of 200 g prothioconazole/ha to cereals (0.8 L prod./ha) and 175 g prothioconazole/ha to oilseed rape (0.7 L prod./ha). For a detailed summary of the GAP uses of ADM.03500.F.2.B, please refer to Table 9.1-1.

For the exposure and risk assessment for non-target arthropods, one spray application at the maximum annual rate of 0.8 L prod./ha in cereals was considered as worst-case application scenario, covering all intended GAP uses of ADM.03500.F.2.B. The exposure of non-target arthropods to ADM.03500.F.2.B expressed as Predicted Environmental Rates (PER) was assessed separately for the in-field area and the off-field area.

### 9.7.2.1 Risk assessment for in-field exposure

The PER for in-field exposure was calculated according to the following formula derived from the ESCORT 2 guidance document.

**Equation 9-3: Calculation of Predicted Environmental Rates in the treated field ($PER_{in\text{-}field}$)**

$$\mathbf{PER_{in\text{-}field} = A \cdot MAF} \quad \text{[L prod./ha } \textit{or} \text{ g a.s./ha]}$$

where A = maximum single application rate [L prod./ha *or* g a.s./ha]
MAF = Multiple Application Factor

According to the ESCORT 2 guidance document, the risk for non-target arthropods other than bees at Tier-1 is assessed by calculating Hazard Quotients (HQ). For this purpose, the maximum Predicted Environmental Rates (PER) is divided by $LR_{50}$ values derived from worst-case laboratory tests conducted with the standard test species *A. rhopalosiphi* and *T. pyri*. If the HQ is below 2 for each of the indicator species, a low risk to non-target arthropods can be concluded, and no further testing is required.

**Table 9.7-2: First-tier assessment of the in-field risk for non-target arthropods due to the use of ADM.03500.F.2.B**

| **Intended use** | Cereals, 1× 200 g a.s./ha (worst-case approach) | | |
|---|---|---|---|
| **Product**<br>**Application rate**<br>**MAF** | ADM.03500.F.2.B<br>1× 200 g a.s./ha (0.8 L prod./ha)<br>1.0 | | |
| **Test species Tier-1** | **Rate with ≤ 50 % effect (g a.s./ha)** | **$PER_{in\text{-}field}$ (g a.s./ha)** | **$HQ_{in\text{-}field}$ < 2?** |
| *Aphidius rhopalosiphi* | > 400 | 200 | Yes (HQ < 0.5) |
| *Typhlodromus pyri* | > 200 | 200 | Yes (HQ < 1.0) |

MAF: Multiple application factor; PER: Predicted environmental rate

In conclusion, an overall acceptable risk for non-target arthropods colonised in-field habitats is indicated by the results of the standard laboratory tests and the maximum seasonal application rate of ADM.03500.F.2.B (*worst-case approach*).

### 9.7.2.2 Risk assessment for off-field exposure

For the predicted exposure of the off-field area, drift deposition was considered by applying the spray drift values according to BBA (2000; cited in the ESCORT 2 guidance document). In view of a downward application of ADM.03500.F.2.B to cereals and oilseed rape, the drift scenario „field crops" was considered as most relevant. Since the maximum seasonal application rate of ADM.03500.F.2.B was considered in the risk assessment, the 90$^{th}$ percentile drift values were implemented in the calculations (MAF = 1).

**Equation 9-4: Calculation of the Predicted Environmental Rates in the off-field ($PER_{off\text{-}field}$) for non-target arthropods**

$$PER_{off\text{-}field} = \frac{A \cdot MAF \cdot f_{drift}}{f_{veg}} \times CF \qquad \text{[L prod./ha or g a.s./ha]}$$

where
- A = maximum single application rate [L prod./ha or g a.s./ha]
- MAF = multiple application factor
- $f_{drift}$ = drift factor; % of the applied rate deposited by spray drift divided by 100
- $f_{veg}$ = vegetation distribution factor; taking into account that spray drift values have been determined for non-vegetated area instead of vegetated area (only for 2-d test systems) (= vdf)
- CF = correction factor (= 5) for higher tier studies

As mentioned above, an acceptable risk for non-target arthropods can be concluded, if the calculated HQ values are below the Tier-1 trigger of 2 (worst-case laboratory tests). In line with ESCORT 2 the corresponding $PER_{off\text{-}field}$ values were multiplied by a correction factor of 10 (Tier-1) in order to extrapolate the effects of the tested species to all other off-field non-target arthropods.

**Table 9.7-3: First-tier assessment of the off-field risk for non-target arthropods due to the use of ADM.03500.F.2.B**

| **Intended use** | Cereals, 1× 200 g a.s./ha (worst-case approach) | | | |
|---|---|---|---|---|
| **Product** | ADM.03500.F.2.B | | | |
| **Application rate** | 1× 200 g a.s./ha (0.8 L prod./ha) | | | |
| **MAF** | 1.0 | | | |
| **vdf** | 5 (2-D) ~~10 (2-D),~~ | | | |
| **Test species Tier-1** | **$LR_{50}$ (lab.) (g a.s./ha)** | **Drift rate (Field crops, 1 m)** | **$PER_{off\text{-}field}$* (g a.s./ha)** | **$HQ_{off\text{-}field}$ < 2?** |
| *Aphidius rhopalosiphi* (2-D) | > 400 | 0.0277 (90$^{th}$) | 11.1 ~~5.54~~ | Yes (HQ < 0.03 ~~0.01~~) |
| *Typhlodromus pyri* (2-D) | > 200 | | 11.1 ~~5.54~~ | Yes (HQ < 0.06 ~~0.03~~) |

MAF: Multiple application factor; vdf: Vegetation distribution factor; PER: Predicted environmental rate; CF: Correction factor
* including a vdf of 5 ~~10~~ for 2-dimensional application test system and a correction factor of 10 (e.g. glass plates or leaf discs)

Unacceptable effects on arthropods are not expected in the nearby off-field area. From this point of view, it is reasonably concluded that even in the case of adverse effects on arthropods colonised the in-field area, a re-colonisation or recovery of the treated in-field area with arthropod species (e.g. by immigration from the off-crop area) can safely be expected within a short time-frame. Therefore, it can be concluded that there is a potential for in-crop re-colonisation/recovery of an affected arthropod population, if any.

**zRMS comments:**

The in - field exposure to the formulated product is amended by the zRMS.
As a worst case the VDF of 5 has been considered, since available investigations indicate that VDF of 10 recommended by ESCORT 2 guidance document is not appropriate and may lead to underestimation of the exposure. It should be, however, noted that according to EFSA Supporting publication 2019:EN-1673, VDF of 5 should be considered as the interim solution that will be reflected in the SANCO/10329/2002 rev 2 final with its implementation considered further. Since use of VDF of 5 was not reflected in the current SANCO terrestrial guidance, its use is not yet mandatory. Nevertheless, the risk assessment performed with VDF of 5 is more protective and is thus agreed by the zRMS.

Based on calculations performed with consideration of the Tier I laboratory data acceptable off-field risk to non-target arthropods from the intended uses of ADM.03500.F.2.B may be concluded with no need for risk mitigation measures.

Overall no unacceptable effects for NTA are expected following application of ADM.03500.F.2.B.

### 9.7.2.3 Additional higher-tier risk assessment

Not considered to be relevant.

### 9.7.2.4 Risk mitigation measures

No risk mitigation are considered to be required.

### 9.7.3 Overall conclusions

Based on the results of the standard laboratory tests on the species *A. rhopalosiphi* and *T. pyri*, an overall acceptable risk for non-target arthropods colonised both in-field and off-field habitats can be concluded, considering the intended GAP uses of ADM.03500.F.2.B in cereals and oilseed rape. Risk mitigation measures are not required.

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

### 9.8.1 Toxicity data

Studies on the toxicity to non-target soil organisms have been carried out with the active substance prothioconazole and its relevant metabolites in soil, i.e. JAU- desthio (M4) and JAU-S-methyl (M1). Full details of these studies are provided in the respective EU DAR and related documents.

Additionally, chronic toxicity studies on earthworms, springtails (*Folsomia candida*) and predatory mites (*Hypoaspis aculeifer*) conducted with ADM.03500.F.2.B, the formulation for which authorisation is sought, have been performed to meet the data requirements set in the Annex to Reg. (EU) 284/2013. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

Endpoints relevant for the risk assessment of soil organisms are listed in the table below.

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

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| **Reproductive toxicity data (Tier-1)** | | | | |
| *Eisenia fetida* | ADM.03500.F.2.B (Prothioconazole EC 250) | 56 d, chronic<br>10 % peat | ~~NOEC = 1000 mg prod./kg soil$_{dw}$ [equivalent to 231.5 mg a.s./kg soil$_{dw}$]~~<br><br>~~NOEC$_{corr}$ = 115.8 mg a.s./kg soil$_{dw}$ 1)~~<br><br>NOEC = 556 mg prod./kg soildw [equivalent to 128.71 mg a.s./kg soildw]<br><br>NOECcorr =64.35 mg a.s./kg soildw 1) | KCP 10.4.1.1/01 Ripperger, D., 2020, report no.: S18-07002A |
| *Eisenia fetida* | JAU-desthio (M4) (*metabolite of prothioconazole*) | 56 d, chronic<br>10 % peat | NOEC = 1.0 mg met./kg soil$_{dw}$<br><br>NOEC$_{corr}$ = 0.5 mg met./kg soil$_{dw}$ 1) | EFSA Scientific Report (2007) 106, 1-98 |
| *Eisenia fetida* | JAU-S-methyl (M1) (*metabolite of prothioconazole*) | 56 d, chronic<br>10 % peat | NOEC = 100 mg met./kg soil$_{dw}$<br><br>NOEC$_{corr}$ = 50 mg met./kg soil$_{dw}$ 1) | EFSA Scientific Report (2007) 106, 1-98 |
| *Folsomia candida* | a.s. | 28 d, chronic<br>5 % peat content | NOEC = 64 mg a.s./kg soil$_{dw}$<br><br>NOEC$_{corr}$ = 32 mg a.s../kg soil$_{dw}$ 1) | EFSA Scientific Report (2007) 106, 1-98 |
| *Folsomia candida* | JAU-desthio (M4) (*metabolite of prothioconazole*) | 28 d, chronic<br>5 % peat content | NOEC > 62.5 mg met. /kg soildw<br><br>NOEC$_{corr}$ > 31.25 mg met../kg soildw | EFSA Scientific Report (2007) 106, 1-98 |
| *Folsomia candida* | JAU-S-methyl (M1) | 28 d, chronic<br>5 % peat content | NOEC >31.6 mg met./kg soildw | EFSA Scientific Report (2007) 106, 1-98 |

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| | (*metabolite of prothio-conazole*) | | $NOEC_{corr}$ >15.8 mg met./kg soildw | |

| Species | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| *Folsomia candida* | ADM.03500.F.2.B (Prothioconazole EC 250) | 28 d, chronic<br>5 % peat content | NOEC = 52.9 mg prod./kg $soil_{dw}$ [equivalent to 12.4 mg a.s./kg $soil_{dw}$]<br><br>$NOEC_{corr}$ = 6.2 mg a.s./kg $soil_{dw}$ [1)] | KCP 10.4.2.1/01: Friedrich, S., 2020, report no.: 19 48 TCC 0033 |
| *Hypoaspis aculeifer* | a.s. | 14 d, chronic<br>LUFA 2.1 soil with 0.9% organic carbon | NOEC = 100 mg a.s./kg $soil_{dw}$ | EFSA Scientific Report (2007) 106, 1-98 |
| *Hypoaspis aculeifer* | ADM.03500.F.2.B (Prothioconazole EC 250) | 14 d, chronic<br>5 % peat content | NOEC = 95.3 ~~52.9~~ mg prod./kg $soil_{dw}$ [equivalent to 22.1 ~~12.4~~ mg a.s./kg $soil_{dw}$]<br><br>$NOEC_{corr}$ =11.05 ~~6.2~~ mg a.s./kg $soil_{dw}$ [1)] | KCP 10.4.2.1/02: Schulz, L., 2020, report no.: 19 48 THC 0026 |

[1)] Corrected value derived by dividing the endpoint by a factor of 2 due to a log Pow > 2

According to the current guidance document SANCO/10239, EC 2002, endpoints ($LC_{50}$, NOEC or $EC_{10}$) considered in the risk assessment for soil macro- and mesofauna should be divided by a factor of 2, if the log Pow is greater than 2, unless it can be demonstrated by soil sorption data or other evidence that the toxicity is independent of organic carbon content in the substrate.

As stated in the EFSA Scientific Report (2007) 106, the log $P_{OW}$ for prothioconazole was determined to be between 2.0 (pH 9) and 4.16 (pH 4) (experimental determination) and thus, a correction factor has to be taken into account. The log $P_{OW}$ values for the major metabolites of prothioconazole in soil were determined to be 4.19 (JAU-S-methyl) and 3.04 (JAU-desthio). As these values are above the relevant threshold of 2, a correction factor of 2 was applied for the metabolite of concern for maximum conservatism.

### 9.8.1.1 Justification for new endpoints

In addition to the active substance and metabolite toxicity data, new endpoints are provided for toxicity of the formulated product ADM.03500.F.2.B. These studies (*Folsomia candida*, *Hypoaspis aculeifer*) are considered to be required according to Regulation (EC) No. 284/2013.

## 9.8.2 Risk assessment

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

The product ADM.03500.F.2.B is an emulsifiable concentrate (EC) containing 250 g/L of the active substance prothioconazole. It is a fungicide applied as spray to infested foliage of spring/winter cereals and oilseed rape. The timing of application is post-emergence (cereal BBCH 30-69 and oilseed rape BBCH 50-73). The worst-case application scenario leading to maximum soil load is a single post-emergence spray application at a rate of 200 g prothioconazole/ha to cereals (0.8 L prod./ha) and 175 g prothioconazole/ha to oilseed rape (0.7 L prod./ha). For a detailed summary of the GAP uses of ADM.03500.F.2.B, please refer to Table 9.1-1.

Exposure levels were calculated based on a worst-case application scenario for ADM.03500.F.2.B resulting in the maximum $PEC_{soil}$ i.e. 1× 200 g a.s./ha (BBCH 30-69, 80 % crop interception) in cereals. For a more comprehensive residue definition and summary of calculations of $PEC_{soil}$ values, please refer to point 8.7.2 of Section 8.

### 9.8.2.1 First-tier risk assessment

Toxicity Exposure Ratios (TER) were calculated with the endpoints for chronic effects on earthworms and other soil organisms (*Hypoaspis aculeifer*, *Folsomia candida*) and the relevant $PEC_{soil}$ values. The TER values are as follows:

**Table 9.8-2: First-tier assessment of the chronic risk for earthworms due to the use of ADM.03500.F.2.B in cereals and oilseed rape**

| **Intended use** | Cereals, 1× 200 g a.s./ha at BBCH 30-69 (worst-case approach) | | |
|---|---|---|---|
| **Product/active substance** | **NOEC (mg/kg dw)** | **$PEC_{soil}$ (mg/kg dw)** | **$TER_{LT}$ (criterion TER ≥ 5)** |
| JAU-desthio (M4) | 0.5 (corr) [1)] | 0.028 (ini)<br>~~0.024 (ini)~~ | 17.9<br>~~20.8~~ |
| JAU-S-methyl (M1) | 50 (corr) [1)] | 0.008 (ini) | 6250 |
| ADM.03500.F.2.B | 64.35 [1)]<br>~~115.8 (a.s., corr)~~ [1)] | 0.053 (a.s., ini) | 1214.2<br>~~2185~~ |

1) Corrected value derived by dividing the endpoint by a factor of 2 due to a log Pow > 2

As outlined above, all $TER_{LT}$ values for the formulated product and the metabolites of prothioconazole potentially relevant in soil are above the trigger of 5, established for long-term exposure, indicating an overall acceptable risk for earthworms at Tier-1 level.

**Table 9.8-3: First-tier assessment of the chronic risk for other non-target soil organisms (meso- and macrofauna) due to the use of ADM.03500.F.2.B in cereals and oilseed rape**

| **Intended use** | Cereals, 1× 200 g a.s./ha at BBCH 30-69 (worst-case approach) | | |
|---|---|---|---|
| **Product/active substance** | **NOEC (mg/kg dw)** | **$PEC_{soil}$ (mg/kg dw)** | **$TER_{LT}$ (criterion TER ≥ 5)** |
| **Chronic effects on *Folsomia candida*** | | | |
| a.s. | 32 (corr) [1)] | 0.053 (ini) | 603.8 |
| ADM.03500.F.2.B | 6.2 (a.s., corr) [1)] | 0.053 (ini) | 117.0 |
| ~~JAU-desthio (M4)~~ | ~~3.2~~ | ~~0.024 (ini)~~ | ~~133.3~~ |
| JAU-desthio (M4) | 31.25 | 0.028 ( ini) | 1 116.07 |
| ~~JAU-S-methyl (M1)~~ | ~~3.2~~ [~~2)~~] | ~~0.008 (ini)~~ | ~~400.0~~ |
| JAU-S-methyl (M1) | 15.8 | 0.008 | 1975 |
| **Chronic effects on *Hypoaspis aculeifer*** | | | |
| a.s. | ~~100~~ 50 [1)] | 0.053 (ini) | ~~1887~~ 943.4 |
| ADM.03500.F.2.B | 11.05-~~6.2~~ (a.s., corr) [1)] | 0.053 (ini) | 208.5-~~117.0~~ |
| JAU-desthio (M4) | 10 [2)] | 0.028 (ini) ~~0.024 (ini)~~ | 357.2 ~~416.7~~ |
| JAU-S-methyl (M1) | 10 [2)] | 0.008 (ini) | 1250 |

[1)] Corrected value derived by dividing the endpoint by a factor of 2 due to a log Pow > 2
[2)] Since no measured toxicity data are available, it was assumed that the metabolite is 10 x more toxic than the parent compound prothioconazole (*unrealistic worst-case approach*)
)

All $TER_{LT}$ values for soil meso- and macrofauna (other than earthworms) are greater than the trigger of 5, established for long-term exposure, indicating an overall acceptable risk at Tier-1 level. Thus, no further considerations have to be taken into account.

**zRMS comments:**

Based on calculations in the Table above, acceptable risk to earthworms can be concluded, from prothioconazole, its metabolites and formulation product.

No toxicity data are available for Hypoapsis aculeifer for metabolites and it was assumed that the metabolite is 10 x more toxic than the parent compound prothioconazole.

Overall no unacceptable effects for earthworm and soil macro-organism are expected following application of ADM.03500.F.2.B.

### 9.8.2.2 Higher-tier risk assessment

Not considered to be required.

## 9.8.3 Overall conclusions

Tier-1 TER values calculated for the active substance prothioconazole and its metabolites potentially of concern in soil are above the trigger value of 5, established for long-term exposure, indicating no unacceptable risk for earthworms and other soil organisms (*Hypoaspis aculeifer, Folsomia candida*).

## 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 prothioconazole and its metabolites potentially relevant in soil, i.e. JAU-desthio (M4) and JAU-S-methyl (M1). Full details of this study are provided in the respective EU DAR and related documents. Endpoints relevant for the risk assessment of soil microorganisms are listed in the table below.

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

| Endpoint | Substance | Exposure System | Results | Reference |
|---|---|---|---|---|
| ~~C~~/N-transformation | a.s. | 28 d, aerobic soil type | No detrimental effects (E < ±25 % of the control) on ~~C~~/N-transformation (28 d) up to 2 kg a.s./ha (= 2.67 mg a.s./kg $soil_{dw}$) | EFSA Scientific Report (2007) 106, 1-98 |
| N-transformation | ADM.03500.F.2.B (Prothioconazole EC 250) | 28 d, aerobic soil type | No detrimental effects (E < ±25 % of the control) on ~~C~~/N-transformation (28 d) up to 4.0 L prod./ha (= 5.76 mg prod./kg $soil_{dw}$) | KCP 10.5/01 Persdorf, M., 2020, report no.: 19 48 SMN 0034 |
| N-transformation | JAU-desthio (M4) (*metabolite of prothioconazole*) | 28 d, aerobic soil type | No detrimental effects (E < ±25 % of the control) on N-transformation (28 d) at 0.2 kg met./ha (= 0.267 mg met./kg $soil_{dw}$) and 1.0 kg met./ha (= 1.33 mg met./kg soildw) | EFSA Scientific Report (2007) 106, 1-98 |
| ~~C~~/N-transformation | JAU-S-methyl (M1) (*metabolite of prothioconazole*) | 28 d, aerobic soil type | No detrimental effects (E < ±25 % of the control) on ~~C~~/N-transformation (28 d) up to 2 kg met./ha (= 2.67 mg met./kg $soil_{dw}$) | EFSA Scientific Report (2007) 106, 1-98 |

**zRMS comments:**

Information on effects of prothioconazole and its relevant metabolites on soil micro-organisms provided in Table 9.9-1 above is in line with EU agreed endpoints reported in EFSA Scientific Report (2007) 106.

Information regarding effects on carbon mineralisation is no longer a data requirement and for this reason is struck through in tables above.

The study performed with ADM.03500.F.2.B was evaluated by the zRMS and considered acceptable.
For the study summary and its evaluation by the zRMS, please refer to Appendix 2.

### 9.9.1.1 Justification for new endpoints

In addition to the active substance data, further endpoints are provided for toxicity of the formulated product ADM.03500.F.2.B to meet the data requirements set forth in the Annex to Reg. (EU) no 284/2013.

## 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).

According to SANCO/10329/2002 rev 2 (final), the outcome of the soil microorganism test is directly assessed in terms of risk. Accordingly, effects within a range of ±25 % observed in the underlying tests are considered to be acceptable in a biological and ecological context provided that the concentrations/rates used in the tests covered the maximum $PEC_{soil}$ / deposit rate.

Exposure levels were calculated based on a worst-case application scenario for ADM.03500.F.2.B resulting in the maximum $PEC_{soil}$, i.e. 1× 200 g a.s./ha (BBCH 30-69, 80 % crop interception) in cereals. For a more comprehensive residue definition and summary of calculations of $PEC_{soil}$ values, please refer to point 8.7.2 of Section 8.

Considering this maximum exposure level an acceptable risk for soil microorganisms with regard to C-/N-transformation is indicated as outlined in the table below.

**Table 9.9-2: Assessment of the risk for effects on soil microorganisms due to the use of ADM.03500.F.2.B**

| **Intended use** | Cereals, 1 × 200 g a.s./ha at BBCH 30-69 (worst-case approach) | | |
|---|---|---|---|
| **C-/N-transformation** | | | |
| **Product/active substance** | **Max. conc. with effects ≤ 25 % (mg/kg dw)** | **$PEC_{soil}$ (mg/kg dw)** | **Risk acceptable?** |
| a.s. | 2.67 (a.s.) | 0.053 (ini) | Yes |
| ADM.03500.F.2.B | 5.67 (prod.) | 0.215 (ini) | Yes |
| JAU-desthio (M4) | 0.267 (met.) | 0.0028 (ini)<br>~~0.024 (ini)~~ | Yes |
| JAU-desthio (M4) | 1.33 (met.) | 0.0028 (ini)<br>~~0.024 (ini)~~ | Yes |
| JAU-S-methyl (M1) | 2.67 (met.) | 0.008 (ini) | Yes |

**zRMS comments:**

The risk assessment presented in Table 9.9-2 above is in general agreed by the zRMS with some minor correction of $PEC_{soil}$ values agreed in the course of evaluation in area of Section 8.

Overall no unacceptable effects on soil microbial activity are expected following application of ADM.03500.F.2.B.

### 9.9.3 Overall conclusions

Effects within a range of ±25 % compared to the control were observed at exposure levels which clearly exceed the maximum PEC values in soil calculated in consideration of the worst-case exposure scenario, i.e. 1× 200 g a.s./ha (BBCH 30-69, 80 % crop interception) in cereals, covering the maximum application rates per crop and year. Thus, an acceptable overall risk for soil microorganisms is indicated for all intended GAP uses of ADM.03500.F.2.B in cereals and oilseed rape.

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

### 9.10.1 Toxicity data

Studies on effects on non-target terrestrial plants have been carried out with the active substance prothioconazole. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on non-target terrestrial plants of ADM.03500.F.2.B, the formulation for which authorisation is sought, were not evaluated as part of the EU assessment of active substance prothioconazole. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

Key studies on effects of formulated prothioconazole on non-target plants were evaluated within the framework of a vegetative vigour test and a seedling emergence test conducted with ADM.03500.F.2.B. The dose-response tests were performed with 10 representative plant species: sugar beet, rape, sunflower, tomato, cucumber, soybean, barley, corn, perennial ryegrass, onion. Endpoints are summarised in Table 9.10-1 below. All $ER_{50}$ values were above the highest concentration tested in the vegetative vigour test and a seedling emergence test and is therefore set at > 0.8 L prod./ha (i.e. > 200 g a.s./ha)

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

| Substance | Exposure System | Most sensitive species | Results | Reference |
|---|---|---|---|---|
| a.s. | Seedling emergence | Pigweed | Lowest $ER_{50}$ > 200 g a.s./ha | EFSA Scientific Report (2007) 106, 1-98 |
| a.s. | Vegetative vigour | Pigweed, sugar beet | Lowest $ER_{50}$ > 250 g a.s./ha | EFSA Scientific Report (2007) 106, 1-98 |
| ADM.03500.F.2.B (Prothioconazole EC 250) | Seedling emergence | --- (NOER of all tested plants is 0.8 L prod./ha) | **Lowest $ER_{50}$ > 200 g a.s./ha** | KCP 10.6.1/01 Klix, V.., 2020a report no. 190403AR / TNK18620 |
| ADM.03500.F.2.B (Prothioconazole EC 250) | Vegetative vigour | Rape | **Lowest $ER_{50}$ > 200 g a.s./ha** | KCP 10.6.1/02 Klix, V.., 2020b report no. 190403AR / TNW18620 |

Bold: Endpoint considered most relevant with respect to risk assessment for non-target terrestrial plants

#### 9.10.1.1 Justification for new endpoints

New endpoints are provided for the formulated product, since the formulation itself is considered to be more relevant in terms of non-target plant exposure under field conditions than effects of the active substance applied as technical grade.

## 9.10.2 Risk assessment

The evaluation of the risk for terrestrial non-target plants 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).

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

According to SANCO/10329/2002 (2002), the risk for non-target plants (defined as *non-crop plants located outside the treatment area*) exposed to fungicides should be considered acceptable if there are no initial screening data indicating more than 50 % effects determined at the maximum single application rate (i.e. Tier-1 risk assessment).

**Table 9.10-2: Screening risk assessment for terrestrial non-target plants based on the results of the vegetative vigour and seedling emergence tests**

| **Intended use** | Cereals, 1 × 200 g a.s./ha at BBCH 30-69 (worst-case approach) | | |
|---|---|---|---|
| **Active substance / product**<br>**Application rate (g a.s./ha)** | ADM.03500.F.2.B<br>1× 200 | | |
| **Test system** | **Lowest $ER_{50}$ [g a.s./ha]** | **Max. single application rate [g a.s./ha]** | **Risk for fungicides according to SANCO/10329/2002 recommendations** |
| Vegetative vigour test | > 200 | 200 | Acceptable risk is indicated since the lowest $ER_{50}$ exceeds the maximum single application rate |
| Seedling emergence test | > 200 | 200 | Acceptable risk is indicated since the lowest $ER_{50}$ exceeds the maximum single application rate |

As outlined in the table above, the $ER_{50}$ values of the two test systems are determinable above the maximum test rate of 200 g a.s./ha (seedling emergence tests and vegetative vigour tests), covering the maximum single application rate of ADM.03500.F.2.B in cereals (i.e. 200 g a.s./ha) and oilseed rape (i.e. 175 g a.s./ha). On this account, an acceptable risk for terrestrial non-target plants exposed to applications of the fungicide ADM.03500.F.2.B is indicated. No mitigation measures need to be applied.

**zRMS comment:**

Based on approach presented by the Applicant above the $ER_{50}$ values (seedling emergence tests and vegetative vigour tests) are above the maximum test rate of 200 g a.s./ha covering the maximum single application rate of ADM.03500.F.2.B in cereals (i.e. 200 g a.s./ha) and oilseed rape (i.e. 175 g a.s./ha).
It should be noted that based on the evaluation of the vegetative vigour study treatment related visual phytotoxic effects such as: chlorosis and/or necrosis were found for onion, tomato, cucumber at rate 0.8/L/ha and for soybean and cucumber (necrosis) at rate 0.4 L/ha at the test end. The % of phytotoxic effects were not reported in details in the study protocol.
For this reason, zRMS estimated $ER_{50}$ value to be 0.4 L/ha for visual phytoxicity effects as a worst case scenario.
PER was derived based on the application rate of 0.8 L product/ha and the drift rate of 2.77%.

The calculations based on estimated value of $E_rC_{50}$ = 0.4 L/ha are presented below.

| **Intended use** | **Cereals, 1 × 200 g a.s./ha at BBCH 30-69 (worst-case approach)** | | | |
|---|---|---|---|---|
| **Active substance / product**<br>**Application rate (g a.s./ha)** | **ADM.03500.F.2.B**<br>**1× 200** | | | |
| **Test system** | **$ER_{50}$visual phytotoxicity (L/ha)** | **PER off field (L/ha)** | **$TER_{Lt}$** | **Trigger** |
| **Vegetative vigour test** | 0.4 | 0.022 | 18.8 | >5 |

Overall, no unacceptable effects on NTP are expected following application of ADM.03500.F.2.B.

It should be indicated that risk assessment based on visual phytotoxicity is left at MSs level.
During commenting period process the following comment from the Applicant was given:
The study report of the vegetative vigour study (KCP 10.6.1/02) does not provide data to calculate an ER50, phytotoxic effects are presented as follows:
- =no effects observed / normal growth
+/- = slight effects
+ = medium effects
++ = strong effects

With these data an accurate and statistically defendable and reliable ER50 calculation is not possible, and the applicant strongly disagrees with the approach just to set the ER50 and use it for the risk assessment. In general, the statistical robustness derived from subjective qualitative visual injury assessments is highly questionable, because effects are not based on exact quantitative measurements compared to the other parameters as weight or length or counts as survival, to be usable for calculations of statistically reliable ERx values. In the current study, effects were not even presented in percentages of scores or visual injury ratings in [%], but are scored by categorisation of symptoms, what makes any reliable ERx calculation impossible. The risk assessment using ER50 based on phytotoxic effects should be removed from the final registration report.

zRMS's answer:

It should be noted that the phytotoxicity visual assessment is now required according to recommendation given during Harmonisation meeting in Central Zone where most of MSs would like to have the information on these effects.
We agree that with data is not possible to calculate the real $ErC_{50}$ because of no information of real % of effects is given in the study report.
Due to that dose-response is declared by the study director , that assumption based on the worst-case scenario is chosen by zRMS. However, the assessment based on this assumption was left for each MSs level.

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

Not considered to be required.

### 9.10.2.3 Higher-tier risk assessment

Not considered to be required.

### 9.10.2.4 Risk mitigation measures

Not considered to be required.

### 9.10.3 Overall conclusions

Based on a screening risk assessment recommended for fungicides, safe uses (with respect to an acceptable risk for terrestrial non-target plants) can be identified for ADM.03500.F.2.B. No mitigation measures need to be applied.

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

Adequate risk assessments were performed for all indicator species relevant in the natural environment. In summary, acceptable acute, short-term or long-term risks were indicated for each of the indicator species including birds, mammals, aquatic organisms, bees and other terrestrial non-target arthropods, soil macro- and meso-organisms, microorganisms, and terrestrial non-target plants, in consideration of the GAP uses intended for ADM.03500.F.2.B. Therefore, further data/studies/calculations on non-target species other than those species mentioned above are not required and thus not provided.

## 9.12 Monitoring data (KCP 10.8)

No monitoring studies assessing ecotoxicological effects of prothioconazole are available and considered to be required.

## 9.13 Classification and Labelling

~~Based on Regulation 1272/2008, product ADM.03500.F.2.B is classified as 'very toxic to aquatic life' (H400) and 'toxic to aquatic life with long lasting effects' (H411).~~

**zRMS comments:**

In all valid acute aquatic toxicity studies formulation L(E)$C_{50}$ values were > 1.0 mg product/L and for this reason is not classified for acute aquatic hazard.

The chronic toxicity studies with the product were available only for algae. In absence of chronic toxicity data for fish and aquatic invertebrates the classification for the chronic aquatic hazard should be thus based on summation method.

