

REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: ADM.03502.F.1.A

(alternative codes: MCW-2091)

Product name(s): see part A

Chemical active substance(s):

Fenpropidin 250 g/L

Prothioconazole 175 g/L

Central zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: Country organisation/representative
as specified in Part A

Submission date: September 2021

MS Finalisation date: December 2022 (initial Core Assessment)

April 2023 (final Core Assessment)

Version history

When	What
2021/09	Version 1 Applicant
December 2022	Initial zRMS assessment 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 .
May 2023	Final report (Core Assessment updated following the commenting period) 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.03502.F.1.A. under Article 59, Regulation 1107/2009/EC. On behalf of the Sponsor Company the applicant claims data protection for the studies conducted with ADM.03502.F.1.A. 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The summaries and evaluations contained in this document may be based on unpublished proprietary data submitted for the purpose of the assessment undertaken by the regulatory authority that prepared it.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authorities should not grant, amend, or renew a registration on the basis of the summaries and evaluation of unpublished proprietary data contained in this document unless they have received the data on which the summaries and evaluation are based, either –

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9.1 Critical GAP and overall conclusions

Table 9.1-1: Table of critical GAPS

[illegible]

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Use-No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g safener/ synergist per ha	Conclusion						
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha prothioconazole / fenpropidin a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms	Non-target plants
Zonal uses (field or outdoor uses, certain types of protected crops)																				
2, 7, 12, 17, 22, 24, 27, 29	DE, AT, BE, NL, CZ, PL,HU, SK	Winter barley (HORVW)	F	<i>Erysiphe graminis</i> , <i>Rhynchosporium secalis</i> , <i>Helminthosporium gramineum</i> , <i>Pyrenophora teres</i> , <i>Puccinia hordei</i> , <i>Blumeria graminis hordei</i>	foliar, spraying, overall	BBCH 30-65 spring	a) 1 b) 1	-	a) 1 L/ha b) 1 L/ha	a) 175 / 250 b) 175 / 250	100 - 400			A	A	R	A	A	A	A
2, 7, 12, 17, 22, 24, 27, 29, 107	DE, AT, BE, CZ, HU, NL, PL, SK, IR	Spring barley (HORVS)	F	<i>Erysiphe graminis</i> <i>Rhynchosporium secalis</i> <i>Helminthosporium gramineum</i> (Pyrenophora teres) <i>Puccinia hordei</i>	foliar, spraying, overall	-/ BBCH 30-65 spring	a) 1 b) 1	-	a) 1 L/ha b) 1 L/ha	a) 175 / 250 b) 175 / 250	100 - 400			A	A	R	A	A	A	A
3, 8, 13, 18,	DE, AT, BE, NL	Rye (SECCW)	F	<i>Erysiphe graminis</i> , <i>Rhynchosporium secalis</i> , <i>Puccinia recondite</i> , <i>Blumeria graminis secalis</i> ,	foliar, spraying, overall	BBCH 30-65 spring	a) 1 b) 1	-	a) 1 L/ha b) 1 L/ha	a) 175 / 250 b) 175 / 250	100 - 400			A	A	R	A	A	A	A
4, 9, 14, 19, 25	DE, AT, BE, NL, PL	Triticale (TTLSS)	F	<i>Erysiphe graminis</i> , <i>Septoria tritici</i> , <i>Puccinia recondite</i> , <i>Puccinia striiformis</i> , <i>Blumeria graminis</i> , <i>Septoria</i>	foliar, spraying, overall	BBCH 30-65 spring	a) 1 b) 1	-	a) 1 L/ha b) 1 L/ha	a) 175 / 250 b) 175 / 250	100 - 400			A	A	R	A	A	A	A

[illegible]

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Use-No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g safener/ synergist per ha	Conclusion						
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha prothioconazole / fenpropidin a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms	Non-target plants
Zonal uses (field or outdoor uses, certain types of protected crops)																				
5, 10, 15, 20,	DE, AT, BE, NL	Oats (AVESS)	F	Erysiphe graminis, Puccinia coronate, Blumeria graminis avenae	foliar, spraying, overall	BBCH 30-65 spring	a) 1 b) 1	-	a) 1 L/ha b) 1 L/ha	a) 175 / 250 b) 175 / 250	100 - 400			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 – 21 “Conclusion”

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

Remarks table:

- Numeration necessary to allow references
- Use official codes/nomenclatures of EU
- 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)
- 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
- 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
- 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
- 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
- The maximum number of application possible under practical conditions of use must be provided
- Minimum interval (in days) between applications of the same product.
- 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
- The dimension (g, kg) must be clearly specified. (Maximum) dose of a.s. per treatment (usually g, kg or L product / ha).
- If water volume range depends on application equipments (e.g. ULVA or LVA) it should be mentioned under “application: method/kind”.
- PHI - minimum pre-harvest interval
- Remarks may include: Extent of use/economic importance/restrictions

9.1.1 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) (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 TERA and TERLT values above the corresponding trigger values, even if considering mixture toxicity. Furthermore, no unacceptable risks are expected arising from other routes of direct exposure or secondary poisoning (residue uptake from drinking water or bio-accumulation 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.03502.F.1.A in cereals.

9.1.1.2 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 (EF-SA Journal 2013;11(7):3290). Based on PEC/RAC calculations for the active substances prothio-conazole and fenpropidin as well as the metabolites potentially relevant in aquatic systems, no un-acceptable risk for aquatic or sediment-dwelling organisms is indicated, if appropriate risk mitigation measures are applied (see table below). However, risk mitigation measures and restrictions should be identified at Member state level due to different national specific requirements (for details, see Part As). The risk arising from bioaccumulation of the active substances and metabolites is considered to be acceptable.

Risk mitigation measures for:

Crop	Application rate [L prod./ha]	BBCH	Risk mitigation measures
Spring cereals	1 × 1.0	30–65	40 m vegetated filter strip (based on R4, stream FOCUS scenario, JAU-desthio).
Winter cereals	1 × 1.0	30–65	40 m vegetated filter strip (based on R1, stream, R3, stream and R4, stream scenarios, JAU-desthio)

Based on the performed calculations following conclusions may be derived:

1. Spring cereals at BBCH 30:

- 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.
- Fenpropidin: acceptable risk with 10 m unsprayed buffer zone with 75% DRT in scenarios (D3, D4, D5).
- Fenpropidin acceptable risk with 20 m VFS in scenario R4
 - Fenpropidin:
 - D3 scenario: risk acceptable: 40 m NBZ+75% DRN
 - D4 scenario (pond): risk acceptable: 10 m NBZ+75% DRN or 20 m NBZ
 - D4 scenario (stream): risk acceptable: 20 m NBZ+75% DRN or 40 m NBZ
 - D5 scenario (pond): risk acceptable: 10 m NBZ+75% DRN or 20 m NBZ
 - D5 scenario (stream): risk acceptable: 10 m NBZ+90% DRN or 20 m NBZ+75% DRN or 40 m DRN
 - R4 scenario (stream): risk acceptable: 40 m NBZ with 10 m VFS

2. Spring cereals at BBCH 65:

- Prothioconazole: acceptable risk with no need for risk mitigation measures
- JAU 6476-desthio:
 - D scenarios and scenario R4: risk acceptable with no need for risk mitigation measures
- ~~• Fenpropidin acceptable risk with 10 m unsprayed buffer zone with 75% DRT in scenarios (D3, D4, D5, R4):~~
 - Fenpropidin:
 - D3 scenarios: risk acceptable: 40 m NBZ+75% DRN
 - D4 scenario (pond): risk acceptable: 10 m NBZ+75% DRN or 20 m NBZ
 - D4 scenario (stream): risk acceptable: 30 m NBZ +75% DRN or 40 m
 - D5 scenario (pond): risk acceptable: 10 m NBZ+75% DRN or 20 m NBZ
 - D5 scenario (stream): risk acceptable: 35 m NBZ+75% DRN or 40 m NBZ
 - R4 scenario (stream): risk acceptable: 25 m +75% DRN or 35 m

It should be noted that 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 at BBCH 30:

- 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.
- ~~• Fenpropidin acceptable risk with 10 m unsprayed buffer zone with 75% DRT in scenarios (D3, D4, D5 and R3):~~
- ~~• Fenpropidin acceptable risk with 20 m VFS in scenarios R1 and R4~~
- Fenpropidin:
 - D3 scenario: risk acceptable: 40 m NBZ+75% DRN
 - D4 scenario (pond): risk acceptable: 10 m NBZ+75% DRN or 20 m NBZ
 - D4 scenario (stream): risk acceptable: 20 m NBZ+75% DRN or 40 m NBZ
 - D5 scenario (pond): risk acceptable: 10 m NBZ+75% DRN or 20 m NBZ
 - D5 scenario (stream): risk acceptable: 10 m NBZ+75% DRN or 25 m NBZ
 - R1 scenario (pond): risk acceptable: 10 m NBZ+75% DRN or 20 m NBZ
 - R1 scenario (stream): risk acceptable: 20 m NBZ+20 m VFS with 90% DRN or 40 m NBZ+10 m VFS
 - R3 scenario (stream): risk acceptable: 35 m NBZ+ 75% DRN or 40 m NBZ
 - R4 scenario (stream): risk acceptable: 35 m NBZ with 10 m VFS

4. Winter cereals at BBCH 65:

- 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.
- ~~• Fenpropidin acceptable risk with 10 m unsprayed buffer zone with 75% DRT in scenarios (D3, D4, D5, R3, R4):~~
- ~~• Fenpropidin acceptable risk with 20 m VFS in scenario R1 and R4 scenarios~~
- Fenpropidin:
 - D3 scenarios: risk acceptable: 40 m NBZ+75% DRN
 - D4 scenario (pond): risk acceptable: 10 m NBZ+75% DRN or 20 m NBZ
 - D4 scenario (stream): risk acceptable: 30 m NBZ +90% DRN or 40 m NBZ
 - D5 scenario (pond): risk acceptable: 10 m NBZ+75% DRN or 20 m NBZ
 - D5 scenario (stream): risk acceptable: 35 m NBZ+75% DRN or 40 m NBZ
 - R1 scenario (pond): risk acceptable: 10 m NBZ+75% DRN or 20 m NBZ
 - R1 scenario (stream): risk acceptable: 25 m NBZ+75% DRN or 35 m NBZ or 40 m NBZ+10 VFS
 - R3 (stream): risk acceptable: 35 m NBZ+75% DRN or 50 m NBZ
 - R4 scenario (stream) risk acceptable: 25 m +75% DRN or 35 m

Based on the performed calculations for the worst-case scenario acceptable risk following application of ADM.03500.F.2.B according to the Central Zone GAP may be concluded.

-that:

-m vegetated filter strip to surface water bodies are respected for spring and winter cereals

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

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

9.1.1.3 Effects on bees (KCP 10.3.1)

The evaluation of the 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.03502.F.1.A in cereals 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.~~

9.1.1.4 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 rhopalosiphii* 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.03502.F.1.A in cereals. Risk mitigation measures are not required.

9.1.1.5 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. $1 \times 1.0 \text{ L prod./ha}$ (BBCH 30-65, 80 % crop interception) in cereals, covering the maximum application rates per crop and year.

Soil macro- and mesofauna

All TERLT values calculated for the active substances and their metabolites potentially relevant in soil are above the trigger values of 5, established for long-term exposure. Thus, an acceptable over-all risk for earthworms and other soil organisms is indicated for the intended GAP uses of ADM.03502.F.1.A in cereals.

Soil microorganisms

Effects within a range of $\pm 25 \%$ 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.03502.F.1.A in cereals.

9.1.1.6 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 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.03502.F.1.A in cereals. Risk mitigation measures are not required.

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

From the comprehensive set of ecotoxicity studies presented for ADM.03502.F.1.A (in addition to the toxicity data for the active substances and metabolites), sufficient data are available for the assessment of the effects of ADM.03502.F.1.A 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.03502.F.1.A is an emulsifiable concentrate (EC) containing 175 g/L of the active substance prothioconazole and 250 g/L of the active substance fenpropidin. It is a fungicide applied as spray to infested foliage of cereals. The timing of application is post-emergence. The worst-case application scenario leading to maximum contamination of the environment is a single spray application at a rate of 1.0 L prod./ha (corresponding to 175 g prothioconazole/ha and 250 g fenpropidin/ha). For a detailed summary of the GAP uses of ADM.03502.F.1.A, please refer to Table 9.1-1.

Table 9.1-2: Critical use pattern of ADM.03502.F.1.A

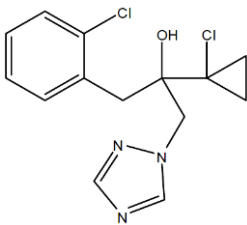
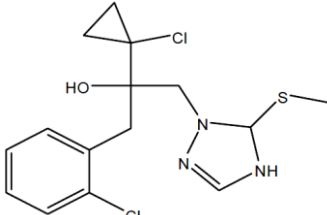
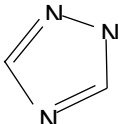
Grouping according to criterion			
Group	Intended uses	Relevant use parameters for grouping	Relevant exposure scenario
Effects on birds and mammals (point CP 9.2 and CP 9.3)			
Field crops	Post-emergence application, Cereals, considering 1× 1.0 L prod./ha, BBCH 30-65	Crop group according to EFSA Journal 2009; 7(12): 1438: Cereals	<p>Maximum application rates, i.e. 1× 1.0 L prod./ha [equivalent to 1× 175 g prothioconazole/ha + 1× 250 g fenpropidin/ha], to cereals, considering indicator species (screening step) and generic focal species (Tier-1/Tier-2) relevant in treated fields according to EFSA exposure scenarios at time of application.</p> <p><i>Most critical routes of exposure:</i> Feeding on food items directly contaminated via spray application; bioaccumulation in food chains; residue uptake from drinking water.</p>
Effects on aquatic organisms (point CP 9.5)			
Field crops	Post-emergence application, Cereals, considering 1× 1.0 L prod./ha, BBCH 30-65	Crop group according to FOCUS (2001 & 2015): (spring/winter) cereals	<p>Maximum application rates, i.e. 1× 1.0 L prod./ha [equivalent to 1× 175 g prothioconazole/ha + 1× 250 g fenpropidin/ha], to cereals, considering all relevant aquatic groups and calculated PEC_{sw} values at FOCUS Step-1 to 4 (if required).</p> <p><i>Most critical routes of exposure:</i> Exposure in surface water and sediment contaminated by spray drift, run-off and drainage</p>

Grouping according to criterion			
Group	Intended uses	Relevant use parameters for grouping	Relevant exposure scenario
Effects on bees (point 9.6)			
Field crops	Post-emergence application, Cereals, considering 1× 1.0 L prod./ha, BBCH 30-65	Crop group according to SANCO/10329/2002 rev.2 (final), October 17, 2002: Field crops	Maximum single application rate, i.e. 1× 1.0 L prod./ha [equivalent to 1× 175 g prothioconazole/ha + 1× 250 g fenpropidin/ha] to cereals <i>Most critical routes of exposure:</i> Contact and oral exposure from spray deposits (overspray, spray drift) and consumption of pollen and nectar from treated crops and weeds
Effects on non-target arthropods (point 9.7)			
Field crops	Post-emergence application, Cereals, considering 1× 1.0 L prod./ha, BBCH 30-65	Crop group according to ESCORT 2 (2000): Field crops	Maximum application rate, i.e. 1× 1.0 L prod./ha, to cereals <i>Most critical routes of exposure:</i> 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, Cereals, considering 1× 1.0 L prod./ha, BBCH 30-65	Crop group according to SANCO/10329/2002 rev 2 (final), October 17, 2002: Cereals	1× 1.0 L prod./ha [equivalent to 1× 175 g prothioconazole/ha + 1× 250 g fenpropidin/ha] to cereals at BBCH 30-65, considering 80 % crop interception <i>Most critical routes of exposure:</i> Exposure in soil contaminated by spray application
Effects on terrestrial non-target plants (point 9.10)			
Field crops	Post-emergence application, Cereals, considering 1× 1.0 L prod./ha, BBCH 30-65	Crop group according to SANCO/10329/2002 rev 2 (final), October 17, 2002: Field crops	Maximum single application rate, i.e. 1× 1.0 L prod./ha, to cereals, as recommended by the guidance document for fungicides <i>Most critical routes of exposure:</i> 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.03502.F.1.A is indicated in the tables below.

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 % water: 32.3 % sediment: 26.9 % whole system: 54.6 %	Yes Secondary poisoning terrestrial vertebrates; Aquatic organisms; Soil macro- and mesofauna; microorganisms
Prothioconazole-S-methyl (M01) (JAU-S-methyl)	358.3 g/mol		soil: 14.6 % water/sediment: 77 % (anaerob)	Yes Secondary poisoning terrestrial vertebrates; Aquatic organisms; Soil macro- and mesofauna; microorganisms
1,2,4-triazole (M13)	69.065 g/mol		water: 37.2 % sediment: 4.6 % whole system: 41.8 %	Yes aquatic organisms

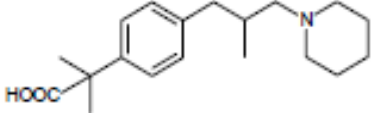
zRMS comments:

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.

Table 9.1-4 Metabolites of fenpropidin potentially relevant in the environment

Metabolite	Molar mass	Chemical structure	Maximum-observed occurrence in compartments	Risk assessment required?
CGA 289267 2-methyl-2-[4-(2-methyl-3-piperidin-1-yl-propyl)-phenyl]-propionic acid	303.4 g/mol		soil: 10.6 % water/sediment: 16.1 % (whereof 14.3 % in water, <5% in sediment)	Yes Secondary poisoning terrestrial vertebrates; Aquatic organisms; Soil macro- and mesofauna; microorganisms

zRMS comments:

Metabolites relevant for soil and water compartment listed in Table 9.1- 4 are the same as indicated in EFSA Journal (2007) 124, 1-84. 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 9.1-4.