Classification of the prothioconazole:

The ATP 17 has been published in March 2021 and should apply from 17 December 2022.
The classification of prothioconazole was changed from Aquatic chronic category 2 to Aquatic Chronic category 1.
Therefore, Prothioconazole is classified as H410 with M factor of 1.
Concentration of prothioconazole in formulationis 23.14% and multiplied by M factor of 1 gives concentration of 23.14 %, <25%
Therefore, the product is classified as Chronic 2 due to Σ (10 × Chronic 1 × M) + Σ (Chronic 2) is ≥ 25.
Based on that, formulation is classified for chronic aquatic hazard in category 2 (H411).
Following phrases must be included in the label:

**Hazard statement:** H411~~0~~
**Signal word:** Warning
**Pictogram:** GHS09
**Safety phrases:** P391, P501

| | |
|---|---|
| **Hazard pictograms:** | GHS09  |
| **Signal word:** | Warning |
| **Hazard statement(s):** | H411~~0~~ - ~~Very~~ Toxic to aquatic life with long lasting effects |
| **Precautionary statement(s):** | P391: Collect spillage<br>P501: Dispose of contents/container to hazardous or special waste collection point, in accordance with local, regional, national and/or international regulation |

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

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

| Data point | Author(s) | Year | **Title**<br>**Company Report No.**<br>**Source (where different from company)**<br>**GLP or GEP status**<br>**Published or not** | Vertebrate study Y/N | Owner |
|---|---|---|---|---|---|
| KCP 10.2.1/01 | xxxxxxxxx | 2020 | ADM.3500.F.2.B: Toxicity to the rainbow trout *Oncorhynchus mykiss* under laboratory conditions (Acute toxicity test – Semi-static)<br>Report no.: S19-03475, Sponsor no.: 000102732<br>xxxxxxxxxxxxxxxxxxxxxxxxxxxxxx<br>GLP<br>Unpublished | Y | ADM |
| KCP 10.2.1/02 | Zetzmann, M. | 2020 | ADM.3500.F.2.B: Toxicity to the water flea *Daphnia magna* Straus under laboratory conditions (Acute immobilisation test – Semi-static)<br>Report no.: S19-03474, Sponsor no.: 000102731<br>Eurofins Agroscience Services Ecotox GmbH<br>GLP<br>Unpublished | N | ADM |
| KCP 10.2.1/03 | Schuler, L. | 2020 | ADM.3500.F.2.B: Toxicity to the single cell green alga *Pseudokirchneriella subcapitata* Hindák under laboratory conditions<br>Report no.: S19-03473, Sponsor no.: 000102730<br>Eurofins Agroscience Services Ecotox GmbH<br>GLP<br>Unpublished | N | ADM |

| Data point | Author(s) | Year | Title<br>Company Report No.<br>Source (where different from company)<br>GLP or GEP status<br>Published or not | Vertebrate study Y/N | Owner |
|---|---|---|---|---|---|
| KCP 10.2.1/04 | Weber, K. | 2020 | ADM.3500.F.2.B: Toxicity to the duckweed *Lemna gibba* under laboratory conditions (Growth inhibition test – Semi-static)<br>Report no.: S19-03476, Sponsor no.: 000102733<br>Eurofins Agroscience Services Ecotox GmbH<br>GLP<br>Unpublished | N | ADM |
| KCP 10.3.1.1/01 | Sekine, T. | 2020a | ADM.3500.F.2.B: Effects (acute contact and oral) on honey bees (*Apis mellifera* L.) in the laboratory<br>Report no.: 137191035, Sponsor no.: 000101260<br>Ibacon GmbH<br>GLP<br>Unpublished | N | ADM |
| KCP 10.3.1.2/01 | Sekine, T. | 2020b | ADM.3500.F.2.B: Chronic oral toxicity test on the honey bee (*Apis mellifera* L.) in the laboratory<br>Report no.: 137191136, Sponsor no.: 000101261<br>Ibacon GmbH<br>GLP<br>Unpublished | N | ADM |
| KCP 10.3.1.3/01 | Colli, M. | 2020 | Effects of ADM.3500.F.2.B on honeybees (*Apis mellifera* L.) 22-day larval toxicity test with repeated exposure<br>Report no.: BT109/19, Sponsor no.: 000101262<br>Biotecnologie BT S.r.l.<br>GLP<br>Unpublished | N | ADM |

| Data point | Author(s) | Year | **Title**<br>**Company Report No.**<br>**Source (where different from company)**<br>**GLP or GEP status**<br>**Published or not** | Vertebrate study Y/N | Owner |
|---|---|---|---|---|---|
| KCP 10.3.2/01 | Röhlig, U. | 2020a | Effects of ADM.3500.F.2.B on the parasitic wasp *Aphidius rhopalosiphi* (DeStefani-Perez) in a laboratory test<br>Report no.: 19 48 NAL 0006, Sponsor no.: 000102735<br>BioChem agrar<br>GLP<br>Unpublished | N | ADM |
| KCP 10.3.2/02 | Röhlig, U. | 2020b | Effects of ADM.3500.F.2.B on the predatory mite *Typhlodromus pyri* Scheuten in a laboratory test (amended)<br>Report no.: 19 48 NTL 0006A, Sponsor no.: 000102734A<br>BioChem agrar<br>GLP<br>Unpublished | N | ADM |
| KCP 10.4.1.1/01 | Ripperger, D. | 2020 | ADM.3500.F.2.B: Effects on the reproduction of the earthworm *Eisenia fetida* (Annelida, Lumbricidae) in artificial soil with 10 % peat (amended)<br>Report no.: S18-07002A, Sponsor no.: 000101433A<br>Eurofins Agroscience Services Ecotox GmbH<br>GLP<br>Unpublished | N | ADM |
| KCP 10.4.2.1/01 | Friedrich, S. | 2020 | Effects of ADM.3500.F.2.B on the reproduction of the collembolan *Folsomia candida*<br>Report no.: 19 48 TCC 0033, Sponsor no.: 000102736<br>BioChem agrar<br>GLP<br>Unpublished | N | ADM |

| Data point | Author(s) | Year | **Title**<br>**Company Report No.**<br>**Source (where different from company)**<br>**GLP or GEP status**<br>**Published or not** | Vertebrate study Y/N | Owner |
|---|---|---|---|---|---|
| KCP 10.4.2.1/02 | Schulz, L. | 2020 | Effects of ADM.3500.F.2.B on the reproduction of the predatory mite *Hypoaspis aculeifer*<br>Report no.: 19 48 THC 0026, Sponsor no.: 000102737<br>BioChem agrar<br>GLP<br>Unpublished | N | ADM |
| KCP 10.5/01 | Persdorf, M. | 2020 | Effects of ADM.3500.F.2.B on the activity of soil microflora (Nitrogen transformation test)<br>Report no.: 19 48 SMN 0034, Sponsor no.: 000102738<br>BioChem agrar<br>GLP<br>Unpublished | N | ADM |
| KCP 10.6.1/01 | Klix, V. | 2020a | ADM.3500.F.2.B - Terrestrial plant test: Seedling emergence and seedling growth test<br>Report no.: 190403AR / TNK18620, Sponsor no.: 000102739<br>Noack Laboratorien GmbH<br>GLP<br>Unpublished | N | ADM |
| KCP 10.6.1/02 | Klix, V. | 2020b | ADM.3500.F.2.B - Terrestrial plant test: Vegetative vigour test<br>Report no.: 190403AR / TNW18620, Sponsor no.: 000102740<br>Noack Laboratorien GmbH<br>GLP<br>Unpublished | N | ADM |

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

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

**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 Y/N | Owner |
|---|---|---|---|---|---|
| - | - | - | - | - | - |

# Appendix 2 Detailed evaluation of the new studies

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

### A 2.1.1 KCP 10.1.1 Effects on birds

#### A 2.1.1.1 KCP 10.1.1.1 Acute oral toxicity

An acute oral toxicity test for birds conducted with ADM.03500.F.2.B is not considered to be required for reasons of animal welfare and since an acceptable acute risk for birds can be concluded indicating that the active substance prothioconazole is of low and acceptable toxicity to birds.

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

Not considered to be required.

### 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

Additional studies are not considered to be required, since sufficient information
is available from studies performed with prothioconazole technical and the formulated prothioconazole (for details refer to the toxicological section). Furthermore, the risk assessment for mammals indicates an acceptable risk for terrestrial vertebrates considering the worst-case application scenarios for ADM.03500.F.2.B and each potential route of exposure.

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

Not considered to be required.

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

According to the new data requirements set forth in the Annex to Reg. (EU) no 283/2013 and 284/2013, at present toxicity tests might be requested for birds and mammals but not for amphibians and reptiles. In addition, it should be noted that no official risk assessment guideline has been developed so far that could be used to estimate the extent of different exposure routes for amphibians and reptiles under natural conditions. Finally, almost no validated standard protocols are yet available for amphibian and reptile testing. Available information from open literature indicates that life stages of amphibians as well as reptiles are covered by the risk assessments for fish (aquatic life stages of amphibians) and birds and mammals (terrestrial life stages of amphibians and reptiles). For details, please refer to point 9.4 (Effects on other terrestrial vertebrate wildlife (reptiles and amphibians)) of this section.

Based on the GAP uses intended for ADM.03500.F.2.B, an acceptable risk for terrestrial vertebrates (including amphibians and reptiles) can be reasonably expected for acute or long-term exposure to food burdened with residues of prothioconazole and its metabolite JAU-desthio (M4), as indicated by $TER_A$ and $TER_{LT}$ values for birds and mammals that are above the corresponding trigger values. For details, please refer to data points 9.2 (Effects on birds) and 9.3 (Effects on terrestrial vertebrates other than birds) of this section. In summary, no additional data are considered to be required.

## A 2.2 KCP 10.2 Effects on aquatic organisms

| | |
|---|---|
| zRMS comments**:** | The study was conducted in line with OECD 203 (2019) with minor deviations such as:<br><br>• The last feeding of fish was three days prior test start. According to guideline the last feeding should be 24 - 48 h prior test start.<br>• The TOC of test medium was 2.7 mg/L. According to guideline the TOC value of test medium should be not higher than 2.0 mg/L.<br><br>These deviations are considered to have no impact on the outcome of the study as all the validity criteria were met.<br><br>The test concentration of active substance was verified in 24 h intervals from fresh and aged test solutions.<br>The recovery rates of the measured prothioconazole concentrations in fresh test solutions ranged between 27 % and 91 % of the nominal concentrations. The recovery rates of the measured prothioconazole concentrations in 24 h aged test solutions ranged between 17 % and 83 % of the nominal concentrations.<br>The endpoints were evaluated based on the geometric mean measured concentrations of the test item and the active substance prothioconazole.<br><br>Overall, the study is considered acceptable with the following endpoint relevant for the risk assessment:<br><br>$LC_{50}$ = 2.49 mg product/L correspond to 0.57 mg a.s./L<br>(based on geometric mean measured concentration) |

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

| | |
|---|---|
| Reference: | KCP 10.2.1/01 |
| Report: | ADM.3500.F.2.B: Toxicity to the rainbow trout *Oncorhynchus mykiss* under laboratory conditions (Acute toxicity test – Semi-static), xxxxxx 2020, report no.: S19-03475, sponsor no.: 000102732 |
| Guideline(s): | OECD 203 (2019) |
| Deviations: | The last feeding of fish was three days prior test start. According to guideline OECD 203 (2019) the last feeding should be 24 - 48 h prior test start.<br>The TOC of test medium was 2.7 mg/L. According to guideline OECD 203 (2019) the TOC value of test medium should be not higher than 2.0 mg/L.<br>Those deviations have no impact on study result. |
| GLP: | Yes (certified laboratory) |
| Acceptability/Reliability: | Yes |
| Duplication<br>(if vertebrate study) | No |

**Executive Summary**

Groups of 7 rainbow trout were exposed to nominal concentrations of 0.17, 0.33, 0.66, 1.31 and 2.63 mg/L ADM.3500.F.2.B (Prothioconazole EC 250, 250 g prothioconazole/L) for 96 hours under semi-static conditions. A control group was run concurrently. Fish were observed at 0 h, 2-3 h, 5-6 h, 24 h, 48 h and 96 h after test start. Water quality parameters pH-value, temperature and oxygen-saturation were determined to be within the acceptable limits after 0, 24, 48, 72 and 96 hours. Test item concentrations were analysed via HPLC-MS/MS. The recovery rates of the measured prothioconazole concentrations in fresh test solutions ranged between 27 % and 91 % of the nominal concentrations. The recovery rates of the measured prothioconazole concentrations in 24 h aged test solutions ranged between 17 % and 83 % of the nominal concentrations. Therefore, the biological endpoints were evaluated based on the geometric mean measured concentrations of the test item and the active ingredient prothioconazole. According to the results of the test, the $LC_{50}$ (96 h) of the test item was determined to be 0.57 mg a.s./L, corresponding to 2.49 mg prod./L (based on geometric mean measured concentrations). The corresponding NOEC (0 % mortality, 96 h) was 0.386 mg a.s./L, corresponding to 1.68 mg prod./L (based on geometric mean measured concentrations). No sublethal effects were observed in the control and test item concentration up to and including 0.704 mg prod./L, corresponding to 0.162 mg a.s./L (geometric mean measured) during the study period of 96 h. Unusual behaviour between day 1 to 3 at 1.68 to 3.69 mg/L was partly observed (swimming at the surface of the aquarium, with seemingly reduced activity).

## I. Materials and methods

### A. Materials

1. Test material: ADM.3500.F.2.B (Prothioconazole EC 250)
   Lot/Batch no.: 1109-210219-01
   Content/Purity: prothioconazole: 23.0 % w/w, 248.2 g/L, (analytical); 250 g/L (nominal)
   Control: dilution medium control
   Toxic reference: none

2. Test organisms -
   Species: rainbow trout (*Oncorhynchus mykiss*)
   Age: not stated
   Mean body length (test start): 43 ± 3 mm
   Mean body weight (test start): 0.57 ± 0.20 g
   Source: Forellenzucht Peter Störk, D-88348 Bad Saulgau, Germany, on 03 July 2019
   Acclimatisation period: > 9 days
   No of fish: 7 fish per group
   Feeding during test: none

3. Test units -
   Type and size: 18 L glass aquaria filled with 15 L of test solution, loading of 0.26 g fish/L
   Test procedure: semi-static test with daily water renewal
   Test duration: 96 hours

4. Test conditions -
   Test medium: dechlorinated drinking water and deionised water
   Water hardness: 10°dH corresponding to 178 mg CaCO3/L
   Water temperature: 12.8 ± 0.4 °C
   Aeration: none
   Photoperiod: 16 hours light/8 hours dark, light intensity780 Lux
   Dissolved oxygen: 91 ± 5 % of oxygen saturation
   pH value: 7.90 ± 0.10.

### B. Study design and method

1. In life dates: August 26 to October 15, 2019 (experimental dates)

2. Test design:

The necessary amount of test item for preparing the stock solution was weighed on a weighing scoop and transferred to a volumetric flask. Test medium was added up to the bench mark and the stock solution was homogenised by shaking. The lower test solutions were prepared by serial dilution of the stock solution and lower test solutions with test medium. Defined volumes of the prepared solutions were transferred into each test aquarium containing defined volumes of test medium. The preparation procedure was repeated every 24 hours. Before any application or dilution step, the glass beakers containing the test solution(s) were thoroughly shaken in order to ensure a homogeneous transmission into either the test aquarium or next dilution step. Test aquaria were then stirred with a whisk in order to ensure a thorough homogenization. The nominal test concentrations were: 0 (control), 0.427, 0.939, 2.07, 4.55 and 10.0 mg/L.

Fish were observed at 0 h, 2-3 h, 5-6 h, 1 day (morning and afternoon), 2 days (morning and afternoon), 3 days (morning and afternoon) and 4 days after test start. Fish were considered dead if there was no visible movement (e.g. gill movement), and if touching of the caudal peduncle produced no reaction. Records were made on visible abnormalities such as: difficulties with maintenance of equilibrium, swimming behaviour, respiratory function, pigmentation and all other observed effects. Dead fish were removed. At termination of the test, all remaining fish were euthanized compliant with Directive 2010/63/EU via anaesthetic overdose, by introducing them in medium containing 10 mg/L phenoxyethanol. Fish were then weighed and measured.

Temperature, pH-value and % oxygen saturation of the test solutions, measured after 0, 24, 48, 72 and 96 hours from fresh and aged test solutions, are reported. Hardness of the test medium and light intensity was measured at the start of the test

3. Analytical verification:

Analytical samples taken from all freshly prepared and 24 h aged test solutions at all medium renewal intervals and from all tested concentrations and the control. The content of the active ingredients in the test solution samples was determined by analysing with HPLC-MS/MS. The analytical method was validated with regards to specificity, linearity, accuracy (recovery), precision and limit of quantification. A description and validation of the analytical method is provided in Part B of Section 5 (*Analytical Methods*).

4. Statistics:

The $LC_{50}$-values are the estimated test item concentrations causing 50 % mortality of the test organisms. The $LC_{50}$-values after 24 h, 72 h and 96 h were determined by the geometric mean between the highest test item concentration causing no mortality and the lowest test item concentration causing 100 % mortality. The $LC_{50}$-value after 48 h was determined by Weibull analysis using linear max. likelihood regression, because at the second highest test item concentration only partial mortality occurred. For data evaluation the statistical programme ToxRat Professional 3.3.0 was used. The level of significance for all statistical procedures was set to $\alpha = 0.05$. Due to a lack of mortality, the $LC_{50}$ value after 5-6 h could not be calculated. The NOEC (mortality) was established based on the highest test concentration at which no mortality above the allowed control mortality (1 fish) was observed.

## II. Results and discussion

### A. Analytical data

The recovery rates of the measured prothioconazole concentrations in fresh test solutions ranged between 27 % and 91 % of the nominal concentrations. The recovery rates of the measured prothioconazole concentrations in 24 h aged test solutions ranged between 17 % and 83 % of the nominal concentrations (see table below). Since the measured concentrations of prothioconazole in several test item solutions were not between 80 and 120 % of nominal concentration, the biological endpoints were evaluated based on the geometric mean measured concentrations of the test item and the active ingredient prothioconazole.

**Table A 1: Determined concentration of prothioconazole**

| Test item nominal (mg/L) | Prothioconazole nominal (mg/L) | Sampling | Prothioconazole | | Test item geometric mean* | | Prothioconazole (mg a.s./L) geometric mean |
|---|---|---|---|---|---|---|---|
| | | | (mg /L) | % of nominal | (%) | mg/L) | |
| Control | 0 | 0 h fresh | < LOD | - | - | - | - |
| | | 24 h aged | | | | | |
| | | 24 h fresh | | | | | |
| | | 48 h aged | | | | | |
| | | 48 h fresh | | | | | |
| | | 72 h aged | | | | | |
| | | 72 h fresh | | | | | |
| | | 96 h aged | | | | | |
| 0.427 | 0.0982 | 0 h fresh | 0.0266 | 27 | 66 | 0.282 | 0.0648 |
| | | 24 h aged[1)] | 0.0164 | 17 | | | |
| | | 24 h fresh | 0.0800 | 81 | | | |
| | | 48 h aged | 0.0685 | 70 | | | |
| | | 48 h fresh | 0.0890 | 91 | | | |
| | | 72 h aged | 0.0800 | 81 | | | |
| | | 72 h fresh | 0.0855 | 87 | | | |
| | | 96 h aged | 0.0735 | 75 | | | |
| 0.939 | 0.216 | 0 h fresh | 0.193 | 89 | 75 | 0.704 | 0.162 |
| | | 24 h aged | 0.177 | 82 | | | |
| | | 24 h fresh | 0.128 | 59 | | | |
| | | 48 h aged | 0.115 | 53 | | | |
| | | 48 h fresh | 0.181 | 84 | | | |
| | | 72 h aged | 0.171 | 79 | | | |
| | | 72 h fresh | 0.170 | 79 | | | |
| | | 96 h aged | 0.171 | 79 | | | |
| 2.07 | 0.476 | 0 h fresh | 0.403 | 85 | 81 | 1.68 | 0.386 |
| | | 24 h aged | 0.390 | 82 | | | |
| | | 24 h fresh | 0.370 | 78 | | | |
| | | 48 h aged | 0.383 | 80 | | | |
| | | 48 h fresh | 0.400 | 84 | | | |
| | | 72 h aged | 0.380 | 80 | | | |
| | | 72 h fresh | 0.383 | 80 | | | |
| | | 96 h aged | 0.385 | 81 | | | |
| 4.55 | 1.05 | 0 h fresh | 0.905 | 86 | 81 | 3.69 | 0851 |
| | | 24 h aged | 0.835 | 80 | | | |
| | | 24 h fresh | 0.820 | 78 | | | |
| | | 48 h aged | 0.815 | 78 | | | |
| | | 48 h fresh | 0.880 | 84 | | | |
| | | 72 h aged | 0.875 | 83 | | | |
| 10.0 | 2.30 | 0 h fresh | 1.93 | 84 | 81 | 8.10 | 1.86 |
| | | 24 h aged | 1.82 | 79 | | | |

LOQ = 0.0427 mg/L test item corresponding to 0.00982 mg/L analyte
LOD = Limit of detection
- = not calculable
[1)] F2/retain sample (analysed given that the first sample was < LOQ).
* calculation based on the geometric mean of means of measured fresh and aged prothioconazole values

## B. Mortality

In the control and at the test item concentrations up to and including nominal 2.07 mg/L (geometric mean measured test item concentration of 1.68 mg/L) no test item related mortality was observed within the period of the test. At nominal 4.55 mg/L (geometric mean measured test item concentration of 3.69 mg/L) five fish were found dead after 48 h (first observation) and all fish were found dead after 72 h (first observation). At 10.0 mg/L (geometric mean measured test item concentration of 8.10 mg/L) all fish were found dead after 24 h (first observation).

**Table A 2: Mortality (%) of fish in the test**

| Nominal test item concentration (mg/L) | Control | 0.427 | 0.939 | 2.07 | 4.55 | 10.0 |
|---|---|---|---|---|---|---|
| time | Mortality (%) | | | | | |
| 2-3 hours | 0 | 0 | 0 | 0 | 0 | 0 |
| 5-6 hours | 0 | 0 | 0 | 0 | 0 | 0 |
| Day 1 (morning) | 0 | 0 | 0 | 0 | 0 | 100 |
| Day 1 (afternoon) | 0 | 0 | 0 | 0 | 0 | 100 |
| Day 2 (morning) | 0 | 0 | 0 | 0 | 71 | 100 |
| Day 2 (afternoon) | 0 | 0 | 0 | 0 | 71 | 100 |
| Day 3 (morning) | 0 | 0 | 0 | 0 | 100 | 100 |
| Day 3 (afternoon) | 0 | 0 | 0 | 0 | 100 | 100 |
| Day 4 | 0 | 0 | 0 | 0 | 100 | 100 |

## C. Toxicological symptoms

No sublethal effects were observed in the control and test item concentration up to and including 0.704 mg prod./L, corresponding to 0.162 mg a.s./L (geometric mean measured) during the study period of 96 h. Unusual behaviour between day 1 to 3 at 1.68 to 3.69 mg/L was partly observed (swimming at the surface of the aquarium, with seemingly reduced activity).

**D. Validity of the test:**

| Validity criterion according to OECD 203 | Results of the study |
|---|---|
| In the control(s) (dilution water control, solvent control), the mortality should not exceed 10% (or one fish, if fewer than 10 control fish are tested) at the end of the exposure. | No fish in the control group died until the end of the test. |
| The dissolved oxygen concentration should be ≥60 % of the air saturation value in all test vessels throughout the exposure. | The dissolved oxygen concentration was ≥ 82 % of the air saturation throughout the test. |
| Analytical measurement of test concentrations is compulsory. | The recovery rates of the measured prothioconazole concentrations in fresh and 24 h aged test solutions ranged between 17 % and 91 % of the nominal concentrations. |

Since the test protocol meets the validity criteria set forth in the most recent test guideline, the study is considered valid.

**III. Assessment and conclusion**

In an acute study with rainbow trout (*Oncorhynchus mykiss*), the acute toxicity of ADM.3500.F.2.B (Prothioconazole EC 250, 250 g prothioconazole/L) was analysed for 96 hours under semi-static conditions. According to the results of the test, the $LC_{50}$ (96 h) of the test item was determined to be 0.57 mg a.s./L and 2.49 mg prod./L (based on geometric mean measured concentrations). The corresponding NOEC (mortality, 96 h) was 0.386 mg a.s./L and 1.68 mg prod./L (based on geometric mean measured concentrations). The study is considered valid (see: "D. Validity of the test" above).

| | |
|---|---|
| zRMS comments**:** | The study was conducted in line with OECD 202 with no deviations.<br><br>The study is considered acceptable as all the validity criteria were met.<br><br>The recovery rate of the measured concentrations of prothioconazole in the freshly prepared test solutions ranged between 83 and 91 % of the nominal concentrations and in the aged 24 h old test solutions between 31 and 85 % of the nominal concentrations.<br>The endpoints were evaluated based on the geometric mean measured concentrations of the test item and the active substance prothioconazole.<br><br>Overall, the study is considered acceptable with the following endpoint relevant for the risk assessment:<br><br>$EC_{50}$ = 8.53 mg test item/L, correspond to 1.96 mg a.s./L<br>(based on geometric mean measured concentrations) |

The following study has not been evaluated during the EU peer review of Prothioconazole.

| | |
|---|---|
| Reference: | KCP 10.2.1/02 |
| Report: | ADM.3500.F.2.B: Toxicity to the water flea *Daphnia magna* Straus under laboratory conditions (Acute immobilisation test – Semi-static), Zetzmann, M., 2020, report no.: S19-03474, sponsor no.: 000102731 |
| Guideline(s): | OECD 202 (2004) |
| Deviations: | one |
| GLP: | Yes (certified laboratory) |
| Acceptability/Reliability: | Yes/ |
| Duplication<br>(if vertebrate study) | Not applicable |

**Executive summary**

The purpose of this study was to evaluate the influence of the test item ADM.3500.F.2.B (Prothioconazole EC 250, 250 g prothioconazole/L) on the immobilisation of *Daphnia magna*. Groups of twenty young daphnids (< 24 hours old) were exposed to ADM.3500.F.2.B at nominal concentrations of 20.0, 9.09, 4.13, 1.88, 0.854 mg/L for 48 hours under semi-static conditions. A control group of *Daphnia* was included in the test containing untreated test medium. In order to check the validity of the results, the toxicity of the reference item potassium dichromate was tested at 0.6 and 2.1 mg/L with 20 test organisms per test concentration. The invertebrates were observed for immobilisation after 24 and 48 hours of exposure. Analytical verification of test item concentrations was performed at test start and test end via HPLC. The recovery rate of the measured concentrations of prothioconazole in the freshly prepared test solutions ranged between 83 and 91 % of the nominal concentrations and in the aged 24 h old test solutions between 31 and 85 % of the nominal concentrations. Therefore, the toxicological endpoints were evaluated using mean measured concentrations of the test item and the active ingredient prothioconazole. Based on the geometric mean measured concentrations, the $EC_{50}$ (48 h) for immobilisation was determined to be 8.53 mg test item/L (95 % confidence interval of 6.73 - 10.5), equivalent to 1.96 mg a.s./L (95 % confidence interval of 1.55 - 2.42). The NOEC (48 h) was determined to be 1.28 mg test item/L and 0.294 mg a.s./L. The corresponding LOEC (48 h) was 3.01 mg test item/L and 0.694 mg a.s./L.

## I. Materials and methods

### A. Materials

| | |
|---|---|
| 1. Test material: | ADM.3500.F.2.B (Prothioconazole EC 250) |
| Lot/Batch no.: | 1109-210219-01 |
| Content/Purity: | prothioconazole: 23.0 % w/w, 248.2 g/L, (analytical); 250 g/L (nominal) |
| Control: | untreated test medium |
| Toxic reference: | potassium dichromate |
| 2. Test organisms - | |
| Species: | *Daphnia magna* Straus, Clone V |
| Age: | max. 24 hours old |
| Source: | bred in the laboratories, obtained from Federal Environment Agency in Berlin/Germany |
| Acclimatisation: | none, the daphnids were reared under test conditions |
| Feeding: | none (during the study) |
| No of organisms: | 20 per treatment, divided in 5 test organisms per replicate |
| 3. Test units and exposure – | |
| Type and size: | glass vessel (100 ml), were filled up with ≥ 50 ml test solution. The test units were covered with a glass plate (thus reducing evaporation) |
| Test procedure: | semi-static, medium renewal after 24 h |
| Test duration: | 48 hours |
| 4. Test conditions - | |
| Test medium: | Elendt M4 |
| Water hardness: | 232 mg/L as $CaCO_3$ |
| Water temperature: | 20.8 ± 0.5 °C |
| Aeration: | none |
| Photoperiod: | 16 hours light/8 hours dark |
| Light intensity: | 941 lux |
| Dissolved oxygen: | ≥ 8.1 mg/L |
| pH value: | 7.51 - 7.99 |

### B. Study design and method

| | |
|---|---|
| 1. In life dates: | July 16 to July 24, 2019 (experimental phase) |

2. Test design:

The daphnids were exposed to five test item concentrations and a control in a semi-static test design for 48 hours, with a medium renewal after 24 h. Two concentrations of the reference item potassium dichromate (0.60 mg/L, 2.1 mg/L) were tested around the same time period as the study. The following nominal concentrations were tested: 20.0, 9.09, 4.13, 1.88, 0.854 mg/L with a spacing factor of 2.2. Untreated test medium was used as a control. Test medium was added up to the bench mark and the solution was homogenised by shaking. Lower test solutions were prepared by dilution of the appropriate solution with test medium. The preparation procedure was repeated after 24 hours. ≥ 50 ml of the prepared solutions were transferred to each test vessel.

After 24 h and 48 h the immobilised daphnids were counted. All daphnids not able to swim within 15 seconds after gentle agitation of the test vessel were considered to be immobilised. If present, behavioural changes of daphnids were recorded 24 and 48 hours after starting the test. Temperature, pH-value and oxygen concentration of the test solutions measured after 0, 24 hours aged and fresh and 48 hours are reported. Hardness of the test medium (untreated control) was measured on the day of application.

3. Analytical verification:

The content of the analyte in the test solution samples was determined by analysing with HPLC-MS/MS. Analytical samples taken at 0 hours (initial value) and 24 hours from fresh and aged test solutions and after 48 hours from aged test solution were analysed from the control and all test item concentrations. A description and validation of the analytical method is provided in Part B of Section 5 (*Analytical Methods*).

4. Statistics:

The values for $EC_{50}$ were determined by Weibull analysis using linear max. likelihood regression. The NOEC was established based on the highest concentration at which the immobilisation is not higher than the allowed control immobilisation (≤ 10 % immobilisation). Additionally, the NOEC and the LOEC were determined using the Step-down Cochran-Armitage Test Procedure.

## II. Results and discussion

### A. Analytical data

The recovery rate of the measured concentrations of prothioconazole in the freshly prepared test solutions ranged between 83 and 91 % of the nominal concentrations and in the aged 24 h old test solutions between 31 and 85 % of the nominal concentrations. The toxicological endpoints were evaluated using mean measured concentrations (based on the geometric mean of each measured concentration) of the test item and the active ingredient prothioconazole.

**Table A 3: Determined concentrations of prothioconazole**

| Test item nominal (mg/L) | Prothioconazole nominal (mg a.s./L) | Sampling (h) | Measured prothioconazole concentration | | Geometric mean measured concentration | | |
|---|---|---|---|---|---|---|---|
| | | | (mg a.s./L) | recovery rate (%) | test item (mg/L) | Prothioconazole (mg a.s./L) | recovery rate (%) |
| Control | 0 | 0 fresh | n.d. | - | - | - | - |
| | | 24 aged | n.d. | - | | | |
| | | 24 fresh | n.d. | - | | | |
| | | 48 fresh | n.d. | - | | | |
| 0.854 | 0.196 | 0 fresh | 0.163 | 83 | 0.461 | 0.106 | 54 |
| | | 24 aged | 0.0616 | 31 | | | |
| | | 24 fresh | 0.166 | 85 | | | |
| | | 48 fresh | 0.0765 | 39 | | | |
| 1.88 | 0.432 | 0 fresh | 0.373 | 86 | 1.28 | 0294 | 68 |
| | | 24 aged | 0.158 | 37 | | | |
| | | 24 fresh | 0.388 | 90 | | | |
| | | 48 fresh | 0.318 | 74 | | | |

| Test item nominal (mg/L) | Prothioconazole nominal (mg a.s./L) | Sampling (h) | Measured prothioconazole concentration | | Geometric mean measured concentration | | |
|---|---|---|---|---|---|---|---|
| | | | (mg a.s./L) | recovery rate (%) | test item (mg/L) | Prothioconazole (mg a.s./L) | recovery rate (%) |
| 4.13 | 0.950 | 0 fresh | 0.820 | 86 | 3.01 | 0.694 | 73 |
| | | 24 aged | 0.540 | 57 | | | |
| | | 24 fresh | 0.865 | 91 | | | |
| | | 48 fresh | 0.610 | 64 | | | |
| 9.09 | 2.09 | 0 fresh | 1.77 | 85 | 7.09 | 1.63 | 78 |
| | | 24 aged | 1.38 | 66 | | | |
| | | 24 fresh | 1.80 | 86 | | | |
| | | 48 fresh | 1.60 | 77 | | | |
| 20.0 | 4.60 | 0 fresh | 3.98 | 87 | 16.8 | 3.86 | 84 |
| | | 24 aged | 3.53 | 77 | | | |
| | | 24 fresh | 4.10 | 89 | | | |
| | | 48 fresh | 3.93 | 85 | | | |

- = not calculated; n.d. = not detectable (< LOD); LOQ = 0.0196 mg/L analyte corresponding to 0.0854 mg/L test item

## B. Immobilisation

The following discussion of the results is based on the geometric mean measured concentrations of the test item and the active ingredient. After 24 hours of exposure no immobilisation was observed in the control and up to and including 3.01 mg test item/L (0.694 mg a.s./L).

No immobilisation higher than the allowed control immobilisation of 10 % was observed up to 7.09 mg test item/L (1.63 mg a.s./L). 60 % immobilisation was observed at the highest test item concentration of 16.8 mg test item/L (3.86 mg a.s./L). After 48 hours of exposure no immobilisation was observed in the control and at test item concentrations 0.461 mg test item/L (0.106 mg a.s./L) and 1.28 mg test item/L (0.294 mg a.s./L). No immobilisation higher than the allowed control immobilisation was observed at 3.01 mg test item/L (0.694 mg a.s./L). 25 % immobilisation was observed at 7.09 mg test item/L (1.63 mg a.s./L).

At the highest test item concentration of 16.8 mg test item/L (3.86 mg a.s./L) all daphnids were immobile. After 24 hours the mobile daphnids in the highest test item concentration of 16.8 mg test item/L (3.86 mg a.s./L) were slightly sticky and showed jerky swimming behaviour. After 48 hours the mobile daphnids in the test item concentration 7.09 mg test item/L (1.63 mg a.s./L) appeared to be sluggish.

**Table A 4: Immobilisation rates in *Daphnia magna* exposed to prothioconazole**

| Immobilised daphnids after 24 h | Geometric mean measured test item concentration (mg/L) | | | | | |
|---|---|---|---|---|---|---|
| | Control | 0.461 | 1.28 | 3.01 | 7.09 | 16.8 |
| Replicate 1 | 0 | 0 | 0 | 0 | 0 | 2 |
| Replicate 2 | 0 | 0 | 0 | 0 | 0 | 2 |
| Replicate 3 | 0 | 0 | 0 | 0 | 1 | 5 |
| Replicate 4 | 0 | 0 | 0 | 0 | 0 | 3 |
| ∑ | 0 | 0 | 0 | 0 | 1 | 12 |
| % | 0 | 0 | 0 | 0 | 5 | 60 |

| Immobilised daphnids after 48 h | Geometric mean measured test item concentration (mg/L) | | | | | |
|---|---|---|---|---|---|---|
| | **Control** | **0.461** | **1.28** | **3.01** | **7.09** | **16.8** |
| Replicate 1 | 0 | 0 | 0 | 0 | 1 | 5 |
| Replicate 2 | 0 | 0 | 0 | 1 | 1 | 5 |
| Replicate 3 | 0 | 0 | 0 | 0 | 2 | 5 |
| Replicate 4 | 0 | 0 | 0 | 1 | 1 | 5 |
| ∑ | 0 | 0 | 0 | 2 | 5 | 20 |
| % | 0 | 0 | 0 | 10 | 25 | 100 |

Toxic reference

In order to check the validity of the results, the toxicity of the reference item potassium dichromate was tested at 0.6 and 2.1 mg/L with 20 test organisms per test concentration. The results are presented in the table below.

**Table A 5: Immobilisation rates in *Daphnia magna* exposed to potassium dichromate**

| $K_2Cr_2O_7$ (mg/L) | 24 h | | 48 h | |
|---|---|---|---|---|
| | Immobilised daphnids | | | |
| | **0.60** | **2.10** | **0.60** | **2.10** |
| Replicate 1 | 0 | 5 | 0 | 5 |
| Replicate 2 | 0 | 5 | 0 | 5 |
| Replicate 3 | 0 | 5 | 3 | 5 |
| Replicate 4 | 0 | 5 | 1 | 5 |
| ∑ | 0 | 20 | 4 | 20 |
| % | 0 | 100 | 20 | 100 |

The results indicate an $EC_{50}$ (24 h) of the reference item potassium dichromate between 0.60 and 2.10 mg/L. Since the results are in accordance with the requirements of the OECD guideline 202 and fall within the historical data generated with the reference item at the testing facility, the daphnids were suitable for the determination of the toxicological effects of the test item.

**C. Validity of the test:**

| Validity criterion according to OECD 202 | Results of the study |
|---|---|
| In the control, including the control containing the solubilising agent, not more than 10 % of the daphnids should have been immobilised or exhibit other signs of disease or stress, for example, discoloration or unusual behaviour such as trapping at surface of water | In the controls 0 % of the daphnids have been immobilised after the 48 hours test duration. |
| The dissolved oxygen concentration at the end of the test should be ≥ 3 mg/L in control and test vessels. | The dissolved oxygen concentration at the end of the test was > 8.1 mg/L. |

Since the test protocol meets the validity criteria set forth in the most recent test guideline, the study is considered valid.

## III. Assessment and conclusion

In a 48-hour acute toxicity test, groups of *Daphnia magna* were exposed to ADM.3500.F.2.B (Prothioconazole EC 250, 250 g prothioconazole/L) under semi-static conditions. A control group exposed to test medium without test item was run concurrently. Based on the geometric mean measured concentrations, the $EC_{50}$ (48 h) for immobilisation was determined to be 8.53 mg test item/L (95 % confidence interval of 6.73 - 10.5), equivalent to 1.96 mg a.s./L (95 % confidence interval of 1.55 - 2.42). The NOEC (48 h) was determined to be 1.28 mg test item/L and 0.294 mg a.s./L. The corresponding LOEC (48 h) was 3.01 mg test item/L and 0.694 mg a.s./L. The study is considered valid (see: "C. Validity of the test" above).

| | |
|---|---|
| zRMS comments**:** | The study was conducted in line with OECD 201 with no deviations.<br><br>The measured concentrations of prothioconazole in the freshly prepared test item solutions ranged from 91 % to 94 % of the nominal concentrations.<br>Over the test period of 24 h and 48 h up to 72 h a concentration dependent decline was observed in the measured samples.<br>In the aged samples after 72 hours the measured concentrations ranged between below LOQ at the lowest test item concentration and 92 % of the nominal at the highest test item concentration.<br><br>The endpoints were evaluated based on the geometric mean measured concentrations of the test item and the active substance prothioconazole.<br><br>Overall, the study is considered acceptable with the following endpoint relevant for the risk assessment:<br><br>$E_rC_{50}$ = 3.27 mg test item/L correspond to 0.752 mg a.s /L<br>(based on geometric mean measured concentrations)<br><br>$E_rC_{20}$ = 2.19 mg test item/L and 0.504 mg a.s./L<br>(based on geometric mean measured concentration)<br><br>$EyC_{10}$ = 1.07 mg test item/L and 0.245 mg a.s./L<br>(based on geometric mean measured concentration)<br><br>$NOE_rC$ = 0.782 mg test item/L and 0.180 mg a.s./L<br>(based on geometric mean measured concentration)<br><br>The $E_yC_{50}$ = 2.10 mg test item/L and 0.484 mg a.s./L<br>(based on geometric mean measured concentration)<br><br>$E_yC_{20}$ = 1.40 mg test item/L (nominal) and 0.322 mg a.s./L<br>(based on geometric mean measured concentration)<br>$E_rC_{10}$ = 1.77 mg test item/L and 0.409 mg a.s./L<br>(based on geometric mean measured concentration)<br><br>$NOE_yC$ = 0.782 mg test item/L and 0.180 mg a.s./L<br>(based on geometric mean measured concentration) |

The following study has not been evaluated during the EU peer review of Prothioconazole.

| | |
|---|---|
| Reference: | KCP 10.2.1/03 |
| Report: | ADM.3500.F.2.B: Toxicity to the single cell green alga *Pseudokirchneriella subcapitata* Hindák under laboratory conditions, Schuler, L., 2020, report no.: S19-03473, sponsor no.: 000102730 |
| Guideline(s): | OECD 201 (2006, Annex 5 corrected 2011) |
| Deviations: | None |
| GLP: | Yes (certified laboratory) |
| Acceptability/Reliability: | Yes |
| Duplication (if vertebrate study) | Not applicable |

**Executive summary**

The purpose of this study was to evaluate the influence of the test item ADM.3500.F.2.B (Prothioconazole EC 250, 250 g prothioconazole/L) on the growth of the unicellular green alga *Pseudokirchneriella subcapitata*. Algae with a cell density of 0.5 x $10^4$/ml were exposed to ADM.3500.F.2.B at nominal concentrations of 0.0954, 0.305, 0.977, 3.13 and 10.0 mg/L for 72 hours under static conditions. A control group was included in the test containing dilution water alone. The number of cells were recorded each day of the test. Analytical verification of test item concentrations was performed at test start and test end via HPLC-MS/MS. The measured concentrations of prothioconazole in the freshly prepared test item solutions ranged from 91 % to 94 % of the nominal concentrations. In the aged samples after 72 hours the measured concentrations ranged between below LOQ at the lowest test item concentration and 92 % of the nominal at the highest test item concentration. Therefore, toxicological endpoints were evaluated based on the geometric mean of each measured concentration of the test item and the active ingredient prothioconazole. Under the conditions of this study, significant inhibitory effects were determined for growth rate and for yield at test item concentrations of 2.78 mg test item/L and 0.640 mg a.s./L (geometric mean measured) and above. The overall LOEC was therefore determined to be 2.78 mg test item/L and 0.640 mg a.s./L (geometric mean measured), the corresponding NOEC was set at 0.782 mg test item/L and 0.180 mg a.s./L (geometric mean measured). The $E_rC_{10}$ was determined to be 1.77 mg test item/L and 0.409 mg a.s./L. The $E_yC_{10}$ was 1.07 mg test item/L and 0.245 mg a.s./L (geometric mean measured). The $E_rC_{20}$ was determined to be 2.19 mg test item/L and 0.504 mg a.s./L. The $E_yC_{20}$ was 1.40 mg test item/L (nominal) and 0.322 mg a.s./L (geometric mean measured). The $E_rC_{50}$ was determined to be 3.27 mg test item/L and 0.752 mg a.s./L. The $E_yC_{50}$ was 2.10 mg test item/L and 0.484 mg a.s./L (geometric mean measured).

## I. Materials and methods

### A. Materials

1. Test material: ADM.3500.F.2.B (Prothioconazole EC 250)
   Lot/Batch no.: 1109-210219-01
   Content/Purity: prothioconazole: 23.0 % w/w, 248.2 g/L, (analytical); 250 g/L (nominal)
   Control: dilution medium control
   Toxic reference: Potassium dichromate, 99.97 % w/w analysed (tested in a separate study twice a year)

2. Test organisms -
   Species: *Pseudokirchneriella subcapitata* (green alga)
   Source: Sciencebridge GmbH, Hans-Adolf-Krebs-Weg 1, D-37077 Göttingen, Germany.
   Cell density: $0.5 \times 10^4$ /ml

3. Test units and exposure –
   Type and size: 100 ml Erlenmeyer flasks loosely covered with aluminium caps each containing approx. 50 ml test solution.
   Test procedure: static, with a shaking speed of 105 rpm
   Test duration: 72 hours

4. Test conditions –
   Test medium: AAP-Medium (according to Annex 3 of OECD 201, composition see Appendix B). The pH was adjusted to 7.5 ± 0.1 with NaOH and HCl
   Water temperature: 22.7 - 23.8 °C
   Photoperiod: constant light
   Light intensity $94.5\ \mu Em^{-2}s^{-1}$
   pH value: 7.13 - 8.16

### B. Study design and method

1. In life dates: September 17 to September 30, 2019 (experimental phase)

2. Test design:

The test organism was exposed to different concentrations (0.0954, 0.305, 0.977, 3.13 and 10.0 mg/L) of the test item set up in a geometrical series with a spacing factor of 3.2 under defined conditions. Initial target cell density was $0.5 \times 10^4$ cells/ml. Three replicates were employed for each of the test item concentrations and six replicates for the control. The necessary amount of test item for preparing the stock solution was weighed on a weighing scoop and transferred to a volumetric flask. Test medium was added up to the bench mark and the solution was homogenised by shaking. Lower test solutions were prepared by dilution of appropriate solutions with test medium. All stock and test solutions were prepared with AAP-medium containing algae.

At test start (0 hours) the number of cells in each control replicate was determined in duplicate. At defined dates (24, 48 and 72 hours), the number of cells in each replicate was determined in duplicate. The determination was performed by fluorescence measurement. The fluorescence measurements were performed with a fluorescence microplate reader (infinite 200Pro) with an emission wavelength of 670 nm and evaluated with Tecan i-control (Software for Tecan Readers Tecan i-control, 1.11.1.0). Additionally, the morphological appearance of the algae cells was observed microscopically at the end of the test.

Measurements of pH-value were performed at t = 0 h and t = 72 h, the temperature was measured continuously and the min/max temperature recorded at hours 0, 24, 48 and 72. The light intensity of all positions of the incubator is measured once a year and was confirmed for one representative position at test start.

Potassium dichromate is tested as the toxic reference item in a separate study twice a year to confirm the sensitivity of the test organism against compounds with known effects under the test conditions.

3. Analytical verification:

Analytical samples were taken and analysed from the control and all test item concentrations at 0 hours (initial value) from fresh test solutions and after 24 hours, 48 hours and 72 hours from aged test solutions. The content of prothioconazole in the test solution samples was determined by analysing with HPLC-MS/MS. The analytical method was validated with regard to specificity, linearity, accuracy (recovery), precision and limit of quantification. A description and validation of the analytical method is provided in Part B of Section 5 (*Analytical Methods*).

4. Statistics

The statistical evaluation for the 72 hours period was performed for growth rate and yield using SAS® (2016). A test for normality of the data was performed by calculating the Shapiro-Wilk statistic and the homogeneity of variance of the data was evaluated by calculating the Levene Test. The NOEC and LOEC were determined by using a multiple comparison method (Dunnetts-test, left sided, for growth rate and for yield). The $EC_{10, 20, 50}$-values for growth rate and yield were determined by probit analysis following the normal and Gompertz distribution, respectively. Due to statistical reasons inhibition-values above 100 % were set to 100 and values below 0 % were set to zero.

## II. Results and discussion

### A. Analytical data

The measured concentrations of prothioconazole in the freshly prepared test item solutions ranged from 91 % to 94 % of the nominal concentrations, demonstrating the correct preparation of the test solutions. Over the test period of 24 h and 48 h up to 72 h a concentration dependent decline was observed in the measured samples. In the aged samples after 72 hours the measured concentrations ranged between below LOQ at the lowest test item concentration and 92 % of the nominal at the highest test item concentration. Therefore, toxicological endpoints were evaluated based on the geometric mean of each measured concentration of the test item and the active ingredient prothioconazole (see table below).

**Table A 6: Determined concentration of prothioconazole**

| Test item nominal (mg/L) | Prothioconazole nominal (mg/L) | Sampling | Prothioconazole found | | | | Actual concentration |
|---|---|---|---|---|---|---|---|
| | | | (mg/L) | % of nominal | Geometric mean[1] (%) | Test item (mg/L) | Prothioconazole (mg a.s./L) |
| Control | 0 | 0 h fresh | < LOD | - | - | - | - |
| | | 24 h aged | | | | | |
| | | 48 h aged | | | | | |
| | | 72 h aged | | | | | |
| 0.0954 | 0.0219 | 0 h fresh | 0.0205 | 94 | 33.7 | 0.0321 | 0.00738 |
| | | 24 h aged | 0.0182 | 83 | | | |
| | | 48 h aged | 0.00714 | 33 | | | |
| | | 72 h aged | 0.00211 | < LOQ[2] | | | |
| 0.305 | 0.0702 | 0 h fresh | 0.0642 | 91 | 66.6 | 0.203 | 0.0468 |
| | | 24 h aged | 0.0636 | 91 | | | |
| | | 48 h aged | 0.0505 | 72 | | | |
| | | 72 h aged | 0.0235 | 33 | | | |
| 0.977 | 0.225 | 0 h fresh | 0.211 | 94 | 80.0 | 0.782 | 0.180 |
| | | 24 h aged | 0.211 | 94 | | | |
| | | 48 h aged | 0.193 | 86 | | | |
| | | 72 h aged | 0.121 | 54 | | | |
| 3.13 | 0.720 | 0 h fresh | 0.666 | 93 | 88.9 | 2.78 | 0.640 |
| | | 24 h aged | 0.647 | 90 | | | |
| | | 48 h aged | 0.640 | 89 | | | |
| | | 72 h aged | 0.604 | 84 | | | |
| 10.0 | 2.30 | 0 h fresh | 2.16 | 94 | 92.7 | 9.27 | 2.13 |
| | | 24 h aged | 2.12 | 92 | | | |
| | | 48 h aged | 2.13 | 93 | | | |
| | | 72 h aged | 2.12 | 92 | | | |

- = not calculated; LOD = 0.000657 mg a.s./L; LOQ = 0.00219 mg a.s./L

[1] calculated as geometric mean of fresh and aged samples

[2] Prothioconazole was detectable but below LOQ in the sample. Therefore, half of the LOQ value (0.001095 mg/L, equals 5 % of nominal) was used for calculation of geometric mean according to OECD Guidance document No. 23 (2000)

## B. Growth rate

Significant inhibitory effects were determined for growth rate and for yield at test item concentrations of 2.78 mg test item/L and 0.640 mg a.s./L (geometric mean measured) and above (see table below). The overall LOEC was therefore determined to be 2.78 mg test item/L and 0.640 mg a.s./L (geometric mean measured), the corresponding NOEC was set at 0.782 mg test item/L and 0.180 mg a.s./L (geometric mean measured).

**Table A 7: Average cell numbers for each sampling time and concentration, the percentage inhibition of growth rate and inhibition of yield**

| Nominal conc. (mg/L) | Average cell numbers [$10^4$/ml] | | | | % Inhibition of growth rate | | | % Inhibition of yield | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 h | 24 h | 48 h | 72 h | 0 - 24 h | 0 - 48 h | 0 - 72 h | 0 - 24 h | 0 - 48 h | 0 - 72 h |
| Control | 0.60 | 2.11 | 10.88 | 55.74 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 0.0954 | 0.60 | 2.13 | 13.18 | 70.30 | 0.1 | -6.4 | -4.9[1)] | -1.3 | -22.4 | -26.4[1)] |
| 0.305 | 0.60 | 2.15 | 13.13 | 68.58 | -1.1 | -6.2 | -4.4[1)] | -2.6 | -21.9 | -23.3[1)] |
| 0.977 | 0.60 | 1.83 | 9.75 | 53.23 | 12.0 | 4.1 | 1.2 | 18.5 | 11.0 | 4.6 |
| 3.13 | 0.60 | 1.04 | 3.83 | 12.97 | 57.0 | 36.2 | 32.3* | 70.9 | 68.6 | 77.6* |
| 10.0 | 0.60 | 0.28 | 0.22 | 0.16 | 160.4 | 134.8 | 128.8*[2)] | 121.2 | 103.7 | 100.8*[2)] |

[1)] value was set to zero for $EC_{10, 20, 50}$-calculation
[2)] value was set to 100 for $EC_{10, 20, 50}$-calculation
* statistically significant different to the control

Toxicological endpoints were evaluated using mean measured concentrations of the test item ADM.3500.F.2.B and the active ingredient prothioconazole. All endpoints are listed in the table below.

**Table A 8: Toxicological endpoints**

| | ADM.3500.F.2.B[4)] (mg test item/L) | Prothioconazole[4)] (mg a.i./L) |
|---|---|---|
| $E_rC_{10}$ (growth rate)[1)] | 1.77 | 0.409 |
| 95 % confidential limits | 1.43 - 2.05 | 0.330 - 0.473 |
| $E_rC_{20}$[1)] | 2.19 | 0.504 |
| 95 % confidential limits | 1.86 - 2.47 | 0.428 - 0.569 |
| $E_rC_{50}$[1)] | 3.27 | 0.752 |
| 95 % confidential limits | 2.92 - 3.68 | 0.673 - 0.847 |
| $E_yC_{10}$ (yield)[2)] | 1.07 | 0.245 |
| 95 % confidential limits | 0.769 - 1.30 | 0.177 - 0.299 |
| $E_yC_{20}$[2)] | 1.40 | 0.322 |
| 95 % confidential limits | 1.10 - 1.62 | 0.254 - 0.372 |
| $E_yC_{50}$[2)] | 2.10 | 0.484 |
| 95 % confidential limits | 1.87 - 2.30 | 0.430 - 0.530 |
| NOEC [3)] | 0.782 | 0.180 |
| LOEC [3)] | 2.78 | 0.640 |

[1)] Probit analysis following normal distribution
[2)] Probit analysis following Gompertz distribution
[3)] Following Dunnetts-t-test (left-sided, $p \leq 0.05$) for growth rate and for yield
[4)] Based on geometric mean of each concentration level

Reference item

Potassium dichromate is tested as the toxic reference item in a separate study twice a year to confirm the sensitivity of the test organism against compounds with known effects under the test conditions with nominal concentrations of 0.0512, 0.128, 0.320, 0.800 and 2.00 mg/L. The $EC_{50}$ values calculated in this reference test were considered to be within an acceptable range therefore it can be considered that the test organism is sensitive (see table below).

**Table A 9: Toxicological endpoints of the reference item potassium dichromate**

| | **Test item nominal (mg test item/L)** |
|---|---|
| $E_rC_{10}$ (growth rate)[1)] | 0.429 |
| $E_rC_{20}$[1)] | 0.603 |
| $E_rC_{50}$[1)] | 1.01 |
| $E_yC_{10}$ (yield)[1)] | 0.128 |
| $E_yC_{20}$[1)] | 0.224 |
| $E_yC_{50}$[1)] | 0.521 |
| $E_bC_{10}$ (biomass)[1)] | 0.161 |
| $E_bC_{20}$[1)] | 0.268 |
| $E_bC_{50}$[1)] | 0.580 |
| NOEC [2)] | 0.128 |
| LOEC [2)] | 0.320 |

[1)] Probit analysis following Gompertz distribution
[3)] Following Bonferroni-Holms corrected Welch test (left-sided, $p \leq 0.05$) for biomass and yield and Dunnetts-t-test (left-sided, $p \leq 0.05$) for growth rate

**C. Validity of the test:**

| **Validity criterion according to OECD 201** | **Results of the study** |
|---|---|
| The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period. This corresponds to a specific growth rate of 0.92 per day. For the most frequently used species the growth rate is usually substantially higher. This criterion may not be met when species that grow slower. In this case, the test period should be extended to obtain at least a 16-fold growth in control cultures, while the growth has to be exponential throughout the test period. The test period may be shortened to at least 48 hours to maintain unlimited, exponential growth during the test as long as the minimum multiplication factor of 16 is reached | Cell numbers, measured in the controls between 0 h and 72 hours, were found to increase by a factor of 92.90. It corresponds to a growth rate of 1.51311 per day. |
| The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3, for 72-hour tests) in the control cultures must not exceed 35 %. This criterion applies to the mean value of coefficients of variation calculated for replicate control cultures. | The mean coefficient of variation for the section-by-section specific growth rates (hours 0 - 24, 24 - 48 and 48 - 72) in the control cultures was 15 %. |
| The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7 % in tests with *Pseudokirchneriella subcapitata* and *Desmodesmus subspicatus*. For other less frequently tested species, the value should not exceed 10 %. | The coefficient of variation of average growth rate in replicate control cultures was 1.9 %. |

Since the test protocol meets the validity criteria set forth in the most recent test guideline, the study is considered valid.

**III. Assessment and conclusion**

In a 96-hour growth rate test, 0.5 x $10^4$ cells/ml of the unicellular green alga *Pseudokirchneriella subcapitata* were exposed to ADM.3500.F.2.B (Prothioconazole EC 250, 250 g prothioconazole/L) under static conditions. A control group exposed to test medium without test item was run concurrently. Significant inhibitory effects were determined for growth rate and for yield at test item concentrations of 2.78 mg test item/L and 0.640 mg a.s./L (geometric mean measured) and above. The overall LOEC was therefore determined to be 2.78 mg test item/L and 0.640 mg a.s./L (geometric mean measured), the corresponding NOEC was set at 0.782 mg test item/L and 0.180 mg a.s./L (geometric mean measured). The $E_rC_{10}$ was determined to be 1.77 mg test item/L and 0.409 mg a.s./L. The $E_yC_{10}$ was 1.07 mg test item/L and 0.245 mg a.s./L (geometric mean measured). The $E_rC_{20}$ was determined to be 2.19 mg test item/L and 0.504 mg a.s./L. The $E_yC_{20}$ was 1.40 mg test item/L (nominal) and 0.322 mg a.s./L (geometric mean measured). The $E_rC_{50}$ was determined to be 3.27 mg test item/L and 0.752 mg a.s./L. The $E_yC_{50}$ was 2.10 mg test item/L and 0.484 mg a.s./L (geometric mean measured). The study is considered valid (see: "C. Validity of the test" above).

| | |
|---|---|
| Comments of zRMS: | The study was conducted in line with OECD 221 with no deviations.<br><br>Analytical samples were taken at 0 hours (initial value) from fresh test solutions, after 2 and 4 days from fresh and aged test solutions and after 7 days from aged test solutions from all test item concentrations and control.<br><br>The measured initial concentrations of prothioconazole ranged from 84 % to 113 % of nominal, except at two timepoints (2d and 4d fresh) at 100 mg/L where 63 and 59 % of nominal were found respectively.<br>In the aged samples the measured concentrations were between <LOQ and 104 % of the nominal. The endpoints based on the time-weighted mean measured concentration of the test item and the prothioconazole.<br><br>Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment:<br><br>$E_rC_{50}$ (frond numbers) = 1.15 mg test item/L, corresponding to 0.264 mg a.s./L (based on time-weighted mean measured concentration)<br><br>$E_r C_{20}$ = 0.0572 mg test item/L corresponding to 0.0132 mg a.s./L (based on time-weighted mean measured concentration)<br>$E_rC_{10}$ = n.d.<br><br>NOER < 0.0122 mg test item /L corresponding to < 0.00281 mg a.s./L (based on time-weighted mean measured concentration)<br><br>$E_rC_{50}$ (dry weight) = 6.68 mg test item/L, corresponding to 1.54 mg a.s./L (based on time-weighted mean measured concentration)<br><br>$E_rC_{20}$ = 0.270 mg test item/L, corresponding to 0.06223 mg a.s./L (based on time-weighted mean measured concentration)<br><br>$E_rC_{10}$ = 0.03233 mg test item/L, corresponding to 0.00744 mg a.s./L (based on time-weighted mean measured concentration)<br><br>NOER = 0.0122 mg test item /L corresponding to 0.00281 mg a.s./L (based on time-weighted mean measured concentration)<br><br>$E_yC_{50}$ (yield for frond number) = 0.0794 mg test item /L corresponding to 0.01831 mg a.s./L (based on time-weighted mean measured concentration) |

| | |
|---|---|
| | $EC_{20}$ = n.d<br>$EC_{10}$=n.d.<br><br>$E_yC_{50}$ (yield for dry weight) = 0.152 mg test item /L corresponding to 0.0500 mg a.s./L (based on time-weighted mean measured concentration)<br>$EC_{20}$ = n.d<br>$EC_{10}$=n.d. |

The following study has not been evaluated during the EU peer review of Prothioconazole.

| | |
|---|---|
| Reference: | KCP 10.2.1/04 |
| Report: | ADM.3500.F.2.B: Toxicity to the duckweed *Lemna gibba* under laboratory conditions (Growth inhibition test – Semi-static), Weber, K., 2020, report no.: S19-03476, sponsor no.: 000102733 |
| Guideline(s): | OECD 221 (2006) |
| Deviations: | None |
| GLP: | Yes (certified laboratory) |
| Acceptability/Reliability: | Yes |
| Duplication (if vertebrate study) | Not applicable |

**Executive summary**

The purpose of this test was to determine the inhibitory effect of the test item ADM.3500.F.2.B (Prothioconazole EC 250, 250 g prothioconazole/L) on the growth of the freshwater aquatic plant *Lemna gibba*. Groups of fronds (3 replicates per treatment/ 6 replicates per treatment control) were exposed to ADM.3500.F.2.B at nominal concentrations of 100, 25.0, 6.25, 1.56; 0.391; 0.0977 and 0.0244 mg test item/L for 7 days under semi-static conditions. A control groups was included in the test containing dilution water alone. 3,5-Dichlorophenol is tested as the toxic reference item in a separate study. Frond numbers in each test vessel were determined at the start of the test. Frond numbers and the appearance of the colonies were checked on t = 0, 2, 4 and 7 days as well as any change in plant development, frond size, necrosis and additional observations of test media or other abnormalities. The content of prothioconazole in the test solution samples was determined by analysing with LC-MS/MS.

The measured initial concentrations of prothioconazole ranged from 59 % to 113 % of nominal. In the aged samples the measured concentrations were between <LOQ and 104 % of the nominal. Toxicological endpoints were evaluated using actual concentrations (based on the time-weighted mean measured concentration of the test item and the a.s.). Under the conditions of this study, the $E_yC_{50}$-value (frond number) was determined to be 0.0794 mg test item/L, corresponding to 0.0183 mg a.s./L (time-weighted mean measured), the $E_rC_{50}$-value (frond numbers) was determined to be 1.15 mg test item/L, corresponding to 0.264 mg a.s./L (time-weighted mean measured). The $E_yC_{50}$-value (dry weight) was determined to be 0.152 mg test item/L, corresponding to 0.0500 mg a.s./L (time-weighted mean measured), the $E_rC_{50}$-value (dry weight) was determined to be 6.68 mg test item/L, corresponding to 1.54 mg a.s./L (time-weighted mean measured). The NOEC (dry weight) was considered to be 0.0122 mg prod./L (equivalent to 0.00281 mg a.s./L). The NOEC (frond number) was considered to be <0.0122 mg prod./L (equivalent to < 0.00281 mg a.s./L).

## I. Materials and methods

### A. Materials

| | |
|---|---|
| 1. Test material: | ADM.3500.F.2.B (Prothioconazole EC 250) |
| Lot/Batch no.: | 1109-210219-01 |
| Content/Purity: | prothioconazole: 23.0 % w/w, 248.2 g/L, (analytical); 250 g/L (nominal) |
| Control: | test medium control |
| Toxic reference: | 3,5-Dichlorophenol (tested in a separate study) |
| 2. Test organisms - | |
| Species: | *Lemna gibba* G3 (duckweed) |
| Source: | in-house culture, originally obtained from Dr. Janet Slovin, Horticulture Crops Quality Laboratory, U.S. Department of Agriculture. BARC-West, Bldg. 050 HH-4, Beltsville, MD 20705, USA. |
| No of plants: | 3 replicates per treatment, 6 replicates for the control were used with 3 - 4 plants with a total of 12 fronds |
| Acclimatisation: | 14 days |
| 3. Test units and exposure – | |
| Type and size: | 250 ml glass beakers each containing 150 ml test solution |
| Test procedure: | semi-static dose-response test, medium renewal after 2 and 4 days |
| Test duration: | 7 days |
| 4. Test conditions – | |
| Test medium: | 20x AAP Medium (according to OECD 221) |
| Temperature. | 24.1 - 24.9 °C |
| Photoperiod: | constant light |
| Light intensity | 7062 lux (mean) |
| pH value: | 7.58 - 7.77 |

### B. Study design and method

| | |
|---|---|
| 1. In life dates: | September 11, 2019 to February 07, 2020 (experimental phase) |

2. Test design:

The following nominal concentrations were tested (spaced by a factor of 4): 100, 25.0, 6.25, 1.56; 0.391; 0.0977 and 0.0244 mg/L and an untreated control (test medium). The necessary amount of test item for preparing the stock solution was weighed on a weighing scoop and transferred to a volumetric flask. Deionised water was added up to the bench mark and the solution was homogenised by shaking. The stock solution was turbid. Lower dilution solutions were prepared by dilution of appropriate solutions with deionised water. Defined volumes of the respective stock solution and dilutions were applied to the respective test vessel containing test medium.

Colonies consisting of 2 - 4 fronds were transferred from the inoculum culture into the test vessels containing a total of 12 fronds each. The size of plants and fronds were similar in appearance in each test vessel. A 7-day semi-static test design was employed at which the colonies were transferred into new test solutions twice (days 2 and 4). Three replicates were used for each test item concentration, six replicates for the control.

Frond numbers in each test vessel were determined at the start of the test. Frond numbers and the appearance of the colonies were checked on t = 0, 2, 4 and 7 days as well as any change in plant development, frond size, necrosis and additional observations of test media or other abnormalities. The dry weight of the fronds was determined at the end of the test. A representative batch of six times 12 fronds from the culture used for the test was dried to determine the dry weight for the test start. The test temperature was measured continuously in a surrogate vessel held under the same conditions as the test vessels and recorded after 0, 2, 4 and 7 days. The pH-value of the test solutions was measured in control and each test concentration in one replicate respectively on t = 0 (fresh solution), 2 (aged and fresh solution), 4 (aged and fresh solution) and 7 days (aged solution).

3,5-Dichlorophenol is tested as the toxic reference item in a separate study twice a year to confirm the sensitivity of the test organism against compounds with known effects under the test conditions.

3. Analytical verification:

Analytical samples were taken at 0 hours (initial value) from fresh test solutions, after 2 and 4 days from fresh and aged test solutions and after 7 days from aged test solutions from all test item concentrations and control. For each sampling also a retain sample was taken. Two sample sets were taken. The content of prothioconazole in the test solution samples was determined by analysing with LC-MS/MS. The analytical method was validated with regards to specificity, linearity, accuracy (recovery), precision and limit of quantification. A description and validation of the analytical method is provided in Part B of Section 5 (*Analytical Methods*).

4. Statistics

The statistical evaluation for day 7 was performed for yield of frond numbers, growth rate of frond numbers, growth rate of dry weight and yield of dry weight. A test for normality of the data was performed by calculating the Shapiro-Wilk's statistic, a test for homogeneity of the data was performed according to Levene. The NOEC and LOEC were determined by using a multiple comparison method (Dunnetts-t-test, left sided, for yield of frond numbers, Bonferroni-Holms corrected Welch test, left sided, for growth rate of frond numbers, yield of dry weight and growth rate of dry weight). The $EC_{10, 20, 50}$-values were determined by probit analysis following normal, logistic and Gompertz distribution. The evaluation of data was performed by SAS® (2016).

## II. Results and discussion

## A. Analytical data

The measured initial concentrations of prothioconazole ranged from 84 % to 113 % of nominal, except at two timepoints (2d and 4d fresh) at 100 mg/L where 63 and 59 % of nominal were found respectively. In the aged samples the measured concentrations were between <LOQ and 104 % of the nominal. Toxicological endpoints were evaluated using actual concentrations (based on the time-weighted mean measured concentration of the test item and the a.s.).

**Table A 10: Determined concentration of prothioconazole and calculated concentration of ADM.3500.F.2.B**

| Test item nominal (mg/L) | Prothioconazole nominal (mg a.s./L) | Sampling (days) | Prothioconazole found | | | Test item actual (mg/L) |
|---|---|---|---|---|---|---|
| | | | (mg a.s./L) | % of nominal | time-weighted mean (mg a.s./L)[1] | |
| Control | 0 | 0 d | < LOD | - | - | - |
| | | 2 d aged | < LOD | - | | |
| | | 2 d fresh | < LOD | - | | |
| | | 4 d aged | < LOD | - | | |
| | | 4 d fresh | < LOD | - | | |
| | | 7 d aged | < LOD | - | | |
| 0.0244 | 0.00561 | 0 d | 0.00582 | 104 | 0.00281 | 0.0122 |
| | | 2 d aged | 0.000397 | <LOQ[2] | | |
| | | 2 d fresh | 0.00558 | 99 | | |
| | | 4 d aged | 0.00267 | 48 | | |
| | | 4 d fresh | 0.00606 | 108 | | |
| | | 7 d aged | 0.000898 | 16 | | |
| 0.0977 | 0.0225 | 0 d | 0.0223 | 99 | 0.0101 | 0.0438 |
| | | 2 d aged | 0.00120 | 5 | | |
| | | 2 d fresh | 0.0251 | 112 | | |
| | | 4 d aged | 0.00475 | 21 | | |
| | | 4 d fresh | 0.0252 | 112 | | |
| | | 7 d aged | 0.00310 | 14 | | |
| 0.391 | 0.0899 | 0 d | 0.0948 | 105 | 0.0498 | 0.217 |
| | | 2 d aged | 0.00872 | 10 | | |
| | | 2 d fresh | 0.0956 | 106 | | |
| | | 4 d aged | 0.0523 | 58 | | |
| | | 4 d fresh | 0.100 | 111 | | |
| | | 7 d aged | 0.0146 | 16 | | |
| 1.56 | 0.359 | 0 d | 0.304 | 85 | 0.265 | 1.15 |
| | | 2 d aged | 0.159 | 44 | | |
| | | 2 d fresh | 0.385 | 107 | | |
| | | 4 d aged | 0.251 | 70 | | |
| | | 4 d fresh | 0.363 | 101 | | |
| | | 7 d aged | 0.180 | 50 | | |

- = not calculated; LOD= 0.000168 mg/L analyte; LOQ = 0.000561 mg/L analyte

[1] time-weighted mean measured concentrations calculated based on formulae in OECD 23, Annex 2 (2019)

[2] ½ LOQ used for calculation of geomean concentration (0.0002805 mg a.s./L)

## B. Growth rate

Significant inhibitory effects were determined for yield of frond numbers and growth rate of frond numbers at test concentrations of 0.0122 mg test item/L, corresponding to 0.00281 mg a.s./L (time-weighted mean measured) and above. For yield of dry weight and growth rate of dry weight significant inhibitory effects were determined at 0.0438 mg test item/L, corresponding to 0.0101 mg a.s./L (time-weighted mean measured) and above. The overall LOEC was therefore determined to be 0.0122 mg test item/L, corresponding to 0.00281 mg a.s./L (time-weighted mean measured), the corresponding NOEC was not determinable within the tested concentration range. The results of the frond numbers and growth rates are summarised in the following tables.