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

Avian toxicity studies have been carried out with the active substances prothioconazole and fenpropidin as well as with the prothioconazole metabolite JAU-desthio. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on birds of ADM.03502.F.1.A were not evaluated as part of the EU assessment of prothioconazole or fenpropidin. However, the provision of further data on the formulation is not considered to be required, because mixture toxicity based on active substance data was addressed in the risk assessment below.

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

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

Species	Substance	Exposure System	Results	Reference
Bobwhite quail (<i>Colinus virginianus</i>)	Prothioconazole technical	Acute oral toxicity	LD₅₀ > 2000 mg a.s./kg bw	EFSA Scientific Report (2007) 106, 1-98
Bobwhite quail (<i>Colinus virginianus</i>)	Prothioconazole technical	Dietary 5 d Short-term	LD ₅₀ > 1413 mg a.s./kg bw	EFSA Scientific Report (2007) 106, 1-98
Mallard duck (<i>Anas platyrhynchos</i>)	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 (<i>Colinus virginianus</i>)	JAU-desthio (M4)	Acute oral toxicity	LD ₅₀ > 2000 mg met./kg bw	EFSA Scientific Report (2007) 106, 1-98
Bobwhite quail (<i>Colinus virginianus</i>)	JAU-desthio (M4)	Dietary 5 d Short-term	LD₅₀ > 297mg met./kg bw	EFSA Scientific Report (2007) 106, 1-98
Mallard duck (<i>Anas platyrhynchos</i>)	Prothioconazole technical	Reproductive toxicity	NOEL = 78 mg a.s./kg bw/d	EFSA Scientific Report (2007) 106, 1-98
Bobwhite quail (<i>Colinus virginianus</i>)	Prothioconazole technical	Dietary 22 weeks Reproductive toxicity	NOEL ≥ 86 mg a.s./kg bw/day	EFSA Scientific Report (2007) 106, 1-98
Bobwhite quail (<i>Colinus virginianus</i>)	JAU-desthio (M4)	Reproductive toxicity	NOEL = 14.8 mg met./kg bw/d	EFSA Scientific Report (2007) 106, 1-98
Pheasant (<i>Phasianus colchicus</i>)	Fenpropidin technical	Acute oral toxicity	LD₅₀ = 369 mg a.s./kg bw	EFSA Scientific Report (2007) 124, 1-84
Mallard duck (<i>Anas platyrhynchos</i>)	Fenpropidin technical	Acute oral toxicity	LD ₅₀ = 1889 mg a.s./kg bw	EFSA Scientific Report (2007) 124, 1-84
Bobwhite quail (<i>Colinus virginianus</i>)	Fenpropidin technical	Dietary 5 d Short-term	LD ₅₀ >1417 mg a.s./kg bw	EFSA Scientific Report (2007) 124, 1-84
Bobwhite quail (<i>Colinus virginianus</i>)	Fenpropidin technical	Reproductive toxicity	NOAEL = 14.6 mg a.s./kg bw/d	EFSA Scientific Report (2007) 124, 1-84

zRMS comments:

Avian toxicity data for fenpropidin, 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 Journal (2007) 124, 1-84 and EFSA Scientific Report (2007) 106, respectively.

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 LD₅₀ 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 has not resulted with increased mortality of tested birds and the acute LD₅₀>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 LD₅₀ >2000 mg pm/kg bw is used by the Applicant, while short-term dietary studies with this compound with lower LD₅₀ of 297 mg pm/kg bw/d should be considered as treatment related mortalities were observed in these short-term dietary studies.

Acute toxicity

According to the recommendations of the current "Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA" (EFSA Journal 2009; 7(12): 1438, acute dietary effects are covered by the acute oral toxicity test resulting in a LD₅₀ as relevant endpoint, which should be used for the TER_A calculations. In contrast, 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 packet.

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 substances 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.03502.F.1.A will be assessed using data on the active substances prothioconazole and fenpropidin (including mixture toxicity, see below). Exposure to ADM.03502.F.1.A 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.

Metabolites

JAU-desthio (M4) was considered to be the only major metabolite in crop foliage (EFSA Scientific Report (2007) 106) and an acute toxicity study is 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 LD₅₀ of > 2000 mg/kg bw in the acute risk assessment for the metabolite JAU-desthio (M4).

Mixture toxicity

The predicted acute mixture toxicity conservatively assuming dose additivity of the active substances (based on the worst-case assumption that the active substances have the same mode of action) was calculated using the following formula, in accordance with the recommendations of Appendix B of EFSA Journal 2009; 7(12): 1438:

Equation 9-1: Calculation of the predicted LD₅₀(mix)

$$LD_{50}(\text{mix}) = \frac{1}{\sum_i \frac{x(\text{a.s.})}{LD_{50}(\text{a.s.})}}$$

where LD₅₀(mix) = the predicted LD₅₀ for the mixture of the active substances

x(a.s.)	= fraction of active substance in the mixture*
LD ₅₀ (a.s.)	= measured LD ₅₀ of the active substances

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

In addition, in order to investigate whether the toxicity to birds is driven by one active substance (or metabolite), the toxicity per fraction (a.s.), defined as LD₅₀(a.s.) divided by x(a.s.), is compared to the predicted LD₅₀(mix). Where this ratio is ≥ 90 % for one of the active substances (or metabolite), this indicates that the compound contained in the formulation will contribute to ≥ 90 % to mixture toxicity, while the other(s) of the mixture will only have a marginal impact on the predicted risk.

In those cases, calculations of TER_A values should be based on endpoints related to the individual compounds only. Accordingly, the toxicity data presented below indicate that none of the compounds contribute to ≥ 90 % to mixture toxicity to birds:

Table 9.2-2: LD₅₀(mix) for birds

Test item	LD ₅₀ (a.s.) [mg/kg bw]	Nominal content in the formulation [g/L]	x(a.s.) *	Toxicity per fraction	LD ₅₀ (mix) [mg/kg bw]	Contribution to mixture toxicity
Prothioconazole	(>) 2000	175	0.41	4857	(>) 555.6	11.4 %
Fenpropidin	369	250	0.59	627.3		88.6 %
JAU-desthio	(>) 2000	175**	0.41	4857	(>) 555.6	11.4 %
Fenpropidin	369	250	0.59	627.3		88.6 %

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

** For prothioconazole metabolite JAU-desthio the application rate of the parent compound was considered – representing an absolute worst-case approach

Although fenpropidin contributes to nearly 90 % to mixture toxicity, it is most appropriate to base the risk assessment for birds on data on the individual compounds as well as on the calculated mixture toxicity endpoints (prothioconazole/fenpropidin as well as JAU-desthio/fenpropidin). Accordingly, the acute oral LD₅₀ > 2000 mg prothioconazole/kg bw, the LD₅₀ > 2000 mg JAU-desthio/kg bw, the LD₅₀ of 369 mg fenpropidin/kg bw and the LD₅₀(mix) of (>) 555.6 mg/kg bw were considered as the most relevant endpoints for the TER_A calculations. This approach is in line with the recommendations from the EFSA Journal 2009; 7(12): 1438.

Reproductive effects

For the long-term risk assessment, the LD₅₀/10 should be used according to EFSA Journal 2009; 7(12): 1438 if it is lower than the reproductive NOEL. However, this is not the case for prothioconazole and fenpropidin. Accordingly, the reproductive NOEL values of 78 mg prothioconazole/kg bw/d, 14.6 mg fenpropidin/kg bw/d are considered as the most relevant endpoints for the TER_{LT} initial calculations.

Metabolites

JAU-desthio (M4) was considered to be the only major metabolite in crop foliage (EFSA Scientific Report (2007) 106) and a chronic toxicity study is 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 NOEL of 14.8 mg/kg bw/d in the reproductive risk assessment for the prothioconazole metabolite JAU-desthio (M4).

Mixture toxicity

With respect to the potential for combined long-term effects, it should be noted that for the approach assuming dose additivity of the active substances, reliable results would only be expected for combinations of effect levels with a defined x, e.g. a LD₅₀, but not for NOELs since the latter effect indicators may rep-

resent varying risk or response levels for different compounds depending on dose-spacing (EFSA Journal 2009; 7(12): 1438, Appendix B). Furthermore, it is considered unlikely that the active substances and all the co-formulants will remain intact over a long-term period in relevant matrices, i.e. plants, animals, soil and water. Therefore, it is unlikely that terrestrial vertebrates could be exposed for a prolonged period to both prothioconazole and fenpropidin at the same time. Accordingly, for the risk assessment based on long-term effects it is not recommended to consider the use of predicted toxicity values.

In addition, it should be noted that the predicted long term mixture toxicity (using the same approach provided in Equation 9-1) indicates that neither the active substances prothioconazole and fenpropidin nor the prothioconazole metabolite JAU-desthio will contribute > 90 % to the mixture toxicity.

Table 9.2-3: NOEL(mix) for birds

Test item	NOEL [mg/kg bw/d]	Nominal content in the formulation [g/L]	x(a.s.) *	Toxicity per fraction	NOEL(mix) [mg/kg bw/d]	Contribution to mixture toxicity
Prothioconazole	78	175	0.41	189.4	21.9	11.6 %
Fenpropidin	14.6	250	0.59	24.8		88.4 %
JAU-desthio	14.8	175**	0.41	35.9	14.7	40.8 %
Fenpropidin	14.6	250	0.59	24.8		59.2 %

Bold: contribution to ≥ 90 % to mixture toxicity

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

** For prothioconazole metabolite JAU-desthio the application rate of the parent compound was considered – representing an absolute worst case approach

For maximum conservatism, the risk assessment for birds was conducted based on the data of the individual compounds as well as on mixture toxicity. Accordingly, the reproductive NOEL values of 78 mg prothioconazole/kg bw/d, 14.8 mg JAU-desthio/kg bw/d, 14.6 mg fenpropidin/kg bw/d as well as the NOEL_{mix} of 21.9 mg/kg bw/d (prothioconazole/fenpropidin) and 14.7 mg/kg bw/d (JAU-desthio/fenpropidin) were considered as the most relevant endpoints for TER_{LT} calculations.

zRMS comments:

Combined acute toxicity

The LD_{50mix} presented in Table 9.2-2 for both a.s.; fenpropidin and prothioconazole has been accepted by the zRMS.

It is noted that for its calculation the acute toxicity endpoints were used and as indicated in the zRMS commenting box in point 9.2.1 it is acceptable.

However, LD_{50mix} for the mixture of fenpropidin and JAU 6476-desthio proposed by the applicant should considered lower toxicity endpoint LD₅₀ of 297 pm./kg bw which was obtained from dietary study for birds.

For this reason, LD_{50mix} was recalculated by zRMS with consideration of this endpoint and a total conversion of prothioconazole to the JAU 6476-desthio metabolite.

zRMS calculations are presented below.

Avian LD₅₀ (mix) for JAU 6476-desthio metabolite and fenpropidin when combined in ADM.03502.F.1.A (step 1 in EFSA GD 2009, Appendix B)

	JAU 6476-desthio	Fenpropidin
Relative amount of a.s. (%)	41 ¹⁾	59
Fraction in the a.s. mixture	0.41	0.59
LD₅₀ of a.s. or met[mg/kg bw]	>297	369
Fraction / LD₅₀	0.0014	0.0016
Sum	0.003	
1/ sum = predicted LD₅₀ (mix)	333.3	

¹⁾ Relative amount of the parent assuming immediate and complete conversion of prothioconazole to JAU 6476-desthio; this in combination with metabolite endpoint represents worst case and covers also contribution of prothioconazole to the mixture toxicity as it is expected that consideration of prothioconazole endpoint of >2000 mg a.s./kg bw in the LD_{50mix} calculation

would give higher combined value

Avian “tox per fraction” for the JAU 6476-desthio metabolite and fenpropidin when combined in ADM.03502.F.1.A (step 1 in EFSA GD 2009, Appendix B)

	JAU 6476-desthio	Fenpropidin	“mix”
Content in the formulation	41	59	
Fraction in mixture	0.41	0.59	
LD ₅₀ (mg/kg bw)	>297	369	LD _{50mix} =333.3
Tox per fraction	724.39	625.42	
Contribution to predicted toxicity	46%	54%	

Fenpropidin contributes 54% to mixture toxicity, while the JAU 6476-desthio metabolite has an impact on the predicted risk of 46 %, therefore, surrogate LD₅₀ of 333.3 mg/kg bw should be used in the acute risk assessment.

Combined long-term toxicity

zRMS agrees that for the approach assuming dose additivity of the active substances, reliable results would only be expected for combinations of effect levels with a defined x, e.g. a LD₅₀, but not for NOELs since the latter effect indicators may represent varying risk or response levels for different compounds depending on dose-spacing according to GD B&M, EFSA Journal 2009; 7(12): 1438, Appendix B. Therefore, for the risk assessment based on long-term effects it is not recommended to consider the use of predicted toxicity values.

Therefore, the calculated NOEL_{mix} provided by the applicant was not considered by zRMS in the current risk assessment.

It should be noted that according to recommendation given in Appendix B of the Guidance Document 2009 for the evaluation of sublethal effects the use of the lowest NO(A)EL of the actives in the formulation, along with the combined exposure estimate from both active substances provides a conservative representation of long-term risks to birds.

Approach taken with regard to the long-term combined risk assessment represents worst case for mixture of active substances and is in general acceptable.

However, the combined long-term risk assessment should also include metabolite JAU 6476-desthio which is more than 5 times more toxic than prothioconazole. Nevertheless, combined long-term risk assessment performed with consideration of the cumulative application rate of fenpropidin and prothioconazole together with the lowest available NOEL of 14.6 mg/kg bw/d covers also exposure to JAU 6476-desthio in the mixture, as even with immediate and complete conversion of prothioconazole to JAU 6476-desthio, its concentration in the mixture will never exceed the concentration of the parent, i.e. 175 g/L. For this reason, combined risk assessment performed with consideration of the cumulative application rate of both compounds and the lowest available toxicity endpoint will cover the long-term combined risk from both active compounds and metabolite.

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.03502.F.1.A is an emulsifiable concentrate (EC) containing 175 g/L of the active substance prothioconazole and 250 g/L of the active substance fenpropidin. It is a fungicide applied as spray to infested foliage of cereals. The timing of application is post-emergence. The worst-case application scenario leading to maximum contamination of the environment is a single spray application at a rate of 1.0 L prod./ha (corresponding to 175 g prothioconazole/ha and 250 g fenpropidin/ha). For a detailed summary of the GAP uses of ADM.03502.F.1.A, 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.03502.F.1.A. A summary of the intended uses and relevant avian indicator species is given in the table below.

Table 9.2-4: Worst-case GAP use of ADM.03502.F.1.A 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, BBCH 30-65, 1×1.0 L prod./ha [equivalent to 1×250 g fenpropidin/ha + 1×175 g prothioconazole/ha]	Small omnivorous bird	158.8 / 64.8

Exposure of terrestrial vertebrates to ADM.03502.F.1.A 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 90th 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.03502.F.1.A. 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 9.2-5: Prothioconazole - screening assessment of the acute and long-term risk for birds due to the use of ADM.03502.F.1.A in cereals

Intended use	Cereals, 1× 1.0 L prod./ha, BBCH ≥ 10					
Active substance	Prothioconazole					
Application rate (g/ha)						
MAF	1.0					
Acute toxicity (mg/kg bw)	> 2000					
TER criterion						
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	> 72.0	
Long-term toxicity (mg/kg bw/d)	78					
TER criterion						

Intended use	Cereals, 1× 1.0 L prod./ha, BBCH ≥ 10				
Active substance	Prothioconazole				
Application rate (g/ha)	1× 175				
MAF	1.0				
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	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 cereals already at screening level. Thus, no further refinements at Tier-1 level are required for prothioconazole.

Fenpropidin

Table 9.2-6: Fenpropidin - screening assessment of the acute and long-term risk for birds due to the use of ADM.03502.F.1.A in cereals

Intended use	Cereals, 1× 1.0 L prod./ha, BBCH ≥ 10				
Active substance	Fenpropidin				
Application rate (g/ha)	1× 250				
MAF	1.0				
Acute toxicity (mg/kg bw)	369				
TER criterion	10				
Crop scenario Growth stage	Indicator species	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_A
BBCH > 10	Small omnivorous bird	158.8	1.0	39.7	9.3

Intended use	Cereals, 1× 1.0 L prod./ha, BBCH ≥ 10				
Active substance	Fenpropidin				
Application rate (g/ha)	1× 250				
MAF	1.0				
Long-term toxicity (mg/kg bw/d)	14.6				
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	8.6	1.7

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

As outlined in the table above, the calculated TER_A and TER_{LT} values for the exposure to fenpropidin are below the trigger of 10 and 5, established for acute and long-term exposure, indicating an unacceptable risk for birds in cereals at screening level. Thus, further refinements at Tier-1 level are required for fenpropidin.

JAU-desthio (M4)

Table 9.2-7: JAU-desthio (M4) - screening assessment of the acute and long-term risk for birds due to the use of ADM.03502.F.1.A in cereals

Intended use	Cereals, 1× 1.0 L prod./ha, BBCH ≥ 10				
Metabolite	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	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_A
BBCH > 10	Small omnivorous bird	158.8	1.0	27.8	>10.68 >72.0
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 acceptable acute risk for birds in cereals already at screening level. By using the parent application rate for the metabolite (assuming 100% conversion into JAU-desthio as an absolute worst-case approach), 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 both active substances is agreed 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.