**Table A 11: Frond numbers: inhibition of yield**

| Test item concentration (mg/L) | | Mean of frond numbers | | | | Yield based on mean frond numbers | Inhibition (%) |
|---|---|---|---|---|---|---|---|
| Nominal | Actual | Days | | | | | |
| | | 0 | 2 | 4 | 7 | 7-0 days | 7-0 days |
| Control | - | 12 | 29 | 79 | 309 | 297 | 0.0 |
| 0.0244 | 0.0122 | 12 | 28 | 69 | 251 | 239* | 19.5 |
| 0.0977 | 0.0438 | 12 | 25 | 57 | 205 | 193* | 35.0 |
| 0.391 | 0.217 | 12 | 23 | 42 | 79 | 67* | 77.4 |
| 1.56 | 1.15 | 12 | 22 | 34 | 45 | 33* | 88.9 |
| 6.25 | 5.49 | 12 | 23 | 30 | 36 | 24* | 91.9 |
| 25.0 | 22.3 | 12 | 21 | 29 | 38 | 26* | 91.2 |
| 100 | 73.8 | 12 | 14 | 15 | 15 | 3* | 99.0 |

* statistically significant different to the control

**Table A 12: Frond numbers: inhibition of growth rates**

| Test item concentration (mg/L) | | Mean growth rate μ ($d^{-1}$) | | | Inhibition growth rate (%) | | |
|---|---|---|---|---|---|---|---|
| Nominal | Actual | Days | | | Days | | |
| | | 2 | 4 | 7 | 2 | 4 | 7 |
| Control | - | 0.4405 | 0.4709 | 0.4641 | 0.0 | 0.0 | 0.0 |
| 0.0244 | 0.0122 | 0.4176 | 0.4356 | 0.4344* | 5.2 | 7.5 | 6.4 |
| 0.0977 | 0.0438 | 0.3667 | 0.3879 | 0.4053* | 16.8 | 17.6 | 12.7 |
| 0.391 | 0.217 | 0.3324 | 0.3130 | 0.2664* | 24.5 | 33.5 | 42.6 |
| 1.56 | 1.15 | 0.3017 | 0.2599 | 0.1850* | 31.5 | 44.8 | 60.1 |
| 6.25 | 5.49 | 0.3176 | 0.2290 | 0.1579* | 27.9 | 51.4 | 66.0 |
| 25.0 | 22.3 | 0.2876 | 0.2228 | 0.1645* | 34.7 | 52.7 | 64.6 |
| 100 | 73.8 | 0.0886 | 0.0500 | 0.0286* | 79.9 | 89.4 | 93.8 |

* statistically significant different to the control

**Table A 13: Frond numbers: mean doubling times**

| Test item concentration (mg/L) | | Days | | | Hours |
|---|---|---|---|---|---|
| Nominal | Actual | | | | |
| | | 2 | 4 | 7 | 7 |
| Control | - | 1.574 | 1.472 | 1.494 | 35.9 |
| 0.0244 | 0.0122 | 1.660 | 1.591 | 1.596 | 38.3 |
| 0.0977 | 0.0438 | 1.890 | 1.787 | 1.710 | 41.0 |
| 0.391 | 0.217 | 2.085 | 2.215 | 2.602 | 62.4 |
| 1.56 | 1.15 | 2.297 | 2.667 | 3.747 | 89.9 |
| 6.25 | 5.49 | 2.182 | 3.027 | 4.390 | 105.4 |
| 25.0 | 22.3 | 2.410 | 3.111 | 4.214 | 101.1 |
| 100 | 73.8 | 7.823 | 13.863 | 24.236 | 581.7 |

**Table A 14: Dry weight: mean values, inhibition of yield, mean growth rate and inhibition of growth rate**

| Test item concentration (mg/L) | | Mean dry weight (g) | | Yield based on mean dry weight (g) | Inhibition [%] | Mean growth rate μ ($d^{-1}$) | Inhibition growth rate (%) |
|---|---|---|---|---|---|---|---|
| Nominal | Actual | Days | | | | | |
| | | 0 | 7 | 7-0 | 7-0 days | Day 7 | Day 7 |
| Control | - | 0.0010 | 0.0428 | 0.0418 | 0.0 | 0.5363 | 0.0 |
| 0.0244 | 0.0122 | | 0.0360 | 0.0350 | 16.3 | 0.5111 | 4.7 |
| 0.0977 | 0.0438 | | 0.0282 | 0.0272* | 34.9 | 0.4766* | 11.1 |
| 0.391 | 0.217 | | 0.0149 | 0.0139* | 66.7 | 0.3848* | 28.2 |
| 1.56 | 1.15 | | 0.0115 | 0.0105* | 74.9 | 0.3483* | 35.1 |
| 6.25 | 5.49 | | 0.0094 | 0.0084* | 79.9 | 0.3195* | 40.4 |
| 25.0 | 22.3 | | 0.0071 | 0.0061* | 85.4 | 0.2791* | 48.0 |
| 100 | 73.8 | | 0.0014 | 0.0004* | 99.0 | 0.0392* | 92.7 |

* statistically significant different to the control

The $EC_{10}$-value for yield of frond numbers, growth rate of frond numbers and yield of dry weight and the $EC_{20}$-value for yield of frond numbers and yield of dry weight were not determinable with reliable results, because the values were outside the tested concentration range (see table below). The $EC_{10}$-value for growth rate of dry weight was determined to be 0.0323 mg test item/L and 0.00744 mg a.s./L (time-weighted mean measured). The $EC_{20}$-value for growth rate of frond numbers was determined to be 0.0572 mg test item/L, corresponding to 0.0132 mg a.s./L (time-weighted mean measured). The $EC_{20}$-value for growth rate of dry weight was determined to be 0.270 mg test item/L and 0.0622 mg a.s./L (time-weighted mean measured). The $EC_{50}$-value for yield of frond numbers was determined to be 0.0794 mg test item/L, corresponding to 0.0183 mg a.s./L (time-weighted mean measured), the $EC_{50}$-value for growth rate of frond numbers was determined to be 1.15 mg test item/L, corresponding to 0.264 mg a.s./L (time-weighted mean measured). The $EC_{50}$-value for yield of dry weight was determined to be 0.152 mg test item/L, corresponding to 0.0500 mg a.s./L (time-weighted mean measured), the $EC_{50}$-value for growth rate of dry weight was determined to be 6.68 mg test item/L, corresponding to 1.54 mg a.s./L (time-weighted mean measured).

**Table A 15:** **$EC_{10, 20, 50}$- and NOEC/LOEC-values of *Lemna gibba* exposed to the test item evaluated using the time-weighted mean measured concentrations of the test item and the a.s.**

| ADM.3500.F.2.B (mg/L) time-weighted mean measured* | | | | | | |
|---|---|---|---|---|---|---|
| **Endpoint** | **$EC_{10}$** | **$EC_{20}$** | **$EC_{50}$** | **95 % confidence limit** | **NOEC** | **LOEC** |
| Yield of frond numbers | n.d. | n.d. | 0.0794[1)] | 0.0218 - 0.207 | <0.0122[4)] | 0.0122[4)] |
| Growth rate of frond numbers | n.d. | 0.0572[2)] | 1.15[2)] | 0.298 - 4.36 | <0.0122[5)] | 0.0122[5)] |
| Yield of dry weight | n.d. | n.d. | 0.152[1)] | 0.0411 - 0.414 | 0.0122[5)] | 0.0438[5)] |
| Growth rate of dry weight | 0.0323[3)] | 0.270[3)] | 6.68[3)] | 1.64 - 28.8 | 0.0122[5)] | 0.0438[5)] |
| **Prothioconazole (mg/L) time-weighted mean measured*** | | | | | | |
| **Endpoint** | **$EC_{10}$** | **$EC_{20}$** | **$EC_{50}$** | **95 % confidence limit** | **NOEC** | **LOEC** |
| Yield of frond numbers | n.d. | n.d. | 0.0183[1)] | 0.00500 - 0.0476 | < 0.00281[4)] | 0.00281[4)] |
| Growth rate of frond numbers | n.d. | 0.0132[2)] | 0.264[2)] | 0.0686 - 1.00 | < 0.00281[5)] | 0.00281[5)] |
| Yield of dry weight | n.d. | n.d. | 0.0500[2)] | 0.0204 - 0.103 | 0.00281[5)] | 0.0101[5)] |
| Growth rate of dry weight | 0.00744[3)] | 0.0622[3)] | 1.54[3)] | 0.378 - 6.61 | 0.00281[5)] | 0.0101[5)] |

[1)] Probit analysis following logistic distribution
[2)] Probit analysis following normal distribution
[3)] Probit analysis following Gompertz distribution
[4)] Following Dunnetts-t-test (left-sided, $p<0.05$)
[5)] Following Bonferroni-Holms corrected Welch test (left-sided, $p<0.05$)
n.d.: not determinable with reliable results, because outside the tested concentration range
* based on time-weighted mean of each concentration level, calculated based on OECD 23, Annex 2 (2019)

## C. Phytotoxic effects

No observations of any morphological differences between fronds of the exposures compared to fronds of the control were made from nominal test item concentration 0.0244 (corresponding to 0.0122 mg test item/L and 0.00281 mg a.s./L (time-weighted mean measured) and 0.0977 mg/L (corresponding to 0.0438 mg test item/L and 0.0101 mg a.s./L (time-weighted mean measured). On day 2 singularized fronds and shortened roots were observed at nominal test item concentration 100 mg/L (corresponding to 73.9 mg test item/L and 17.0 mg a.s./L (time-weighted mean measured). On day 4 gibbosity were noted from test item concentration 0.391 to 25.0 mg/L (corresponding to 0.217 mg test item/L and 0.0498 mg a.s./L (time-weighted mean measured) to 22.3 mg test item/L and 5.13 mg a.s./L (time-weighted mean measured)). Shortened roots were observed at concentrations of 1.56 to 100 mg/L (corresponding to 1.15 mg test item/L and 0.265 mg a.s./L (time-weighted mean measured) to 73.9 mg test item/L and 17.0 mg a.s./L (time-weighted mean measured)). At test item concentration 100 mg/L (corresponding to 73.9 mg test item/L and 17.0 mg a.s./L (time-weighted mean measured)) chlorosis was observed. On day 7 gibbosity were noted from test item concentration 0.391 to 25.0 mg/L (corresponding to 0.217 mg test item/L and 0.0498 mg a.s./L (time-weighted mean measured) to 22.3 mg test item/L and 5.13 mg a.s./L (time-weighted mean measured)). Shortened roots were observed at concentrations of 1.56 to 100 mg/L (corresponding to 1.15 mg test item/L and 0.265 mg a.s./L (time-weighted mean measured).

From test item concentration 0.391 to 6.25 mg/L (corresponding to 0.217 mg test item/L and 0.0498 mg a.s./L (time-weighted mean measured) to 5.49 mg test item/L and 1.26 mg a.s./L (time-weighted mean measured)) deformed fronds were found. Singularized fronds and chlorosis were observed at test item concentration 100 mg/L (corresponding to 73.9 mg test item/L and 17.0 mg a.s./L (time-weighted mean measured).

Reference item

The reference item 3,5-Dichlorophenol (100 % w/w, analysed) was used in a semi-static test at 5 different concentrations (20.0, 8.00, 3.20, 1.28, 0.512 mg/L). The results of the toxic reference test are summarised in the table below.

**Table A 16: $EC_{10, 20, 50}$- and NOEC/LOEC-values of *Lemna gibba* exposed to 3,5-Dichlorophenol**

| Endpoint | Test item (mg/L) nominal | | | | |
|---|---|---|---|---|---|
| | **$EC_{10}$** | **$EC_{20}$** | **$EC_{50}$** | **NOEC** | **LOEC** |
| Yield of frond Numbers[1)] | 3.75 | 4.63 | 6.93 | 3.20 | 8.00[3)] |
| Growth rate of frond numbers[1)] | 5.06 | 6.35 | 9.79 | 3.20 | 8.00[3)] |
| Yield of dry weight[2)] | 7.42 | 7.61 | 7.90 | 3.20 | 8.00[3)] |
| Growth rate of dry weight[2)] | 7.63 | 7.83 | 8.14 | 3.20 | 8.00[3)] |

[1)] Probit analysis following normal distribution
[2)] Probit analysis following Gompertz distribution
[3)] Following Dunnetts-t-test (left-sided, $p<0.05$)

The $EC_{50}$ values calculated in this reference test were considered to be within an acceptable range, therefore it can be considered that the test organisms are sensitive

**D. Validity of the test:**

| Validity criterion according to OECD 221 | Results of the study |
|---|---|
| The doubling time of frond number in the control must be less than 2.5 days (60 h), corresponding to approximately a seven-fold increase in seven days and an average specific growth rate of 0.275/d. | The doubling time of frond numbers in the control was 1.494 days (corresponding to 35.9 hours. |

Since the test protocol meets the validity criteria set forth in the most recent test guideline, the study is considered valid.

## III. Assessment and conclusion

In a 7-day growth rate test, the freshwater aquatic plant *Lemna gibba* was exposed to ADM.3500.F.2.B (Prothioconazole EC 250, 250 g prothioconazole/L) under semi-static conditions. An untreated control was also run in parallel. Under the conditions of this study, the $E_yC_{50}$-value (frond number) was determined to be 0.0794 mg test item/L, corresponding to 0.0183 mg a.s./L (time-weighted mean measured), the $E_rC_{50}$-value (frond numbers) was determined to be 1.15 mg test item/L, corresponding to 0.264 mg a.s./L (time-weighted mean measured).

The $E_yC_{50}$-value (dry weight) was determined to be 0.152 mg test item/L, corresponding to 0.0500 mg a.s./L (time-weighted mean measured), the $E_rC_{50}$-value (dry weight) was determined to be 6.68 mg test item/L, corresponding to 1.54 mg a.s./L (time-weighted mean measured). The NOEC (dry weight) was considered to be 0.0122 mg prod./L (equivalent to 0.00281 mg a.s./L). The NOEC (frond number) was considered to be <0.0122 mg prod./L (equivalent to < 0.00281 mg a.s./L). The study is considered valid (see: "D. Validity criteria" above).

## A 2.2.2 KCP 10.2.2 Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms

No long-term and chronic studies with the formulation ADM.03500.F.2.B were conducted, as the results of the performed studies indicate no undue toxicity of the formulated product in comparison with the active substance prothioconazole.

## A 2.2.3 KCP 10.2.3 Further testing on aquatic organisms

Not required for reasons given under point A 2.2.2 above.

## 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

| | |
|---|---|
| zRMS comments**:** | The study was conducted in line with OECD 213 and 214 with no deviations.<br><br>All validity criteria were met.<br><br>Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment:<br><br>$LD_{50}$ 48 h > 106.6 µg a.s./bee (oral)<br>$LD_{50}$ 48 h = 133.3 µg a.s./bee (contact)<br>$LD_{50}$ 96 h = 125.3 µg a.s./bee (contact) |

| | |
|---|---|
| Reference: | KCP 10.3.1.1/01 |
| Report: | ADM.3500.F.2.B: Effects (acute contact and oral) on honey bees (*Apis mellifera* L.) in the laboratory, Sekine, T., 2020a, report no.: 137191035, sponsor no.: 000101260 |
| Guideline(s): | OECD 213 and 214 (1998) |
| Deviations: | None |
| GLP: | Yes (certified laboratory) |
| Acceptability/Reliability: | Yes/ |
| Duplication (if vertebrate study) | Not applicable |

**Executive summary**

The purpose of this study was to determine the acute contact and oral toxicity of ADM.3500.F.2.B to the honey bee. For the oral toxicity test, groups of 10 bees (10 bees per replicate, 5 replicates per concentration) were exposed to ADM.3500.F.2.B (Prothioconazole EC 250, 250 g prothioconazole/L) at a nominal concentration of 100.0 µg test item/bee (106.6 µg a.s./ bee measured) for 48 hours. For the contact toxicity test, groups of 10 bees (10 bees per replicate, 3 replicates per concentration ) were exposed to ADM.3500.F.2.B (Prothioconazole EC 250) at a nominal concentration of 200.0, 100.0, 50.0, 25.0 and 12.5 µg a.s./bee for 96 hours. Several control groups were included in the test containing tap water (contact with tap water + 0.5 % Adhäsit) or sugar solution (oral). As a toxic reference, BAS 152 11 I (dimethoate: 400.0 g/L) was used. The number of dead bees and behavioural abnormalities were recorded after 4, 24 and 48 hours (contact and oral test), 72 and 96) hours (contact test). Analytical verification of test item concentrations was not performed. Under the conditions of this study, the acute oral $LD_{50}$ after 48 h was > 106.6 µg a.s./bee. The acute contact $LD_{50}$ after 48 h was determined to be 133.3 µg a.s./bee (95 % confidence interval of 100 - 200.0 µg a.s./bee) and the $LD_{50}$ after 96 h was determined to be125.3 µg a.s./bee (95 % confidence interval of 25 - 200.0 µg a.s./bee).

**I. Materials and methods**

**A. Materials**

| | | |
|---|---|---|
| 1. | Test material: | ADM.3500.F.2.B (Prothioconazole EC 250) |
| | Lot/Batch no.: | 1109-210219-01 |
| | Content/Purity: | prothioconazole: 23.0 % w/w, 248.2 g/L, (analytical); 250 g/L (nominal) |

| | |
|---|---|
| Control: | 50 % w/v sucrose solution: 500 g sucrose/L tap water (oral), tap water with 0.5 % Adhäsit (contact) |
| Toxic reference: | BAS 152 11 I, dimethoate: 400.0 g/L (nominal) dimethoate: 429.0 g/L (analysed) |
| Wetting agent: | Adhäsit (ibacon080317, 100 g/L Marlopon nominal) |

2. Test organisms -

| | |
|---|---|
| Species: | *Apis mellifera* L. |
| Age: | young adult worker bees |
| Source | own breeding at Ibacon |
| No. of organisms: | oral: 5 replicates, each consisting of 10 bees per cage per treatment, contact: 3 replicates, each consisting of 10 bees per cage per treatment |
| Feeding: | *ad libitum* with 50 % (w/v) sucrose solution |
| Acclimatisation | not stated |

3. Test units and exposure –

| | |
|---|---|
| Type and size: | Stainless steel chambers (ca. 8 cm x 6 cm x 4 cm (length x height x width)) with a removable glass sheet at the front side. The bottom is perforated with 98 ventilation holes (ø 1 mm) |
| Test procedure: | oral and contact exposure |
| Test duration: | 48 h (oral) and 96 h (contact) |

4. Test conditions –

| | |
|---|---|
| Temperature: | 23 - 25 °C |
| Relative humidity: | 61 - 65 % |
| Photoperiod: | constant darkness |

**B. Study design and method**

1. In-life dates: July 15 to August 24, 2019 (experimental phase)

2. Test design:

Contact toxicity testing

In a 96 h contact toxicity test, bees were exposed to different concentrations (200.0, 100.0, 50.0, 25.0 and 12.5 µg a.s./bee) of ADM.3500.F.2.B. A single 5 µL droplet of ADM.3500.F.2.B diluted in an appropriate carrier (tap water + 0.5 % Adhäsit) was placed onto each dorsal bee thorax using a calibrated pipette (Multipette©, Eppendorf). For the control group, one 5 µL droplet of tap water containing 0.5 % Adhäsit was applied to each bee thorax. The reference item (0.30, 0.20, 0.15 and 0.10 µg dimethoate per bee) was also applied in 5 µL tap water (dimethoate made up in tap water containing 0.5 % Adhäsit).

Oral toxicity testing

In a 48 h limit acute oral toxicity test, bees were exposed to a nominal concentration of 100.0 µg test item/bee (106.6 µg a.s./ bee measured). ADM.3500.F.2.B and the reference item (0.30, 0.15, 0.08 and 0.05 µg dimethoate per bee) were both applied in 50 % w/v sucrose solution, which was used as the carrier (food) in the oral test. For the control group, pure 50 % w/v sucrose solution was offered to the bees. The treated food was offered in syringes, which were weighed before and after introduction into the cages (duration of uptake was 3 hours and 45 minutes for the test item treatment).

After a maximum of 3 hours and 45 minutes, the food uptake was completed and the syringes containing the treated food were removed, weighed, and replaced by ones containing fresh, untreated food. The target dose level (*e.g.* 100 µg a.s./bee nominal) would have been obtained if exactly 20 mg/bee of the treated food were ingested. In practice, uptake of the treated sugar solutions differed slightly from the nominal 20 mg/bee and results are given based on the measured consumption.

The number of dead bees were recorded after 4 (± 0.5 h) hours (first day); 24 and 48 (± 2 h) hours (contact and oral test); 72 and 96 (± 2 h) hours (contact test).

Behavioural abnormalities were assessed after 4 (± 0.5 h) hours (first day); 24 and 48 (± 2 h) hours (contact and oral test); 72 and 96 (± 2 h) hours (contact test).

3. Statistics:

Results obtained from the bees treated with the test item and the reference item were compared to those obtained from the control in both the contact and oral tests. As no test item treatment group showed mortality above 50.0 %, no statistical evaluation on the oral $LD_{50}$ has been carried out. The contact v values of the test item were determined using the binomial distribution (95 % -confidence limits) (according to Stephan, 1977). The contact $LD_{50}$ determination took into account the control mortality by correcting according to Abbott's formula (1925). The contact and oral $LD_{50}$values of the reference item were also determined using the binomial distribution (95 % -confidence limits) and took control mortality into account according to Abbott's formula. The software used to perform the statistical analysis was ToxRat Professional, Version 3.2.1, ToxRat Solutions GmbH .

## II. Results and discussion

### A. Mortality

Contact toxicity

The contact test was extended up to 96 hours due to increasing mortality between 24, 48, and 72 hours. Mortality occurred in all treatment groups in a dose related pattern. The dose levels between 200 and 12.5 µg/bee resulted in mortality ranging from 100 % to 6.7 % at the end of the test (96 hours after application). Control mortality was 10.0 % (see table below).

During the first 24 hours, behavioural impairments such as discoordinated movements, moribundity and apathy were observed in the three highest treatment groups. Between 24 and 48 h, a few bees were found apathetic, moribund or showed discoordinated movements. One moribund bee was observed at 96 hours in the 25.0 µg/bee treatment group

**Table A 17: Mortality and behavioural abnormalities of the bees in the contact toxicity test**

| Dose µg a.s./bee | 4 hours | | 24 hours | | 48 hours | | 72 hours | | 96 hours | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean mortality % | Mean b. a. % | Mean mortality % | Mean b. a. % | Mean mortality % | Mean b. a. % | Mean mortality % | Mean b. a. % | Mean mortality % | Mean b. a. % |
| 200 | 0.0 | 93.3 | 63.3 | 36.7 | 83.3 | 10.0 | 100.0 | 0.0 | 100.0 | 0.0 |
| 100 | 0.0 | 23.3 | 20.0 | 16.7 | 26.7 | 0.0 | 26.7 | 0.0 | 33.3 | 0.0 |
| 50 | 0.0 | 6.7 | 10.0 | 13.3 | 20.0 | 3.3 | 26.7 | 0.0 | 40.0 | 0.0 |
| 25 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 0.0 | 10.0 | 0.0 | 13.3 | 3.3 |
| 12.5 | 0.0 | 0.0 | 3.3 | 0.0 | 3.3 | 0.0 | 6.7 | 0.0 | 6.7 | 0.0 |
| Water control | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 10.0 | 0.0 |
| Reference item µg a.s./bee | | | | | | | | | | |
| 0.30 | 0.0 | 6.7 | 73.3 | 3.3 | 73.3 | 0.0 | 73.3 | 0.0 | 80.0 | 0.0 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 0.20 | 0.0 | 3.3 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 |
| 0.15 | 0.0 | 0.0 | 40.0 | 0.0 | 56.7 | 0.0 | 60.0 | 0.0 | 60.0 | 0.0 |
| 0.10 | 0.0 | 0.0 | 0.0 | 0.0 | 20.0 | 0.0 | 23.3 | 0.0 | 23.3 | 0.0 |

results are averages from three replicates (ten bees each) per dose/control
b. a. = behavioural abnormalities; water = $CO_2$/water-treated control

Oral toxicity

In the oral toxicity test, the maximum nominal test level of 100 µg a.s./bee corresponded to an actual intake of 106.6 µg a.s./bee. No mortality was observed at this dose level after 48 hours. 6.0 % mortality occurred in the control (see table below).

One apathetic bee was observed 4 hours after study start. No further test item induced behavioural effects could be observed during the study conduct.

**Table A 18: Mortality and behavioural abnormalities of the bees in the oral toxicity test**

| **Dose µg a.s./bee** | **4 hours** | | **24 hours** | | **48 hours** | |
|---|---|---|---|---|---|---|
| | **Mean mortality %** | **Mean b. a. %** | **Mean mortality %** | **Mean b. a. %** | **Mean mortality %** | **Mean b. a. %** |
| 10.6 | 0.0 | 2.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Water control | 0.0 | 0.0 | 0.0 | 0.0 | 6.0 | 0.0 |
| Reference item µg a.s./bee | | | | | | |
| 0.33 | 0.0 | 52.0 | 94.0 | 0.0 | 98.0 | 0.0 |
| 0.17 | 0.0 | 8.0 | 66.0 | 2.0 | 74.0 | 0.0 |
| 0.08 | 0.0 | 0.0 | 12.0 | 0.0 | 20.0 | 0.0 |
| 0.06 | 0.0 | 0.0 | 0.0 | 0.0 | 2.0 | 0.0 |

results are averages from five replicates (ten bees each) per dose/control
b. a. = behavioural abnormalities

**B. Validity of the test:**

| **Validity criterion according to OECD 213 and 214** | **Results of the study** |
|---|---|
| The average mortality for the total number of controls must not exceed 10 % at the end of the test (for contact and oral). | The average mortality in the control were ≤ 10 % (contact) and ≤ 6.0 % (oral). |
| The $LD_{50}$ of the toxic standard meets the specified range of 0.10 - 0.30 µg a.s./bee (contact) and 0.10 - 0.35 µg a.s./bee (oral). | The 48 h contact $LD_{50}$ of the toxic standard was 0.16 µg a.s./bee and the 72 h oral $LD_{50}$ of the toxic standard was 0.14 µg a.s./bee. |

Since the test protocol meets the validity criteria set forth in the most recent test guideline, the study is considered valid.

**III. Assessment and conclusion**

In a 48-hour acute oral and a 96 h contact toxicity test, honey bees (*Apis mellifera L.*) were exposed to ADM.3500.F.2.B (Prothioconazole EC 250, 250 g prothioconazole/L). Under the conditions of this study, the acute oral $LD_{50}$ after 48 h was > 106.6 µg a.s./bee. The acute contact $LD_{50}$ after 48 h was determined to be 133.3 µg a.s./bee (95 % confidence interval of 100 - 200.0 µg a.s./bee) and the $LD_{50}$ after 96 h was determined to be 125.3 µg a.s./bee (95 % confidence interval of 25 - 200.0 µg a.s./bee). The study is considered valid (see: "B. Validity of the test" above).

## A 2.3.1.1.1 KCP 10.3.1.1.1 Acute oral toxicity to bees

Please refer to point A 2.3.1.1.1 above.

## A 2.3.1.1.2 KCP 10.3.1.1.2 Acute contact toxicity to bees

Please refer to point A 2.3.1.1.1 above.

## A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

| | |
|---|---|
| Comments of zRMS: | The study was conducted in line with OECD 245 with no deviations.<br><br>All validity criteria were met.<br><br>Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment:<br><br>$LC_{50}$ (10 days) = 862.7 mg a.s./kg feeding solution<br>(equivalent to 3751 mg test item/kg feeding solution)<br><br>$LDD_{50}$ = (10 days) = 14.4 µg a.s./bee/day<br>(equivalent to 62.6 µg test item/bee/day).<br><br>NOEC (10 days) = 500 mg a.s./kg feeding solution<br>(equivalent to 2174 mg test item/kg feeding solution)<br><br>NOEDD = 8.76 µg a.s./bee/day<br>(equivalent to 38.1 µg test item/bee/day) |

| | |
|---|---|
| Reference: | KCP 10.3.1.2/01 |
| Report: | ADM.3500.F.2.B: Chronic oral toxicity test on the honey bee (*Apis mellifera* L.) in the laboratory, Sekine, T., 2020b, report no.: 137191136, sponsor no.: 000101261 |
| Guideline(s): | OECD 245 (2017) |
| Deviations: | None |
| GLP: | Yes (certified laboratory) |
| Acceptability/Reliability: | Yes |
| Duplication<br>(if vertebrate study) | Not applicable |

**Executive summary**

The purpose of this study was to determine the chronic oral toxicity of ADM.3500.F.2.B (Prothioconazole EC 250, 250 g prothioconazole/L) to the honey bee (*A. mellifera* L.) for a period of 10 days. Groups of 10 bees (10 bees per replicate, 3 replicates per concentration ) were exposed to ADM.3500.F.2.B at a nominal concentration of 100, 50, 25.0, 12.5 and 6.3 µg a.s./bee. A control (50 % (w/v) sucrose/water solution) was

included in the test containing. As a toxic reference, BAS 152 11 I (dimethoate: 400.0 g/L) was used. The number of dead bees and behavioural abnormalities were assessed daily until test end, 10 days following start of exposure. Analytical verification of test item concentrations was performed via UPLC-method with UV-detection with the highest (4000 ppm) and the lowest (250 ppm) concentration of the feeding solution.

The recovery rates in the sample solutions were between 78 and 117 % of nominal value. Under the conditions of this study, the $LC_{50}$ value (10 days) was 862.7 mg a.s./kg feeding solution (equivalent to 3751 mg test item/kg feeding solution). The $LDD_{50}$ value (10 days) was 14.4 µg a.s./bee/day (equivalent to 62.6 µg test item/bee/day). The NOEC and NOEDD values (10 days) were 500 mg a.s./kg feeding solution (equivalent to 2174 mg test item/kg feeding solution) and 8.76 µg a.s./bee/day (equivalent to 38.1 µg test item/bee/day), respectively.

## I. Materials and methods

### A. Materials

| | |
|---|---|
| 1. Test material: | ADM.3500.F.2.B (Prothioconazole EC 250) |
| Lot/Batch no.: | 1109-210219-01 |
| Content/Purity: | prothioconazole: 23.0 % w/w, 248.2 g/L, (analytical); 250 g/L (nominal) |
| Control: | 50 % w/v sucrose solution: 500 g sucrose/L water |
| Toxic reference: | BAS 152 11 I, dimethoate: 400.0 g/L (nominal) dimethoate: 429.0 g/L (analysed) |
| 2. Test organisms - | |
| Species: | *Apis mellifera* L. |
| Age: | young adult worker bees (2 days old) |
| Source | own breeding at Ibacon |
| No. of organisms: | 3 replicates per concentration, each consisting of 10 bees per cage per treatment |
| Feeding: | *ad libitum* with 50 % (w/v) sucrose solution containing either the test item, the reference item or sucrose solution only |
| Acclimatisation | after hatch, the bees were collected and thereafter acclimatised under test conditions for one day |
| 3. Test units and exposure – | |
| Type and size: | stainless steel chambers (ca. 8.2 cm x 5.9 cm x 4.2 cm (length x height x width)) with a removable glass sheet at the front side. The bottom is perforated with 98 ventilation holes (ø 1 mm) |
| Test procedure: | chronic oral exposure |
| Test duration: | 10 days |
| 4. Test conditions – | |
| Temperature: | 31 - 33 °C |
| Relative humidity: | 51 - 70 % |
| Photoperiod: | constant darkness |

### B. Study design and method

| | |
|---|---|
| 1. In-life dates: | August 13 to August 23, 2019 (experimental phase) |

2. Test design:

The test item was dissolved in 50 % (w/v) sucrose solution (treated feeding solution) in order to receive the targeted final concentrations. The test item feeding solution(s) was prepared freshly every day. Every day the feeding syringes containing the feeding solutions were replaced by fresh ones. 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 replacement with fresh food). The following concentrations of the test item were tested: 4000, 2000, 1000, 500 and 250 ppm (mg a.s./kg feeding solution). The nominal doses per bee/day were: 100, 50, 25.0, 12.5 and 6.3 µg a.s./bee. The actual doses per bee/day were: 47.5, 26.5, 17.2, 8.76 and 5.79 µg a.s./bee/day

A stock solution of the reference item was prepared on day 0 and was stored in the refrigerator at 0 - 8°C for 3 days. Deionised water was used as the solvent. The reference item feeding solution was prepared with 50 % (w/v) sucrose solution. One concentration with 1 ppm dimethoate (1 mg dimethoate/kg feeding solution) was used. The nominal dose per bee/day was: 0.03 µg as./bee per day (taking into account an expected mean uptake of feeding solution of 25 mg/bee per day. The exact dose per bee per day was calculated after determination of the definitive food uptake by the bees at test end taking into consideration loss by evaporation). The actual dose per bee/day was: 0.02 µg a.s./bee per day (based on the actual intake of treated food). For the water control a 50 % (w/v) sucrose solution was used.

The number of dead bees was assessed daily until test end, ten days following start of exposure. Behavioural abnormalities were assessed daily until test end (day 1 to day 10). Sub-lethal effects such as symptoms of poisoning or any abnormal behaviour in comparison to the control were recorded. The food consumption per bee was calculated by the number of surviving bees per assessment and the amount of food consumed on the following assessment day

3. Analytical verification:

The concentration of the active ingredient prothioconazole was determined via UPLC-method with UV-detection with the highest (4000 ppm) and the lowest (250 ppm) concentration of the feeding solution.

4. Statistics:

The $LC_{50}$ and $LDD_{50}$ values of the test item were determined using Probit Analysis (according to Finney 1971). The NOEDD / NOEC of the test item was determined using a Step-down Cochran-Armitage Test Procedure (one-sided greater, $\alpha = 0.05$). A qualitative trend analysis and Tarone's Test Procedure to test for extra-binomial variance had been performed in advance. The software used to perform the statistical analysis was ToxRat Professional, Version 3.2.1, ® ToxRat Solutions GmbH .

## II. Results and discussion

### A. Analytical data

The recovery rates in the sample solutions were between 78 and 117 % of nominal value (see table below).

**Table A 19: Analytical results**

| Sample (days after application) | Concentration | | | | |
|---|---|---|---|---|---|
| | Found (mg a.s./L) | D.F. | Calculated (mg a.s./L) | nominal (mg a.s./L) | % of nominal |
| Control (0) | < LOD | 25 | n.a. | 0 | n.a. |
| Control (3) | < LOD | 25 | n.a. | 0 | n.a. |
| Control (7) | < LOD | 25 | n.a. | 0 | n.a. |
| 250 ppm (0) | 12.363 | 25 | 309.075 | 297.260 | 104 |
| 250 ppm (1) | 11.083 | 25 | 277.071 | 297.260 | 93 |
| 250 ppm (2) | 12.436 | 25 | 310.893 | 297.260 | 105 |
| 250 ppm (3) | 11.236 | 25 | 280.904 | 297.260 | 94 |
| 250 ppm (4) | 11.691 | 25 | 292.280 | 297.260 | 98 |
| 250 ppm (5) | 13.946 | 25 | 348.657 | 297.260 | 117 |
| 250 ppm (6) | 12.203 | 25 | 305.079 | 297.260 | 103 |
| 250 ppm (7) | 12.073 | 25 | 301.834 | 297.260 | 102 |
| 250 ppm (8) | 11.474 | 25 | 286.857 | 297.260 | 97 |
| 250 ppm (9) | 12.128 | 25 | 303.206 | 297.260 | 102 |
| 4000 ppm (0) | 18.144 | 250 | 4536.058 | 4756.167 | 95 |
| 4000 ppm (1) | 17.873 | 250 | 4468.308 | 4756.167 | 94 |
| 4000 ppm (2) | 17.635 | 250 | 4408.778 | 4756.167 | 93 |
| 4000 ppm (3) | 17.519 | 250 | 4379.862 | 4756.167 | 92 |
| 4000 ppm (4) | 17.694 | 250 | 4423.547 | 4756.167 | 93 |
| 4000 ppm (5) | 14.770 | 250 | 3692.516 | 4756.167 | 78 |
| 4000 ppm (6) | 17.210 | 250 | 4302.410 | 4756.167 | 90 |
| 4000 ppm (7) | 18.978 | 250 | 4744.541 | 4756.167 | 100 |
| 4000 ppm (8) | 19.268 | 250 | 4816.975 | 4756.167 | 101 |
| 4000 ppm (9) | 18.902 | 250 | 4725.447 | 4756.167 | 99 |

LOD Limit of Detection = 0.12 mg a.i./L, n.a. not applicable, D.F. Dilution factor

## B. Mortality

Mortality occurred in all test item treated dose levels ranging from 3.3 % in the 250 mg a.s./kg feeding solution group to 100 % in the 4000 mg a.s./kg feeding solution group at test end (10 days following the start of chronic exposure). In the three highest concentration levels (4000, 2000 and 1000 mg a.s./kg feeding solution) 100.0, 86.7 and 80.0 % mortality occurred, respectively, which were statistically significantly different to the control (Step-down Cochran-Armitage Test Procedure, one-sided greater, $\alpha = 0.05$). There was no mortality in the control (50 % w/v sucrose solution). The reference item (dimethoate) at a concentration of 1 ppm (1 mg dimethoate/kg feeding solution) corresponding to an actual dose of 0.02 µg a.s./bee/day caused 100 % mortality on day 4 (see table below).

On days 2, 5 and 9 one moribund bee was found in the 47.5 µg a.s./bee/day dose level (4000 mg a.s./kg) and on day 4 in the 26.4 µg a.s./bee/day dose level (2000 mg a.s./kg). One apathetic bee was found on day 6 in the 17.2 and 8.76 µg a.s./bee/day dose level and one on day 9 in the 17.2 µg a.s./bee/day dose level. No further test item induced behavioural abnormalities were found during the 10 days of study duration (see table below).

**Table A 20: Mortality and behavioural abnormalities of the bees in the chronic oral toxicity test**

| Group | Conc. (mg/kg) | Mean dose per/bee/day* (µg/bee) | Day 1 | | Day 2 | | Day 3 | | Day 4 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | M. m. % | Mean b. a. % | M. m. % | Mean b. a. % | M. m. % | Mean b. a. % | M. m. % | Mean b. a. % |
| Test item (mg a.s./kg) (µg a.s./bee) | 4000 | 47.5 | 0.0 | 0.0 | 6.7 | 3.3 | 13.3 | 0.0 | 33.3 | 0.0 |
| | 2000 | 26.5 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 0.0 | 3.3 | 3.3 |
| | 1000 | 17.2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 500 | 8.76 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 250 | 5.79 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Ref.item (mg/µg a.s.) | 1.0 | 0.017 | 0.0 | 0.0 | 6.7 | 0.0 | 56.7 | 0.0 | 100.0 | 0.0 |
| control | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | | 0.0 | 0.0 | 0.0 |

| Group | Conc. (mg/kg) | Mean dose per/bee/day* (µg/bee) | Day 5 | | Day 6 | | Day 7 | | Day 8 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | M. m. % | Mean b. a. % | M. m. % | Mean b. a. % | M. m. % | Mean b. a. % | M. m. % | Mean b. a. % |
| Test item (mg a.s./kg) (µg a.s./bee) | 4000 | 47.5 | 63.3 | 3.3 | 80.0 | 0.0 | 80.0 | 0.0 | 96.7 | 0.0 |
| | 2000 | 26.5 | 13.3 | 0.0 | 43.3 | 0.0 | 53.3 | 0.0 | 56.7 | 0.0 |
| | 1000 | 17.2 | 0.0 | 0.0 | 6.7 | 3.3 | 13.3 | 0.0 | 33.3 | 0.0 |
| | 500 | 8.76 | 0.0 | 0.0 | 0.0 | 3.3 | 3.3 | 0.0 | 3.3 | 0.0 |
| | 250 | 5.79 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Ref.item (mg/µg a.s.) | 1.0 | 0.017 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 |
| control | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

| Group | Conc. (mg/kg) | Mean dose per/bee/day* (µg/bee) | Day 9 | | Day 10 | |
|---|---|---|---|---|---|---|
| | | | M. m. % | Mean b. a. % | M. m. % | Mean b. a. % |
| Test item (mg a.s./kg) (µg a.s./bee) | 4000 | 47.5 | 96.7 | 3.3 | 100.0 | 0.0 |
| | 2000 | 26.5 | 76.7 | 0.0 | 86.7 | 0.0 |
| | 1000 | 17.2 | 60.0 | 3.3 | 80.0 | 0.0 |
| | 500 | 8.76 | 3.3 | 0.0 | 3.3 | 0.0 |
| | 250 | 5.79 | 0.0 | 0.0 | 3.3 | 0.0 |
| Ref.item (mg/µg a.s.) | 1.0 | 0.017 | 100.0 | 0.0 | 100.0 | 0.0 |
| control | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

results are averages from three replicates (ten bees each) per concentration or control
M. m. = mean mortality, b. a. = behavioural abnormalities
* at test end, 10 days following application

**C. Validity of the test:**

| Validity criterion according to OECD 245 | Results of the study |
|---|---|
| The average mortality across replicates for the untreated control and solvent control groups is ≤15 % at the end of the test (10 days following start of exposure); when a solvent control is included, the average mortality across replicates for the solvent control should also be ≤ 15 % | The average mortality across replicates for the untreated control was 0 % on day 10. |
| The average mortality in the reference substance treated group is ≥ 50 % at the end of the test (10 days following start of exposure). | The average mortality in the reference substance treated group was 100 % at the end of the test |

Since the test protocol meets the validity criteria set forth in the most recent test guideline, the study is still considered valid.

**III. Assessment and conclusion**

In a 10-day chronic toxicity test, honey bees (*Apis mellifera L.*) were exposed to ADM.3500.F.2.B (Prothioconazole EC 250, 250 g prothioconazole/L). Under the conditions of this study, the $LC_{50}$ value (10 days) was 862.7 mg a.s./kg feeding solution (equivalent to 3751 mg test item/kg feeding solution). The $LDD_{50}$ value (10 days) was 14.4 µg a.s./bee/day (equivalent to 62.6 µg test item/bee/day). The NOEC and NOEDD values (10 days) were 500 mg a.s./kg feeding solution (equivalent to 2174 mg test item/kg feeding solution) and 8.76 µg a.s./bee/day (equivalent to 38.1 µg test item/bee/day), respectively. The study is considered valid (see: "C. Validity of the test" above).

## A 2.3.1.3 KCP 10.3.1.3 Effects on honey bee development and other honey bee life stages

| | |
|---|---|
| Comments of zRMS: | The study was conducted in line with OECD 239 with no deviations.<br><br>All validity criteria were met.<br><br>The analysis of the stock solutions used to treat the diet administered to the larvae showed that the test item content was in the range of ± 20 % of nominal concentration (mean recovery rates of 97.24 - 102.71 %).<br>Therefore, the endpoints were calculated on the basis of the nominal doses of test item.<br><br>Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment:<br><br>NOED larvae on D8 = 40.00 µg prod./larva/developmental period (9.20 µg a.s./larva/developmental period)<br><br>NOEC larvae on D8 = 259.74 mg prod./kg diet (59.74 mg a.s./kg diet)<br><br>22 d NOED adult emergence rate = 40.00 µg prod./larva (9.20 µg a.s./larva)<br><br>22 d NOEC adult emergence rate = 259.74 mg prod./kg diet (59.74 mg a.s./kg diet)<br><br>The mortality data did not allow the extrapolation of the $ED/EC_{10}$, $ED/EC_{20}$ and $ED/EC_{50}$ because the effects were < 10 % for each endpoint. |

| | |
|---|---|
| Reference: | KCP 10.3.1.3/01 |
| Report: | Effects of ADM.3500.F.2.B on honeybees (*Apis mellifera* L.) 22-day larval toxicity test with repeated exposure, Colli, M., 2020, report no.: BT109/19, sponsor no.: 000101262 |
| Guideline(s): | OECD Guidance Document, No. 239 (2016) ENV/JM/MONO(2016)34 guidance document no. 239 |
| Deviations: | None |
| GLP: | Yes (certified laboratory) |
| Acceptability/Reliability: | Yes/ |
| Duplication (if vertebrate study) | Not applicable |

**Executive summary**

The purpose of this study was to determine the chronic oral toxicity of ADM.3500.F.2.B (Prothioconazole EC 250, 250 g prothioconazole/L) to 3 days old (D3) honey bee larvae (*A. mellifera* L.) for a period of 22 days. Groups of 12 bees (12 bees per replicate, 3 replicates per concentration ) were exposed to ADM.3500.F.2.B at a nominal concentration of 2.5, 5.0, 10.0, 20.0 and 40.0 µg prod./larva (equivalent to 16.23, 32.47, 64.94, 129.87 and 259.74 mg prod./kg diet).

A control group received diet with water only. As a toxic reference, Dimethoate: (99.5% purity) was used. Assessments on mortality and any developmental/behavioral abnormality were performed from D4 to D8 and on D15 and on D22. The pupal mortality and the adults' emergence rate were assessed on D22. Analytical verification of test item concentrations was performed with the highest and the lowest concentration of the diet solution. The analysis of the stock solutions used to treat the diet administered to the larvae showed that the test item content was in the range of ± 20 % of nominal concentration (mean recovery rates of 97.24 - 102.71 %). Therefore, the endpoints were calculated on the basis of the nominal doses of test item. Under the conditions of this study, the NOED for larvae on D8 was determined to be 40.00 µg prod./larva/developmental period (9.20 µg a.s./larva/developmental period) equivalent to 259.74 mg prod./kg diet (59.74 mg a.s./kg diet). The NOED and the NOEC for adult emergence rate were determined to be 40.00 µg prod./larva (9.20 µg a.s./larva) and 259.74 mg prod./kg diet (59.74 mg a.s./kg diet) respectively. The mortality data did not allow the extrapolation of the $ED/EC_{10}$, $ED/EC_{20}$ and $ED/EC_{50}$ because the effects were < 10 % for each endpoint.

## I. Materials and methods

### A. Materials

| | |
|---|---|
| 1. Test material: | ADM.3500.F.2.B (Prothioconazole EC 250) |
| Lot/Batch no.: | 1109-210219-01 |
| Content/Purity: | prothioconazole: 23.0 % w/w, 248.2 g/L, (analytical); 250 g/L (nominal) |
| Control: | water mixed to the diet |
| Toxic reference: | Dimethoate: (99.5% purity) |
| 2. Test organisms - | |
| Species: | *Apis mellifera* L. |
| Age: | 3 days old larvae (D3) |
| Source | colony maintained at BioTecnologie BT S.r.l. |
| No. of organisms: | 3 replicates per concentration, each consisting of 12 larvae per treatment |
| Feeding: | diets as recommended by the OECD guidance document no. 239<br>D1: 20 µL diet A for each larva (50 % weight of fresh royal jelly + 50 % weight of an aqueous solution containing 2 % weight of yeast extract, 12 % weight of glucose and 12% weight of fructose)<br>D2 = no diet was administered |

| | |
|---|---|
| | D3: 20 µL diet B for each larva (50 % weight of fresh royal jelly + 50 % weight of an aqueous solution containing 3 % weight of yeast extract, 15% weight of glucose and 15 % weight of fructose)<br>D4 = 30 µL, D5 = 40 µL and D6 = 50 µL diet C for each larva<br>D4 to D6: 50 % weight of fresh royal jelly + 50 % weight of an aqueous solution containing 4 % weight of yeast extract, 18 % weight of glucose and 18 % weight of fructose |
| Acclimatisation | none |

3. Test units and exposure –

| | |
|---|---|
| Type and size: | the larvae were reared in crystal polystyrene grafting cells with an internal diameter of 9 mm and a depth of 8 mm. Each cell was placed into a well of a 48-well plate. The top of the grafting cell was maintained at the level of the plate by placing a piece of dental roll wetted with 500 µL of the sterilizing solution enhanced with 15 % weight/volume glycerol at the bottom of the wells |
| Test procedure: | larval toxicity test, repeated exposure |
| Test duration: | 22 days |

4. Test conditions –

| | |
|---|---|
| Temperature: | 34.0 - 35.0°C |
| Relative humidity: | from D1 to D8 = 90.0 - 100.0 % (average 95.6 %), from D8 to D15: 75.0 - 85.0 % (average 81.9 %), from D15 to D22: 50.0 - 80.0 % (average 64.1 %) |
| Photoperiod: | constant darkness |

**B. Study design and method**

1. In-life dates: July 10 to February 28, 2020 (experimental phase)

2. Test design:

A minimum of twelve larvae from each of three colonies were allocated on the same plate. Each plate corresponded to a treatment level, to the control or to the reference item. As the test item is a formulated product, stock solutions of the test item were prepared daily in ultrapure water. The test item was administered daily to the larvae with the diet in a range of five test item concentrations (2.5, 5.0, 10.0, 20.0 and 40.0 µg prod./larva, equivalent to 16.23, 32.47, 64.94, 129.87 and 259.74 mg prod./kg diet). A control group received diet with water only. The stock solutions were used to prepare the treated diets. At day 1 (D1), the comb containing first instar larvae were transferred from the hive to the laboratory. A volume of 20 µL of diet A was dropped into each cell, then one larva was grafted from the comb to the cell, onto the surface of the diet, using a grafting tool. All larvae were fed once per day from D1 to D6 (except D2), and food was added even if the previous food was not totally consumed. From D3 to D6, the test and reference item solutions, respectively, were mixed into the diet at the targeted concentration, just prior to its administration. On D3, twelve well-fed larvae from each of the three replicates were selected per plate: the grafting cells containing an alive larva were transferred from the plates prepared on D1 to new plates (one plate per treatment) and arranged, so that a clear assignment to the replicates (colonies) is possible. All larvae on one plate received the same treatment. On each feeding day, the position of the plates was changed within the desiccator to ensure eliminating potential spatial bias. The diet was warmed before use. All larvae were fed once a day, except on D2, on a warming plate at about 34 - 35°C, with a multi stepper pipette following the below schedule:

The reference item (a.s. Dimethoate) was tested at the constant concentration of 48 mg a.s./kg of diet (equivalent to a total cumulative amount of 7.39 µg/larva). The test and reference item were dissolved in water and were mixed with the diet just prior to administration to the larvae and provided on D3, D4, D5 and D6.

Mortalities were assessed and recorded from D4 to D8 and on D15. An immobile larva or a larva which did not react to the contact of the grafting tool or paintbrush was recorded as dead. On D15 larvae that had not transformed into pupae were recorded as dead and removed. Hatched adults were recorded on D22. Adult emergence rate was calculated as a percentage by comparing the number of bees emerged on D22 to the number of larvae on D3 when dosing started. The pupal mortality was evaluated on D15 and on D22:- on D15 as a percentage by comparing the number of dead pupae from D8 to D15 to the number of larvae entering in pre-pupa stage on D8; - on D22 as a percentage by comparing the number of pupae that failed to emerge, including those bees without emergence on D22 and dead pupae removed during pupa stage (from D8 to D22) to the number of larvae entering in pre-pupa stage on D8.

The larvae mortality was calculated as a percentage by comparing the number of larvae which died during larval stages (from D3 to D8) to the number of larvae on D3 when dosing started. Other observation, e.g. larval appearance and size, behaviour, morphological differences and any other adverse effects after emergence (in comparison with controls) were recorded qualitatively.

3. Analytical verification:

Each day of administration, samples of the stock solutions (at lowest and highest concentrations, 0.25 and 4.00 mg/ml) and a sample of the water used for the untreated control, were analysed for the determination of the actual concentration of the test chemicals. A description and validation of the analytical method is provided in Part B of Section 5 (*Analytical Methods*).

4. Statistics:

To evaluate the data for statistical significance and to determine the NOED/NOEC, a $Chi^2$ 2x2 table test with Bonferroni correction (one-sided greater) was performed. The software ToxRatPro Version 3.3.0 was used for the statistical analysis.

## II. Results and discussion

## A. Analytical data

The analysis of the stock solutions used to treat the diet administered to the larvae showed that the test item content was in the range of ± 20 % of nominal concentration, so was demonstrated that the larvae were treated with the corresponding nominal dose of test item and the endpoints were calculated on the basis of the nominal doses of test item (see table below).

**Table A 21: Analytical results**

| Day of diet | Stock solution (mg/ml) | Recovery (%) | Mean recovery % | SD | RSD (%) |
|---|---|---|---|---|---|
| D3 | S1: 0.25 | 102.07 | 100.87 | 1.70 | 1.69 |
| | | 99.66 | | | |
| | S5: 4.00 | 100.32 | 101.48 | 1.63 | 1.61 |
| | | 102.63 | | | |
| D4 | S1: 0.25 | 97.24 | 98.02 | 1.10 | 1.12 |
| | | 98.79 | | | |
| | S5: 4.00 | 101.99 | 102.71 | 1.01 | 0.98 |
| | | 103.42 | | | |
| D5 | S1: 0.25 | 97.24 | 97.24 | 0.00 | 0.00 |
| | | 97.24 | | | |
| | S5: 4.00 | 100.10 | 99.22 | 1.24 | 1.25 |
| | | 98.34 | | | |
| D6 | S1: 0.25 | 98.45 | 98.37 | 0.12 | 0.12 |
| | | 98.28 | | | |
| | S5: 4.00 | 99.54 | 99.93 | 0.55 | 0.55 |
| | | 100.32 | | | |

## B. Mortality

Regarding the effects on larvae on D8 (developmental period), the test item ADM.3500.F.2.B did not cause statistically significant mortality when administered up to the dose of 40.00 µg test item/larva (see table below). Therefore, the NOED for larvae on D8 was determined to be 40.00 µg prod./larva/developmental period (9.20 µg a.s./larva/developmental period) equivalent to 259.74 mg prod./kg diet (59.74 mg a.s./kg diet).

**Table A 22: Mortality (%) and corrected mortality (CM%) of larvae (on D8)**

| Dose (µg prod./larva) | Concentration (mg prod./kg diet) | Larvae mortality on D8 | | |
|---|---|---|---|---|
| | | Mean % | CM - Mean % | Significant |
| 0.0 | 0.00 | 5.56 | n.a. | n.a. |
| 2.5 | 16.23 | 2.78 | 0.0 | - |
| 5.0 | 32.47 | 2.78 | 0.0 | - |
| 10.0 | 64.94 | 8.33 | 2.94 | - |
| 20.0 | 129.87 | 8.33 | 2.94 | - |
| 40.0 | 259.74 | 11.11 | 5.88 | - |

n.a. = not applicable
+ : significant; - : non-significant ($Chi^2$ 2x2 table test with Bonferroni correction - $\alpha = 0.05$, one-sided greater).

**Table A 23: Pupal mortality**

| Dose (µg prod./larva) | Concentration (mg prod./kg diet) | Pupal mortality from D8 to D15* | Pupal mortality from D8 to D22** |
|---|---|---|---|
| | | Mean % | Mean % |
| 0.0 | 0.00 | 8.82 | 14.71 |
| 2.5 | 16.23 | 8.57 | 11.43 |
| 5.0 | 32.47 | 2.86 | 5.71 |
| 10.0 | 64.94 | 9.09 | 9.09 |
| 20.0 | 129.87 | 15.15 | 15.15 |
| 40.0 | 259.74 | 12.50 | 12.50 |

*calculated in percentage comparing the number of dead pupae from D8 to D15 to the number of alive pupae on D8
**calculated in percentage comparing the number of dead pupae from D8 to D22 to the number of alive pupae on D8

Regarding the effects on adult emergence on D22, the test item ADM.3500.F.2.B did not cause statistically significant reduction in emergence rate at each tested dose. The NOED and the NOEC for adult emergence rate were determined to be 40.00 µg prod./larva (9.20 µg a.s./larva) and 259.74 mg prod./kg diet (59.74 mg a.s./kg diet) respectively (see table below). The mortality data did not allow the extrapolation of the $EC_{10}$, $EC_{20}$ and $EC_{50}$ because the effects were < 10 % for each endpoint.

**Table A 24: Total mortality (M%) and corrected mortality (CM%) from D3 to D22**

| Dose (µg prod./larva) | Concentration (mg prod./kg diet) | Mortality (larvae + pupae) on D22 | | | Adult emergence on D22 | |
|---|---|---|---|---|---|---|
| | | Mean % | CM - Mean % | Signifi-cant | Mean % | Significant |
| 0.0 | 0.00 | 19.44 | n.a. | n.a. | 80.56 | n.a. |
| 2.5 | 16.23 | 13.89 | 0.0 | - | 86.11 | - |
| 5.0 | 32.47 | 8.33 | 0.0 | - | 91.67 | - |
| 10.0 | 64.94 | 16.67 | 0.0 | - | 83.33 | - |
| 20.0 | 129.87 | 22.22 | 3.45 | - | 77.78 | - |
| 40.0 | 259.74 | 22.22 | 3.45 | - | 77.78 | - |

n.a. = not applicable
+ : significant; - : non-significant ($Chi^2$ 2x2 table test with Bonferroni correction - $\alpha = 0.05$, one-sided greater).

Reference item

The reference item (a.s. Dimethoate) was tested at the constant concentration of 48 mg a.s./kg of diet (equivalent to a total cumulative amount of 7.39 µg/larva). The test and reference item were dissolved in water and were mixed with the diet just prior to administration to the larvae and provided on D3, D4, D5 and D6. The reference item caused a mortality of 100 % on D8 (see table below).

**Table A 25: Reference item - mean mortality**

| Dose (µg a.s./larva) | Concentration (mg a.s./kg diet) | Mortality on D8 mean % |
|---|---|---|
| 7.39 | 48.00 | 100.00 |

## C. Validity of the test:

| Validity criterion according to the OECD guidance document no. 239 | Results of the study |
|---|---|
| In the control plate(s), cumulative larval mortality from D3 to D8 should be ≤ 15 % across all replicates. | In the control plate(s), cumulative larval mortality from D3 to D8 was 5.56 % across all replicates. |
| In the control plate(s), the adult emergence rate on D22 should be ≥ 70 % across all replicates. | In the control plate(s), the adult emergence rate on D22 was 80.56 %. |
| Positive control: if the dimethoate is used, larval mortality should be ≥ 50 % on D8 across all replicates; if the fenoxycarb is used, the emergence rate should be ≤ 20 % on D22 across all replicates. | The positive control dimethoate cause larval mortality of 100 % on D8. |

Since the test protocol meets the validity criteria set forth in the most recent test guideline, the study is still considered valid.

## III. Assessment and conclusion

In a 22-day larval toxicity test with repeated exposure, 3 days old larvae (D3) (*Apis mellifera L.*) were exposed to ADM.3500.F.2.B (Prothioconazole EC 250, 250 g prothioconazole/L). Under the conditions of this study, the NOED for larvae on D8 was determined to be 40.00 µg prod./larva/developmental period (9.20 µg a.s./larva/developmental period) equivalent to 259.74 mg prod./kg diet (59.74 mg a.s./kg diet). The NOED and the NOEC for adult emergence rate were determined to be 40.00 µg prod./larva (9.20 µg a.s./larva) and 259.74 mg prod./kg diet (59.74 mg a.s./kg diet) respectively. The mortality data did not allow the extrapolation of the ED/$EC_{10}$, ED/$EC_{20}$ and ED/$EC_{50}$ because the effects were < 10 % for each endpoint. The study is considered valid (see: "C. Validity of the test" above).

## A 2.3.1.4 KCP 10.3.1.4 Sub-lethal effects

Not considered to be required.

## A 2.3.1.5 KCP 10.3.1.5 Cage and tunnel tests

Not considered to be required.

## A 2.3.1.6 KCP 10.3.1.6 Field tests with honeybees

Not considered to be required.

## A 2.3.2 KCP 10.3.2 Effects on arthropods (other than bees)

| | |
|---|---|
| Comments of zRMS: | The study was conducted in line with Mead-Briggs et al. (2000), Grimm, C. et al. (2001) method with no deviations.<br><br>All validity criteria were met.<br><br>Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment:<br><br>$LR_{50}$ 48 h > 400 g a.s./ha (equivalent to > 1582 ml prod./ha) |

| | |
|---|---|
| Reference: | KCP 10.3.2/01 |
| Report: | Effects of ADM.3500.F.2.B on the parasitic wasp *Aphidius rhopalosiphi* (DeStefani-Perez) in a laboratory test, Röhlig, U., 2020a, report no.: 19 48 NAL 0006, sponsor no.: 000102735 |
| Guideline(s): | Mead-Briggs *et al*. (2000), Grimm, C. *et al*. (2001) |
| Deviations: | None |
| GLP: | Yes (certified laboratory) |
| Acceptability/Reliability: | Yes/ |
| Duplication (if vertebrate study) | Not applicable |

**Executive summary**

Groups of 7 females + 3 males (4 replicates/group) of the parasitic wasp species *Aphidius rhopalosiphi* were exposed to freshly dried residues of ADM.3500.F.2.B (Prothioconazole EC 250, 250 g prothioconazole/L) after spray application onto glass plates at rates of 25, 50, 100, 200 and 400 g a.s./ha (equivalent to 98.9, 197.8, 395.6, 791 and 1582 ml product/ha, based on analysed content of a.s). A control group exposed to tap deionised water without only and a toxic reference (Dimethoate EC 400) were run concurrently. Mortality and behaviour of the wasps was recorded at approximately 2, 24 and 48 h after exposure to the product. After 48 hours, 15 surviving females of the control group, as well as each of the treated groups were randomly selected for a following reproduction test. Under the conditions of the present study, the $LR_{50}$ after 48 hours was > 400 g a.s./ha (equivalent to > 1582 ml prod./ha). The NOER for mortality was determined to be 400 g a.s./ha (equivalent to 1582 ml prod./ha). The $ER_{50}$ was estimated to be > 400 g a.s./ha (equivalent to > 1582 ml prod./ha). The NOER for reproduction was determined to be 400 g a.s./ha (equivalent to 1582 ml prod./ha).

### I. Materials and methods

### A. Materials

| | | |
|---|---|---|
| 1. | Test material: | ADM.3500.F.2.B (Prothioconazole EC 250) |
| | Lot/Batch no.: | 3178-010519-01 |
| | Active substance content: | prothioconazole, 250 g /L (nominal), 252.8 g/L (analysed) |
| | Control: | deionised water (200 L/ha). |
| | Toxic reference: | Dimethoate EC 400 (BAS 152 11 I) / Dimethoate 400 g/L (nominal), 429.0 g/L (analysed) |
| 2. | Test organisms - | |
| | Species: | *Aphidius rhopalosiphi* (De Stefani-Perez) |
| | Age: | adult, ≤ 48 hours |
| | Source | Katz Biotech AG, Baruth, Germany |
| | No. of organisms: | mortality phase: 7 females + 3 males per replicate (4 repli- |

| | |
|---|---|
| | cates/group), reproduction phase: 1 female per replicate (15 replicates per group) |
| Feeding: | *ad libitum* with 33 % honey-water |
| Acclimatisation: | under controlled laboratory conditions |

3. Test units and exposure –

| | |
|---|---|
| Type and size: | mortality test: 2 square glass plates (13 cm x 13 cm), held apart by an aluminium frame (13 cm x 13 cm x 1.4 cm) with gauze covered holes for forced air ventilation (blowing air; flow rate: 2.5 L/min).<br>reproduction test: acrylic cylinder (about 11 cm Ø, 20 cm high) with approx. 20 wheat seedlings (*Triticum*) e.g. variety "Tambor" (8 days old) planted in a pot containing potting soil, infested with > 100 adult and nymphal aphids (reared in the laboratory of the test facility) and covered at the top of the cylinder with gauze. |
| Test procedure: | laboratory test under worst-case conditions, rate-response test |
| Test substrate: | wheat seedlings (*Triticum*) planted in potting soil |
| Test duration. | mortality test: 48 h<br>reproduction test: further 12 days, (24 h for parasitisation + 11 days for development of wasps) |

4. Test conditions -

| | |
|---|---|
| Temperature: | 19 - 22°C |
| Relative humidity: | 65 - 73 % |
| Photoperiod: | 16 h light/8 h dark |
| Light intensity: | 1190 lux (exposure phase), 2340 lux (parasitisation phase), 6810 lux (reproduction phase) |

**B. Study design and method**

1. In-life dates: September 09 to September 23, 2019 (experimental phase)

2. Test design:

The study encompassed 7 treatment groups (5 test item rates, control and reference item), each with 4 replicates. Seven females and 3 males per replicate were exposed to ADM.3500.F.2.B sprayed on glass plates at application rates of 25, 50, 100, 200 and 400 g a.s./ha (equivalent to 98.9, 197.8, 395.6, 791 and 1582 ml product/ha, based on analysed content of a.s.). Additional test units were treated with deionised water for the water control and with Dimethoate EC 400 (active substance 429.0 g/L) as the reference item. Mortality assessments were carried out 2, 24 and 48 hours after test initiation.

After 48 hours, to determine the parasitisation capacity, a sufficient number of surviving females of the control group, as well as each of the treated groups were randomly selected (approximately the same number of surviving females from each replicate) and individually confined in acrylic cylinders containing untreated potted wheat plants infested with > 100 adult and nymphal cereal aphids (*Rhopalosiphum padi*). The wasps were removed 24 hours later and the parasitisation units were maintained in the climatic room for further 11 days. After that, the number of parasitised aphids (aphid mummies) was recorded and the parasitisation rate per wasp was determined.

3. Statistics:

Shapiro-Wilk's test and Levene's test were used, respectively, to test the data for normality and homogeneity of variance. As the data were normally distributed and variance homogeneous a multiple test (Williams-t-test) was performed. The Williams-t-test compares a set of treatments against a single control mean.

$Chi^2$ 2x2 Table Test ($\alpha = 0.05$) was used for mortality: $Chi^2$-2 x 2 Test with Bonferroni Correction with survival at 48 h: Comparisons between treatment and control were done with the multiple significance level (Alpha is 0.050; one-sided greater). Two-sample comparisons are performed sequentially using the adjusted Alpha* (= alpha/(k-1) and the standard normal variable z; k: number of comparisons (after Holm 1979)). Ho (no effect) is accepted, if the probability p(z) > Alpha*; p(z) is the probability that the increase in category "Dead" observed in the treatment(s) is due to chance. Note that the step-down test terminates after the first non-significant treatment is encountered.

## II. Results and discussions

## A. Mortality

The results of the control group indicated that the test organisms were in a good condition (mortality: 2.5 %, reproduction: 20.3 mummies per female). The results of the reference item group indicated that the test system was sensitive to harmful substances (corrected mortality: 100 %). Concerning mortality and reproduction in the control group as well as the susceptibility of the test organisms to the reference item, the study is proved to be valid. After 48 hours, a mortality rate of 2.5 % was observed in the control. In the test item treatments, mortality ranged between 0 % and 2.5 %. This resulted in corrected mortality rates of -2.6 % and 0 %. No statistically significant effects on mortality were determined at any test item treatment groups ($Chi^2$ 2x2 Table Test, $\alpha = 0.05$). The $LR_{50}$ was estimated to be > 400 g a.s./ha (equivalent to > 1582 ml product/ha). The NOER (no observed effect rate) for mortality was determined to be 400 g a.s./ha (equivalent to 1582 ml product/ha).

**Table A 26: Mortality of *Aphidius rhopalosiphi***

| **Treatment group** | | **Dead wasps (number)** | **Moribund wasps (number)** | **Surviving wasps (number)** | **Mortality[1] (%)** | **Corrected mortality (Abbott) [%]** |
|---|---|---|---|---|---|---|
| Control | deionised water | 1 | 0 | 39 | 2.5 | - |
| ADM.3500.F.2.B (g a.s./ha) | 25 | 1 | 0 | 39 | 2.5 (n.s.) | 0 |
| | 50 | 0 | 0 | 40 | 0 (n.s.) | -2.6 |
| | 100 | 1 | 0 | 39 | 2.5 (n.s.) | 0 |
| | 200 | 1 | 0 | 39 | 2.5 (n.s.) | 0 |
| | 400 | 1 | 0 | 39 | 2.5 (n.s.) | 0 |
| Dimethoate EC 400 (ml prod./ha) | 0.3 | 39 | 1 | 0 | 100 | 100 |

10 wasps per replicate were introduced (4 replicates per treatment)
[1] mortality including dead and moribund wasps 48 hours after exposure
n.s. not statistically significant different compared to the control: $Chi^2$ 2x2 Table Test ($\alpha = 0.05$) for test item

## B. Reproduction

The mean number of mummies per female per day in the test item treatment groups were between 20.7 and 22.2, compared to the control with 20.3 mummies/female. No statistically significant effects on reproductive capacity were determined at all test item treatment groups (WILLIAMS-t-test, $\alpha = 0.05$). The $ER_{50}$ for reproduction was estimated to be > 400 g a.s./ha ha (equivalent to > 1582 ml product/ha). The NOER (no observed effect rate) for reproduction was determined to be 400 g a.s./ha ha (equivalent to 1582 ml product/ha).