TER_A and TER_{LT} values for the exposure to fenpropidin are below the trigger of 10 and 5 for acute and long-term exposure, indicating an unacceptable risk for birds. Therefore, further refinements at Tier-1 level are required for fenpropidin.

It should be noted that the acute risk for metabolite JAU 6476-desthio was performed by the Applicant with consideration of the acute LD₅₀ 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 9.2-7 above was amended accordingly with consideration of the LD₅₀ of 297 mg pm/kg bw/d.

Thus, TER_A value with LD₅₀ of >297 mg pm/kg bw/d for the exposure to JAU-desthio (M4) is above the trigger of 10, indicating an acceptable acute risk for birds at the screening level.

In case of 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_{LT} value is below the trigger of 5, and Tier-1 long-term risk assessment for the metabolite of concern was required.

Mixture toxicity

Since the acute and long-term risk for birds exposed to fenpropidin is not acceptable at screening step, no mixture toxicity was addressed at this level.

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.03502.F.1.A. A summary of the critical GAP uses and relevant avian indicator species is given in the table below.

Table 9.2-8: Critical use pattern of ADM.03502.F.1.A and corresponding avian generic focal species relevant for Tier-1 assessments

	Worst-case application scenario	EFSA crop group	EFSA Tier-1 scenario	Generic focal species (Representative)	Shortcut value for TER _A /TER _{LT}
Cereals	Post-emergence, BBCH 30-65, 1× 1.0 L prod./ha	Cereals	BBCH 30-39	Small omnivorous bird (lark)	12.0 / 5.4
			BBCH ≥ 40	Small omnivorous bird (lark)	7.2 / 3.3

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.

Fenpropidin

Table 9.2-9: Fenpropidin - Tier-1 assessment of the acute and long-term risk for birds due to the use of ADM.03502.F.1.A in cereals

Intended use	Cereals, 1× 1.0 L prod./ha, BBCH 30–65				
Metabolite	Fenpropidin				
Application rate (g/ha)	1× 250				
MAF	1.0				
Acute toxicity (mg/kg bw)	369				
TER criterion	10				
Crop scenario Growth stage	Indicator species	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_A
Cereals, BBCH 30-39	Small omnivorous bird (lark)	12.0	1.0	3.0	123.0
Cereals, BBCH ≥ 40	Small omnivorous bird (lark)	7.2	1.0	1.8	205.0
Cereals late season seed heads	Small granivorous	4.0	1.0	1	369

Intended use	Cereals, 1× 1.0 L prod./ha, BBCH 30–65				
Metabolite	Fenpropidin				
Application rate (g/ha)	1× 250				
MAF	1.0				
Long-term toxicity (mg/kg bw/d)	14.6				
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 30-39	Small omnivorous bird (lark)	5.4	0.53	0.7	20.4
Cereals, BBCH ≥ 40	Small omnivorous bird (lark)	3.3	0.53	0.4	33.4
Cereals late season seed heads	Small granivorous	4.7	0.53	0.58	25.17

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 fenpropidin are well above the respective trigger, indicating an acceptable acute and 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 fenpropidin.

JAU-desthio (M4)

Table 9.2-10: JAU-desthio (M4) - Tier-1 assessment of the long-term risk for birds due to the use of ADM.03502.F.1.A in cereals

Intended use	Cereals, 1× 1.0 L prod./ha, BBCH 30–65				
Metabolite	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}
Cereals, BBCH 30-39	Small omnivorous bird (lark)	5.4	0.53	0.5	29.5
Cereals, BBCH ≥ 40	Small omnivorous bird (lark)	3.3	0.53	0.3	48.4
Cereals late season seed heads	Small granivorous	4.7	0.53	0.44	33.63

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 considering the parent application rate). Thus, no further refinements are considered to be required for the metabolite.

zRMS comments:

Tier 1 risk assessment

The Tier 1 risk assessment for fenpropidin and prothioconazole metabolite JAU 6476-desthio is agreed by zRMS. TER_A and TER_{LT} values are above the trigger of 10 and 5 for acute and long-term exposure, indicating acceptable risk for birds.

Overall, acceptable acute and long-term risk may be concluded for birds exposed to prothioconazole, fenpropidin and metabolite JAU 6476-desthio in ADM.03502.F.1.A.

Prothioconazole/fenpropidin - Mixture toxicity

Table 9.2-11: Mixture toxicity (prothioconazole/ fenpropidin) - Tier-1 assessment of the acute and long-term risk for birds due to the use of ADM.03502.F.1.A in cereals

Intended use	Cereals, 1× 1.0 L prod./ha [equivalent to 1× 175 g prothioconazole/ha + 1× 250 g fenpropidin/ha], BBCH 30 – 65				
Metabolite/active substance	Prothioconazole + fenpropidin				
Application rate (g/ha)	1× 425 (sum of a.s.)				
MAF	1.0				
Acute toxicity (mg/kg bw)	(>) 555.6				
TER criterion	10				
Crop scenario Growth stage	Indicator species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _A
Cereals, BBCH 30-39	Small omnivorous bird (lark)	12.0	1.0	5.1	(>) 108.9
Cereals, BBCH ≥ 40	Small omnivorous bird (lark)	7.2	1.0	3.1	(>) 181.6
Cereals late season seed heads	Small granivorous	4.0	0.53	0.9	617.33
Long-term toxicity (mg/kg bw/d)	21.9				
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 30-39	Small omnivorous bird (lark)	5.4	0.53	1.2	18.0
Cereals, BBCH ≥ 40	Small omnivorous bird (lark)	3.3	0.53	0.7	29.5

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 this mixture toxicity scenario (prothioconazole/fenpropidin) are well above the respective trigger, indicating an acceptable acute and 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 mixture toxicity.

Prothioconazole/JAU-desthio/fenpropidin - Mixture toxicity

Table 9.2-12: Mixture toxicity (Prothioconazole/JAU-desthio/fenpropidin) - Tier-1 assessment of the acute and long-term risk for birds due to the use of ADM.03502.F.1.A in cereals

Intended use	Cereals, 1× 1.0 L prod./ha [equivalent to 1× 175 g prothioconazole/ha + 1× 250 g fenpropidin/ha], BBCH 30 – 65				
Metabolite/active substance	Prothioconazole/JAU-desthio + fenpropidin				
Application rate (g/ha)	1× 425*				
MAF	1.0				
Acute toxicity (mg/kg bw)	(>) 333.3 555.6				
TER criterion	10				
Crop scenario Growth stage	Indicator species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _A
Cereals, BBCH 30-39	Small omnivorous bird (lark)	12.0	1.0	5.1	(>) 108.9 63.35

Intended use	Cereals, 1× 1.0 L prod./ha [equivalent to 1× 175 g prothioconazole/ha + 1× 250 g fenpropidin/ha], BBCH 30 – 65				
Metabolite/active substance	Prothioconazole/JAU-desthio + fenpropidin				
Application rate (g/ha)	1× 425*				
MAF	1.0				
Cereals, BBCH ≥ 40	Small omnivorous bird (lark)	7.2	1.0	3.1	(>)107.52 181.6
Long-term toxicity (mg/kg bw/d)	14.6 ^{a)}				
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 30-39	Small omnivorous bird (lark)	5.4	0.53	1.2	12.16
Cereals, BBCH ≥ 40	Small omnivorous bird (lark)	3.3	0.53	0.7	20.85 19.8
Cereals late season seed heads	Small granivorous	4.7	0.53	1.059	13.78

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

^{a)} The lowest NOEL of the two substances and JAU 6476-desthio is 14.6 mg a.s./kg bw reported for fenpropidin. This endpoint is applied to the reproductive risk assessment for the mixture of all relevant compounds

As outlined in the table above, the calculated TER_A and TER_{LT} values for this mixture toxicity scenario (JAU-desthio/fenpropidin) are well above the respective trigger, indicating an acceptable acute and 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 mixture toxicity.

zRMS comments:

Combined acute risk assessment

The zRMS calculated the LD₅₀mix with consideration of relevant toxicity endpoint for the metabolite JAU-desthio and fenpropidin (for details, see commenting box in point 9.2.1.1 above) and the acute risk assessment in Table 9.2-12 has been amended accordingly.

With regard to the exposure, assumed application rate of prothioconazole accounts also for its conversion to JAU 6476-desthio, as even with immediate and complete conversion the its concentration in the mixture will never exceed the concentration of the parent, i.e. 175 g/L.

Combined long-term risk assessment

As already indicated in the zRMS comments in point 9.2.1.1 above, consideration of the lowest toxicity endpoint of 14.6 mg/kg bw/d together with cumulative application rate of both active substances represents worst case and accounts also for conversion of prothioconazole to metabolite JAU 6476-desthio.

Based on performed calculations acceptable acute and long-term risk may be concluded for birds exposed to the mixture of fenpropidin, prothioconazole and JAU 6476-desthio following application of ADM.03502.F.1.A.

For request of cMS the Combi-TER approach is presented below:

Tier-1 risk assessment

The risk assessment for birds and mammals was performed according to the current "Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA" (EFSA Journal 2009; 7(12): 1438. For further details, please refer to the core dossier of ADM.03502.F.1.A, Part B – Section 9.

The calculated TER_{LT} values for the single active substances and the representative indicator species are summarised in the table below.

Intended use	ADM.03502.F.1.A, cereals, 1× 1.0 L prod./ha, BBCH 30–65		
Crop scenario, Growth stage and Indicator species	TER _{LT}		
	Fenpropidin	Prothioconazole	JAU-desthio (M4)
Cereals, BBCH 30-39, Small omnivorous bird (lark)	20.4	156	29.5
Cereals, BBCH ≥ 40, Small omnivorous bird (lark)	33.4	255	48.4

Combi-TER approach

The Combi-TER approach was performed in accordance with the CZ Evaluation Manual ecotoxicology (May 2021) and the guidance document on work-sharing in the northern zone in the authorisation of plant protection products (June 2021), based on the model of concentration addition using the following equation:

$$Trigger_A - value / TER_A + Trigger_B - value / TER_B + \dots = SUM$$

If $SUM < 1$ the risk assessment is acceptable

Where:

- "Trigger-value" represents the uncertainty factor of chemical A, B etc.

- TER is the Toxicity Exposure Ratio calculated from the substance specific effect concentration (e.g. EC50, EC10 or NOEC) divided by the expected environmental exposure.

The results of the Combi-TER approach are presented in the following table.

Intended use	ADM.03502.F.1.A, cereals, 1× 1.0 L prod./ha, BBCH 30–65	
Crop scenario, Growth stage and Indicator species	Combi-TER _{LT}	
	Fenpropidin + Prothioconazole	Fenpropidin + JAU-desthio (M4)
Cereals, BBCH 30-39, Small omnivorous bird (lark)	0.28	0.41
Cereals, BBCH ≥ 40, Small omnivorous bird (lark)	0.17	0.25

As outlined in the table above, all Combi-TER_{LT} values are below the relevant trigger of 1, indicating an acceptable risk for the long-term exposure of birds for the intended use of ADM.03502.F.1.A in cereals.

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.03502.F.1.A 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 ($K_{oc} < 500$ L/kg) or 3000 in the case of more sorptive substances ($K_{oc} \geq 500$ L/kg).

With $K(f)_{oc} > 500$ L/kg, the active substances prothioconazole ($K_{OC} = 1765$ L/kg) and fenpropidin ($K_{OC} = 3808$ L/kg, mean) as well as the metabolite JAU-desthio (M4) ($K_{OC} = 523-625$ L/kg) belong to the group of less 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 1×175 g prothioconazole/ha, 1×175 g JAU-desthio (M4)/ha and 1×250 g fenpropidin/ha) covers the risk for water-drinking birds from all intended GAP uses of ADM.03502.F.1.A (for details, see point 9.1.1).

Prothioconazole

Effective application rate (g/ha)	=	1×175		
Acute toxicity (mg/kg bw)	=	> 2000	quotient =	< 0.1
Reprod. toxicity (mg/kg bw/d)	=	78	quotient =	2.2

Fenpropidin

Effective application rate (g/ha)	=	1×250		
Acute toxicity (mg/kg bw)	=	369	quotient =	0.7
Reprod. toxicity (mg/kg bw/d)	=	14.6	quotient =	17.1

JAU-desthio (M4)

Effective application rate (g/ha)	=	1×175		
Acute toxicity (mg/kg bw)	=	>297 2000	quotient =	< 0.59 0.1
Reprod. toxicity (mg/kg bw/d)	=	14.8	quotient =	11.8

In order to apply consistent approach, the drinking water risk assessment was performed also for metabolite JAU 6476-S-methyl and is presented below. Calculations were performed with assumption of 10 times toxicity of the parent.

JAU 6476-S-methyl effective application rate 1×175 g/ha

Acute toxicity (mg/kg bw)	>200	quotient	= 0.875	Trigger: 3000
Reprod. toxicity (mg/kg bw/d)	7.8	quotient	=22.43	

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:

Since is not a for-spray-applications -> 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. Therefore, The leaf scenario does not have to be considered taking into account the proposed uses (cereals). The evaluation of the risk resulting from uptake of contaminated water in Puddle scenario was not required since ratio between effective application rate and endpoint relevant for acute risk and long-term assessment is <3000.

9.2.2.4 Effects of secondary poisoning

Active substances

Based on a $\log P_{ow} > 3$ for prothioconazole (i.e. $\log P_{ow}$ of 3.82 at pH 7; for details, see EFSA Scientific report (2007) 106, 1- 98) and fenpropidin (i.e. $\log P_{ow}$ of 4.5 at pH 9.0; for details, see EFSA Scientific report (2007) 124, 1- 84) a potential for bioaccumulation has to be considered for these compounds on a hypothetical basis. Thus, for both active substances a risk assessment was performed for exposure from accumulation in food chains in agreement with the current guidance document.

Prothioconazole 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 Pow
JAU-desthio	Soil, surface water	3.04
JAU-S-methyl	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.

Fenpropidin metabolites

For this metabolite potentially of concern in soil and surface water, i.e. no experimentally determined log Pow value is available from the DAR. On this account, model calculation using KOWWIN (version 1.68, 2010) was performed. Based on this model calculation, the logPow values were determined at 1.61, indicating no potential for bioaccumulation of CGA 289263:

Compound	Major metabolite in	SMILE CODE	Log Pow
CGA 289267	Soil, surface water	<chem>c1cc(C(C(=O)O)(C)C)ccc1CC(C)CN2CCCCC2</chem>	1.61

In conclusion, a potential for bioaccumulation may be expected only for the active substance fenpropidin. Consequently, a deterministic risk assessment by calculating TER values was performed only for this compound 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 2: Calculation of Daily Dietary Dose for earthworm-eating birds and mammals

DDD = PEC_{worm} · f_{conv}		[mg/kg bw/d]
where	(1) PEC _{worm} = PEC _{soil} · 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	
	Pow = 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-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 0.009 mg a.s./kg soil_{dw} was calculated for the intended GAP uses of ADM.03502.F.1.A 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 9.2-13: 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
Bird, 100 g	Prothio- conazole	0.009	2.270	0.020	1.05	0.021	NOEL	78	3636	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 birds can be concluded. No further considerations have to be taken into account.

JAU-desthio (M4)

A log P_{OW} of 3.04 was determined for the metabolite JAU-desthio (M4) corresponding to a P_{OW} of 1096.5. 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 $PEC_{tw(21-d)}$ 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 $PEC_{tw(21-d)}$ in soil of 0.018 mg met./kg soil_{dw} was calculated for the intended GAP uses of ADM.03502.F.1.A 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 9.2-14: Daily Dietary Dose (DDD) and Tier-1 TER_{LT} for earthworm-eating birds

Species	PEC _{(tw(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
Bird, 100 g	JAU-desthio (M4)	0.022 0.018	1.216	0.026 0.022	1.05	0.028 0.024	NOEL	14.8	528.6 643.8	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 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 $PEC_{tw(21-d)}$ 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 $PEC_{tw(21-d)}$ in soil of 0.006 mg met./kg soil_{dw} was calculated for the intended GAP uses of ADM.03502.F.1.A 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 9.2-15: Daily Dietary Dose (DDD) and Tier-1 TER_{LT} for earthworm-eating birds

Species	PEC _{tw(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
Bird, 100 g	JAU-S-methyl (M1)	0.007 ₆	3.652	0.025 ₂	1.05	0.026 ₃	NOEL	7.8 ²⁾	300 _{339.0}	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.