**Table A 27: Reproduction of *Aphidius rhopalosiphi***

| **Treatment group** | | **Number of females used for reproduction phase** | **Mean number of mummies/female[1]** | **Effect on reproduction (%)[2]** |
|---|---|---|---|---|
| Control | deionised water | 15 | 20.3 | - |
| ADM.3500.F.2.B (g a.s./ha) | 25 | 15 | 20.7 (n.s.) | -2.0 |
| | 50 | 15 | 20.8 (n.s.) | -2.5 |
| | 100 | 15 | 21.4 (n.s.) | -5.4 |
| | 200 | 15 | 20.7 (n.s.) | -2.0 |
| Dimethoate EC 400 (g a.s./ha) | 400 | 15 | 20.7 (n.s.) | -4.4 |

[1] the mean number of mummies/female was calculated from the number of mummies per surviving female
[2] Change in mean number of mummies per female, relative to control. A negative value indicates an increase and a positive value indicates a decrease relative to the control.
n.s. not statistically significantly different compared to the control (Williams-t-test, $\alpha = 0.05$)

**C. Validity of the test:**

| **Validity criterion according to Mead-Briggs *et al.* (2000)** | **Results of the study** |
|---|---|
| The mortality in the control treatment should not exceed 13 %. | The mean mortality in the control treatment was 2.5 %. |
| The level of mortality in the toxic reference treatment should be specified in the study protocol and should be based on the previous experience of the test laboratory. | In the toxic reference treatment, 100 % mortality after 48 h was observed, which met the validity criterion imposed for this treatment. |
| Wasps in the control group should produce a minimum of 5 mummies per female. In the control group there should be no more than 2 wasps producing zero values to determine true treatment effects. | The mean mummy production in the control group was 20.3 per female. No wasps in the control group produced zero mummies. |

Since the test protocol meets the validity criteria set forth in the most recent test guideline, the study is considered valid.

## III Assessment and conclusion

In a 48-hour mortality test and a following reproduction test, groups of *Aphidius rhopalosiphi* were exposed to freshly dried residues of product ADM.3500.F.2.B (Prothioconazole EC 250, 250 g prothioconazole/L) applied to glass plates. After exposure to 25, 50, 100, 200 and 400 g a.s./ha (equivalent to 98.9, 197.8, 395.6, 791 and 1582 ml prod./ha, based on analysed content of a.s.), the $LR_{50}$ after 48 hours was > 400 g a.s./ha (equivalent to > 1582 ml prod./ha). The NOER for mortality was determined to be 400 g a.s./ha ha (equivalent to 1582 ml prod./ha). The ER50 was estimated to be > 400 g a.s./ha ha (equivalent to > 1582 ml prod./ha). The NOER for reproduction was determined to be 400 g a.s./ha ha (equivalent to 1582 ml prod./ha). The study is considered valid (see: “C. Validity criteria” above).

| | |
|---|---|
| Comments of zRMS: | The study was conducted in line with IOBC (BLÜMEL et al. 2000) guideline with no deviations.<br><br>All validity criteria were met.<br><br>Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment:<br><br>$LR_{50}$ for *Typhlodromus pyri* > 200 g a.s./ha (equivalent to > 791 ml prod./ha). |

| | |
|---|---|
| Reference: | KCP 10.3.2/02 |
| Report: | Effects of ADM.3500.F.2.B on the predatory mite *Typhlodromus pyri* Scheuten in a laboratory test, Röhlig, U., 2020b, report no.: 19 48 NTL 0006A, sponsor no.: 000102734A |
| Guideline(s): | IOBC (BLÜMEL *et al.* 2000) |
| Deviations: | None |
| GLP: | Yes (certified laboratory) |
| Acceptability/Reliability: | Yes |
| Duplication (if vertebrate study) | Not applicable |

**Executive summary**

Groups of 20 protonymphs (5 replicates/group) of the predatory mite *Typhlodromus pyri* were exposed to freshly dried residues of ADM.3500.F.2.B (Prothioconazole EC 250, 250 g prothioconazole/L) after spray application onto glass plates at rates of 12.5, 25, 50, 100 and 200 g a.s./ha (equivalent to 49.4, 98.9, 197.8, 395.6 and 791 ml prod./ha, based on analysed content of a.s.). A control group exposed to purified water without test item and a toxic reference (Dimethoate EC 400) were run concurrently. On day 3, 7, 9, 11 and 14 after the application, the number of surviving predatory mites were counted (from the 7th day onward differentiated according to sex). Under the conditions of the present study, the 7-day $LR_{50}$ for *Typhlodromus pyri* was estimated to be > 200 g a.s./ha (equivalent to > 791 ml prod./ha). The NOER for mortality was determined to be 200 g a.s./ha (equivalent to 791 ml prod./ha). The $ER_{50}$ for reproduction was estimated to be > 200 g a.s./ha (equivalent to > 791 ml prod./ha). The NOER for reproduction was determined to be 200 g a.s./ha (equivalent to 791 ml prod./ha).

## I. Materials and methods

### A. Materials

1. Test material: ADM.3500.F.2.B (Prothioconazole EC 250)
   - Lot/Batch no.: 3178-010519-01
   - Active substance content: prothioconazole, 250 g /L (nominal), 252.8 g/L (analysed)
   - Control: deionised water
   - Toxic reference: Dimethoate EC 400 (Dimethoate 400 g/L (nominal), 429.0 g/L (analysed)

2. Test organisms -
   - Species: *Typhlodromus pyri* (Scheuten)
   - Age: protonymphs, ≤ 24 hours
   - Source: Katz Biotech AG", An der Birkenpfuhlheide 10, 15837 Baruth, Germany
   - No. of organisms: 20 protonymphs/replicate (5 replicates/group)
   - Feeding: at test start and at each assessment day with pollen (pine, *Pinus nigra*) and birch (*Betula pendula*), 1:1
   - Acclimatisation: not stated

3. Test units and exposure –

| | |
|---|---|
| Type and size: | 2 glass plates (cover glasses: 50 mm x 22 mm stuck together along their longitudinal sides) with a barrier of sticky material on moistened filter paper on a sponge placed in a plastic tray (inside dimensions: about 165 mm x 120 mm x 60 mm) filled with tap water up to a height of approx. 15 mm |
| Test procedure: | laboratory test under worst-case conditions, rate-response test |
| Test substrate: | glass plates |
| Test duration. | mortality test: 7 days<br>reproduction test: further 7 days |

4. Test conditions -

| | |
|---|---|
| Temperature: | 23 °C - 27 °C |
| Relative humidity: | 67 % - 71 % |
| Photoperiod: | 16 h light/8 h dark |
| Light intensity: | 2010lux |

**B. Study design and method**

1. In-life dates: September 03 to September 17, 2019 (experimental phase)

2. Test design:

Protonymphs were exposed to dried spray residues of different application rates (12.5, 25, 50, 100 and 200 g a.s./ha, equivalent to 49.4, 98.9, 197.8, 395.6 and 791 ml prod./ha, based on analysed content of a.s.) of the test item applied on glass plates. All substances were applied in 200 L water/ha, sprayed on glass plates, via laboratory spraying equipment and air dried afterwards. 7 treatment groups (5 test item rates, water treated control and reference item) were set up with 5 replicates (consisting of 20 protonymphs) per treatment. Exposure lasted until 14 days after application.

On day 3, 7, 9, 11 and 14 after the application, the number of surviving predatory mites were counted (from the 7th day onward differentiated according to sex), dead mites were recorded and removed; mites that were missing or trapped (in the insect glue) were separately recorded. The number of eggs laid and hatched juveniles present were determined on days 9, 11 and 14, these were removed on days 9 and 11. Any eggs found on day 7 were removed and not counted in the reproduction assessment. The final assessment for mortality was performed on day 7 after treatment and the final assessment for reproduction was made on day 14 after treatment. From these data, the cumulative juvenile and adult mortality on day 7 (in %) corrected for control mortality according to Abbott (1925) and the cumulative mean reproduction per female (during 7 days - day 7-14) were calculated.

3. Statistics:

For statistical calculation of the results, the computer program ToxRat Professional 3.3.0 (Ratte, 2018) was used. Mortality was analysed for statistical significance using the Multiple Sequentially-rejective $Chi^2$-2x2 Table test after Bonferroni-Holm as distribution-free test which do not require testing for normality or homoscedasticity prior to analysis. The accepted significance level was $\alpha = 0.05$. Reproduction was analysed for statistical significance using Williams-t-test, following Shapiro-Wilks's test on normal distribution and Levene's test procedure on variance homogeneity and a trend analysis by contrasts to test the data for monotonicity of rate/response. Since there were only slight effects on mortality and reproduction in the test item treatment groups, a calculation of the $LR_{50}$ (median lethal rate) and $ER_{50}$ (median effect rate) was not possible.

**II. Results and discussions**

**A. Mortality**

After 7 days in the control a mortality rate of 1.0 % was observed. In the test item treatments, mortality

ranged between 1.0 % and 2.0 %. This resulted in corrected mortality rates between 0 % and 1.0 %. No statistically significant effects on mortality were determined at any test item rates, which were tested, compared to the control (see table below). The $LR_{50}$ was estimated to be > 200 g a.s./ha (equivalent to > 791 ml prod./ha). The NOER for mortality was determined to be 200 g a.s./ha (equivalent to 791 ml prod./ha). The results of the reference item group indicated that the test system was sensitive to harmful substances (corrected mortality: 73.7 %).

**Table A 28: Mortality in *Typhlodromus pyri* after 7 days of exposure to treated glass plates**

| Treatment | Rate [1] (g a.s./ha) | Mortality [2] (%) | Corrected mortality [3] (%) |
|---|---|---|---|
| Control | - | 1.0 | - |
| ADM.3500.F.2.B | 12.5 | 2.0 (n.s) | 1.0 |
| | 25 | 2.0 (n.s) | 1.0 |
| | 52 | 2.0 (n.s) | 1.0 |
| | 100 | 1.0 (n.s) | 0.0 |
| | 200 | 1.0 (n.s) | 0.0 |
| Toxic reference | 15 | 74.0 | 73.7 |

[1] application rate in 200 L water/ha
[2] Mortality after 7 days of exposure to residues on treated glass plates. The results for mortality in individual test item treatments were compared to that in the control using Multiple Sequentially-rejective $Chi^2$ -2x2 Table test after Bonferroni-Holm ($\alpha = 0.05$)
[3] Mortality corrected according to ABBOTT (1925)
n.s. not statistically significant different compared to the control

## B. Reproduction

The reproduction rate amounted to 7.59 eggs/female in the control treatment. The reproduction rate in the test item treated groups were between 7.32 eggs/female and 8.09 eggs/female (see table below). Thus, an effect on reproduction between 3.6 % and -6.6 % was calculated for the test item treated groups compared to the control. No statistically significant effects on reproduction were determined at all test item rates. The $ER_{50}$ for reproduction was estimated to be > 200 g a.s./ha (equivalent to > 791 ml product/ha). The NOER for reproduction was determined to be 200 g a.s./ha (equivalent to 791 ml product/ha). No unusual observations regarding behaviour were noted in the control and the test item treatment groups at any observation point during the test.

**Table A 29: Effects on reproduction in *Typhlodromus pyri* after 7 days of exposure to treated glass plates**

| Treatment | Rate [1] (g a.s./ha) | Mean number eggs per female [2] (7 - 14 day) | Effects on reproduction [3] (%) |
|---|---|---|---|
| Control | - | 7.59 | - |
| ADM.3500.F.2.B | 12.5 | 7.98 (n.s.) | -5.1 |
| | 25 | 8.09 (n.s.) | -6.6 |
| | 50 | 7.64 (n.s.) | -0.7 |
| | 100 | 7.32 (n.s.) | 3.6 |
| | 200 | 7.41 (n.s.) | 2.4 |

[1] Application rate in 200 L water/ha
[2] Results for reproduction compared by Williams-t-test ($\alpha = 0.05$)
[3] Change in mean number of eggs per female, relative to control. A positive value indicates a decrease and a negative value indicates an increase relative to the control.
n.s. not statistically significantly different compared to the control

## C. Validity of the test:

| Validity criterion according to Blümel *et al.* (2000) | Results of the study |
|---|---|
| The arithmetic mean mortality (dead and escaped individuals) in the control should not exceed 20 % on day after treatment application. | The mean mortality in the control was 1 %. |
| The cumulative mean number of eggs per females in the control (from day 7 to day 14) should be ≥ 4 eggs/female. | The cumulative mean number of eggs per females in the control (from day 7 to day 14) was 7.59 eggs/female. |
| The cumulative means mortality (control corrected) of protonymphs on day 7 exposed to the toxic reference item should range between 50 and 100 %. | The means mortality of protonymphs on day 7 exposed to the toxic reference item was 73.7%. |

Since the test protocol meets the validity criteria set forth in the most recent test guideline, the study is considered.

## III. Assessment and conclusion

In a 7-day mortality test followed by a 7-day reproduction test, groups of *Typhlodromus pyri* were exposed to freshly dried residues of the product ADM.3500.F.2.B (Prothioconazole EC 250, 250 g prothioconazole/L) applied to glass plates. After exposure to 12.5, 25, 50, 100 and 200 g a.s./ha (equivalent to 49.4, 98.9, 197.8, 395.6 and 791 ml prod./ha), the 7-day $LR_{50}$ for *Typhlodromus pyri* was estimated to be > 200 g a.s./ha (equivalent to > 791 ml prod./ha). The NOER for mortality was determined to be 200 g a.s./ha (equivalent to 791 ml prod./ha). The $ER_{50}$ for reproduction was estimated to be > 200 g a.s./ha (equivalent to > 791 ml prod./ha). The NOER for reproduction was determined to be 200 g a.s./ha (equivalent to 791 ml prod./ha). The study is considered valid (see: “C. Validity criteria” above).

## A 2.4 KCP 10.4 Effects on non-target soil meso- and macrofauna

### A 2.4.1 KCP 10.4.1 Earthworms

### A 2.4.1.1 KCP 10.4.1.1 Earthworms - sub-lethal effects

| | |
|---|---|
| Comments of zRMS: | The study was conducted in line with OECD 222 with minor deviations:<br><br>The moisture contents on day 0 were slightly higher than the required upper limit of 60 % of the maximum water holding capacity in the test soil of eight treatment groups as well as two treatment groups at the end of the exposure phase.<br><br>The deviation is considered to have no impact on the quality of the study, because all validity criteria are met.<br><br>Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment:<br><br>NOEC body weight change, ~~reproduction~~ ≥1000 mg/kg soil dw<br><br>Since no clear dose-response relationship was observed and the difference compared to the control was not higher than 30 %, a calculation of reliable ECx values was not possible.<br><br>EC50 reproduction > 1000 mg test item/kg soil dw,<br><br>During commenting period process, the following comment from AT was provided.<br>At 1000 mg/kg soil dw, there is an effect of 29.4% on reproduction. Even if it is not statisitcally significant, it should be considered biologically significant (we consider the limit for biological relevance at 15%). The NOEC would therefore be 556 mg/kg soil dw.<br>Although there is no clear dose response in the lower concentrations, in this test, in general values indicating higher reproduction than the control should also be considered and discussed as potential effects, for endpoint derivation.<br><br>zRMS agrees with this comment and updated the NOEC, reproduction to NOEC =556 mg/kg dws |

| | |
|---|---|
| Reference: | KCP 10.4.1.1/01 |
| Report: | ADM.3500.F.2.B: Effects on the reproduction of the earthworm *Eisenia fetida* (Annelida, Lumbricidae) in artificial soil with 10 % peat, Ripperger, D., 2020, report no.: S18-07002A, sponsor no.: 000101433A |
| Guideline(s): | OECD 222 (2016) |
| Deviations: | The moisture contents on day 0 were slightly higher than the required upper limit of 60 % of the maximum water holding capacity in the test soil of eight treatment groups as well as two treatment groups at the end of the exposure phase. The deviation is considered to have no impact on the quality of the study, because all validity criteria are met. |
| GLP: | Yes (certified laboratory) |
| Acceptability/Reliability: | Yes |
| Duplication (if vertebrate study) | Not applicable |

**Executive summary**

In a 56-day reproduction toxicity test groups of earthworms (10 worms per replicate, 4 replicates per test item group and 8 replicates per control) were exposed for 28 days to ADM.3500.F.2.B (Prothioconazole EC 250, 250 g prothioconazole/L) mixed into artificial soil (peat content: 10 %; w/w) at concentrations of 16.3, 29.4, 52.9, 95.3, 171, 309, 556 and 1000 mg test item/kg $soil_{dw}$. Mortality was assessed after 28 days

of exposure. The body weights of the adult earthworms were determined on day 0 and day 28. The number of juveniles was determined at test end on day 56. All treatment data was compared to a control treatment of water (mixed into the soil). Based on the results of the study, the 28-day NOEC for mortality, body weight change and reproduction was considered to be ≥1000 mg/kg $soil_{dw}$, the highest concentration tested. Since no clear dose-response relationship was observed and the difference compared to the control was not higher than 30 %, a calculation of reliable ECx values was not possible. The $EC_{50}$ of the reproduction is > 1000 mg test item/kg $soil_{dw}$, the highest concentration tested.

## I. Materials and methods

### A. Materials

| | |
|---|---|
| 1. Test material: | ADM.3500.F.2.B (Prothioconazole EC 250) |
| Lot/Batch no.: | 1109-210219-01 |
| Content/Purity: | prothioconazole: 23.0 % w/w, 248.2 g/L, (analytical); 250 g/L (nominal) |
| Control: | water mixed into the artificial soil |
| Toxic reference: | boric acid (purity analysed: 100.1 %) |
| 2. Test organisms - | |
| Species: | *Eisenia fetida* |
| Age: | adult worms (with clitellum) |
| Source: | in-house culture |
| Weight at test start: | 308 - 600 mg |
| No. of organisms: | 40 test organisms divided into four replicates (10 worms/replicate), 8 replicates for the control |
| Feeding: | 4 g of finely ground cow manure on day 1, 8 ,15, 22 and 28 after application |
| 3. Test units and exposure – | |
| Type and size: | plastic vessels (approx. 17 cm x 12.5 cm x 6 cm; 1150 $cm^3$), filled with artificial soil to a height of approx. 5 cm, corresponding to 500 g dry soil. A plastic lid with holes covered the vessel to prevent test organisms from escaping and to allow for gaseous exchange, whilst limiting evaporation. |
| Test procedure: | reproductive toxicity test using artificial soil with 10 % peat |
| Test duration: | 56 days |
| Test substrate: | artificial soil according to OECD 222 with 10 % peat |
| Composition: | 70 % industrial sand (fine sand dominant with more than 50 % of the particles between 50 and 200 microns)<br>20 % kaolin, kaolinite content > 30 %<br>< 1 % calcium carbonate (CaCO3) to achieve a pH of 6.0 ± 0.5 |
| 4. Test conditions – | |
| pH value: | 5.9 - 6.0 (test start), 6.2 - 6.4 (test end) |
| Soil moisture: | 58.5 - 62.2 % (test start), 58.7 - 61.4 % (test end) of the $WHC_{max}$ |
| Temperature: | 20.64 - 21.27 °C |
| Photoperiod: | 16 hours light/8 hours dark |
| Light intensity: | 450 - 600 lux |

### B. Study design and method

| | |
|---|---|
| 1. In-life dates: | July 08 to September 04, 2019 (experimental phase) |

2. Test design:

The study was conducted as a dose-response test with 8 test item concentrations, with 4 replicates per concentration as well as a control with 8 replicates. Each replicate contained 10 adult worms. Application of the test item (16.3, 29.4, 52.9, 95.3, 171, 309, 556 and 1000 mg test item/kg $soil_{dw}$) was performed by diluting in water and mixing it into the artificial test soil. The total duration of the exposure phase was 56 days. After 28 days mortality and body weight change of the adult earthworms was assessed. On day 8, 15 and 22 after test start, food consumption of the adult earthworms was estimated. After 56 days effects on the reproduction were assessed. The toxic reference item boric acid was tested in a separate study to confirm the sensitivity of the test organism against compounds with known effects under the applied test conditions.

3. Statistics:

Calculation of treatment means and standard deviations. Analysis of mortality data using Multiple Chi²-test with Bonferroni-Holm adjustment (one-sided greater, $\alpha = 0.05$). Analysis of biomass for normality (Shapiro-Wilk's test, $\alpha = 0.01$) and homoscedasticity (Levene's test: $\alpha = 0.01$), followed by Multiple Welch's t-test with Bonferroni-Holm adjustment (two-sided, $\alpha = 0.05$). Analysis of the reproduction for normality (Shapiro-Wilk's test: $\alpha = 0.01$), homoscedasticity (Levene's test: $\alpha = 0.01$) and data monotonicity (trend analysis by contrasts: $\alpha = 0.05$), followed by Williams' test (one-sided smaller, $\alpha = 0.05$). Since no clear dose-response relationship was observed and the difference compared to the control was not higher than 30%, a calculation of reliable ECx values was not possible.

## II. Results and discussions

### A. Mortality

When compared to the control group, no statistically significant increase in adult mortality of *E. fetida* could be determined for any of the test item concentrations after 28 days of exposure (see table below). Accordingly, the LOEC for mortality could not be determined and the NOEC was considered to be ≥ 1000 mg test item/kg $soil_{dw}$, the highest concentration tested (see table below).

**Table A 30: Adult mortality of *Eisenia fetida* after 28 days of exposure**

| Test item concentration ([mg test item /kg $soil_{dw}$) | Total number of adult earthworms introduced | Total number of dead/not recovered adult earthworms after 28 days of exposure | Mean mortality (%) |
|---|---|---|---|
| Control | 80 | 1 | 1.3 |
| 16.3 | 40 | 1 | 2.5 |
| 29.4 | 40 | 0 | 0.0 |
| 52.9 | 40 | 0 | 0.0 |
| 95.3 | 40 | 0 | 0.0 |
| 171 | 40 | 0 | 0.0 |
| 309 | 40 | 0 | 0.0 |
| 556 | 40 | 0 | 0.0 |
| 1000 | 40 | 0 | 0.0 |

### B. Body weight change

No statistically significant difference in the body weight change of the adult earthworms was determined at any of the test item concentrations compared to the control group (see table below). Accordingly, the LOEC for body weight change could not be determined and the NOEC was considered to be ≥ 1000 mg test item/kg $soil_{dw}$, the highest concentration tested.

Food consumption of the adult earthworms was observed to be similar in all test item groups compared to the control group during the first four exposure weeks.

**Table A 31: Mean body weight and weight change of adult *Eisenia fetida* at start and after 28 days of exposure**

| Test item concentration (mg test item /kg $soil_{dw}$) | at test start | | after 28 days | | | |
|---|---|---|---|---|---|---|
| | Mean weight (mg/worm) | ±SD | Mean weight (mg/worm) | ±SD | Mean weight change [a] | |
| | | | | | (mg/worm) | (%) |
| Control | 439.9 | 37.6 | 536.0 | 30.1 | +96.1 | +22.4 |
| 16.3 | 447.9 | 42.1 | 489.4 | 19.8 | +41.6 | +9.9 |
| 29.4 | 448.1 | 33.0 | 492.3 | 63.6 | +44.2 | +9.8 |
| 52.9 | 445.7 | 29.9 | 536.7 | 16.8 | +91.0 | +20.7 |
| 95.3 | 447.1 | 25.6 | 510.0 | 29.8 | +62.9 | +14.1 |
| 171 | 443.2 | 32.1 | 503.5 | 33.9 | +60.3 | +14.2 |
| 309 | 448.8 | 24.4 | 522.3 | 7.0 | +73.6 | +16.7 |
| 556 | 446.4 | 22.6 | 536.2 | 16.8 | +89.8 | +20.2 |
| 1000 | 444.3 | 31.0 | 513.5 | 28.3 | +69.3 | +15.7 |

SD: standard deviation
[a] body weight changes were first calculated for each replicate and then averaged to determine the treatment group

## C. Reproduction

No statistically significant reduction in the number of juveniles was found at any of the test item groups compared to the control group (see table below). Accordingly, the LOEC for reproduction could not be determined and the NOEC was determined as ≥ 1000 mg test item/kg $soil_{dw}$, the highest concentration tested. Since no clear dose-response relationship was observed and the difference compared to the control was not higher than 30 %, a calculation of reliable ECx values was not possible. The $EC_{50}$ of the reproduction is greater than 1000 mg test item/kg $soil_{dw}$, the highest concentration tested.

**Table A 32: Mean number of juveniles of *Eisenia fetida* after 56 days of exposure**

| Test item Concentration (mg test item /kg $soil_{dw}$) | Mean number of juveniles per replicate | ±SD | CV (%) | Reduction in reproduction [1] (%) |
|---|---|---|---|---|
| Control | 98.9 | 20.4 | 20.6 | - |
| 16.3 | 158.0 | 40.1 | 25.4 | -59.8 |
| 29.4 | 104.0 | 34.4 | 33.1 | -5.2 |
| 52.9 | 114.8 | 32.2 | 28.0 | -16.1 |
| 95.3 | 157.0 | 29.5 | 18.8 | -58.7 |
| 171 | 175.0 | 44.4 | 25.4 | -76.9 |
| 309 | 132.8 | 20.9 | 15.7 | -34.3 |
| 556 | 87.0 | 5.0 | 5.7 | 12.0 |
| 1000 | 69.8 | 13.6 | 19.5 | 29.4 |

SD: standard deviation; CV: coefficient of variation
[1] negative values indicates higher reproduction compared to the control

Behavioural abnormalities and pathological symptoms

One day after application, a total of 13 out of the 40 earthworms (3, 3, 5 and 2 earthworms in the respective replicates) in the highest test item concentration (1000 mg test item/kg $soil_{dw}$) were on the soil surface. Thereafter, the worms re-buried into the soil and no other behavioural abnormalities and no pathological symptoms of the adult earthworms were observed during the four weeks of adult earthworm exposure.

Reference item

The toxic reference item boric acid (purity analysed: 100.1 %) was tested in a separate toxicity study with test item concentrations of 150, 212, 300, 424 and 600 mg test item/kg $soil_{dw}$. The $EC_{50}$ of the reproduction was determined to be 174 mg/kg soil dry weight (95 % confidence limit of 133 and 215 mg/kg soil dry weight, respectively). Hence, suitable sensitivity of the test system was demonstrated.

**D. Validity of the test:**

| **Validity criterion according to OECD 222** | **Results of the study** |
|---|---|
| Each replicate of the controls (containing 10 adults) should produce ≥ 30 juveniles by the end of the test. | Each replicate (containing 10 adults) produced 120 - 70 to 135 juveniles by the end of the test. |
| The coefficient of variation of reproduction in the controls should be ≤ 30 %. | The coefficient of variation of reproduction is 20.6 %. |
| The adult mortality in the controls over the initial 4 weeks of the test to be ≤ 10 %. | The adult mortality over the initial 4 weeks of the test was 1.3 %. |

Since the test protocol meets the validity criteria set forth in the most recent test guideline, the study is considered valid.

**III. Assessment and conclusion**

In a 56-day reproduction test, groups of earthworms (*Eisenia andrei*) were exposed to ADM.3500.F.2.B (Prothioconazole EC 250, 250 g prothioconazole/L) mixed into artificial soil (peat content: 10 %). Based on the results of the study, the 28-day NOEC for mortality, body weight change and reproduction was considered to be ≥1000 mg/kg $soil_{dw}$, the highest concentration tested. Since no clear dose-response relationship was observed and the difference compared to the control was not higher than 30 %, a calculation of reliable ECx values was not possible. The $EC_{50}$ of the reproduction is > 1000 mg test item/kg $soil_{dw}$, the highest concentration tested. The study is considered valid (see: “D. Validity criteria” above).

## A 2.4.1.2 KCP 10.4.1.2 Earthworms - field studies

Not considered to be required.

## 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 in line with OECD 232 with minor no deviations.<br><br>All validity criteria are met.<br><br>Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment:<br><br>NOEC $_{\text{mortality, reproduction}}$ = 52.9 mg prod./kg soil dw (equivalent to 12.4 mg a.s./kg soil dw)<br><br>$EC_{10}$ = 71.0 mg prod./kg soil dw (equivalent to 16.6 mg prod./kg soil dw)<br>$EC_{20}$ = 121 mg prod./kg soil dw (equivalent to 28.2 mg prod./kg soil dw)<br>$EC_{50}$ = 332 mg prod./kg soil dw (equivalent to 77.6 mg prod./kg soil dw) |

| | |
|---|---|
| Reference: | KCP 10.4.2.1/01 |
| Report: | Effects of ADM.3500.F.2.B on the reproduction of the collembolan *Folsomia candida*, Friedrich, S., 2020, report no.: 19 48 TCC 0033, sponsor no.: 000102736 |
| Guideline(s): | OECD 232 (2016) |
| Deviations: | None |
| GLP: | Yes (certified laboratory) |
| Acceptability/Reliability: | Yes |
| Duplication (if vertebrate study) | Not applicable |

**Executive summary**

In a laboratory study with springtails (*Folsomia candida*), the reprotoxic potential of ADM.3500.F.2.B (Prothioconazole EC 250, 250 g prothioconazole/L) was analysed. For this, 40 juvenile (9 - 12 day old) springtails per group were exposed to nominal concentrations of 16.3, 29.4, 52.9, 95.3, 171, 309, 556, 1000 mg prod./kg $soil_{dw}$, mixed into artificial soil (5 % sphagnum peat) for 28 days. Following the exposure, the number of surviving adults and the number of their offspring were assessed. This was compared to a control containing untreated substrate. In a separate study, the sensitivity of the test system was verified using boric acid. Under the conditions of the study, the $LC_{50}$ was calculated to be 282 mg prod./kg $soil_{dw}$ (equivalent to 65.9 mg a.s./kg $soil_{dw}$). The NOEC for mortality and reproduction was determined to be 52.9 mg prod./kg $soil_{dw}$ (equivalent to 12.4 mg a.s./kg $soil_{dw}$). The $EC_{10}$, $EC_{20}$ and $EC_{50}$ values for reproduction were calculated to be 71.0, 121 and 332 mg prod./kg $soil_{dw}$ (equivalent to 16.6, 28.2 and 77.6 mg a.s./kg $soil_{dw}$), respectively.

**I. Materials and methods**

**A. Materials**

| | | |
|---|---|---|
| 1. | Test material: | ADM.3500.F.2.B (Prothioconazole EC 250) |
| | Lot/Batch no.: | 3178-010519-01 |
| | Content/Purity: | prothioconazole: 23.0 % w/w, 252.8 g/L, (analytical); 250 g/L (nominal) |
| | Control: | untreated substrate |
| | Toxic reference: | Boric Acid (purity: 100.8 %, analysed) |

2. Test organisms

| | |
|---|---|
| Species: | *Folsomia candida* (Collembola, Isotomidae) |
| Age: | juvenile, 9 - 12 day old |
| Source: | in-house culture |
| No. of organisms: | 10 springtails per replicate (4 replicates per group, 8 replicates for the control) |
| Acclimatisation: | none |
| Feeding: | at the start of the test and after 14 days with approximately 2 mg of granulated dry yeast |

3. Test units and exposure -

| | |
|---|---|
| Type and sizes: | glass container (approximately 150 ml) covered with a lid, surface area of soil: 18.9 $cm^2$ |
| Test procedure: | reproductive toxicity test using artificial soil with 5 % peat |
| Test duration: | 28 days |
| Test substrate: | artificial soil with 5 % peat |
| Composition: | 74.7 % industrial quartz sand, predominantly fine sand with more than 50 % of the particles between 50 and 200 µm<br>20 % kaolin, kaolinite content > 30 %<br>0.3 % calcium carbonate |

4. Test conditions

| | |
|---|---|
| pH value: | 5.90 - 6.01 (test start), 5.73 - 5.81 (test end) |
| Soil moisture: | 58.6 - 58.8 % (test start), 56.9 - 57.9 % (test end) of WHC |
| Temperature: | 19.3 - 21.8 °C |
| Photoperiod: | 16 hours light/8 hours dark |
| Light intensity: | 600 lux |

**B. Study design and method**

1. In life dates: September 03 to October 01, 2019 (experimental phase)

2. Test design:

An exact weighed amount of the test item was dispersed in deionised water to make a stock solution without addition of solubility mediators, immediately before application. This stock solution was diluted with deionised water to prepare further test solutions (serial dilution). Afterwards the test solutions were thoroughly mixed with the artificial soil separately for each treatment group by intensive stirring in a laboratory mixer. 16.3, 29.4, 52.9, 95.3, 171, 309, 556, 1000 mg test item/kg $soil_{dw}$ were used in this study. The control substrate contained the corresponding amount of deionised water only. After thorough mixing, 30 g (dry weight) of the test substrate was placed into each vessel. The test was started using juvenile collembolans, *Folsomia candida*, well-fed and 9 - 12 days old. 10 test organisms, were introduced to each vessel.

4 weeks after introducing the test organisms, the parental and juvenile collembolans in the test and control vessels were counted. Missing parental collembolans were assumed to have died during the test period. Surviving adults and juveniles were counted using a digital image processing system (LemnaTec Scanalyzer), an automated counting technique based on a video camera connected to a digital image storage and analysis system. The pH and water content of the test substrate were determined at the start and at the end of the test. The water content was checked weekly by reweighing the additional test vessels. Water loss was compensated if exceeding 2 % of the initial water content.

3. Statistics:

Mortality (number of dead adults) in % for each treatment group was calculated. Missing collembolans were counted as dead. The reproductive output for each test item treatment group was calculated in % compared to control.

R (%) = (Rt / Rc) * 100 %
Rt and Rc are the absolute values observed in the treatment and control groups.

The statistical analysis was performed with the software ToxRat Professional 3.2.1 (2015). Step-down Cochran-Armitage test and Williams-t-test were used to compare the control with the independent test item groups. The LCx and ECx values were calculated by Probit analysis using linear maximum likelihood regression and using the 3-parametric normal cumulative distribution function (CDF), respectively.