Fenpropidin

A log P_{OW} of 4.5 at pH 9 was determined for fenpropidin corresponding to a P_{OW} of 31623. Using this P_{OW} , the K_{OC} of 3808 (for details, see EFSA Scientific report (2007) 124, 1-84) and a default value of 0.02 for f_{OC} , the calculated bioconcentration factor in worms is 4.994. 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_{accum} in soil of 0.069 mg a.s./kg soil_{dw} (absolute worst case) was calculated for the intended GAP uses of ADM.03502.F.1.A 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 9.2-16: Daily Dietary Dose (DDD) and Tier-1 TER_{LT} for earthworm-eating birds

Species	PEC_{accum} [mg/kg]	BCF	PEC_{worm} [mg/kg]	f_{conv}	DDD [mg/kg bw/d]	Endpoint [mg/kg bw/d]	TER_{LT} ¹⁾	TER trigger
Bird, 100 g	Fenpropidin	0.069	4.994	0.345	1.05	0.362	NOEL 14.6	40.4 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 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 3: Calculation of Daily Dietary Dose for fish-eating birds and mammals

DDD = $PEC_{fish} \cdot f_{conv}$		[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

Prothioconazole and its metabolites

The maximum FOCUS Step-2 PEC_{tw,21d} (absolute worst-case approach) 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) 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 9.2-17: Daily Dietary Dose (DDD) and Tier-1 TER_{LT} for fish-eating birds

Species	Max. FOCUS Step-2 PEC _{tw, 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
Bird, 1000 g	Prothioconazole 0.17 ²⁾ 0.24²⁾	19.7	0.0033 ²⁾ 0.0035	0.159	0.0005 ²⁾ 0.0005	NOEL 78	156.00 103758	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_{tw,21d} for the parent compound in winter cereals at BBCH 30

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 9.2-18: Daily Dietary Dose (DDD) and Tier-1 TER_{LT} for fish-eating birds

Species	Max. FOCUS Step-2 PEC _{tw, 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
Bird, 1000 g	JAU-desthio (M4) 2.70 ²⁾ 5.8²⁾	65	0.175 ²⁾ 0.175	0.159	0.028 ²⁾ 0.060	NOEL 14.8	528.57 246.9	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_{tw,21d} for the metabolite in winter cereals at BBCH 30

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 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 9.2-19: 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 9.2-20: BCF model outputs

Compound	Estimated BCF (EPISUITE/BCFBAF v3.01) (L/kg wet wt)	Reference
JAU-S-methyl (M1)	Regression based: BCF = 319.3 Arnot-Grobas, mid-trophic: BCF = 800.1	EPISUITE 4.1

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 9.2-21: 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
Bird, 1000 g	JAU-S-methyl (M1)	319.3	0.198 0.037	0.159	0.031 0.006	NOEL 7.8 ³⁾	251.61 1336	5
		800.1	0.50 0.092		0.08 0.015		97.7 533.2	

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 winter cereals at BBCH 30

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.

Fenpropidin

The maximum Step-2 PEC_{twa,21d} for fenpropidin as well as the BCF value of 163 was considered for the calculation of the corresponding PEC_{fish} values. 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.

Table 9.2-22: 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
Bird, 1000 g	Fenpropidin	1.99 4.54²⁾	163	0.32 0.74	0.159	0.05 0.118	NOEL	14.6	292 124.1	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 parent compound in winter cereals at BBCH 30

As outlined in the table above, the TER_{LT} value for fenpropidin 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 PEC_s 21 d TWA 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.03502.F.1.A in cereals, 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.

The acute and long-term combined risk from mixture of both active substances as well as a.s-fenpropidin and prothioconazole metabolite JAU-desthio (M4) was considered acceptable.

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.03502.F.1.A.

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 substances prothioconazole and fenpropidin as well as the prothioconazole metabolite JAU-desthio (M4). Full details of these studies are provided in the respective EU DAR and related documents.

Effects on mammals of ADM.03502.F.1.A were not evaluated as part of the EU assessment of the active substances. New data submitted with this application are listed in Appendix 1 and summarised in Section 6 (Mammalian Toxicology) of this report.

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

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
Rat	Fenpropidin technical	Acute toxicity	LD ₅₀ = 1452 mg a.s./kg bw	EFSA Scientific Report (2007) 124, 1-84
Rat	Fenpropidin technical	Reproductive toxicity	NOAEL = 500 mg a.s./kg feed corresp. 60.25 mg a.s./kg bw/d	EFSA Scientific Report (2007) 124, 1-84

zRMS comments:

Mammal toxicity data for fenpropidin, prothioconazole and prothioconazole metabolite JAU 6476-desthio provided in Table 9.3-1 above were validated by zRMS and confirmed that they are in line with EU agreed endpoints reported in EFSA Journal (2007) 124, 1-84 and EFSA Scientific Report (2007) 106, respectively.

Acute toxicity

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 substances are used in preference to data from tests with the formulated material. Exposure to ADM.03502.F.1.A 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 addition, an acute toxicity study with the product is performed for purposes of classification and labelling of the product and is thus not suitable for the derivation of a precise LD₅₀ used for the ecotoxicological risk assessment. Therefore, and for the reason given in the paragraph above, the EU agreed endpoints determined for the active substances should preferably be used as key endpoints for the risk assessment.

Metabolites

JAU-desthio (M4) was considered to be the only major metabolite in crop foliage (EFSA Scientific Report (2007) 106) and an acute toxicity study is 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 LD₅₀ of 2235 mg/kg bw in the acute risk assessment for the metabolite JAU-desthio (M4).

Mixture toxicity

The predicted acute mixture toxicity conservatively assuming dose additivity of the active substances (based on the worst-case assumption that the active substances have the same mode of action) was calculated using the formula already considered in the risk assessment for birds (for details, see Equation 9-1). In addition, in order to investigate whether the toxicity to mammals is driven by one active substance, the toxicity per fraction (a.s.), defined as LD₅₀(a.s.) divided by x(a.s.), was compared to the predicted LD₅₀(mix).

Where this ratio is ≥ 90 % for one of the active substances (or metabolites), this indicates that the compounds contained in the formulation will contribute to ≥ 90 % to mixture toxicity, while the other(s) of the mixture will only have a marginal impact on the predicted risk. In those cases, calculations of TER_A values should be based on endpoints related to the individual compounds only. Accordingly, the toxicity data presented below indicate that none of the compounds contribute to ≥ 90 % to mixture toxicity to mammals:

Table 9.3-2: LD₅₀(mix) for mammals

Test item	LD ₅₀ (a.s.) [mg/kg bw]	Nominal content in the formulation [g a.s./L]	x(a.s.) *	Toxicity per fraction	LD ₅₀ (mix) [mg/kg bw]	Contribution to mixture toxicity
Prothioconazole	> 6200	175	0.41	15057.1	> 2121	14.1 %
Fenpropidin	1452	250	0.59	2468.4		85.9 %
JAU-desthio	2235	175	0.41	5427.9	1697	31.3 %
Fenpropidin	1452	250	0.59	2468.4		68.7 %

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

** For prothioconazole metabolite JAU-desthio the application rate of the parent compound was considered – representing an absolute worst-case approach

In conclusion, the risk assessment for mammals should be based on data on the individual active substances as well as on mixture toxicity (prothioconazole/fenpropidin as well as JAU-desthio/fenpropidin). Accordingly, the acute oral LD₅₀ > 6200 mg prothioconazole/kg bw, the LD₅₀ = 2235 mg JAU-desthio/kg bw, the LD₅₀ of 1452 mg fenpropidin/kg bw as well as the LD₅₀(mix) > 2121 mg/kg bw (prothioconazole/fenpropidin) and 1697 mg/kg bw (JAU-desthio/fenpropidin) were considered as the most relevant endpoints for the TER_A calculations. This approach is in line with the recommendations from Appendix B of the EFSA Journal 2009; 7(12): 1438.

Reproductive effects

Metabolites

JAU-desthio (M4) was considered to be the only major metabolite in crop foliage (EFSA Scientific Report (2007) 106) and a chronic toxicity study is 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 NOAEL of 10 mg/kg bw/d in the reproductive risk assessment for the prothioconazole metabolite JAU-desthio (M4).

Mixture toxicity

With respect to the potential for combined long-term effects, it should be noted that for the approach assuming dose additivity of the active substances, reliable results would only be expected for combinations of effect levels with a defined x, e.g. a LD₅₀, but not for NOELs since the latter effect indicators may represent varying risk or response levels for different compounds depending on dose-spacing (EFSA Journal 2009; 7(12): 1438, Appendix B).

Furthermore, it is considered unlikely that the active substances and all the co-formulants will remain intact over a long-term period in relevant matrices, i.e. plants, animals, soil and water. Therefore, it is unlikely that terrestrial vertebrates could be exposed for a prolonged period to both prothioconazole and fenpropidin at the same time. Accordingly, for the risk assessment based on long-term effects it is not recommended to consider the use of predicted toxicity values.

Nevertheless, for maximum conservatism and because the predicted long-term mixture toxicity (using the same approach provided in Equation 9-1) indicates that none of the compounds contribute > 90 % to the mixture toxicity, the risk assessment for mammals was performed based on data of the individual compounds as well as on mixture toxicity. Accordingly, the reproductive NOAEL of 95.6 mg prothioconazole/kg bw/d, 10 mg JAU-desthio/kg bw/d, 60.25 mg fenpropidin/kg bw/d as well as the NO(A)EL_(mix) of 71.1 mg/kg bw/d (prothioconazole/fenpropidin) and 19.6 mg/kg bw/d (JAU-desthio/fenpropidin) were considered as the most relevant endpoints for TER_{LT} calculations.

Table 9.3-3: NO(A)EL_(mix) for mammals

Test item	NO(A)EL (a.s.) [mg/kg bw/d]	Nominal content in the formulation [g a.s./L]	x(a.s.)*	Toxicity per fraction	NO(A)EL _(mix) [mg/kg bw/d]	Contribution to mixture toxicity
Prothioconazole	95.6	175	0.41	232.2	71.1	30.6 %
Fenpropidin	60.25	250	0.59	102.4		69.4 %
JAU-desthio	10	175	0.41	24.3	19.6	80.8 %
Fenpropidin	60.25	250	0.59	102.4		19.2 %

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

** For prothioconazole metabolite JAU-desthio the application rate of the parent compound was considered—representing an absolute worst-case approach

zRMS comments:

Combined acute toxicity

The LD_{50mix} presented in Table 9.3-2 has been validated by the zRMS and it is confirmed to be correct.

Combined long-term toxicity

zRMS agrees that for the approach assuming dose additivity of the active substances, reliable results would only be expected for combinations of effect levels with a defined x, e.g. a LD₅₀, but not for NOELs since the latter effect indicators may represent varying risk or response levels for different compounds depending on dose-spacing according to GD B&M, EFSA Journal 2009; 7(12): 1438, Appendix B.

Therefore, for the risk assessment based on long-term effects it is not recommended to consider the use of predicted toxicity values.

Therefore, the calculated NOEL_{mix} was not considered by zRMS in the current risk assessment.

It should be noted that according to recommendation given in Appendix B of the Guidance Document 2009 for the evaluation of sublethal effects, the use of the lowest NO(A)EL of the actives in the formulation, along with the combined exposure estimate from both active substances provides a conservative representation of long-term risks to mammals.

Approach taken with regard to the long-term combined risk assessment represents worst case for mixture of active substances and is in general acceptable.

However, the combined long-term risk assessment should also include metabolite JAU 6476-desthio which is more than 5 times more toxic than prothioconazole. Taking this into account, the combined chronic risk to all three compounds would be covered when based on NOAEL of 10 mg pm/kg bw/d, derived for metabolite JAU 6476-desthio, and cumulative application rate of both active compounds (i.e. 425 g/ha). It is noted that as even with immediate and complete conversion of prothioconazole to JAU 6476-desthio, its concentration in the mixture will never exceed the concentration of the parent, i.e. 175 g/L. The combined risk assessment was amended accordingly in points below.

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.03502.F.1.A is an emulsifiable concentrate (EC) containing 175 g/L of the active substance prothioconazole and 250 g/L of the active substance fenpropidin. It is a fungicide applied as spray to infested foliage of cereals. The timing of application is post-emergence. The worst-case application scenario leading to maximum contamination of the environment is a single spray application at a rate of 1.0 L prod./ha (corresponding to 175 g prothioconazole/ha and 250 g fenpropidin/ha). For a detailed summary of the GAP uses of ADM.03502.F.1.A, 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.03502.F.1.A. A summary of the intended uses and relevant mammalian indicator species is given in the table below.

Table 9.3-4: Worst-case GAP use of ADM.03502.F.1.A 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, BBCH 30-65, 1× 1.0 L prod./ha [equivalent to 1× 250 g fenpropidin/ha + 1× 175 g prothioconazole/ha]	Small herbivorous mammal	118.4 / 48.3

Exposure of terrestrial vertebrates to ADM.03502.F.1.A 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 90th 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 com-

pared 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.03502.F.1.A. 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 9.3-5: Prothioconazole - screening assessment of the acute and long-term risk for mammals due to the use of ADM.03502.F.1.A in cereals

Intended use	Cereals, 1×1.0 L prod./ha, BBCH ≥ 10				
Active substance	Prothioconazole				
Application rate (g/ha)	1×175				
MAF	1.0				
Acute toxicity (mg/kg bw)	> 6200				
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	299.2
Long-term toxicity (mg/kg bw/d)	95.6				
TER criterion	5				
Crop scenario Growth stage	Indicator species	SV_m	$MAF_m \times TWA$	DDD_m (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 cereals already at screening level. Thus, no further refinements at Tier-1 level are required for prothioconazole.

Fenpropidin

Table 9.3-6: Fenpropidin - screening assessment of the acute and long-term risk for mammals due to the use of ADM.03502.F.1.A in cereals

Intended use	Cereals, 1×1.0 L prod./ha, BBCH ≥ 10				
Active substance	Fenpropidin				
Application rate (g/ha)	1×250				
MAF	1.0				
Acute toxicity (mg/kg bw)	1452				
TER criterion	10				
Crop scenario Growth stage	Indicator species	SV_{90}	MAF_{90}	DDD_{90} (mg/kg bw/d)	TER_A

Intended use	Cereals, 1 × 1.0 L prod./ha, BBCH ≥ 10					
Active substance	Fenpropidin					
Application rate (g/ha)	1 × 250					
MAF	1.0					
BBCH > 10	Small herbivorous mammal	118.4	1.0	29.6	49.1	
Long-term toxicity (mg/kg bw/d)	60.25					
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	6.4	9.4	

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 fenpropidin 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 fenpropidin.

JAU-desthio (M4)

Table 9.3-7: JAU-desthio (M4) - screening assessment of the acute and long-term risk for mammals due to the use of ADM.03502.F.1.A in cereals

Intended use	Cereals, 1 × 1.0 L prod./ha, BBCH ≥ 10					
Metabolite	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	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_A	
BBCH > 10	Small herbivorous mammal	118.4	1.0	20.7	107.9	

Intended use	Cereals, 1× 1.0 L prod./ha, BBCH ≥ 10				
Metabolite	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	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 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.

zRMS comments:

Screening step in the risk assessment

The screening step risk assessment for both active substances and prothioconazole metabolite JAU 6476-desthio is agreed 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 mammals.

TER_A and TER_{LT} values for the exposure to fenpropidin are above the trigger of 10 and 5 for acute and long-term exposure, indicating acceptable risk for mammals.

Based on the calculation provided above the TER_A for acute exposure for prothioconazole metabolite JAU 6476-desthio is above trigger value of 10 but long-term exposure from this metabolite needs Tier 1 risk assessment.

Prothioconazole/fenpropidin – Mixture toxicity

Table 9.3-8: Mixture toxicity (prothioconazole/fenpropidin) – screening assessment of the acute and long-term risk for mammals due to the use of ADM.03502.F.1.A in cereals

Intended use	Cereals, 1× 1.0 L prod./ha [equivalent to 1× 175 g prothioconazole + 250 g fenpropidin/ha], BBCH ≥ 10				
Active substance	Prothioconazole + fenpropidin				
Application rate (g/ha)	1× 425 (sum a.s.)				
MAF	1.0				
Acute toxicity (mg/kg bw)	> 2121 (LD _{50, mixt} prothioconazole/fenpropidin)				
TER criterion	10				
Crop scenario Growth stage	Indicator species	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_A
BBCH > 10	Small herbivorous mammal	118.4	1.0	50.3	> 42.2
Long-term toxicity (mg/kg bw/d)	71.1 (NOAEL _{mixt} prothioconazole/fenpropidin)				
TER criterion	5				

Intended use	Cereals, 1 × 1.0 L prod./ha [equivalent to 1 × 175 g prothioconazole + 250 g fenpropidin/ha], BBCH ≥ 10				
Active substance	Prothioconazole + fenpropidin				
Application rate (g/ha)	1 × 425 (sum a.s.)				
MAF	1.0				
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	10.9	6.5

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/fenpropidin are above the trigger of 10 and 5, established for acute and long-term exposure, indicating an acceptable risk for mammals in cereals already at screening level. Thus, no further refinements are considered to be required for mixture toxicity.

Prothioconazole /JAU-desthio/fenpropidin - Mixture toxicity

Table 9.3-9: Mixture toxicity (prothioconazole/ JAU 6476-desthio /fenpropidin) - screening assessment of the acute risk for mammals due to the use of ADM.03502.F.1.A in cereals

Intended use	Cereals, 1 × 1.0 L prod./ha [equivalent to 1 × 175 g JAU-desthio* + 250 g fenpropidin/ha], BBCH ≥ 10				
Active substance	Prothioconazole/JAU-desthio + fenpropidin				
Application rate (g/ha)	1 × 425				
MAF	1.0				
Acute toxicity (mg/kg bw)	1697 (LD _{50, mix} ; JAU-desthio/fenpropidin)				
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	50.3	33.7

SV: shortcut value; MAF: multiple application 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 value for the exposure to /JAU-desthio/fenpropidin are above the trigger of 10, indicating an acceptable acute risk for mammals in cereals already at screening level. Thus, no further refinements are considered to be required for acute mixture toxicity.

Since the long-term risk for mammals exposed to JAU-desthio is not acceptable at screening step, no long-term mixture toxicity was addressed at this level.

zRMS comments:

Combined acute risk assessment

LD₅₀ mix with consideration of relevant toxicity endpoint for the metabolite (for details, see commenting box in point 9.3.1 above) and the acute risk assessment in Table 9.3-9 has been accepted by zRMS.

With regard to the exposure, assumed application rate of prothioconazole accounts also for its conversion to JAU 6476-desthio, as even with immediate and complete conversion the its concentration in the mixture will never exceed the concentration of the parent, i.e. 175 g/L.

It should be noted that calculation of mixture toxicity with regard acute combined toxicity endpoint for metabolite and fenpropidin covers the acute risk assessment for acute combined risk from prothioconazole and fenpropidin.

Based on performed calculation, acceptable combined acute risk to mammals exposed to the mixture of both active compounds and metabolite JAU 6476-desthio may be concluded.

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.03502.F.1.A. A summary of the critical GAP uses and relevant mammalian indicator species is given in the table below.

Table 9.3-10: Critical use pattern of ADM.03502.F.1.A 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 30-65, 1× 1.0 L prod./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

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 9.3-11: JAU-desthio (M4) - Tier-1 assessment of the long-term risk for mammals due to the use of ADM.03502.F.1.A in cereals

Intended use	Cereals, 1× 1.0 L prod./ha, BBCH 30–65				
Metabolite	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}
Cereals, BBCH ≥ 20	Small insectivorous mammal (shrew)	1.9	0.53	0.17 0.2	58.82 56.7
Cereals, BBCH ≥ 40	Small herbivorous mammal (vole)	21.7	0.53	2.0	5.0
Cereals, BBCH 30-39	Small omnivorous mammal (mouse)	3.9	0.53	0.36 0.4	27.76
Cereals, BBCH ≥ 40	Small omnivorous mammal (mouse)	2.3	0.53	0.2	50 46.9

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

Table 9.3-12-1: Prothioconazole - Tier-1 assessment of the long-term risk for mammals due to the use of ADM.03502.F.1.A in cereals

Intended use	Cereals, 1 × 1.0 L prod./ha, BBCH 30–65				
Metabolite	Prothioconazole				
Application rate (g/ha)	1 × 175				
MAF	1.0				
Long-term toxicity (mg/kg bw/d)	95.6				
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	0.18	531.1
Cereals, BBCH ≥ 40	Small herbivorous mammal (vole)	21.7	0.53	2.01	47.6
Cereals, BBCH 30-39	Small omnivorous mammal (mouse)	3.9	0.53	0.36	265.5
Cereals, BBCH ≥ 40	Small omnivorous mammal (mouse)	2.3	0.53	0.21	455.2

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

Table 9.3-13-2: Fenpropidin - Tier-1 assessment of the long-term risk for mammals due to the use of ADM.03502.F.1.A in cereals

Intended use	Cereals, 1 × 1.0 L prod./ha, BBCH 30–65				
Metabolite	Fenpropidin				
Application rate (g/ha)	1 × 250				
MAF	1.0				
Long-term toxicity (mg/kg bw/d)	60.25				
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	0.25	241
Cereals, BBCH ≥ 40	Small herbivorous mammal (vole)	21.7	0.53	2.87	21
Cereals, BBCH 30-39	Small omnivorous mammal (mouse)	3.9	0.53	0.51	118.1
Cereals, BBCH ≥ 40	Small omnivorous mammal (mouse)	2.3	0.53	0.30	200.3

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

zRMS comments:

Tier 1 risk assessment

The Tier 1 risk a long-term risk assessment for prothioconazole metabolite JAU 6476-desthio was verified by the zRMS and then considered acceptable.

The Tier 1 risk assessment has been added by the zRMS as being necessary for evaluation of the long-term combined risk.

As outlined in the table above, the calculated TER_{LT} values for the exposure to JAU-desthio (M4) are above the trigger of 5 (or for the vole: meet the trigger of 5), established for long-term exposure, indicating an acceptable risk for mammals in cereals at Tier-1 (even under still absolute worst-case exposure assumptions). Thus, no further refinements are required for the metabolite.

Mixture toxicity

Table 9.3-14: Mixture toxicity (JAU-desthio/fenpropidin/prothioconazole) - Tier-1 assessment of the long-term risk for mammals due to the use of ADM.03502.F.1.A in cereals

Intended use	Cereals, 1× 1.0 L prod./ha [equivalent to 1× 175 g prothioconazole/ha + 1× 250 g fenpropidin/ha], BBCH 30 – 65				
Metabolite/active substance	JAU-desthio + fenpropidin/ prothioconazole				
Application rate (g/ha)	1× 425				
MAF	1.0				
Long-term toxicity (mg/kg bw/d)	10 (JAU 6476-desthio) ^a 49.6				
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	0.4	25 45.8
Cereals, BBCH ≥ 40	Small herbivorous mammal (vole)	21.7	0.53	4.9	2.04 4.0
Cereals, BBCH 30-39	Small omnivorous mammal (mouse)	3.9	0.53	0.9	11.11 22.3
Cereals, BBCH ≥ 40	Small omnivorous mammal (mouse)	2.3	0.53	0.5	20 37.8

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

^a) The lowest NOEL of the two substances and JAU 6476-desthio is 10 mg pm/kg bw reported for JAU 6476-desthio. This endpoint is applied to the reproductive risk assessment for the mixture of all relevant compounds.

As outlined in the table above, almost all calculated TER_{LT} values for this mixture toxicity scenario (JAU-desthio/fenpropidin/prothioconazole) are above the trigger of 5, established for long-term exposure, indicating an acceptable long-term risk for mammals in cereals at Tier-1 level. By contrast, the TER_{LT} value for the small herbivorous mammal “vole” is below the trigger. Thus, further refinements are considered to be required for mixture toxicity.

However, it should be noted that TER calculations above were conducted for the metabolite JAU-desthio (M4) 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 9.3-15: Mixture toxicity (JAU-desthio/fenpropidin) - Tier-1 assessment of the long-term risk for mammals due to the use of ADM.03502.F.1.A in cereals based on actual percentage of JAU-desthio (M4) in monocotyledonous plants

Intended use	Cereals, 1× 1.0 L prod./ha [equivalent to 1× 175 g prothioconazole/ha + 1× 250 g fenpropidin/ha], BBCH 30 – 65
Metabolite/active substance	JAU-desthio + fenpropidin
Application rate (g/ha)	1× 311.25*
MAF	1.0

Long-term toxicity (mg/kg bw/d)	19.6				
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	3.6	5.5

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 $175 \times 0.35 = 61.25$ g/ha was considered

Taking this into account, the TER value for the small herbivorous mammal “vole” is 5.5 and thus above the trigger. In conclusion, an acceptable risk can also be concluded for this JAU-desthio/fenpropidin scenario. 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.

zRMS comments:

The application dose refinement for the metabolite JAU-6476-desthio was not considered by zRMS. TER_{LT} value for the originally proposed application dose as the worst case is preferred by zRMS to refine the combined risk to vole at BBCH >40.

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: Deposition values reported in the latest ‘Generic Guidance for Tier-1 FOCUS Ground Water Assessment’ (vers. 2.2; May 2014) are used for refinement purposes. Based on the updated crop interception values, it is deemed acceptable to consider a f_{dep} of 0.1 instead of 0.3 for cereal crop stages at BBCH 40-65 (growth stages relevant for the risk assessment of the common vole).

Table 9.3-16: Mixture toxicity (JAU-desthio/fenpropidin) - Tier-2 assessment of the long-term risk for mammals due to the use of ADM.03502.F.1.A in cereals

Intended use	Cereals, 1×1.0 L prod./ha [equivalent to 1×175 g prothioconazole/ha + 1×250 g fenpropidin/ha], BBCH 30 – 65								
Metabolite/active substance	JAU-desthio + fenpropidin/Prothioconazole								
Application rate (g/ha)	$1 \times 425^*$								
MAF	1.0								
Long-term toxicity (mg/kg bw/d)	10 19.6								
TER criterion	5								
Generic focal species	Food item	FIR/bw	RUD	A_{MAF}	TWA	f_{dep}	PT	DDD_m (mg/kg bw/d)	TER_{LT}
Small herbivore (vole), BBCH ≥ 40	100 % grass	1.33	54.2	0.425	0.53	0.1	1	1.6	6.25 12.1

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 this mixture toxicity scenario (JAU-

desthio/fenpropidin/Prothioconazole) is above the trigger of 5, established for long-term exposure, indicating an acceptable long-term risk for the small mammal “vole” in cereals. No further considerations have to be taken into account.

Furthermore, it should be noted that 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 DT₅₀ of 10 d) was determined for JAU-desthio (M4) indicated by a mean foliar DT₅₀ of 3.2 days (n = 8 trials). Thus, it would be deemed acceptable to use this refined DT₅₀ for the re-calculation of the Time-Weighted Average Factor (f_{twa}). In conclusion, the use of a f_{twa} of 0.22 instead of the default value of 0.53 would be deemed appropriate.

zRMS comments:

Refined of combined long-term risk assessment

The refinement of combined long-term risk assessment presented in the Table above for fenpropidin, prothioconazole and prothioconazole metabolite JAU 6476-desthio together with the cumulative application rate of the active substances with the lowest of NOEL value of 10 mg pm /kg and with consideration fdep of 0.1 instead of 0.3 value for cereals crop stages at BBCH 40-65 (growth stages relevant for the common vole) indicated an acceptable risk.

For concerned Member States preferring simplified for each active substance approach (TER_{mix}), respective calculation based on the lowest TER_{LT} values is presented below.

TER_{mix} values based on TER_{LT} (Tier 1) values for each active substance.

TER values based on TER1 (TER1) values for each active substance:						Σ1/TER	Σ1/TER ⁻¹	Trigger
Fenpropidin		Prothioconazole		JAU 6476-desthio				
21 ¹⁾	0.047	47.6 ¹⁾	0.021	5 ¹⁾	0.2	0.268	3.73 ¹	5

¹⁾ the lowest TER_{LT} at Tier 1 for vole BBCH >40

Based on the calculations of TER_{mix} with consideration of TER_{LT} values at Tier 1, the trigger value is below 5.

The refinement of combined long-term risk assessment for fenpropidin, prothioconazole and prothioconazole metabolite JAU 6476-desthio together with consideration of fdep of 0.1 (reported in the latest ‘Generic Guidance for Tier-1 FOCUS Ground Water Assessment’ (vers. 2.2; May 2014)) instead of 0.3 value for cereals crop stages at BBCH 40-65 has been considered by zRMS.

The relevant calculations are provided below:

Tier-2 assessment of the long-term risk for vole due to the use of fenpropidin in ADM.03502.F.1.A in cereals.

Intended use	Cereals, 1× 1.0 L prod./ha [equivalent to 1× 175 g prothioconazole/ha + 1× 250 g fenpropidin/ha], BBCH 30 – 65								
Metabolite/active substance	Fenpropidin								
Application rate (g/ha)	1× 250								
MAF	1.0								
Long-term toxicity (mg/kg bw/d)	60.25								
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	0.25	0.53	0.1	1	0.95	63.42

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

Tier-2 assessment of the long-term risk for vole due to the use prothioconazole in ADM.03502.F.1.A in cereals.

Intended use	Cereals, 1× 1.0 L prod./ha [equivalent to 1× 175 g prothioconazole/ha + 1× 250 g fenpropidin/ha], BBCH 30 – 65
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Metabolite/active substance	Prothioconazole								
Application rate (g/ha)	1× 175								
MAF	1.0								
Long-term toxicity (mg/kg bw/d)	95.6								
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	0.175	0.53	0.1	1	0.67	142.6

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

Tier-2 assessment of the long-term risk for vole due to the use of JAU-desthio in ADM.03502.F.1.A in cereals.

Intended use	Cereals, 1× 1.0 L prod./ha [equivalent to 1× 175 g prothioconazole/ha + 1× 250 g fenpropidin/ha], BBCH 30 – 65								
Metabolite/active substance	JAU-desthio								
Application rate (g/ha)	1× 175								
MAF	1.0								
Long-term toxicity (mg/kg bw/d)	10								
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	0.25	0.53	0.1	1	0.67	14.92

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

TER_{mix} for vole at BBCH> 40 based on refined TER_{LT} values.

TERmix for vol at BBCH 40 based on refined TER1 values.						Σ1/TER	Σ1/TER ⁻¹	Trigger
Fenpropidin		Prothioconazole		JAU 6476-desthio				
63.42 ¹⁾	0.015	142.6 ¹⁾	0.007	14.92 ¹⁾	0.067	0.09	11.11 ¹⁾	5

¹⁾ TER_{LT} values calculated with fdep=0.1 at BBCH >40

The refinement of combined long-term risk assessment for fenpropidin, prothioconazole and prothioconazole metabolite JAU 6476-desthio together with consideration of fdep of 0.1 instead of 0.3 value for cereals crop stages at BBCH 40-65 (growth stages relevant for the common vole) indicating an acceptable risk.

Overall, based on performed calculations of refined TER_{mix} value acceptable combined long-term risk may be concluded for mammals.

9.3.2.3 Drinking water exposure

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

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

With $K(f)_{oc} > 500$, the active substances prothioconazole ($K_{oc} = 1765$) and fenpropidin ($K_{oc} = 3808$) as well as the metabolite JAU-desthio (M4) ($K_{oc} = 523$ - 625) belong to the group of less 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 1×175 g prothioconazole/ha, 1×175 g JAU-desthio (M4)/ha and 1×250 g fenpropidin/ha) covers the risk for water-drinking mammals from all intended GAP uses of ADM.03502.F.1.A (for details, see point 9.1.1).

Prothioconazole

Effective application rate (g/ha)	=	1×175		
Acute toxicity (mg/kg bw)	=	> 6200	quotient =	< 0.1
Reprod. toxicity (mg/kg bw/d)	=	95.6	quotient =	1.8

Fenpropidin

Effective application rate (g/ha)	=	1×250		
Acute toxicity (mg/kg bw)	=	1452	quotient =	0.2
Reprod. toxicity (mg/kg bw/d)	=	60.25	quotient =	4.1

JAU-desthio (M4)

Effective application rate (g/ha)	=	1×175		
Acute toxicity (mg/kg bw)	=	2235	quotient =	0.1
Reprod. toxicity (mg/kg bw/d)	=	10	quotient =	17.5

In order to apply consistent approach, the drinking water risk assessment was performed also for metabolite JAU 6476-S-methyl and is presented below. Calculations were performed with assumption of 10 times toxicity of the parent.

JAU 6476-S-methyl effective application rate 1×175 g/ha

Acute toxicity (mg/kg bw)	620	quotient	=	0.28	Trigger: 3000
Reprod. toxicity (mg/kg bw/d)	9.56	quotient	=	18.30	

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:

~~Since is not a for-spray-applications / 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.~~
Therefore, The leaf scenario does not have to be considered taking into account the proposed uses (cereals).
The evaluation of the risk resulting from uptake of contaminated water for Puddle scenario was not required since ratio between effective application rate and endpoint relevant for acute risk and long-term assessment is <3000.

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 substances prothioconazole and fenpropidin as well as for the prothioconazole metabolites JAU-desthio (M4) and JAU-S-methyl (M1) ($\log P_{ow} > 3$). By contrast, no potential is indicated for the fenpropidin metabolite CGA 289263 with respect to a $\log P_{ow} < 3$. 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 2 (p. 34), 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 9.3-17: 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
Mammal, 10 g	Prothio- conazole	0.009	2.270	0.020	1.28	0.026	NOAEL	96.5	3691	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 9.3-18: 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
Mammal, 10 g	JAU-desthio (M4)	0.022 0.018	1.216	0.027 0.023	1.28	0.034 0.028	NOAEL	10.0	294.1 256.8	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 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 9.3-19: 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
Mammal, 10 g	JAU-S-methyl (M1)	0.0075 0.007	3.652	0.022	1.28	0.032 0.028	NOAEL	9.65 ²⁾	301.56 344.1	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.

Fenpropidin

Table 9.3-20: 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
Mammal, 10 g	Fenpro- pidin	0.069	4.994	0.345	1.28	0.441	NOEL	60.25	136.6	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.

Food chain from fish to fish-eating mammals

Estimated theoretical exposure of fish-eating mammals was calculated with Equation 3 (p. 37), 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 9.3-21: 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
Mammals, 3000 g	Prothioconazole 0.17 ²⁾ 0.24²⁾	19.7	0.0033 0.005	0.142	0.0005 0.001	NOEL 96.5	191200 143735	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 parent compound in winter cereals at BBCH 30

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 9.3-22: 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
Mammals, 3000 g	JAU-desthio (M4) 2.70 5.8²⁾	65	0.1755 0.377	0.142	0.025 0.054	NOEL 10	400 186.8	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 winter cereals at BBCH 30

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 9.3-23: 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
Mammals, 3000 g	JAU-S-methyl (M1) 0.62 ²⁾ 0.115²⁾	319.3	0.198 0.037	0.142	0.028 0.005	NOEL 9.65 ³⁾	344.64 1851	5
		800.1	0.50 0.092		0.070 0.013		137.85 738.6	

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 winter cereals at BBCH 30

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.

Fenpropidin

Table 9.3-24: 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
Mammals, 3000 g	Fenpropidin 1.99 ²⁾ 4.54 ²⁾	163	0.32 0.74	0.142	0.046 0.105	NOEL 60.25	1309.8 573.4	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 parent compound in winter cereals at BBCH 30

As outlined in the table above, the TER_{LT} value for fenpropidin 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 PEC_s 21 d TWA 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.03502.F.1.A, 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. The acute and long-term combined risk from mixture of both active substances as well as for a.s-fenpropidin and prothioconazole metabolite JAU-desthio (M4) was considered acceptable.

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.03502.F.1.A.

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 substances 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.03502.F.1.A. 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.03502.F.1.A, 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, 2009³).

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, fenpropidin and JAU-desthio (M4) are sufficiently conservative for the terrestrial phase of amphibians and reptiles.