## II. Results and discussions

### A. Mortality

Mortality of 0.0 % - 87.5 % was recorded in the test item treatment groups. 2.5 % parental mortality was observed in the control (see table below). Statistically significant effects (Step-down Cochran-Armitage test, $\alpha = 0.05$, one-sided greater) on parental mortality were recorded at 95.3, 171, 309, 556 and 1000 mg test item/kg $soil_{dw}$. No effects on behaviour of the collembolans were observed during the test. The NOEC for mortality was determined to be 52.9 mg test item/kg $soil_{dw}$. The $LC_{50}$ was calculated to be 282 mg test item/kg $soil_{dw}$.

**Table A 33: Effects of ADM.3500.F.2.B on mortality of parental collembolans**

| Treatment group | mg test item/kg $soil_{dw}$ | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Control** | **16.3** | **29.4** | **52.9** | **95.3** | **171** | **309** | **556** | **1000** |
| **Replicate** | **Number of surviving parental collembolans per replicate (4 weeks after test initiation)** | | | | | | | | |
| 1 | 9 | 10 | 10 | 10 | 8 | 8 | 4 | 2 | 1 |
| 2 | 10 | 9 | 9 | 10 | 5 | 7 | 3 | 5 | 2 |
| 3 | 10 | 10 | 10 | 10 | 6 | 6 | 4 | 3 | 1 |
| 4 | 9 | 10 | 10 | 10 | 7 | 5 | 6 | 4 | 1 |
| 5 | 10 | - | - | - | - | - | - | - | - |
| 6 | 10 | - | - | - | - | - | - | - | - |
| 7 | 10 | - | - | - | - | - | - | - | - |
| 8 | 10 | - | - | - | - | - | - | - | - |
| Mean | 9.8 | 9.8 | 9.8 | 10.0 | 6.5 | 6.5 | 4.3 | 3.5 | 1.3 |
| SD | 0.5 | 0.5 | 0.5 | 0.0 | 1.3 | 1.3 | 1.3 | 1.3 | 0.5 |
| CV (%) | 4.7 | 5.1 | 5.1 | 0.0 | 19.9 | 19.9 | 29.6 | 36.9 | 40.0 |
| Mortality (%) | 2.5 | 2.5 | 2.5 | 0.0 | 35.0* | 35.0* | 57.5* | 65.0* | 87.5* |

* statistically significantly different compared to control (Step-down Cochran-Armitage test for mortality, $\alpha = 0.05$, one-sided greater)
SD: standard deviation, CV %: coefficient of variation

### B. Reproduction

The mean number of juvenile collembolans counted four weeks after introduction of the parental collembolans into the test vessels was 1552 in the control and 1552, 1511, 1521, 1229, 1010, 932, 631 and 250 at concentrations of 16.3, 29.4, 52.9, 95.3, 171, 309, 556 and 1000 mg test item/kg $soil_{dw}$, respectively. Statistically significant effects (Williams-t-test, $\alpha = 0.05$, one-sided smaller) on the number of juveniles produced compared to the control group were recorded at 95.3, 171, 309, 556 and 1000 mg test item/kg $soil_{dw}$ (see table below).

The NOEC for reproduction was determined to be 52.9 mg test item/kg soil dry weight. The $EC_{10}$, $EC_{20}$ and $EC_{50}$ values for reproduction were calculated to be 71.0, 121 and 332 mg test item/kg $soil_{dw}$, respectively.

**Table A 34: Effects of ADM.3500.F.2.B on number of juvenile collembolans**

| Treatment group | mg test item/kg $soil_{dw}$ | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Control** | **16.3** | **29.4** | **52.9** | **95.3** | **171** | **309** | **556** | **1000** |
| **Replicate** | **Number of juveniles per replicate (4 weeks after test initiation)** | | | | | | | | |
| 1 | 1351 | 1447 | 1522 | 1622 | 1366 | 1195 | 882 | 459 | 273 |
| 2 | 1593 | 1472 | 1647 | 1490 | 1135 | 703 | 751 | 711 | 316 |
| 3 | 1713 | 1603 | 1674 | 1378 | 1052 | 987 | 1061 | 765 | 139 |
| 4 | 1232 | 1685 | 1201 | 1593 | 1364 | 1153 | 1033 | 588 | 271 |
| 5 | 1522 | - | - | - | - | - | - | - | - |
| 6 | 1750 | - | - | - | - | - | - | - | - |
| 7 | 1563 | - | - | - | - | - | - | - | - |
| 8 | 1689 | - | - | - | - | - | - | - | - |
| Mean | 1552 | 1552 | 1511 | 1521 | 1229* | 1010* | 932* | 631* | 250* |
| SD | 181.0 | 112.1 | 217.0 | 110.7 | 160.4 | 223.2 | 143.9 | 136.4 | 76.7 |
| CV (%) | 11.7 | 7.2 | 14.4 | 7.3 | 13.0 | 22.1 | 15.4 | 21.6 | 30.7 |
| Reduction of reproduction (% compared to control) | - | 0.0 | 2.6 | 2.0 | 20.8 | 34.9 | 40.0 | 59.3 | 83.9 |

* statistically significantly different compared to control (Williams-t-test for reproduction, $\alpha = 0.05$, one-sided smaller)
Calculations were done using unrounded values
SD: standard deviation, CV %: coefficient of variation
Percent reduction: (1-Rt/Rc) * 100 %
Rt = mean number of juveniles observed in the treated groups
Rc = mean number of juveniles observed in the control group

A summary with the endpoints derived from this study is presented in the table below.

**Table A 35: Endpoints derived from the study**

| | **Endpoints** | |
|---|---|---|
| | [mg test item/kg soil dry weight] | [mg a.s./kg soil dry weight][3] |
| NOEC (mortality) | 52.9 | 12.4 |
| NOEC (reproduction) | 52.9 | 12.4 |
| $LC_{50}$ (mortality) [1] | 282<br>(95 % confidence limits 192 - 413) | 65.9<br>(95 % confidence limits 44.9 - 96.7) |
| $EC_{10}$ (reproduction) [2] | 71.0<br>(95 % confidence limits 64.7 - 78.2) | 16.6<br>(95 % confidence limits 15.2 - 18.3) |
| $EC_{20}$ (reproduction) [2] | 121<br>(95 % confidence limits 112 - 130) | 28.2<br>(95 % confidence limits 26.2 - 30.5) |
| $EC_{50}$ (reproduction)[2] | 332<br>(95 % confidence limits 319 - 345) | 77.6<br>(95 % confidence limits 74.8 - 80.8) |

[1] based on Probit analysis
[2] based on 3-parametric normal CDF
[3] calculated by correcting by analysed content of active ingredient (23.4 %)

Reference item

To verify the sensitivity of the test system the reference item boric acid (analysed purity: 100.8 %) was routinely tested at concentrations of 44, 67, 100, 150 and 225 mg/kg $soil_{dw}$. The collembolans of the reference test were from the same source culture as those used in the definitive test. In a separate study, the $EC_{50}$ was determined to be 103 mg/kg $soil_{dw}$. The $LC_{50}$ was determined to be 161 mg/kg $soil_{dw}$. The NOEC for mortality and for reproduction was determined to be 44 mg/kg $soil_{dw}$. The $EC_{50}$ value for the reproduction was close to the value of 100 mg/kg $soil_{dw}$ as stated in OECD 232 (2016). The $EC_{50}$ therefore showed that the test system is suitably sensitive.

**C. Validity of the test:**

| **Validity criterion according to OECD 232** | **Results of the study** |
|---|---|
| The mean adult mortality in the controls should not exceed 20 % at the end of the test. | The mean adult mortality in the control was 2.5 % at the end of the test. |
| The mean number of juveniles per vessel in the controls should be at least 100 at the end of the test. | The mean number of juveniles per vessel in the control was 1552 juveniles per vessel at the end of the test. |
| The coefficient of variation calculated for the number of juveniles in the controls should be less than 30 % at the end of the definitive test. | The coefficient of variation calculated for the number of juveniles in the control was 11.7 % at the end of the definitive test. |

Since the test protocol meets the validity criteria set forth in the most recent test guideline, the study is considered valid.

## III. Assessment and conclusion

In a laboratory study with springtails (*Folsomia candida*), the reprotoxic potential of ADM.3500.F.2.B (Prothioconazole EC 250, 250 g prothioconazole/L) was analysed. Under the conditions of the study, the $LC_{50}$ was calculated to be 282 mg prod./kg $soil_{dw}$ (equivalent to 65.9 mg a.s./kg $soil_{dw}$). The NOEC for mortality and reproduction was determined to be 52.9 mg prod./kg $soil_{dw}$ (equivalent to 12.4 mg a.s./kg $soil_{dw}$). The $EC_{10}$, $EC_{20}$ and $EC_{50}$ values for reproduction were calculated to be 71.0, 121 and 332 mg prod./kg $soil_{dw}$ (equivalent to 16.6, 28.2 and 77.6 mg a.s./kg $soil_{dw}$), respectively. The study is considered valid (see: “C. Validity of the test” above).

| | |
|---|---|
| Comments of zRMS: | The study was conducted in line with OECD 226 with no deviations.<br><br>All validity criteria are met.<br><br>Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment:<br><br>$NOEC_{reproduction}$ = 95.3 mg prod./kg soil dw (equivalent to 22.1 mg a.s./kg soil dw)<br>$EC_{10}$ > 100 mg prod./kg soil dw (equivalent to 22.1 mg a.s./kg soil dw) |

| | |
|---|---|
| Reference: | KCP 10.4.2.1/02 |
| Report: | Effects of ADM.3500.F.2.B on the reproduction of the predatory mite *Hypoaspis aculeifer*, Schulz, L., 2020, report no.: 19 48 THC 0026, sponsor no.: 000102737 |
| Guideline(s): | OECD 226 (2016) |
| Deviations: | None |
| GLP: | Yes (certified laboratory) |
| Acceptability/Reliability: | Yes/ |
| Duplication (if vertebrate study) | Not applicable |

**Executive summary**

In a laboratory study with the predatory mite *Hypoaspis aculeifer*, the reprotoxic potential of ADM.3500.F.2.B (Prothioconazole EC 250, 250 g prothioconazole/L) was analysed. For this, 10 adult female mites per group (4 replicates per group, 8 replicates for the control) were exposed to nominal concentrations of 16.3, 29.4, 52.9, 95.3, 171, 309, 556 and 1000 mg prod./kg $soil_{dw}$, mixed into artificial soil (5 % sphagnum peat) for 14 days. Following the exposure, the number of surviving adults and the number of their offspring were assessed. This was compared to a control containing untreated substrate. In a separate study, the sensitivity of the test system was verified using Dimethoate. Under the conditions of the study, the $LC_{50}$ and the $EC_{50}$ values could not be calculated, but it can be concluded that these values are > 1000 mg prod./kg $soil_{dw}$ (equivalent to > 231.5 mg a.s./kg $soil_{dw}$), the highest concentration tested. The $EC_{10}$ and $EC_{20}$ values for reproduction were calculated to be 100.0 and 433.2 mg prod./kg $soil_{dw}$, respectively (equivalent to 23.1 and 100.3 mg a.s./kg $soil_{dw}$). The NOEC for mortality and reproduction was determined to be 556 and 95.3 mg prod./kg $soil_{dw}$, respectively (equivalent to 128.6 and 22.1 mg a.s./kg $soil_{dw}$).

## I. Materials and methods

### A. Materials

1. Test material: ADM.3500.F.2.B (Prothioconazole EC 250)
   Lot/Batch no.: 3178-010519-01
   Content/Purity: prothioconazole: 23.0 % w/w, 252.8 g/L, (analytical); 250 g/L (nominal)
   Control: untreated substrate
   Toxic reference: Dimethoate (98.8 % ± 0.5 %, analysed)

2. Test organisms
   Species: *Hypoaspis aculeifer* (Canestrini)
   Age: adult female mites with an age difference of 2 days
   Source: in-house culture
   No. of organisms: 10 soil mites per replicate (4 replicates per group, 8 replicates for the control)

| | |
|---|---|
| Acclimatisation: | none |
| Feeding: | every 2 - 3 days with *Tyrophagus putrescentiae* (Schrank), originally obtained from "Bayer CropScience AG", Monheim am Rhein, Germany, reared in the test facility |

3. Test units and exposure –

| | |
|---|---|
| Type and sizes: | 160 ml WECK-jar with glass lid (inside dimensions: 4.7 cm in diameter, 8 cm high), filled with 20 g soil dry weight (height of soil approximately 1.7 cm) |
| Test procedure: | mortality and reproductive toxicity test using artificial soil with 5 % peat |
| Test duration: | 14 days |
| Test substrate: | artificial soil according to OECD 226 with 5 % peat |
| Composition: | 74.8 % industrial quartz sand, predominantly fine sand with more than 50 % of the particles between 50 and 200 µm<br>20 % kaolin, kaolinite content > 30 %<br>0.2 % calcium carbonate |

4. Test conditions

| | |
|---|---|
| pH value: | 5.9 - 6.1 (test start), 5.4 - 5.5 (test end) |
| Soil moisture: | 45.90 - 49.47 % (test start), 45.92 - 49.42 % (test end) of WHC |
| Temperature: | 20.0 - 21.1 °C |
| Photoperiod: | 16 hours light/8 hours dark |
| Light intensity: | 582 lux |

**B. Study design and method**

1. In life dates: September 11 to October 11, 2019 (experimental phase)

2. Test design:

An exact weighed amount of the test item was mixed with deionised water, immediately prior to application. The test item solution was then thoroughly mixed with the prepared artificial soil by means of a hand stirrer. 8 different concentrations of the test item were used (16.3, 29.4, 52.9, 95.3, 171, 309, 556 and 1000 mg prod./kg $soil_{dw}$). At test start (within 2 h after treatment of the soil), adult females of the synchronised culture were transferred to the prepared test vessels which contained untreated (control) or test item treated artificial soil (20 g soil dry weight). The water content of the soil substrate in the test vessels was determined at test start (after application) and at day 14. The water content was maintained throughout the test by reweighing the additional test vessels and any water loss was compensated. The vessels were briefly opened every 2 - 3 days for aeration and feeding. On day 14, surviving *Hypoaspis aculeifer* mites and juveniles were extracted from each test replicate using a MacFadyen high-gradient extractor (heat/light extraction method). Any adult mites not found after extraction were recorded as dead. From these data the mortality of the adult females and the reproductive output were calculated. The extraction efficiency of the extractor was determined to be 97.5 % in a separate extraction run.

3. Statistics:

The statistical analysis was performed with the software ToxRat Professional 3.2.1 (2015) and ToxRat Professional 3.3.0 (RATTE 2018). Multiple Sequentially-rejective Fisher Test and the Williams-t-test were used to compare the control with the independent test item group. Probit analysis using linear maximum likelihood regression was used for ECx determination.

## II. Results and discussions

### A. Mortality

Mortality rates of 2.5 - 17.5 % were recorded in the test item treatment groups. In the control group the mortality rate was 2.5 %. The observed mortality rates for adult mortality in the test item treatment groups compared to control were not statistically significant up to and including 556 mg test item/kg $soil_{dw}$ (see table below).

However, the test item caused statistically significant effects on mortality at 1000 mg test item/kg $soil_{dw}$ (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, $\alpha = 0.05$, one-sided greater). Differences in the behaviour and the morphology of the mites between the control and the test item treatment groups could not be observed.

**Table A 36: Effects of the test item on mortality of adult mites**

| Treatment group (mg prod./$soil_{dw}$) | Number of surviving adult mites per replicate | | | | | | | | Mean | Standard deviation | Mortality (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | | | |
| Control | 9 | 10 | 10 | 9 | 10 | 10 | 10 | 10 | 9.8 | 0.5 | 2.5 |
| 16.3 | 9 | 10 | 9 | 9 | | | | | 9.3 | 0.5 | 7.5 |
| 29.4 | 10 | 9 | 10 | 9 | | | | | 9.5 | 0.6 | 5.0 |
| 52.9 | 10 | 10 | 9 | 9 | | | | | 9.5 | 0.6 | 5.0 |
| 95.3 | 10 | 10 | 10 | 9 | | | | | 9.8 | 0.5 | 2.5 |
| 171 | 9 | 10 | 10 | 10 | | | | | 9.8 | 0.5 | 2.5 |
| 309 | 10 | 10 | 9 | 8 | | | | | 9.3 | 1.0 | 7.5 |
| 555 | 10 | 9 | 10 | 9 | | | | | 9.5 | 0.6 | 5.0 |
| 1000 | 8 | 7 | 9 | 9 | | | | | 8.3* | 1.0 | 17.5 |

The calculations were performed with unrounded values.
* statistically significant compared to control (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm for mortality, $\alpha = 0.05$, one-sided greater)

### B. Reproduction

Fourteen days after introduction of the parental mites into the test vessels, the mean number of juveniles was 288.0, 253.3, 272.3, 269.5, 233.8, 262.3, 223.8 and 214.3 at 16.3, 29.4, 52.9, 95.3, 171, 309, 556 and 1000 mg test item/kg $soil_{dw}$., respectively (see table below). The mean number of juveniles in the control was 294.6. The test item showed no statistically significantly adverse effects on reproduction up to and including 95.3 mg test item/kg soil dry weight.

However, the test item caused statistically significant effects on reproduction at 171, 309, 556, and 1000 mg test item/kg soil dry weight (Williams-t-test, $\alpha = 0.05$, one-sided smaller).

**Table A 37: Effects of the test item on number of juvenile mites**

| Treatment group (mg prod./soil$_{dw}$) | Number of juvenile mites per replicate | | | | | | | | Mean | SD | CV (%) | Reproduction (% of control) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | | | | |
| Control | 237 | 272 | 267 | 290 | 278 | 351 | 327 | 335 | 294.6 | 39.2 | 13.3 | 100 |
| 16.3 | 337 | 248 | 266 | 301 | - | - | - | - | 288.0 | 39.4 | 13.7 | 98 |
| 29.4 | 282 | 275 | 299 | 285 | - | - | - | - | 285.3 | 10.1 | 3.5 | 97 |
| 52.9 | 265 | 279 | 278 | 267 | - | - | - | - | 272.3 | 7.3 | 2.7 | 92 |
| 95.3 | 281 | 283 | 264 | 250 | - | - | - | - | 269.5 | 15.5 | 5.8 | 91 |
| 171 | 308 | 177 | 193 | 257 | - | - | - | - | 233.8* | 60.4 | 25.8 | 79 |
| 309 | 318 | 259 | 272 | 200 | - | - | - | - | 262.3* | 48.6 | 18.5 | 89 |
| 555 | 241 | 176 | 231 | 247 | - | - | - | - | 223.8* | 32.5 | 14.5 | 76 |
| 1000 | 216 | 127 | 282 | 232 | - | - | - | - | 214.3* | 64.6 | 30.2 | 73 |

* statistically significant compared to control (Williams-t-test for reproduction, $\alpha = 0.05$, one-sided smaller)
CV: Coefficient of variation, SD: Standard deviation

All relevant endpoints derived from the study are listed in the table below.

**Table A 38: Endpoints of the study**

| | Endpoint | |
|---|---|---|
| | mg test item/kg soil$_{dw}$ | mg a.s./kg soil$_{dw}$[3] |
| NOEC (mortality) | 556 | 128.6 |
| NOEC (reproduction) | 953 | 22.1 |
| LOEC (mortality) | 1000 | 231.5 |
| LOEC (reproduction) | 171 | 39.7 |
| $LC_{50}$[1] | > 1000 | > 231.5 |
| $EC_{10}$[2] | 100.0<br>(95 % confidence limit 41.3 - 242.0) | 23.1<br>(95 % confidence limit 9.6 - 56.0) |
| $EC_{20}$[2] | 433.2<br>(95 % confidence limit 244.5 - 767.6) | 100.3<br>(95 % confidence limit 56.6 - 177.7) |
| $EC_{50}$[1] | > 1000 | > 231.5 |

[1] based on estimation of the data, no mortality or inhibition of reproduction, respectively, of ≥ 50 % occurred
[2] based on probit analysis using linear max. likelihood regression
[3] based on nominal content of a.s.

Reference item

In a separate study, the $EC_{50}$ (reproduction) of the reference item dimethoate (98.8 % ± 0.5 %, analysed) was calculated to be 6.3 mg a.s./kg soil$_{dw}$. The results of the reference test demonstrate the sensitivity of the test system.

**C. Validity of the test:**

| Validity criterion according to OECD 226 | Results of the study |
|---|---|
| Mean adult female mortality in the control should not exceed 20 % at the end of the test. | The mean adult female mortality in the control was 2.5 % at the end of the test. |
| The mean number of juveniles in the control per replicate (with 10 adult females introduced) should be at least 50 at the end of the test. | The mean number of juveniles in the control per replicate was 294.6 at the end of the test. |
| The coefficient of variation calculated for the number of juvenile mites in the control per replicate should not be higher than 30 % at the end of the definitive test. | The coefficient of variation calculated for the number of juvenile mites in the control per replicate was 13.3 % at the end of the definitive test. |

Since the test protocol meets the validity criteria set forth in the most recent test guideline, the study is considered valid.

**III. Assessment and conclusion**

In a laboratory study with the predatory mite *Hypoaspis aculeifer*, the reprotoxic potential of ADM.3500.F.2.B (Prothioconazole EC 250, 250 g prothioconazole/L) was analysed. The $LC_{50}$ and the $EC_{50}$ values could not be calculated, but it can be concluded that these values are > 1000 mg prod./kg $soil_{dw}$ (equivalent to > 231.5 mg a.s./kg $soil_{dw}$), the highest concentration tested. The $EC_{10}$ and $EC_{20}$ values for reproduction were calculated to be 100.0 and 433.2 mg prod./kg $soil_{dw}$, respectively (equivalent to 23.1 and 100.3 mg a.s./kg $soil_{dw}$). The NOEC for mortality and reproduction was determined to be 556 and 95.3 mg prod./kg $soil_{dw}$, respectively (equivalent to 128.6 and 22.1 mg a.s./kg $soil_{dw}$). The study is considered valid (see: “C. Validity of the test” above).

## A 2.4.2.2 KCP 10.4.2.2 Higher tier testing

Not considered to be required.

## A 2.5 KCP 10.5 Effects on soil nitrogen transformation

| | |
|---|---|
| Comments of zRMS: | The study was conducted in line with OECD 216 with no deviations.<br><br>All validity criteria are met.<br><br>Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment:<br><br>ADM.3500.F.2.B caused no adverse effects on soil nitrogen transformation (deviation from control < 25 %, measured as NO3-N production) at the end of the 28-day incubation period at concentrations up to 5.76 mg test item/kg soil dw (equivalent to 4 L test item/ha). |

| | |
|---|---|
| Reference: | KCP 10.5/01 |
| Report: | Effects of ADM.3500.F.2.B on the activity of soil microflora (Nitrogen transformation test), Persdorf, M., 2020, report no.: 19 48 SMN 0034, sponsor no.: 000102738 |
| Guideline(s): | OECD 216 (2000) |
| Deviations: | one |
| GLP: | Yes (certified laboratory) |
| Acceptability/Reliability: | Yes/ |
| Duplication (if vertebrate study) | Not applicable |

**Executive summary**

The effects of ADM.3500.F.2.B (Prothioconazole EC 250, 250 g prothioconazole/L) on the activity of soil microorganisms was assessed in a test that measured nitrogen turnover using agriculturally utilised soil. The test item was incorporated into the soil at an application rate of 0.8 L /ha and 4 L /ha (equivalent to 1.15 mg test item/kg $soil_{dw}$ and 5.76 mg test item/kg $soil_{dw}$). The control consisted of untreated soil and was run concurrently. As a toxic reference, Dicyandiamide was tested in a separate study. Soil samples were taken at test start of (3 hours), and 7, 14 and 28 days after application and the $NH_4$-N-, $NO_3$-N- and $NO_2$-N-contents were determined. Under the conditions of this test, ADM.3500.F.2.B caused no adverse effects on soil nitrogen transformation (deviation from control < 25 %, measured as $NO_3$-N production) at the end of the 28-day incubation period at concentrations up to 5.76 mg test item/kg $soil_{dw}$ (equivalent to 4 L test item/ha).

**I. Materials and methods**
**A. Materials**

1. Test material: ADM.3500.F.2.B (Prothioconazole EC 250)
   Lot/Batch no.: 3178-010519-01
   Content/Purity: prothioconazole: 23.4 % w/w, 252.8 g/L, (analytical); 250 g/L
   Control: untreated soil
   Toxic reference: Dicyandiamide (99.6 % analysed, tested in a separate study)

2. Test units and exposure -
   Type and size: wide-mouth glass flasks (500 ml)
   Filling: 200 g $soil_{dw}$
   Test duration: 28 days
   Replicates: 3 replicates for each test point

3. Test conditions –

| | |
|---|---|
| Test procedure: | N-transformation test |
| Test substrate: | agriculturally utilised soil obtained from Wassergut Canitz, Schlag 34/3, Saxony, Germany |
| Test type: | sandy loam, silty-loamy sand (DIN 4220) |
| Sand content: | 50.5 - 54 % |
| Silt content: | 37.0 - 40.1 % |
| Clay content: | 9.0 - 9 .5 % |
| pH-value: | 6.0 |
| Organic carbon content: | 1.48 % |
| Microbial biomass (C content) | 4.13 % of total organic carbon content. |
| Soil moisture: | 42.99 - 44.03 % of WHC |
| Temperature: | 19.0 - 21.0 °C in a climatic room |
| Photoperiod: | dark |

**B. Study design and method**

1. In life dates: August 28 to September 25, 2019

2. Test design:

200 g of soil (dry weight, one sub-sample) was weighed per replicate. The soil was mixed with 0.5 % (i.e. 1.0 g/200 g $soil_{dw}$) lucerne meal by means of a hand-stirrer (the C/N ratio of the lucerne meal was 13.2/1). One additional soil sample (without lucerne meal) was used for determination of the initial $NH_4$-N-content and $NO_3$-N-content. The $NO_3$-N-content was 5.81 mg/100 g $soil_{dw}$. The test item was mixed with deionised water and the test solution was subsequently mixed with the soil by means of a hand stirrer. Water was added to the soil to achieve a water content of approximately 45 % of WHC. The test item was applied at a rate of 0.8 L/ha and 4 L/ha (equivalent to 1.15 mg test item/kg $soil_{dw}$ and 5.76 mg test item/kg $soil_{dw}$).

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. The pH-values of the soil used in the tests were measured at test start (after application) and at the sampling on day 28.

Soil samples (10 g $soil_{dw}$ per replicate) were taken at test start of (3 hours), and 7, 14 and 28 days after application and the $NH_4$-N-, $NO_3$-N- and $NO_2$-N-contents were determined.

3. Statistics:

The mean nitrogen-content, standard deviation and coefficient of variation were calculated for each treatment group and sampling date. Furthermore, the nitrogen transformation rate per time interval and the nitrogen transformation rate/time interval/day (day 0-7, 7-14, 14-28) were calculated for each treatment group. The % deviations in the quantities of nitrogen formed between the control and the test item treatment groups were determined as follows: % deviation to control = ((test item rate - control rate)/control rate) x 100 %.

**II. Results and discussions**

**A. Nitrogen turnover**

No adverse effects of the test item on nitrogen transformation in soil were observed at either test concentrations (1.15 and 5.76 mg test item/kg soil dry weight) after 28 days (see table below).

**Table A 39: Effects on nitrogen transformation rate (nitrate/day) after treatment with ADM.3500.F.2.B**

| Time interval (days) | Control | 1.15 mg test item/kg $soil_{dw}$ equivalent to 0.8 L test item/ha | | 5.76 mg test item/kg $soil_{dw}$ equivalent to 4 L test item/ha | |
|---|---|---|---|---|---|
| | $NO_3$-N/day (mg/kg $soil_{dw}$) | $NO_3$-N/day (mg/kg $soil_{dw}$) | Deviation from control (%)[1)] | $NO_3$-N/day (mg/kg $soil_{dw}$) | Deviation from control (%)[1)] |
| 0-7 | 4.23 | 4.48 | +5.9 | 4.20 | -0.6 |
| 7-14 | 1.97 | 2.04 | +3.6 | 2.07 | +5.3 |
| 14-28 | 1.13 | 1.15 | +1.7 | 1.32 | +17.3 |

[1)] based on NO3-N-production; - = inhibition; + = stimulation

Reference item

The test with the reference item Dicyandiamide was carried out from in a separate study using application rates of 37.5 kg/ha, 75 kg/ha, 150 kg/ha. The toxic standard Dicyandiamide caused effects of -71.7 %, -80.6 % and -87.1 % (required ≥ 25 %) on the nitrogen transformation in a field soil at the tested concentrations of 50 mg, 100 mg and 200 mg Dicyandiamide per kg $soil_{dw}$, respectively, 28 days after application and thus demonstrated the sensitivity of the test system. The results are summarised in the table below.

**Table A 40: Effects of the reference item dicyandiamide on the nitrogen transformation/time interval/day**

| Time interval (days) | Control | Dicyandiamide 50 mg/kg $soil_{dw}$ | | Dicyandiamide 100 mg/kg $soil_{dw}$ | | Dicyandiamide 200 mg/kg $soil_{dw}$ | |
|---|---|---|---|---|---|---|---|
| | $NO_3$-N/day (mg/kg $soil_{dw}$) | $NO_3$-N/day (mg/kg $soil_{dw}$) | Deviation from control (%)[1)] | $NO_3$-N/day (mg/kg $soil_{dw}$) | Deviation from control (%)[1)] | $NO_3$-N/day (mg/kg $soil_{dw}$) | Deviation from control (%)[1)] |
| 0-7 | 3.92 | 0.80 | -79.5 | 0.17 | -95.6 | -0.11 | -102.8 |
| 7-14 | 1.50 | 0.21 | -85.7 | 0.08 | -94.9 | -0.02 | -101.6 |
| 14-28 | 1.03 | 0.29 | -71.7 | 0.20 | -80.6 | -0.13 | -87.1 |

[1)] based on NO3-N-production; - = inhibition; + = stimulation

**B. Validity of the test:**

| Validity criterion according to OECD 216 | Results of the study |
|---|---|
| The variation between replicate control samples should be less than ± 15 %. | The coefficients of variation in the control group of the nitrogen test were maximum 1.8 %. |

Since the test protocol meets the validity criteria set forth in the most recent test guideline, the study is considered valid.

## III. Assessment and conclusion

The effects on the activity of soil micro-organisms following application of ADM.3500.F.2.B were investigated with agriculturally utilised soil. The test item was mixed into the soil at concentrations equivalent to application rates of 0.8 L /ha and 4 L /ha (equivalent to 1.15 mg test item/kg $soil_{dw}$ and 5.76 mg test item/kg $soil_{dw}$). Under the conditions of this test, ADM.3500.F.2.B caused no adverse effects on soil nitrogen transformation (deviation from control < 25 %, measured as $NO_3$-N production) at the end of the 28-day incubation period at concentrations up to 5.76 mg test item/kg $soil_{dw}$ (equivalent to 4 L test item/ha). The study is considered valid (see: “B. Validity of the test”).

## 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 was conducted in line with OECD 208 with minor deviation:<br>• Short term decrease of the relative humidity to < 40 % at the end of daylight periods took place but had no negative impact on plant growth. Good plant growth was judged by the growth of the control plants.<br><br>The measured concentrations of prothioconazole were 97 to 99% of nominal concentration.<br><br>All validity criteria are met.<br><br>Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment:<br><br>The $ER_{50}$ of shoot height, shoot fresh weight and emergence > 0.8 L prod./ha for all tested plant species.<br><br>No treatment related visual phytotoxic effects were observed for all tested plant species. |

| | |
|---|---|
| Reference: | KCP 10.6.1/01 |
| Report: | ADM.3500.F.2.B - Terrestrial plant test: Seedling emergence and seedling growth test, Klix, V., 2020a, report no.: 190403AR / TNK18620, sponsor no.: 000102739 |
| Guideline(s): | OECD 208 (2006) |
| Deviations: | None |
| GLP: | Yes (certified laboratory) |
| Acceptability/Reliability: | Yes |
| Duplication (if vertebrate study) | Not applicable |

**Executive summary**

The effects of ADM.3500.F.2.B (Prothioconazole EC 250, 250 g prothioconazole/L) on non-target plants were recorded in a seedling emergence test with 6 Dicotyledonous and 4 Monocotyledonous species, i.e. sugar beet, rape, sunflower, tomato, cucumber, soybean, barley, corn, perennial ryegrass, onion. ADM.3500.F.2.B was applied to the soil at application rates of 0.8, 0.4, 0.2, 0.1 and 0.05 L prod./ha after the seeds were sown in untreated soil. Control groups treated with distilled water were run concurrently. The plants were observed for number of emerged seedlings, visual phytotoxic effects and number of dead plants on study day 7, 14 and 21. Due to longer germination period for onion and sunflower the rate of emergence of the control plants primary reached > 50 % within 14 days. Therefore, onion and sunflower were additionally observed on study day 28. The content of prothioconazole in the test solution samples was determined by analysing with LC-MS/MS. The measured concentrations of prothioconazole were 97 to 99 % indicating the correct preparation of the spray solutions. During this study, no treatment related visual phytotoxic effects were observed for all tested plant species at test end. The NOER of shoot height, shoot fresh weight and emergence for all tested plants is set at 0.8 L prod./ha. The $ER_{25}$ and $ER_{50}$ of shoot height, shoot fresh weight and emergence was determined to be > 0.8 L prod./ha.