In conclusion, based on the uses intended for ADM.03502.F.1.A, 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 fenpropidin (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 substances and relevant metabolites in aquatic systems. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on aquatic organisms of ADM.03502.F.1.A were not evaluated as part of the EU assessment of the active substance. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

Table 9.5-1: Prothioconazole and relevant metabolite(s) in aquatic systems - endpoints and effect values relevant for the risk assessment for aquatic organisms

Species	Substance	Time scale	Results	Reference
Toxicity to fish				
<i>Oncorhynchus mykiss</i>	Prothioconazole technical	acute	LC ₅₀ = 1.83 mg a.s./L _{nom}	EFSA Scientific Report (2007) 106, 1-98
<i>Lepomis macrochirus</i>	Prothioconazole	96 h, s	LC ₅₀ = 4.59 mg a.s./L _{mm}	EFSA Scientific Report (2007) 106, 1-98
<i>Cyprinus carpio</i>	Prothioconazole	96 h, s	LC ₅₀ = 6.91 mg a.s./L _{mm}	EFSA Scientific Report (2007) 106, 1-98
<i>Oncorhynchus mykiss</i>	JAU-desthio (M4) (metabolite of prothioconazole)	acute	LC ₅₀ = 6.63 mg met./L _{nom}	EFSA Scientific Report (2007) 106, 1-98
<i>Leuciscus idus melanotus</i>	JAU 6476-desthio	96 h, s	LC ₅₀ = 13.2 mg met./L _{mm}	EFSA Scientific Report (2007) 106, 1-98
<i>Oncorhynchus mykiss</i>	JAU-S-methyl (M1) (metabolite of prothioconazole)	acute	LC ₅₀ = 1.8 mg met./L _{nom}	EFSA Scientific Report (2007) 106, 1-98
<i>Oncorhynchus mykiss</i>	1,2,4-Triazole (metabolite of prothioconazole)	acute	LC ₅₀ = 498 mg met./L _{nom}	EFSA Scientific Report (2007) 106, 1-98
<i>Oncorhynchus mykiss</i>	Prothioconazole technical	chronic, ELS	NOEC = 0.308 mg a.s./L	EFSA Scientific Report (2007) 106, 1-98
<i>Oncorhynchus mykiss</i>	JAU-desthio (M4) (metabolite of prothioconazole)	chronic, ELS	NOEC = 0.00334 mg met./L	EFSA Scientific Report (2007) 106, 1-98
<i>Oncorhynchus mykiss</i>	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				
<i>Daphnia magna</i>	Prothioconazole technical	acute	EC ₅₀ = 1.3 mg a.s./L _{nom}	EFSA Scientific Report (2007) 106, 1-98
<i>Daphnia magna</i>	JAU-desthio (M4) (metabolite of prothioconazole)	acute	EC ₅₀ > 10 mg met./L _{nom}	EFSA Scientific Report (2007) 106, 1-98

Species	Substance	Time scale	Results	Reference
<i>Daphnia magna</i>	JAU-S-methyl (M1) (metabolite of prothioconazole)	acute	EC₅₀ = 2.8 mg met./L_{nom}	EFSA Scientific Report (2007) 106, 1-98
<i>Daphnia magna</i>	1,2,4-Triazole (metabolite of prothioconazole)	acute	EC₅₀ = 900 mg met./L_{nom}	EFSA Scientific Report (2007) 106, 1-98
<i>Daphnia magna</i>	Prothioconazole technical	chronic	NOEC = 0.56 mg a.s./L_{nom}	EFSA Scientific Report (2007) 106, 1-98
<i>Daphnia magna</i>	JAU-desthio (M4) (metabolite of prothioconazole)	chronic	NOEC = 0.10 mg met./L_{nom}	EFSA Scientific Report (2007) 106, 1-98
Toxicity to sediment-dwelling organisms				
<i>Chironomus riparius</i>	Prothioconazole technical	chronic	NOEC = 9.14 mg a.s./L_{nom}	EFSA Scientific Report (2007) 106, 1-98
<i>Chironomus riparius</i>	JAU-desthio (M4) (metabolite of prothioconazole)	chronic	NOEC = 2.0 mg met./L_{nom}	EFSA Scientific Report (2007) 106, 1-98
Toxicity to algae				
<i>Pseudokirchneriella subcapitata</i>	Prothioconazole technical	Sub-chronic	E _b C ₅₀ = 1.1 mg a.s./L E_rC₅₀ = 2.18 mg a.s./L	EFSA Scientific Report (2007) 106, 1-98
<i>Scenedesmus subspicatus</i>	JAU-desthio (M4) (metabolite of prothioconazole)	Sub-chronic	E _b C ₅₀ = 0.073 mg met./L E_rC₅₀ = 0.55 mg met./L	EFSA Scientific Report (2007) 106, 1-98
<i>Pseudokirchneriella subcapitata</i>	JAU-S-methyl (M1) (metabolite of prothioconazole)	Sub-chronic	E _b C ₅₀ = 3.77 mg met./L E_rC₅₀ = 47.4 mg met./L	EFSA Scientific Report (2007) 106, 1-98
<i>Pseudokirchneriella subcapitata</i>	1,2,4-Triazole (metabolite of prothioconazole)	Sub-chronic	E _b C ₅₀ = 8.2 mg met./L* E_rC₅₀ = 22.5 mg met./L*	EFSA Scientific Report (2007) 106, 1-98
Fish bioconcentration				
<i>Lepomis macrochirus</i>	Prothioconazole	Bioconcentration	BCF 19.7 (Whole fish wet weight) Clearance time (CT ₅₀ days):0.8 Level of residues (%) after 14 days depuration phase: 9%	EFSA Scientific Report (2007) 106, 1-98
<i>Lepomis macrochirus</i>	JAU 6476-desthio	Bioconcentration	BCF 65 (Whole fish wet weight) Clearance time (CT ₅₀ days):0.4-0.5 Level of residues (%) after 14 days depuration phase: 4%	EFSA Scientific Report (2007) 106, 1-98

values **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).

Toxicity data presented above indicate that the prothioconazole metabolites 1,2,4-triazole and JAU-S-methyl (M1) which were considered potentially of concern in surface water show less toxicity compared to the parent compound. Concurrently, relevant PEC_{sw} values for these metabolites do not exceed the predicted concentrations in surface water calculated for the parent compound. Thus, either way (from both the toxicity and exposure point of view), it is reasonably concluded that the risk for aquatic organisms arising from 1,2,4-triazole and JAU-S-methyl (M1) is covered by prothioconazole. Consequently, separate TER calculations for these metabolites potentially of concern in surface water are not considered to be required. By contrast, toxicity studies show that JAU-desthio (M4) is of higher toxicity to algae and fish and thus this metabolite was addressed in the following risk assessment.

Table 9.5-2: Fenpropidin and relevant metabolite(s) in aquatic systems - endpoints and effect values relevant for the risk assessment for aquatic organisms

Species	Substance	Time scale	Results	Reference
Toxicity to fish				
<i>Lepomis macrochirus</i>	Fenpropidin technical	acute	LC ₅₀ = 1.9 mg a.s./L	EFSA Scientific Report (2007) 124, 1-84
<i>Oncorhynchus mykiss</i>	Fenpropidin technical	acute	LC ₅₀ = 2.6 mg a.s./L	EFSA Scientific Report (2007) 124, 1-84
<i>Cyprinus carpio</i>	Fenpropidin technical	acute	LC ₅₀ = 3.6 mg a.s./L	EFSA Scientific Report (2007) 124, 1-84
<i>Oncorhynchus mykiss</i>	CGA 289267 (metabolite of fenpropidin)	acute	LC ₅₀ > 100 mg met./L	EFSA Scientific Report (2007) 124, 1-84
<i>Oncorhynchus mykiss</i>	Fenpropidin technical	chronic	NOEC = 0.32 mg a.s./L	EFSA Scientific Report (2007) 124, 1-84
Toxicity to aquatic invertebrates				
<i>Daphnia magna</i>	Fenpropidin technical	acute	EC ₅₀ = 0.54 mg a.s./L	EFSA Scientific Report (2007) 124, 1-84
<i>Daphnia magna</i>	CGA 289267 (metabolite of fenpropidin)	acute	EC ₅₀ > 100 mg met./L	EFSA Scientific Report (2007) 124, 1-84
<i>Daphnia magna</i>	Fenpropidin technical	chronic	NOEC = 0.32 mg a.s./L	EFSA Scientific Report (2007) 124, 1-84
Toxicity to sediment-dwelling organisms				
<i>Chironomus riparius</i>	Fenpropidin technical	chronic	NOEC = 1.0 mg a.s./L (spiked-water)	EFSA Scientific Report (2007) 124, 1-84
<i>Chironomus riparius</i>	Fenpropidin technical	chronic	NOEC = 40 mg a.s./kg (spiked-sediment)	EFSA Scientific Report (2007) 124, 1-84
Toxicity to algae				
<i>Scenedesmus subspicatus</i>	Fenpropidin technical	Sub-chronic	E _b C ₅₀ = 0.0057 mg a.s./L E _r C ₅₀ = 0.0076 mg a.s./L	EFSA Scientific Report (2007) 124, 1-84 DAR (2006) for fenpropidin
<i>Navicula pelliculosa</i>	Fenpropidin technical	Sub-chronic	E _b C ₅₀ = 0.0008 – 0.002 mg a.s./L E _r C ₅₀ not reported	EFSA Scientific Report (2007) 124, 1-84
<i>Scenedesmus subspicatus</i>	Fenpropidin (applied as TERN 750 EC)	Sub-chronic	E _b C ₅₀ = 0.00016 mg a.s./L E _r C ₅₀ = 0.00033 mg a.s./L	EFSA Scientific Report (2007) 124, 1-84 DAR (2006) for fenpropidin
<i>Scenedesmus subspicatus</i>	CGA 289267 (metabolite of fenpropidin)	Sub-chronic	E _b C ₅₀ = 31 mg met./L E _r C ₅₀ = 69 mg met./L	EFSA Scientific Report (2007) 124, 1-84 DAR (2006) for fenpropidin

Species	Substance	Time scale	Results	Reference
Higher-tier studies (micro- or mesocosm studies)				
Multi species mesocosm with fenpropidin (applied as MCW-273 750 EC (750 fenpropidin/L) Due to clear temporary effects of the test item on some primary producers and photosynthesis in total at all test concentration, the general NOEC on the community and population level is < 0.3 µg as/L. The NOEAEC is considered to be 30 µg as/L because the only long-term effect observed at this concentration was a higher abundance of macrophytes which is likely a result of the experimental design but representative for the field situation			NOEAEC = 0.03 mg a.s./L NOEC on community level: < 0.3 µg a.s./L	KCP 10.2.3/01 Wellmann et al., 2006 Report no. FEI-010/4-52
Evaluation of all three submitted Mesocosm studies with the active substance fenpropidin to derive an overall NOEAEC <u>Neumann study 1997:</u> proposed NOEAEC = 0.39 µg a.s./L* If a class 3B is considered acceptable and a trend to class 5A is acceptable as well, the NOEAEC might be set at 6.8 µg fenpropidin a.s./L. * the lowest available NOEAEC is expressed in terms on measured concentrations 6 h post the first and second application, and consequently can be considered a worst-case estimate since the dissipation of fenpropidin from water is relatively fast (DT50 approximately 3.6 days). <u>Huber study:</u> Based on Effect class 3A and taking into account the trends in effects and recovery, the proposed NOEAEC of 0.55 µg fenpropidin a.s./L. These conclusions are only indicative for the evaluation of the effects of fenpropidin. <u>Wellmann study:</u> Based on class 3A effects on other endpoints than macrophytes are considered acceptable a NOEAEC of 1.0 µg fenpropidin/L is proposed. Note: delayed effects on several zooplankton taxa and Chironomidae observed at treatment levels up to 30 µg as/L and resulting in Effect class 3B-4 effects might also be explained as an indirect effect due to the shift from a filamentous to a macrophyte-dominated test system. Safety assessment factor of 2 proposed.			proposed NOEAEC = 1 µg a.s./L	KCP 10.2.3/02 Arts, G.H.P and Brock, T.C.M., 2009

Toxicity data presented above indicate that the metabolite CGA 289267 which was considered potentially of concern in surface water shows less toxicity compared to the parent compound. Concurrently, relevant PEC_{sw} values for the metabolite do not exceed the predicted concentrations in surface water calculated for the parent compound. Thus, either way (from both the toxicity and exposure point of view), it is reasonably concluded that the risk for aquatic organisms arising from the metabolite is covered by the parent fenpropidin. Consequently, separate TER calculations for CGA 289267 potentially of concern in surface water are not considered to be required.

Species	Substance	Time scale	Results	Reference
<i>Oncorhynchus mykiss</i>	ADM.03502.F.1.A (250 g fenpropidin + 175 g prothioconazole/L)	acute	LC ₅₀ = 6.23 mg prod./L nom	KCP 10.2.1/01
<i>Daphnia magna</i>	ADM.03502.F.1.A (250 g fenpropidin + 175 g prothioconazole/L)	acute	EC ₅₀ = 5.57 mg prod./L nom	KCP 10.2.1/02 Renner, P. 2020b, report no 20 48 ADL 0008
<i>Desmodesmus subspicatus</i>	ADM.03502.F.1.A (250 g fenpropidin + 175 g prothioconazole/L)	Sub-chronic	E _y C ₅₀ = 0.472 µg prod./L geomean E _r C ₅₀ = 0.895 µg prod./L geomean	KCP 10.2.1/03 Scheerbaum, D. 2021 Report no.: SO21519 / SSO19707
<i>Lemna gibba</i>	ADM.03502.F.1.A (250 g fenpropidin + 175 g prothioconazole/L)	Sub-chronic	E _y C ₅₀ (frond no.) = 0.148 mg prod./L twa E _y C ₅₀ (biomass) = 0.192 mg prod./L twa E _r C ₅₀ (frond no.) = 0.596 mg prod./L nom twa E _r C ₅₀ (biomass) = 1.242 mg prod./L twa	KCP 10.2.1/04 Renner, P., 2021 report no.: 2048ALE0006

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.

Table 9.5-4: Prothioconazole/fenpropidin Toxicity per fraction assessment and MDR calculation for additive mixture toxicity

[illegible]

Test-item	LC ₅₀ /EC ₅₀ measured for the a.s. [µg/L]	Actual-con- tent-in product [g a.s./L]	%-of-a.s. in-mixture	LC ₅₀ /EC ₅₀ theoretical (mix) [µg/L]	%-of-mix- ture-toxicity	LC ₅₀ /EC ₅₀ (measured) [µg/L] ^{a)}	MDR ^{b)}
Prothioconazole	1300	175	0.41	711.2	22.5	2276	0.3
Fenpropidin	540	250	0.59		77.5		
Algae							
Prothioconazole	2180	175	0.41	0.561	0.0	0.366	1.5
Fenpropidin	0.33	250	0.59		100.0		

Bold: contribution to ≥ 90 % to mixture toxicity

* MDR: Model Deviation Ratio = (L/EC₅₀-theoretical) / (L/EC₅₀-measured)

^{a)} Product endpoint corrected for active substance content; product density is 1.04 g/cm³

For fish and aquatic invertebrates, the observed and calculated mixture toxicities are considered in agreement, since the MDR is between 0.2 and 5. In such a case, measured mixture toxicity should be used in the risk assessment, at least if mixture compositions in the study and at PEC_{SW(mix)} are compatible.

Since it is obvious that algae are by far the most sensitive species for fenpropidin and higher tier data might be required anyway to conclude an acceptable risk for algae, a RQ_{mix} approach according to EFSA guidance document would be the more relevant approach to address mixture toxicity. However, it should be noted that the contribution of fenpropidin to the mixture toxicity is 100 %, regardless of whether Tier-1 data for the active substances (see above) or higher tier data for fenpropidin NOEAEC = 1 µg/L were considered. Thus, it is deemed acceptable to perform the risk assessment individually on the basis of the data of the active substances which is in line with the recommendations of the EFSA guidance document.

Since for aquatic macrophytes only data for the formulated product are available which could be used for mixture toxicity, the product endpoint corrected for active substance content (i.e., 243.6 µg a.s._{sum}/L, based on product density of 1.04 g/cm³) was used in the risk assessment.

Table 9.5-5: JAU-desthio/fenpropidin – Toxicity per fraction assessment and MDR calculation for additive mixture toxicity (via spray drift)

Test-item	LC ₅₀ /EC ₅₀ measured [µg/L]	Actual-con- tent [g/L]	%-in mixture	LC ₅₀ /EC ₅₀ theoretical (mix) [µg/L]	%-of-mixture toxicity	LC ₅₀ /EC ₅₀ (measured) [µg/L] ^{a)}	MDR [±]
Fish							
JAU-desthio	6630	51.3 ^{±±}	0.17	2163	5.4	—	—
Fenpropidin	1900	250	0.83		94.4		
Aquatic invertebrate							
JAU-desthio	≥ 10000	51.3 ^{±±}	0.17	643.7	1.1	—	—
Fenpropidin	540	250	0.83		98.9		

Test-item	LC ₅₀ /EC ₅₀ measured [µg/L]	Actual-con- tent [g/L]	% in mixture	LC ₅₀ /EC ₅₀ theoretical (mix) [µg/L]	% of mixture toxicity	LC ₅₀ /EC ₅₀ (measured) [µg/L] ^{a)}	MDR [*]
Algae							
JAU-desthio	550	51.3**	0.17	0.398	0.0	—	—
Fenpropidin	0.33	250	0.83		100.0		

Bold: contribution to ≥ 90 % to mixture toxicity

* MDR: Model Deviation Ratio = (L/EC₅₀ theoretical) / (L/EC₅₀ measured)

** based on the maximum occurrence via spray drift at FOCUS Step 1 (for details, see Part B of Section 8)

Table 9.5-6: JAU-desthio/fenpropidin – Toxicity per fraction assessment and MDR calculation for additive mixture toxicity (via run-off)

Test item	LC ₅₀ /EC ₅₀ measured [µg/L]	Actual con- tent [g/L]	% in mixture	LC ₅₀ /EC ₅₀ theoretical (mix) [µg/L]	% of mixture toxicity	LC ₅₀ /EC ₅₀ (measured) [µg/L] ^{a)}	MDR [®]
Fish							
JAU-desthio	6630	90.3**	0.27	2344	5.4	—	—
Fenpropidin	1900	250	0.73		94.4		
Aquatic invertebrate							
JAU-desthio	≥ 10000	90.3**	0.27	721.0	1.1	—	—
Fenpropidin	540	250	0.73		98.9		
Algae							
JAU-desthio	550	90.3**	0.27	0.449	0.0	—	—
Fenpropidin	0.33	250	0.73		100.0		

Bold: contribution to ≥ 90 % to mixture toxicity

* MDR: Model Deviation Ratio = (L/EC₅₀ theoretical) / (L/EC₅₀ measured)

** based on the maximum occurrence via run-off entry at FOCUS Step 1 (for details, see Part B of Section 8)

As the contribution of fenpropidin to the mixture toxicity for all aquatic groups is ≥ 90 %, it is therefore deemed acceptable to perform the risk assessment individually on the basis of the data of the active substance and the metabolite JAU-desthio. Thus, no additional acute mixture toxicity calculations are considered to be required.

Chronic mixture toxicity

It should be noted that for the approach assuming dose additivity of the active substances reliable results would only be expected for combinations of effect levels with a defined x, e.g. a LC₅₀, but not for NOECs since the latter effects indicators may represent varying risk or response levels for different compounds, depending on dose-spacing. Furthermore, it is considered unlikely that the active substances will remain intact over a long-term period, and it is unlikely that aquatic organisms could be exposed for a prolonged period to both active substances at the same time.

Table 9.5-7: Prothioconazole/fenpropidin - Toxicity per fraction assessment for additive mixture toxicity

Test item	NOEC measured for the a.s. [µg/L]	Actual con- tent in product [g a.s./L]	% of a.s. in mixture	NOEC theoretical (mix) [µg/L]	% of mixture toxicity	NOEC (measured) [µg/L] ^{a)}	MDR*
Fish							
Prothioconazole	308	175	0.41	304	40.7	---	---
Fenpropidin	302	250	0.59		59.3		
Aquatic invertebrates							
Prothioconazole	560	175	0.41	388.6	28.6	---	---
Fenpropidin	320	250	0.59		71.4		

Bold: contribution to ≥ 90 % to mixture toxicity

* MDR: Model Deviation Ratio = (L/EC₅₀ theoretical) / (L/EC₅₀ measured)

As outlined in the table above, the contribution of prothioconazole or fenpropidin to the mixture toxicity for fish and aquatic invertebrates is not $\geq 90\%$. Therefore, a NOEC_{mix} of 304 $\mu\text{g/L}$ and 388.6 should be considered in addition to the individual active substance data.

Table 9.5-8: ~~JAU-desthio/fenpropidin – Toxicity per fraction assessment for additive mixture toxicity (via spray drift)~~

Test-item	NOEC measured [µg/L]	Actual content [g/L]	% of a.s. in mixture	NOEC theoretical (mix) [µg/L]	% of mixture toxicity	NOEC (measured) [µg/L] ^{a)}	MDR ^{b)}
Fish							
JAU-desthio	3.34	51.3 ^{25.2}	0.17	18	90.0	—	—
fenpropidin	147	250	0.83		10.0		
Aquatic invertebrates							
JAU-desthio	100	51.3 ^{25.2}	0.17	11.3	1.9	—	—
fenpropidin	9.54	250	0.83		98.1		

Bold: contribution to $\geq 90\%$ to mixture toxicity

* MDR: Model Deviation Ratio = $(\text{L}/\text{EC}_{50} \text{ theoretical}) / (\text{L}/\text{EC}_{50} \text{ measured})$

** based on the maximum occurrence via spray drift at FOCUS Step 1 (for details, see Part B of Section 8)

Table 9.5-9: ~~JAU-desthio/fenpropidin – Toxicity per fraction assessment for additive mixture toxicity (via run-off)~~

Test-item	NOEC measured [$\mu\text{g/L}$]	Actual content [g/L]	% of a.s. in mixture	NOEC theoretical (mix) [$\mu\text{g/L}$]	% of mixture toxicity	NOEC (measured) [$\mu\text{g/L}$] ^{a)}	MDR*
Fish							
JAU-desthio	3.34	90.3**	0.27	11.8	94.1	—	—
fenpropidin	147	250	0.73		5.9		

Test-item	NOEC measured [µg/L]	Actual content [g/L]	% of a.s. in mixture	NOEC theoretical (mix) [µg/L]	% of mixture toxicity	NOEC (measured) [µg/L] ^(a)	MDR [*]
Aquatic invertebrates							
JAU-desthio	100	90.3**	0.27	12.6	3.3	—	—
fenpropidin	9.54	250	0.73		96.7		

Bold: contribution to ≥ 90 % to mixture toxicity

* MDR: Model Deviation Ratio = $(L/EC_{50} \text{ theoretical}) / (L/EC_{50} \text{ measured})$

** based on the maximum occurrence via run-off entry at FOCUS Step 1 (for details, see Part B of Section 8)

As outlined in the table above, the contribution of JAU-desthio (M4) and fenpropidin to the mixture toxicity for fish and aquatic invertebrates, respectively, is ≥ 90 %, therefore the risk assessment for these aquatic groups is covered by the assessment for the individual active substances. No additional acute mixture toxicity calculations are considered to be required.

9.5.1.1 Justification for new endpoints

In addition to the active substances and metabolite toxicity data, new endpoints are provided for acute toxicity of the formulated product ADM.03502.F.1.A. These studies are considered to be required according to Regulation (EC) No. 284/2013.

In the EU review process for fenpropidin, no studies with the relevant formulation were evaluated. Hence, new studies were performed to assess the acute effects of MCW-273 750 EC in daphnia, algae, and fish.

Mesocosm studies stimulate environmentally more realistic exposure regimes of water bodies to plant protection products. Two mesocosm studies were EU-approved, and to support the risk assessment, an additional mesocosm study was performed with formulated fenpropidin (Wellmann, 2006). The endpoints of the three independent studies were as follow: the Neumann study (1997) proposed a NOEAEC of 0.39 µg a.s./L, the Huber study stated a NOEAEC of 0.55 µg a.s./L, whereas the Wellmann study proposed a NOEAEC of 30 µg a.s./L.

To support the submission, an external evaluation of all three available mesocosm studies with formulations containing the active substance fenpropidin was performed by Arts and Brock (2009), who classified the observed treatment-related effects in the three studies according to the “Effect classes” described in De Jong et al. (2008)⁴ adapted after Brock et al. (2000)⁵. Thereafter, the Huber study was shown to have some serious drawbacks and hence the study is only used indicatively, whereas the studies by Neumann and Wellmann can be considered of high quality.

In case short-term class 3A effects on other endpoints than macrophytes are considered acceptable, a NOEAEC of 1.0 mg fenpropidin/L can be derived from the Wellmann study. Based on Effect class 3A and taking into account the trends in effects and recovery, a NOEAEC of 0.39 µg fenpropidin a.s./L was derived by the re-evaluation of the results of the Neumann study. If a class 3B is considered acceptable and a trend to class 5A is acceptable as well, the NOEAEC might be set at 6.8 µg fenpropidin/L. However, this suggested endpoint has been rejected by the EFSA committee as explained in the EFSA conclusion 124 (2007), due to long-lasting effects on Chlorophyceae at 1.4 µg fenpropidin/L (LOEAEC).

The range of derived NOEAEC values for fenpropidin from the three experimental pond studies was found to be relatively small (0.39 to 1.0 µg a.s./L). The difference between the lowest and highest value is less than a factor of 3. In this context it should be noted that the lowest available NOEAEC from the Neumann study is expressed in terms of measured concentrations 6 h post the first and second applica-

⁴ De Jong F.M.W, Brock T.C.M., Foekema E.M. & Leeuwangh P. (2008): Guidance for summarizing and evaluating aquatic micro- and mesocosms. RIVM Report 601506009

⁵ Brock, T.C.M., R.P.A. van Wijngaarden & G.J. van Geest (2000): Ecological risks of pesticides in freshwater ecosystems. Part 2: Insecticides. Alterra-rapport 089, 142 pp.

tion, and consequently can be considered a worst-case estimate since the dissipation of fenpropidin from water is relatively fast (DT_{50} approximately 3.6 days). In addition, the highest NOEAEC of 1.0 $\mu\text{g a.s./L}$ (in the Wellmann study from 2006) is lower than the LOEAEC of 1.4 $\mu\text{g a.s./L}$ (in the Neumann study from 1997).

All 3 studies were considered as highly sensitive to detect effects on the taxonomic groups which are susceptible to fenpropidin (i.e. green algae and other primary producers) as well as any resulting indirect effects. Therefore, the results from all three studies should be taken into account to deduce an appropriate Assessment Factor. Because several micro- and mesocosm studies are available, a “case-by-case decision” is warranted and an Assessment Factor of less than 3 is justified. The NOEAECs were based on Effect Class 3A effects, i.e. full recovery was observed within 8 weeks after the 1st application (i.e. approximately 6 weeks after the last application).

As a conclusion, the external evaluation of the observations found in those three studies suggest an overall NOEAEC of 1.0 $\mu\text{g a.s./L}$ to be used in the risk assessment. An overall Assessment Factor of 2 is therefore regarded as appropriate to derive an overall Regulatory Acceptable Concentration.

zRMS comment:

All three mesocosm studies presented in this application have been evaluated by the RMS Sweden for fenpropidin.

The endpoints of the three independent studies were as follow: the Neumann study (1997) proposed a NOEAEC of 0.39 $\mu\text{g a.s./L}$, the Huber study stated a NOEAEC of 0.55 $\mu\text{g a.s./L}$, whereas the Wellmann (2006) study proposed a NOEAEC of 30 $\mu\text{g a.s./L}$.

To support the submission, an external evaluation of all three available mesocosm studies with formulations containing the active substance fenpropidin was performed by Arts and Brock (2009), who classified the observed treatment-related effects in the three studies according to the “Effect classes” described in De Jong et al. (2008)⁶ adapted after Brock et al. (2000)⁷. Thereafter, the Huber study was shown to have some serious drawbacks and hence the study is only used indicatively, whereas the studies by Neumann and Wellmann can be considered of high quality.

As stated in the fenpropidin DAR addendum (Addendum following the evaluation of new Annex II data Post-Annex I inclusion, Fenpropidin, Volume 3, Annex B Ecotoxicology (Sept 2011)): “The RMS agrees with the conclusion by Arts and Brock (2009) that no NOEC could be demonstrated in the study by Wellman et al. (2006) submitted after the Annex I inclusion and therefore cannot support a change of the NOEC agreed in LoEP (i.e. NOEC of 0.39 $\mu\text{g a.s./L}$ which was based on phytoplankton effects, long time recovery within the phytoplankton community and uncertainty regarding possible effects on zooplankton.

According to the EFSA conclusion 2007, an AF of 1-3 should be decided by MSs and their national level.

The Central Zone Ecotoxicology Harmonisation Group generally recommended an ETO approach to be used to set the regulatory acceptable concentration (RAC) along with an appropriate assessment factor of 2-3 for authorisation of products in Central Zone.

In zRMS’s opinion it is more appropriate in terms of protectiveness to select the most conservative of the 2 different endpoints derived from these two mesocosm studies Wellman 2006 with NOEC of 1 $\mu\text{g a.s./L}$ and Neumann 1997 with NOEC of 0.39 $\mu\text{g a.s./L}$ (agreed at EU level).

Therefore, a more conservative value of 0.39 $\mu\text{g /L}$ and AF 3 is proposed to use in the risk assessment by zRMS.

The study by Wellmann 2006 does not have the same value as the study by Neumann, 1997 considering the ecological relevance and richness of species of the community tested, since the study by Neumann includes much more sensitive/vulnerable taxa (i.e. algal taxa) than the study by Wellmann.

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

⁶ De Jong F.M.W, Brock T.C.M., Foekema E.M. & Leeuwangh P. (2008): Guidance for summarizing and evaluating aquatic micro- and mesocosms. RIVM Report 601506009

⁷ Brock, T.C.M., R.P.A. van Wijngaarden & G.J. van Geest (2000): Ecological risks of pesticides in freshwater ecosystems. Part 2: Insecticides. Alterra-rapport 089, 142 pp.

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 (LC₅₀, EC₅₀, 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.03502.F.1.A 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 9.5-10: 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.03502.F.1.A: 1× 175 g a.s./ha post-emergence to spring / winter cereals at BBCH 30-65

Group		Tier-1 assessment (based on laboratory data)					
		Fish acute	Fish chronic	Invertebrates acute	Invertebrates chronic	Sediment-dweller chronic	Algae sub-chronic
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>C. riparius</i>	<i>P. subcapitata</i>
Endpoint (µg/L)		LC ₅₀ 1830	NOEC 308	EC ₅₀ 1300	NOEC 560	NOEC 9140	E _r C ₅₀ 2180
AF		100	10	100	10	10	10
RAC (µg/L)		18.3	30.8	13	56.0	914	218
FOCUS Scenario	PEC _{sw} max (µg/L)						
Step 1							
	19.01	1.04	0.6	1.5	0.3	< 0.1	0.1
Step 2							
March-May / June-Sept. / Oct. – Feb.	1.61	0.1	---	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**

~~As outlined in the table above, for the maximum application to spring / winter cereals at BBCH 30-65, 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-desthio (M4):

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.03502.F.1.A: 1× 175 g a.s./ha post-emergence to spring cereals at BBCH 30-65

Group		Tier-1 assessment (based on laboratory data)					
		Fish acute	Fish chronic	Invertebrates acute	Invertebrates chronic	Sediment-dweller chronic	Algae sub-chronic
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>C. riparius</i>	<i>S. subspicatus</i>
Endpoint (µg/L)		LC ₅₀ 6630	NOEC 3.34	EC ₅₀ (>) 10000	NOEC 100	NOEC 2000	E _r C ₅₀ 550
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 27.23	0.51 0.4	102.48 81.5	>0.34 0.3	3.42 2.7	0.17 0.1	0.62 0.5
Step 2							
March-May / June – Sept.	3.18 2.87	---	9.52 8.6	---	0.32	---	---
Step 3							
D3, ditch	0.035	---	0.1	---	---	---	---
D4, pond	0.007	---	< 0.1	---	---	---	---
D4, stream	0.024	---	0.1	---	---	---	---
D5, pond	0.007	---	< 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**; NSB = Non-spraying buffer; VS = Vegetative strip

Group	PEC _{SW} max (µg/L)	Tier-1 assessment (based on laboratory data)					
		Fish acute	Fish chronic	Invertebrates acute	Invertebrates chronic	Sediment-dweller chronic	Algae sub-chronic
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>C. riparius</i>	<i>S. subspicatus</i>
Endpoint		LC ₅₀	NOEC	EC ₅₀	NOEC	NOEC	ErC 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							
Step 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		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>C. riparius</i>	<i>S. subspicatus</i>
Endpoint (µg/L)		LC ₅₀ 6630	NOEC 3.34	EC ₅₀ (>) 10000	NOEC 100	NOEC 2000	E _r C ₅₀ 550
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)						
	34.23	0.51	102.48	>0.34	3.42	0.17	0.62
Step 2							
March-May / June – Sept.	3.18	---	9.52	---	0.32	---	---
Step 3							
D3, ditch	0.038	---	0.11	---	---	---	---
D4, pond	0.007	---	0.020	---	---	---	---
D4, stream	0.025	---	0.075	---	---	---	---
D5, pond	0.007	---	0.020	---	---	---	---

Group		Tier-1 assessment (based on laboratory data)					
		Fish acute	Fish chronic	Invertebrates acute	Invertebrates chronic	Sediment-dweller chronic	Algae sub-chronic
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>C. riparius</i>	<i>S. subspicatus</i>
Endpoint (µg/L)		LC ₅₀ 6630	NOEC 3.34	EC ₅₀ (>) 10000	NOEC 100	NOEC 2000	E _r C ₅₀ 550
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	---	0.11	---	---	---	---
R4, stream	0.020	---	0.06	---	---	---	---

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**; NSB = Non-spraying buffer; VS = Vegetative strip

~~As outlined in the table above, for the maximum application to spring cereals at BBCH 30-65, all PEC/RAC ratios for the metabolite JAU desthio (M4) are below the relevant trigger of 1 at FOCUS Step 4, 10 m NSB (+VS) at the latest. Thus, no further considerations have to be taken into account.~~

zRMS comment:

Based on the performed calculations following conclusions may be derived:

- **Spring cereals at BBCH 30:**
 - 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.
- **Spring cereals at BBCH 65:**
 - Prothioconazole: acceptable risk with no need for risk mitigation measures
 - JAU 6476-desthio:
 - D scenarios and scenario R4 : risk acceptable with no need for risk mitigation measures

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.03502.F.1.A: 1× 175 g a.s./ha post-emergence to winter cereals at BBCH 30 -65

Group		Tier-1 assessment (based on laboratory data)					
		Fish acute	Fish chronic	Invertebrates acute	Invertebrates chronic	Sediment-dweller chronic	Algae sub-chronic
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>C. riparius</i>	<i>S. subspicatus</i>
Endpoint (µg/L)		LC ₅₀ 6630	NOEC 3.34	EC ₅₀ (>) 10000	NOEC 100	NOEC 2000	E _r C ₅₀ 550
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 27.23	0.51 0.4	102.48 81.5	>0.34 0.3	3.42 2.7	0.17 0.1	0.62 0.5
Step 2							
Oct-Feb	3.18 6.73	---	9.52 20.1	---	0.32 0.7	---	---
Step 3							
D3, ditch	0.018	---	0.1	---	---	---	---
D4, pond	0.005	---	< 0.1	---	---	---	---
D4, stream	0.021	---	0.1	---	---	---	---
D5, pond	0.006	---	< 0.1	---	---	---	---
D5, stream	0.031	---	0.1	---	---	---	---
R1, pond	0.050	---	0.1	---	---	---	---
R1, stream	0.431	---	1.3	---	---	---	---
R3, stream	0.377	---	1.1	---	---	---	---
R4, stream	0.558	---	1.7	---	---	---	---
Step 4, 10-m NSB (+ VS)							
R1, stream	0.196	---	0.6	---	---	---	---

Group		Tier-1 assessment (based on laboratory data)					
		Fish acute	Fish chronic	Invertebrates acute	Invertebrates chronic	Sediment-dweller chronic	Algae sub-chronic
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>C. riparius</i>	<i>S. subspicatus</i>
Endpoint		LC ₅₀	NOEC	EC ₅₀	NOEC	NOEC	E _r C 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	0.172	---	0.5	---	---	---	---
R4, stream	0.254	---	0.8	---	---	---	---

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**; NSB = Non-spraying buffer; VS = Vegetative strip

Table 9.5-14-1: 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.03502.F.1.A: 1× 175 g a.s./ha post-emergence to winter cereals at BBCH 65.