**I. Materials and methods**

**A. Materials**

1. Test material: ADM.3500.F.2.B (Prothioconazole EC 250)

Lot/Batch no.: 3178-010519-01
Content/Purity: prothioconazole: 23.4 % w/w, 252.8 g/L, (analytical); 250 g/L
Control: tap water at 200 L/ha
Solvent/vehicle: water
Toxic reference: none

2. Test organisms -
Dicotyledonous species: *Beta vulgaris* (sugar beet, Amaranthaceae)
*Brassica napus* (rape, Brassicaceae)
*Helianthus annuus* (sunflower, Asteraceae)
*Lycopersicon esculentum* (tomato, Solanaceae)
*Cucumis sativus* (cucumber, Cucurbitaceae)
*Glycine max* (soybean, Fabaceae)
Monocotyledonous species: *Hordeum vulgare* (barley, Poaceae)
*Zea mays* (corn, Poaceae)
*Lolium perenne* (perennial ryegrass, Poaceae)
*Allium cepa* (onion, Liliaceae)
Growth stage at treatment: seeds
No. of plants: 8 replicates per application rate and control

3. Test units and exposure –
Test system: seedling emergence
Type and size: plastic containers (standard flower pots with drainage holes in the bottom) with a height of 9 cm, a diameter of ca. 12 cm and a surface area of approximately 113 $cm^2$, filled with approximately 700 g soil.
Test duration: 14 days after 50 % emergence of the seedlings in the control group (for each species)

4. Test conditions –
Test substrate: a 2:1 mixture of natural soil LUFA 2.2, origin: Landwirtschaftliche Untersuchungs- und Forschungsanstalt Speyer (LUFA), Obere Langgasse 40, 67346 Speyer, Germany Dörentrup Quarz GmbH Co. KG, An der Sandgrube 1, 31089 Duingen, Germany
Test type: loamy sand (84.2 % sand, 7.0 % clay and 8.8 % silt)
Grain size: ≤ 2 mm
pH-value: 5.63 ± 0.03
Temperature: 16.4 - 23.8 °C
Photoperiod: 16 h
Light intensity: 4713 ± 453 lux
Relative humidity: 35.8 - 93.9 %

| | Temperature [°C] | Relative humidity [%] | Photoperiod [h] | Illumination* [lx] |
|---|---|---|---|---|
| Nominal | 22 ± 10 | 70 ± 30 | 16 | > 3500 |
| Actual | 16.4 - 23.8 | 35.8 - 93.9 | | 4713 ± 453 |

* The illumination is given in lux. The factor to convert lux to µE/$m^2$/s is 0.0135 (cool white fluorescent tubes). The used tubes are special light sources for plants. They emit most of their light at the blue and red ends of the spectrum and are perfectly matching to that required for photo-biological processes.

Watering: bottom watering of the test containers

| | |
|---|---|
| Number of seeds per replicate: | Nominal: 3 - 10 seeds per 100 $cm^2$ per replicate<br>Actual:<br>Barley, perennial ryegrass, onion, sugar beet, rape, tomato:<br>5 seeds per 113 $cm^2$<br>Corn, sunflower, cucumber, soybean: 3 seeds per 113 $cm^2$ |

**B. Study design and method**

1. In life dates: September 25 to October 23, 2019

2. Test design:

The number of seeds per replicate were 3 - 10 seeds per 100 $cm^2$ (nominal). The test was conducted with application rates of 0.8, 0.4, 0.2, 0.1 and 0.05 L prod./ha (factor 2). The application apparatus of the test facility is constructed like a fixed field sprayer, under which a conveyor belt transported the test containers containing test medium and seeds. Before application, the apparatus was adjusted and calibrated to guarantee the required volume of tap water (200 L/ha). The test item was applied at test start on the soil surface after the seeds were sown. During the observation period the plants were observed for number of emerged seedlings, visual phytotoxic effects and number of dead plants on study day 7, 14 and 21. Due to longer germination period for onion and sunflower the rate of emergence of the control plants primary reached > 50 % within 14 days.

Therefore, onion and sunflower were additionally observed on study day 28. The rating of the treated plants was done in relation to the untreated control plants. Observations included all variations, either inhibitory or stimulatory, between the treated test replicates and the untreated control replicates. Such variations were phytotoxic symptoms (e.g. chlorosis, necrosis, wilting), formative effects and growth and development rates. At the end of the study, shoot height (in cm), measured after cutting the plants, and shoot fresh weight of the shoots (in mg) were measured additionally.

The room temperature and relative humidity were recorded throughout the test with a temperature and moisture datalogger. The illumination is determined twice per year.

3. Analytical verification:

The concentration of the active ingredient of the test item ADM.3500.F.2.B was confirmed by analytical verification of the spray solutions (highest application rate and control) and calibration of the application equipment. The samples were analysed with a LC-MS/MS method. A description and validation of the analytical method is provided in Part B of Section 5 (*Analytical Methods*).

4. Statistics:

One Way Analysis of Variance (ANOVA) and Dunnett's test were carried out for the determination of statistically significant differences compared to control replicates. When running a One Way Analysis of Variance, a Normality test (Shapiro-Wilk) and an Equal Variance test (Brown-Forsythe) were done first. P-values for both, Normality and Equal Variance test, are 0.05. The α-value for ANOVA and Dunnett's test (acceptable probability of incorrectly concluding that there is a difference) is $\alpha = 0.05$. Failure of the normality test can be caused by extremely homogeneous emergence and growth patterns as opposed to higher variances in other treatments. Due to the high and even number of replicates in the control and treatment groups, the failure had no influence on the robustness of the calculations. No effects ≥ 25 % occurred at the end of the study. Therefore, no growth ER-values were calculable.

**II. Results and discussion**

**A. Analytical data**

The spray solutions were sampled prior to application and subsequently analytically verified. The measured concentrations of prothioconazole were 97 to 99 % indicating the correct preparation of the spray solutions (see table below).

**Table A 41: Measured concentrations and percent of nominal concentrations of prothioconazole**

| Nominal concentration | | Prothioconazole | | | |
|---|---|---|---|---|---|
| **Test item (L prod./ha)** | **Prothioconazole (mg a.s./L)** | **Meas. conc. (mg a.s./L)** | **%** | **Meas. conc. (mg a.s./L)** | **%** |
| 0.800 | 1011 | 999 | 99 | 958 | 97 |
| QC-Standard | 117[1)] | 120 | 102 | 120 | 100 |
| Control | | < LOQ | | < LOQ | |

Meas. conc. a.s. = Measured concentration of the active ingredient, mean value of 2 injections (dilution factor taken into account)
% = Percent of nominal concentration of the active ingredient
LOQ = Limit of quantification (117 mg a.s./L)
QC-Standard = 500 mg test item/L, corresponding to 117 mg a.s./L
1) = weighing factor taken into account: 118 mg a.s./L on 2019-09-25, 120 mg a.s./L on 2019-09-27

## B. Visual phytotoxicity

The plants were observed for visual phytotoxic effects and number of dead plants on day 7, 14 and 21 (onion and sunflower additionally on day 28). No treatment related visual phytotoxic effects were observed for all tested plant species at test end.

## C. Effects on seedling emergence

For barley, corn, perennial ryegrass, sugar beet, rape, tomato, cucumber and soybean > 50 %of the control plants were emerged after 7 days. For onion and sunflower, the rate of emergence of the control plants reached > 50 % within 14 days. The test with onion and sunflower was prolonged until 28 days. The results of the individual emergence rates are listed in the table below.

**Table A 42: Emerged seedlings on day 21 or day 28 (test end for onion, sunflower)**

| Application rate (L prod./ha) | Emergence (%) | | | | |
|---|---|---|---|---|---|
| | Barley | Corn | Perennial ryegrass | Onion | Sugar Beet |
| control | 95 | 96 | 93 | 90 | 98 |
| 0.05 | 95 | 96 | 93 | 93 | 93 |
| 0.1 | 100 | 100 | 93 | 98 | 98 |
| 0.2 | 95 | 88 | 93 | 88 | 100 |
| 0.4 | 98 | 92 | 90 | 90 | 95 |
| 0.8 | 100 | 96 | 88 | 88 | 95 |
| | | | | | |
| Application rate (L prod./ha) | Emergence (%) | | | | |
| | Rape | Sunflower | Tomato | Cucumber | Soybean |
| control | 90 | 92 | 90 | 96 | 88 |
| 0.05 | 85 | 92 | 83 | 100 | 92 |
| 0.1 | 88 | 92 | 95 | 96 | 100 |
| 0.2 | 88 | 96 | 75 | 96 | 92 |
| 0.4 | 85 | 92 | 93 | 88 | 100 |
| 0.8 | 93 | 79 | 85 | 92 | 92 |

## D. Shoot height

The results of the shoot heights of the plants exposed to ADM.3500.F.2.B are listed in the table below.

**Table A 43: Shoot height at test end**

| Application rate (L prod./ha) | Shoot height at test end (mean ± SD) | | | | |
|---|---|---|---|---|---|
| | Barley | Corn | Perennial ryegrass | Onion | Sugar Beet |
| control | 39.7 ± 2.0 | 33.8 ± 3.7 | 18.7 ± 2.0 | 16.7 ± 2.7 | 8.4 ± 0.7 |
| 0.05 | 38.7 ± 2.9 | 35.9 ± 2.3 | 18.7 ± 1.4 | 15.1 ± 3.0 | 9.2 ± 0.6# |
| 0.1 | 37.3 ± 4.8 | 35.4 ± 2.0 | 18.7 ± 1.5 | 16.9 ± 2.0 | 7.7 ± 0.6 |
| 0.2 | 37.9 ± 1.4 | 35.9 ± 2.0 | 17.0 ± 2.8 | 15.6 ± 2.4 | 8.9 ± 0.6 |
| 0.4 | 37.7 ± 1.8 | 36.6 ± 2.2 | 17.6 ± 1.7 | 16.2 ± 2.1 | 9.0 ± 0.4 |
| 0.8 | 39.0 ± 2.1 | 35.0 ± 2.4 | 18.3 ± 2.0 | 15.1 ± 2.8 | 9.1 ± 0.5 |
| | | | | | |

| Application rate (L prod./ha) | Shoot height at test end (mean ± SD) | | | | |
|---|---|---|---|---|---|
| | Rape | Sunflower | Tomato | Cucumber | Soybean |
| control | 13.8 ± 1.7 | 8.3 ± 0.8 | 10.8 ± 0.5 | 8.0 ± 0.6 | 21.0 ± 1.7 |
| 0.05 | 13.8 ± 1.7 | 8.1 ± 1.4 | 10.3 ± 2.0 | 8.2 ± 1.1 | 22.5 ± 0.9 |
| 0.1 | 13.6 ± 1.0 | 7.5 ± 0.8 | 10.6 ± 1.0 | 8.6 ± 0.9 | 22.2 ± 1.4 |
| 0.2 | 14.1 ± 1.1 | 8.6 ± 1.2 | 10.9 ± 1.0 | 8.3 ± 1.1 | 22.3 ± 0.6 |
| 0.4 | 14.3 ± 1.2 | 8.2 ± 1.3 | 11.0 ± 0.7 | 8.3 ± 1.4 | 22.0 ± 1.5 |
| 0.8 | 13.6 ± 1.1 | 8.7 ± 1.3 | 10.6 ± 1.1 | 8.8 ± 0.9 | 22.0 ± 1.8 |

#) statistically significant promoted growth

## E. Biomass (shoot fresh weight)

The results of the plant biomass exposed to ADM.3500.F.2.B are listed in the table below.

**Table A 44: Shoot fresh weight at test end**

| Application rate (L prod./ha) | Shoot height at test end (mean ± SD) | | | | |
|---|---|---|---|---|---|
| | Barley | Corn | Perennial ryegrass | Onion | Sugar Beet |
| control | 1311 ± 131 | 1854 ± 449 | 99 ± 25 | 238 ± 61 | 680 ± 120 |
| 0.05 | 1246 ± 125 | 2135 ± 278 | 103 ± 18 | 192 ± 57 | 759 ± 147 |
| 0.1 | 120 ± 156 | 2112 ± 266 | 99 ± 17 | 238 ± 55 | 461# ± 124 |
| 0.2 | 1186 ± 142 | 2148 ± 278 | 86 ± 21 | 207 ± 49 | 699 ± 100 |
| 0.4 | 1137 ± 114 | 2140 ± 190 | 82 ± 18 | 214 ± 54 | 771 ± 150 |
| 0.8 | 1224 ± 139 | 2057 ± 256 | 84 ± 30 | 198 ± 64 | 752 ± 122 |
| | | | | | |
| Application rate (L prod./ha) | Shoot height at test end (mean ± SD) | | | | |
| | Rape | Sunflower | Tomato | Cucumber | Soybean |
| control | 1190 ± 263 | 1233 ± 167 | 571 ± 69 | 1328 ± 170 | 2622 ± 311 |
| 0.05 | 1221 ± 364 | 1162 ± 370 | 496 ± 147 | 1319 ± 351 | 2570 ± 213 |
| 0.1 | 1172 ± 209 | 975 ± 215 | 523 ± 50 | 1395 ± 251 | 2523 ± 120 |
| 0.2 | 1275 ± 177 | 1228 ± 282 | 556 ± 85 | 1328 ± 270 | 2571 ± 217 |
| 0.4 | 1273 ± 241 | 1169 ± 234 | 554 ± 30 | 1285 ± 418 | 2580 ± 89 |
| 0.8 | 1217 ± 223 | 1268 ± 319 | 531 ± 93 | 1465 ± 280 | 2610 ± 273 |

# statistically significant inhibition, but not test item related (as inhibition does not increase with the concentration);the result is not considered to set endpoints

## F. Validity of the test:

| Validity criterion according to OECD 208 | Results of the study |
|---|---|
| The seedling emergence in the control is at least 70 %. | The seedling emergence in the control was ≥ 88 %. |
| The seedlings of the control shall not exhibit visible phyto-toxic effects (e.g. chlorosis, necrosis, wilting, leaf and stem deformations) and the plants exhibit only normal variation in growth and morphology for that particular specie. | The seedlings of the control did not exhibit visible phytotoxic effects and the plants exhibited only normal variation in growth and morphology for that particular specie. |
| The mean plant survival in the control is at least 90 % for the duration of the study. | The mean survival of the plants in the control group was ≥ 97 % at the end of the test. |
| Environmental conditions for a particular species shall be identical and growing media contain the same amount of soil matrix, support media, or substrate from the same source. | Environmental conditions and growing media were identical for each plant species. |

Since the test protocol meets the validity criteria set forth in the most recent test guideline, the study is considered valid.

**III. Assessment and conclusion**

In a seedling emergence test with 10 plant species, the effect of an application of ADM.3500.F.2.B on seedling emergence, phytotoxic effects and biomass reduction was tested at test termination two weeks after 50 % of the control seedlings had emerged. In this study, no treatment related visual phytotoxic effects were observed for all tested plant species at test end. The NOER of shoot height, shoot fresh weight and emergence for all tested plants is set at 0.8 L prod./ha. The $ER_{25}$ and $ER_{50}$ of shoot height, shoot fresh weight and emergence was determined to be > 0.8 L prod./ha. The study is considered valid (see: "F. Validity of the test").

| | |
|---|---|
| Comments of zRMS: | The study was conducted in line with OECD 227 with minor deviation:<br>• Short term decrease of the relative humidity to < 40 % at the end of daylight periods took place but had no negative impact on plant growth. Good plant growth was judged by the growth of the control plants.<br><br>The measured concentrations of prothioconazole were 97 to 98% of nominal test concentration.<br><br>All validity criteria are met.<br><br>Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment:<br><br>The $ER_{50}$ of shoot height, shoot fresh weight > 0.8 L prod./ha for all tested plant species.<br><br>Visual phytotoxicity:<br><br>The treatment-related visual toxicity was observed in onion (necrosis), tomato (chlorosis) and cucumber (chlorosis) at an application rate of 0.8 L prod./ha and for cucumber and soybean at rate 0.4 L/ha (necrosis). No details of % effects are provided in the study.<br><br>For this reason, zRMS estimated theoretically $E_rC_{50}$ = 0.4 L/ha for visual phytotoxicity effects until relevant information/explanation will be added by the applicant. |

| | |
|---|---|
| Reference: | KCP 10.6.1/02 |
| Report: | ADM.3500.F.2.B - Terrestrial plant test: Vegetative vigour test, Klix, V., 2020b, report no.: 190403AR / TNW18620, sponsor no.: 000102740 |
| Guideline(s): | OECD 227 (2006) |
| Deviations: | None |
| GLP: | Yes (certified laboratory) |
| Acceptability/Reliability: | Yes |
| Duplication<br>(if vertebrate study) | Not applicable |

**Executive summary**

The effects of ADM.3500.F.2.B (Prothioconazole EC 250, 250 g prothioconazole/L) on non-target plants were recorded in a vegetative vigour test with 6 Dicotyledonous and 4 Monocotyledonous species, i.e. rape, sugar beet, sunflower, tomato, cucumber, soybean, onion, barley, corn, and perennial ryegrass. At 2-3 leaf stage, the plants were treated with an application of Mirage 45 EC sprayed at a rate of 0.8, 0.4, 0.2, 0.1 and 0.05 L/ha. The sunflower was treated with concentrations of 0.8, 0.267, 0.0889, 0.0296 and 0.00988 L/ha. Control groups treated with distilled water were run concurrently. At test start, the growth stage of the plants was documented according to BBCH code. During the observation period the plants were observed on day 7, 14 and 21 for visual phytotoxic effects and number of dead plants. At the end of the study, the shoot height (in cm), measured after cutting the plants, and the fresh weights of the shoots (in g) were measured additionally. The concentration of prothioconazole was confirmed by analytical verification of

the spray solutions (highest application rate and control) with a LC-MS/MS method. The measured concentrations of prothioconazole were 97 to 98 % indicating the correct preparation of the spray solutions. Under the conditions of the test, symptoms of phytotoxicity were observed in onion (necrosis), tomato (chlorosis) and cucumber (chlorosis) at an application rate of 0.8 L prod./ha. The symptoms of phytotoxicity were observed in cucumber and soybean (necrosis) at an application rate of ≥ 0.4 L prod./ha. The NOER of shoot height and shoot fresh weight for all tested plants is set at 0.8 L prod./ha, except for rape, where the NOER for shoot fresh weight was determined to be 0.4 L prod./ha. The $ER_{25}$ and $ER_{50}$ of shoot height and shoot fresh weight was above the highest concentration tested and is therefore set at > 0.8 L prod./ha.

**I. Materials and methods**
**A. Materials**

| | |
|---|---|
| 1. Test material: | ADM.3500.F.2.B (Prothioconazole EC 250) |
| Lot/Batch no.: | 3178-010519-01 |
| Content/Purity: | prothioconazole: 23.4 % w/w, 252.8 g/L, (analytical); 250 g/L |
| Control: | tap water |
| Solvent/vehicle: | tap water |
| Toxic reference: | none |
| 2. Test organisms - | |
| Dicotyledonous species: | *Brassica napus* (rape, Brassicacea)<br>*Beta vulgaris* (sugar beet, Chenopodiaceae)<br>*Helianthus annuus* (sunflower, Asteraceae)<br>*Lycopersicon esculentum* (tomato, Solanaceae)<br>*Cucumis sativus* (cucumber, Cucurbitaceae)<br>*Glycine max* (soybean, Leguminosae) |
| Monocotyledonous species: | *Allium cepa* (onion, Liliaceae)<br>*Hordeum vulgare* (barley, Poaceae)<br>*Zea mays* (corn, Poaceae)<br>*Lolium perenne* (perennial ryegrass, Poaceae) |
| Growth stage at treatment: | 2 - 3 leaf stage |
| No. of plants: | 8 replicates per limit application rate and control, 3 plants per pot |
| 3. Test units and exposure – | |
| Test system: | vegetative vigour, limit test |
| Type and size: | plastic containers (standard flower pots with drainage holes in the bottom) with a height of 9 cm, a diameter of ca. 12 cm and a surface area of approximately 113 $cm^2$ were filled with approximately 700 g soil. |
| Test duration: | 21 days |
| 4. Test conditions – | |
| Test substrate: | a 2:1 mixture of natural soil LUFA 2.2, origin: Landwirtschaftliche Untersuchungs- und Forschungsanstalt Speyer (LUFA), Obere Langgasse 40, 67346 Speyer, Germany Dörentrup Quarz GmbH Co. KG, An der Sandgrube 1, 31089 Duingen, Germany |
| Test type: | loamy sand (93.4 % sand, 3.7 % clay and 3.0 % silt) |
| Grain size: | ≤ 2 mm |
| pH-value: | 4.86 ± 0.08 |
| Temperature: | 16.4 - 27.1 °C |
| Photoperiod: | 16 h |
| Light intensity: | 5213 ± 769 lux |

Relative humidity: 32.7 - 93.4 %
Watering: bottom watering of the test containers

| | Temperature [°C] | Relative humidity [%] | Photoperiod [h] | Illumination* [lx] |
|---|---|---|---|---|
| Nominal | 22 ± 10 | 70 ± 30 | 16 | > 3500 |
| Actual | 16.4 - 27.1 | 32.7 - 93.4 | | 5213 ± 769 |

* The illumination is given in lux. The factor to convert lux to µE/m²/s is 0.0135 (cool white fluorescent tubes). The used tubes are special light sources for plants. They emit most of their light at the blue and red ends of the spectrum and are perfectly matching to that required for photo-biological processes.

**B. Study design and method**

1. In life dates: September 05 to October 01, 2019

2. Test design:

Prior to experimental starting the seeds were sown in sowing container. After homogeneous and sufficient emergence (≥ 70 %, assessed by visual observation) and complete opening of the cotyledons the plants were transplanted into the test containers. During cultivation the plants were bottom watered and fertilized as necessary. The climatic conditions were the same as in the test. The cultivation period depends on the rate of growth. The test was started when the plants had reached a 2-3 true leaf stage.

Based on the results of the preliminary study the application rates were selected as follows: Barley, corn, perennial ryegrass, onion, sugar beet, rape, tomato, cucumber, soybean: 0.8, 0.4, 0.2, 0.1 and 0.05 L/ha (factor 2) Sunflower: 0.8, 0.267, 0.0889, 0.0296 and 0.00988 L/ha (factor 3) The test solutions for the highest application rates by direct weighing of the test item followed by dilution in tap water. Further application rates were prepared by dilution out of these solutions.

The control and test item solutions were applied once to test containers containing test medium and plants of each plant species at the start of exposure. The application apparatus of the test facility is constructed like a fixed field sprayer under which a conveyor belt transported the test container containing test medium and plants. Before application, the apparatus was adjusted and calibrated to guarantee the required volume of spray solution. At test start, the growth stage of the plants was documented according to BBCH code. During the observation period the plants were observed on day 7, 14 and 21 for visual phytotoxic effects and number of dead plants. The rating of the treated plants was done in relation to the untreated control plants. Observations included all variations, either inhibitory or stimulatory, between the treated replicates and the untreated controls. Such variations were phytotoxic symptoms (e.g. chlorosis, necrosis, wilting), formative effects of growth and development rates. At the end of the study, the shoot height (in cm), measured after cutting the plants, and the fresh weights of the shoots (in g) were measured additionally. The room temperature and relative humidity were recorded continuously throughout the test with a temperature and moisture datalogger. The illumination is determined twice per year.

3. Analytical verification:

The concentration of the active ingredient of the test item ADM.3500.F.2.B was confirmed by analytical verification of the spray solutions (highest application rate and control) and calibration of the application equipment. The samples were analysed with a LC-MS/MS method. A description and validation of the analytical method is provided in Part B of Section 5 (*Analytical Methods*).

4. Statistics:

The percentage of inhibition of biomass growth (shoot height, shoot fresh weight) for each plant species was calculated in relation to the control. All dead plants were observed and weighed and included in the calculation as possible. No determination of the shoot height was carried out for dead plants. One Way Analysis of Variance (ANOVA) and Dunnett's test were carried out for the determination of statistically significant differences compared to control replicates. When running a One Way Analysis of Variance, a Normality test (Shapiro-Wilk) and an Equal Variance test (Brown-Forsythe) were done first. P-values for both, Normality and Equal Variance test, are 0.05. The α- value for ANOVA and Dunnett's test (acceptable probability of incorrectly concluding that there is a difference) is $\alpha = 0.05$. Failure of the normality or equal variance test can be caused by extremely homogeneous emergence and growth patterns as opposed to higher variances in other treatments. Due to the high and even number of replicates in the control and treatment groups, the failure had no influence on the robustness of the calculations. No effects ≥ 25 % occurred at the end of the study. Therefore, no ER-values were calculable

## II. Results and discussion

## A. Analytical data

The spray solutions were sampled prior to application and subsequently analytically verified. The measured concentrations of prothioconazole were 97 to 98 % indicating the correct preparation of the spray solutions. The analytical results are presented in the table below.

**Table A 45: Measured concentrations and percent of nominal concentrations of prothioconazole**

| Nominal concentration | | Prothioconazole | | | |
|---|---|---|---|---|---|
| **Test item (L prod./ha)** | **Prothioconazole (mg a.s./L)** | **Meas. conc. (mg a.s./L)** | **%** | **Meas. conc. (mg a.s./L)** | **%** |
| 0.800 | 1011 | 992 | 98 | 982 | 97 |
| QC-Standard | 117[1)] | 122 | 103 | 110 | 95 |
| Control | | < LOQ | | < LOQ | |

Meas. conc. a.s. = Measured concentration of the active ingredient, mean value of 2 injections (dilution factor taken into account)
% = Percent of nominal concentration of the active ingredient
LOQ = Limit of quantification (117 mg a.s./L)
QC-Standard = 500 mg test item/L, corresponding to 117 mg a.s./L
1) = weighing factor taken into account: 119 mg a.s./L on 2019-09-05, 116 mg a.s./L on 2019-09-10

## B. Visual phytotoxicity

Symptoms of phytotoxicity were observed in onion (necrosis), tomato (chlorosis) and cucumber (chlorosis) at an application rate of 0.8 L prod./ha. The symptoms of phytotoxicity were observed in cucumber and soybean (necrosis) at an application rate of ≥ 0.4 L prod./ha (see table below).

**Table A 46: Treatment related visual phytotoxic effects at test end**

| Species | Main observed visual effects* | Appearance at application rates (L product/ha) |
|---|---|---|
| Barley | None | - |
| Corn | None | - |
| Perennial ryegrass | None | - |
| Onion | Necrosis | 0.8 |
| Sugar beet | None | - |
| Rape | None | - |
| Sunflower | None | - |
| Tomato | Chlorosis | 0.8 |
| Cucumber | Necrosis<br>Chlorosis | ≥ 0.4<br>0.8 |
| Soybean | Necrosis | ≥ 0.4 |

* Effects were considered as main effect when > 2 replicates were influenced.

## C. Shoot height

The results of the shoot heights of the plants exposed to ADM.3500.F.2.B are listed in the table below.

**Table A 47: Shoot height at test end**

| Application rate (L prod./ha) | Shoot height at test end (mean ± SD) | | | | |
|---|---|---|---|---|---|
| | **Barley** | **Corn** | **Perennial ryegrass** | **Onion** | **Sugar Beet** |
| control | 54.9 ± 3.9 | 83.3 ± 1.4 | 44.0 ± 2.8 | 35.9 ± 2.3 | 19.9 ± 1.2 |
| 0.05 | 56.4 ± 4.1 | 83.0 ± 5.1 | 45.7 ± 2.6 | 37.6 ± 2.2 | 21.2 ± 1.1 |
| 0.1 | 54.5 ± 2.8 | 80.5 ± 5.3 | 46.3 ± 3.9 | 36.2 ± 1.6 | 20.4 ± 0.5 |
| 0.2 | 55.5 ± 2.2 | 79.2 ± 6.1 | 45.7 ± 2.9 | 38.5 ± 2.5 | 20.6 ± 1.8 |
| 0.4 | 55.4 ± 3.8 | 80.2 ± 2.9 | 46.5 ± 3.0 | 37.8 ± 2.6 | 20.9 ± 2.2 |
| 0.8 | 52.4 ± 2.4 | 80.3 ± 3.9 | 44.6 ± 3.0 | 36.6 ± 3.0 | 20.1 ± 1.1 |
| | | | | | |
| **Application rate (L prod./ha)** | **Shoot height at test end (mean ± SD)** | | | | |
| | **Rape** | **Sunflower** | **Tomato** | **Cucumber** | **Soybean** |
| control | 27.3 ± 0.9 | 14.6 ± 1.2 | 42.0 ± 2.6 | 24.9 ± 5.4 | 48.4 ± 5.2 |
| 0.05 (0.00988)* | 28.6 ± 1.0 | 14.5 ± 1.1 | 45.0 ± 2.8 | 26.1 ± 4.5 | 46.8 ± 4.8 |
| 0.1 (0.0296)* | 27.9 ± 1.2 | 14.7 ± 1.2 | 43.8 ± 3.0 | 26.5 ± 4.5 | 48.8 ± 4.5 |
| 0.2 (0.0889)* | 29.3 ± 0.9# | 13.9 ± 1.2 | 45.0 ± 3.7 | 24.4 ±3.7 | 46.9 ± 4.6 |
| 0.4 (0.267)* | 28.5 ± 1.2 | 14.5 ± 1.0 | 44.2 ± 2.7 | 24.9 ± 5.9 | 46.3 ± 4.1 |
| 0.8 | 28.8 ± 1.5 | 13.5 ± 0.6 | 44.4 ± 2.8 | 24.3 ± 6.3 | 44.6 ± 3.6 |

* for the test with sunflower, different applications rates were used
# statistically significant promoted growth

## D. Biomass (shoot fresh weight)

The results of the plant biomass exposed to ADM.3500.F.2.B are listed in the table below.

**Table A 48: Shoot fresh weight at test end**

| Application rate (L prod./ha) | Shoot height at test end (mean ± SD) | | | | |
|---|---|---|---|---|---|
| | **Barley** | **Corn** | **Perennial ryegrass** | **Onion** | **Sugar Beet** |
| control | 14.25 ± 2.91 | 38.81 ± 2.61 | 8.07 ± 1.81 | 8.40 ± 1.88 | 19.24 ± 4.76 |
| 0.05 | 14.89 ± 290 | 37.93 ± 7.92 | 9.66 ± 1.54 | 9.87 ± 2.55 | 24.53 ± 3.67 |
| 0.1 | 14.85 ± 2.64 | 35.62 ± 5.17 | 8.22 ± 1.80 | 9.59 ± 1.84 | 20.47 ± 2.82 |
| 0.2 | 15.57 ± 2.41 | 33.68 ± 5.46 | 9.00 ± 1.77 | 11.13 ± 2.28 | 22.64 ± 4.67 |
| 0.4 | 14.74 ± 2.00 | 34.89 ± 4.09 | 8.56 ± 2.11 | 10.24 ± 2.28 | 22.50 ± 3.74 |
| 0.8 | 15.50 ± 2.79 | 34.08 ± 2.45 | 8.12 ± 2.11 | 9.46 ± 2.53 | 19.66 ± 3.36 |
| | | | | | |
| **Application rate (L prod./ha)** | **Shoot height at test end (mean ± SD)** | | | | |
| | **Rape** | **Sunflower** | **Tomato** | **Cucumber** | **Soybean** |
| control | 46.62 ± 3.42 | 9.47 ± 2.48 | 73.32 ± 6.74 | 51.73 ± 15.54 | 22.08 ± 2.12 |
| 0.05 (0.00988)* | 45.02 ± 3.90 | 11.68 ± 1.77 | 68.29 ± 6.68 | 54.93 ± 13.35 | 21.93 ± 2.00 |
| 0.1 (0.0296)* | 42.71 ± 3.69 | 12.45 ± 1.90 | 64.55 ± 4.90 | 54.27 ± 11.79 | 22.26 ± 2.28 |
| 0.2 (0.0889)* | 64.81 ± 3.42 | 11.18 ± 2.52 | 69.87 ± 6.26 | 50.08 ± 11.30 | 21.81 ± 2.72 |
| 0.4 (0.267)* | 42.03 ± 5.05 | 11.55 ± 1.45 | 67.07 ± 9.12 | 48.07 ± 15.55 | 23.41 ± 3.76 |
| 0.8 | 39.89 ± 4.08 | 10.01 ± 1.82 | 64.86 ± 11.64 | 46.95 ± 15.68 | 21.28 ± 3.67 |

* for the test with sunflower, different applications rates were used
# statistically significant inhibition, but not test item related (as inhibition does not increase with the concentration);the result is not considered to set endpoints

**E. Validity of the test:**

| Validity criterion according to OECD 227 | Results of the study |
|---|---|
| The seedling emergence is at least 70 %. | The seedling emergence was ≥ 70 %. |
| The plants of the control group do not exhibit visible phytotoxic effects (e.g., chlorosis, necrosis, wilting, leaf and stem deformations). Plants exhibit only normal variation in growth and morphology for that particular specie | The plants in the control group exhibited no visible phytotoxic effects. The mean growth and morphology in the control group were within the normal variation for the particular plant species. |
| The mean plant survival in the control is at least 90 % for the duration of the study. | The mean survival of the plants in the control group was at least 90 % at the end of the test (100 % for barley, corn, perennial ryegrass, onion, rape, sunflower, tomato, cucumber, soybean, 96 % for sugar beet. |
| Environmental conditions for a particular species are identical and growing media contain the same amount of soil matrix, support media, or substrate from the same source. | For each species, all organisms were from the same source. All test chambers or rooms used for particular species were identical and had the same conditions and contained the same amount of soil matrix, support media or substrate from the same source. |

Since the test protocol meets the validity criteria set forth in the most recent test guideline, the study is considered valid.

**III. Assessment and conclusion**

In a vegetative vigour screening test with 10 plant species, the effect of an application of 5 different concentrations of ADM.3500.F.2.B on phytotoxicity and inhibition of growth was tested over 21 days. Control groups treated with distilled water were run concurrently. Under the conditions of the test, symptoms of phytotoxicity were observed in onion (necrosis), tomato (chlorosis) and cucumber (chlorosis) at an application rate of 0.8 L prod./ha. The symptoms of phytotoxicity were observed in cucumber and soybean (necrosis) at an application rate of ≥ 0.4 L prod./ha. The NOER of shoot height and shoot fresh weight for all tested plants is set at 0.8 L prod./ha, except for rape, where the NOER for shoot fresh weight was determined to be 0.4 L prod./ha. The $ER_{25}$ and $ER_{50}$ of shoot height and shoot fresh weight was above the highest concentration tested and is therefore set at > 0.8 L prod./ha. The study is considered valid (see: “E. Validity of the test”).

## A 2.6.2 KCP 10.6.2 Testing on non-target plants

Further data is not considered to be required, since toxicity of ADM.03500.F.2.B to terrestrial non-target plants is adequately addressed under point KCP 10.6.1 within the framework of the vegetative vigour and seedling emergence tests.

## A 2.6.3 KCP 10.6.3 Extended laboratory studies on non-target plants

Submission of such information is not required, since an acceptable risk for the non-target flora can be concluded from the results of laboratory studies as outlined in the risk assessment above (for details, please refer to point 9.10).

## A 2.7 KCP 10.7 Effects on other terrestrial organisms (flora and fauna)

Adequate risk assessments were performed for all indicator species relevant in the natural environment. In summary, acceptable acute, short-term, or long-term risks were indicated for each of the indicator species including birds, mammals, aquatic organisms, bees and other terrestrial non-target arthropods, soil macro- and mesofauna, microorganisms, and terrestrial non-target plants in consideration of the uses intended for ADM.03500.F.2.B. Therefore, further data/studies/calculations on non-target species other than those species mentioned above are not required and thus not provided.

## A 2.8 KCP 10.8 Monitoring data

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