Application rate of 100 mg a.i./ha (1000 L/ha) post-emergence to winter cereals at 25-31°C							
Group	PEC _{SW} max (µg/L)	Tier-1 assessment (based on laboratory data)					
		Fish acute	Fish chronic	Invertebrates acute	Invertebrates chronic	Sediment-dweller chronic	Algae sub-chronic
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>C. riparius</i>	<i>S. subspicatus</i>
Endpoint (µg/L)		LC ₅₀ 6630	NOEC 3.34	EC ₅₀ (>) 10000	NOEC 100	NOEC 2000	E _r C ₅₀ 550
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	0.51 0.4	102.48 81.5	>0.34 0.3	3.42 2.7	0.17 0.1	0.62 0.5
Step 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		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>C. riparius</i>	<i>S. subspicatus</i>
Endpoint (µg/L)		LC ₅₀ 6630	NOEC 3.34	EC ₅₀ (>) 10000	NOEC 100	NOEC 2000	E _r C ₅₀ 550
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)						
Oct-Feb	3.18	---	9.52	---	0.32	---	---
Step 3							
D3, ditch	0.049	---	0.146	---	---	---	---
D4, pond	0.007	---	0.020	---	---	---	---
D4, stream	0.026	---	0.078	---	---	---	---
D5, pond	0.007	---	0.02	---	---	---	---
D5, stream	0.038	---	0.11	---	---	---	---
R1, pond	0.068	---	0.20	---	---	---	---
R1, stream	0.262	---	0.784	---	---	---	---
R3, stream	0.387	---	1.158	---	---	---	---
R4, stream	0.020	---	0.06	---	---	---	---
Step 4, 10-m NSB (+ VS)							
R3, stream	0.171	---	0.51	---	---	---	---

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**; NSB = Non-spraying buffer; VS = Vegetative strip

As outlined in the table above, for the maximum application to winter cereals at BBCH 30-65, all PEC/RAC ratios for the metabolite JAU-desthio (M4) are below the relevant trigger of 1 at FOCUS Step 4, 10-m NSB (+VS) at the latest. Thus, no further considerations have to be taken into account.

zRMS comment:

Based on the performed calculations following conclusions may be derived:

1. Winter cereals at BBCH 30:

- 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.

2. Winter cereals at BBCH 65:

- 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.

Table 9.5-15: Fenpropidin: 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.03502.F.1.A: 1× 250 g a.s./ha post-emergence to spring cereals at BBCH 30-65

Group		Tier-1 assessment (based on laboratory data)							Tier-3 assessment
		Fish acute	Fish chronic	Invertebrates acute	Invertebrates chronic	Sediment-dweller chronic		Algae sub-chronic	Evaluation of all three available mesocosm studies with fenpropidin
Test species		<i>L. macrochirus</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>C. riparius</i>		<i>S. subspicatus</i>	
Endpoint (µg/L)		LC ₅₀	NOEC	EC ₅₀	NOEC	NOEC	NOEC	ErC ₅₀	RAC
		1900	320	540	320	1000	40000	0.33	1.0
AF		100	10	100	10	10	10	10	2
RAC (µg/L)	19	32	5.4	32	100	4000 µg/kg sed.	0.033	0.5	
FOCUS Scenario	PEC _{sw} ^{max} (µg/L)								
Step 1									
µg/L	16.01	0.8	0.5	3.0	0.5	0.2	---	485.2	32
µg/kg sed.	526.61	---	---	---	---	---	0.1	---	
Step 2									
March-May / June – Sept.	2.60	---	0.08	0.5	---	---	---	78.8	5.2
Step 3									
D3, ditch	1.555	---	---	---	---	---	---	47.1	3.1
D4, pond	0.052	---	---	---	---	---	---	1.6	0.1
D4, stream	1.270	---	---	---	---	---	---	38.5	2.5
D5, pond	0.052	---	---	---	---	---	---	1.6	0.1
D5, stream	1.305	---	---	---	---	---	---	39.6	2.6
R4, stream	1.026	---	---	---	---	---	---	31.1	2.1

Group		Tier-1 assessment (based on laboratory data)							Tier-3 assessment
		Fish acute	Fish chronic	Invertebrates acute	Invertebrates chronic	Sediment-dweller chronic		Algae sub-chronic	Evaluation of all three available mesocosm studies with fenpropidin
Test species		<i>L. macrochirus</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>C. riparius</i>		<i>S. subspicatus</i>	
Endpoint (µg/L)		LC ₅₀ 1900	NOEC 320	EC ₅₀ 540	NOEC 320	NOEC 1000	NOEC 40000	ErC ₅₀ 0.33	
AF		100	10	100	10	10	10	10	
RAC (µg/L)		19	32	5.4	32	100	4000 µg/kg sed.	0.033	
FOCUS Scenario	PEC _{sw} max (µg/L)								
Step 4, 10-m NSB (+VS)									
D3, ditch	0.22	—	—	—	—	—	—	6.7	0.4
+90 % DRT	0.021	—	—	—	—	—	—	0.6	—
D4, pond	0.032	—	—	—	—	—	—	0.97	0.1
D4, stream	0.243	—	—	—	—	—	—	7.4	0.5
+90 % DRT	0.024	—	—	—	—	—	—	0.7	—
D5, pond	0.032	—	—	—	—	—	—	0.97	0.1
D5, stream	0.25	—	—	—	—	—	—	7.6	0.5
+90 % DRT	0.024	—	—	—	—	—	—	0.7	—
R4, stream	0.205	—	—	—	—	—	—	6.2	0.4
+VS	0.196	—	—	—	—	—	—	5.9	—

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**; NSB = Non-spraying buffer; VS = Vegetative strip; DRT = Drift-reducing techniques

Group		Tier-1 assessment (based on laboratory data)						
		Fish acute	Fish chronic	Invertebrates acute	Invertebrates chronic	Sediment-dweller chronic		Algae sub-chronic
Test species		<i>L. macrochirus</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>C. riparius</i>		<i>S. subspicatus</i>
Endpoint		LC ₅₀	NOEC	EC ₅₀	NOEC	NOEC	NOEC	E _r C ₅₀
(µg/L)		1900	320	540	320	1000	40000	0.33
AF		100	10	100	10	10	10	10
RAC (µg/L)		19	32	5.4	32	100	4000 µg/kg sed.	0.033
FOCUS Scenario	PEC _{SW} max (µg/L)							
Step 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		<i>L. macrochirus</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>C. riparius</i>		<i>S. subspicatus</i>
Endpoint (µg/L)		LC ₅₀ 1900	NOEC 320	EC ₅₀ 540	NOEC 320	NOEC 1000	NOEC 40000	E _r C ₅₀ 0.33
AF		100	10	100	10	10	10	10
RAC (µg/L)		19	32	5.4	32	100	4000 µg/kg sed.	0.033
FOCUS Scenario	PEC _{SW} max (µg/L)							
µg/L	16.01	0.8	0.5	3.0	0.5	0.2	---	485.2
µg/kg sed.	526.61	---	---	---	---	---	0.1	---
Step 2								
March-May / June – Sept.	2.60	---	0.08	0.5	---	---	---	78.8
Step 3								
D3, ditch	1.555	---	---	---	---	---	---	47.12
D4, pond	0.052	---	---	---	---	---	---	1.57
D4, stream	1.366	---	---	---	---	---	---	41.4
D5, pond	0.052	---	---	---	---	---	---	1.57
D5, stream	1.455	---	---	---	---	---	---	44.1
R4, stream	1.031	---	---	---	---	---	---	31.24
Step 4, 10-m NSB								
D3, ditch	0.22	---	---	---	---	---	---	6.66
+90 % DRT	0.021	---	---	---	---	---	---	32.25
D4, pond	0.035	---	---	---	---	---	---	0.96
D4, stream	0.250	---	---	---	---	---	---	7.84
+90 % DRT	0.024	---	---	---	---	---	---	0.72
D5, pond	0.032	---	---	---	---	---	---	0.96

Group		Tier-1 assessment (based on laboratory data)						
		Fish acute	Fish chronic	Invertebrates acute	Invertebrates chronic	Sediment-dweller chronic		Algae sub-chronic
Test species		<i>L. macrochirus</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>C. riparius</i>		<i>S. subspicatus</i>
Endpoint (µg/L)		LC ₅₀ 1900	NOEC 320	EC ₅₀ 540	NOEC 320	NOEC 1000	NOEC 40000	E _r C ₅₀ 0.33
AF		100	10	100	10	10	10	10
RAC (µg/L)		19	32	5.4	32	100	4000 µg/kg sed.	0.033
FOCUS Scenario	PEC _{SW max} (µg/L)							
D5, stream	0.280	—	—	—	—	—	—	0.15
+ 90 % DRT	0.054	—	—	—	—	—	—	0.73
R4, stream	0.108	—	—	—	—	—	—	6
+ VS	0.102	—	—	—	—	—	—	3.09
R4, stream,								
+75 %	0.08							0.15

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**; NSB = Non-spraying buffer; VS = Vegetative strip; DRT = Drift-reducing techniques

As outlined in the table above, for the maximum application to spring cereals at BBCH 30-65, all Tier 1 PEC/RAC ratios for fenpropidin are below the relevant trigger of 1 at FOCUS Step 4, 10-m NSB + 90% DRT at the latest, except for the R4 scenario. Nevertheless, if considering Tier 3 data relevant for algae (i.e., a RAC of 0.5 µg a.s./L), an acceptable risk and aquatic groups can be concluded with a 10-m NSB.

zRMS comment:

Based on the calculation in Table above, for the maximum application to spring cereals at BBCH 30-65, all Tier 1 PEC/RAC ratios for fenpropidin are below the relevant trigger of 1 at FOCUS Step 4, 10-m NSB + 90% DRT, except for the R4 scenario.

Further refinement with Tier 3 data relevant for algae with RAC of 0.13 µg a.s./L based on NOEC = 0.39 µg/L value with AF = 3 has been considered by zRMS for all FOCUS scenarios.

Fenpropidin: Acceptability of risk (PEC/RAC < 1): 1 × 250 g a.s./ha post-emergence to spring cereals at BBCH 30.

Group		Tier 3
Test species		Mesocosmos
Endpoint		NOEC=0.39
AF		3
RAC (µg/L)		0.13
	PEC _{sw} (µg/L)	PEC/RAC
STEP 1	16.01	123.15
STEP 2	2.60	20
STEP 3		
D3, ditch	1.555	11.96
D4, pond	0.052	0.4
D4, stream	1.270	9.76
D5, pond	0.052	0.4
D5, stream	1.305	10.03
R4, stream	1.026	7.90
STEP 4, 10-m NSB (+VS), STEP 4 10-m NSB +DRT		
D3, ditch	0.220	1.70
+75 % DRT	0.054	0.41
D4, pond	0.032	0.25
D4, stream	0.243	1.86
+75% DRT	0.060	0.46
D5, pond	0.032	0.25
D5, stream	0.25	1.92
+75 % DRT	0.061	0.46
R4 stream	0.270	1.58
+VES	0.270	1.58

Step 4, 20-m NSB (+VS)		
R4-stream,	0.270	1.56
+VS	0.101	0.78
Based on the performed calculations following conclusions may be derived:		
1. Spring cereals at BBCH 30:		
<ul style="list-style-type: none"> Fenpropidin acceptable risk with 10-m unsprayed buffer zone with 75% DRT in scenarios (D3, D4, D5). Fenpropidin acceptable risk with 20-m VFS in scenario R4 		
Fenpropidin: Acceptability of risk (PEC/RAC < 1): 1× 250 g a.s./ha post-emergence to spring cereals at BBCH 65.		
Group		Tier 3
Test species		Mesocosmos
Endpoint		NOEC = 0.39
AF		3
RAC (µg/L)		0.13
	PEC _{sw} (µg/L)	PEC/RAC
STEP 1	16.01	
STEP 2	2.60	
STEP 3		
D3, ditch		11.96
D4, pond		0.40
D4, stream		10.50
D5, pond		0.4
D5, stream		11.20
R4, stream		7.93
STEP 4, 10-m NSB (+VS), STEP 4 10-m NSB +DRT		
D3, ditch		1.69
+75% DRT	54	0.41
D4, pond		0.24
D4, stream		2.0
+75% DRT	64	0.49

D5_pond			0.24
D5_stream			2.15
+75% DRT	0.069		0.53
R4_stream			1.52
+VFS			0.78
R4_stream			1.52
+75%			0.61

Based on the performed calculations following conclusions may be derived:

2. Spring cereals at BBCH 65:

- Fenpropidin acceptable risk with 10 m unsprayed buffer zone with 75% DRT in scenarios (D3, D4, D5, R4).
- Fenpropidin acceptable risk with 10 m VFS in scenario R4

Commenting period process (April 2023)

The new STEP 4 PEC_{sw} calculations were in Section 8 for spring and winter cereals. Accordingly, risk assessment for aquatic organisms was updated by the the Applicant based on new Step 4 PEC_{sw} values.

The new calculations submitted by the Applicant are provided by zRMS in the Tables below:

It should be indicated that PEC_{sw} values above the **RAC of 0.130 µg/L are shown in bold** indicated an unacceptable risk.

Spring cereals

FOCUS STEP 4 PEC_{sw} for fenpropidin following 1 × 250 g a.s./ha to spring cereals at BBCH 30 considering EVA derived deposition rates and a worst-case interception of 100 %

STEP 4		Max PEC _{sw} (µg/L) considering following mitigation:											
No spray buffer (m)		10	10	10	20	20	20	10	20	10	10	20	20
Vegetative strip (m)		none	none	none	none	none	none	10	20	10	10	20	20
Nozzle reduction		none	75%	90%	none	75%	90%	none	none	75%	90%	75%	90%
D3	Ditch	0.678	0.572	0.554	0.382	0.328	0.319	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
D4	Pond	0.142	0.118	0.113	0.084	0.068	0.065	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
D4	Stream	0.384	0.206	0.170	0.207	0.115	0.097	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
D5	Pond	0.142	0.118	0.113	0.084	0.068	0.065	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
D5	Stream	0.348	0.159	0.121	0.185	0.088	0.068	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
R4	Stream	0.416	0.281	0.254	0.229	0.205	0.205	0.416	0.229	0.281	0.254	0.159	0.145

No spray buffer (m)		25	25	25	30	30	30	35	35	35	25	30	35
Vegetative strip (m)		none	none	none	none	none	none	none	none	none	10	10	10
Nozzle reduction		none	75%	90%	none	75%	90%	none	75%	90%	none	none	none
D3	Ditch	0.295	0.251	0.244	0.228	0.190	0.184	0.180	0.147	0.142	n.r.	n.r.	n.r.
D4	Pond	0.066	0.053	0.050	0.052	0.040	0.038	0.042	0.031	0.029	n.r.	n.r.	n.r.
D4	Stream	0.163	0.089	0.074	0.132	0.070	0.057	0.108	0.054	0.044	n.r.	n.r.	n.r.
D5	Pond	0.066	0.053	0.050	0.052	0.040	0.038	0.042	0.031	0.029	n.r.	n.r.	n.r.
D5	Stream	0.147	0.068	0.052	0.120	0.054	0.041	0.100	0.043	0.031	n.r.	n.r.	n.r.
R4	Stream	0.205	0.205	0.205	0.205	0.205	0.205	0.205	0.205	0.205	0.178	0.142	0.113
No spray buffer (m)		40	40	40	50	50	50	-	-	-	-	40	50
Vegetative strip (m)		none	none	none	none	none	none	-	-	-	-	10	10
Nozzle reduction		none	75%	90%	none	75%	90%	-	-	-	-	none	none
D3	Ditch	0.142	0.113	0.109	0.091	0.066	0.062	-	-	-	-	n.r.	n.r.
D4	Pond	0.034	0.024	0.022	0.023	0.014	0.013	-	-	-	-	n.r.	n.r.
D4	Stream	0.091	0.044	0.034	0.066	0.028	0.021	-	-	-	-	n.r.	n.r.
D5	Pond	0.034	0.024	0.022	0.023	0.014	0.013	-	-	-	-	n.r.	n.r.
D5	Stream	0.085	0.035	0.025	0.064	0.023	0.016	-	-	-	-	n.r.	n.r.
R4	Stream	0.205	0.205	0.205	0.205	0.205	0.205	-	-	-	-	0.093	0.093

PEC_{sw} values above the RAC of 0.130 µg/L are shown in **bold**.

FOCUS STEP 4 PEC_{sw} for fenpropidin following 1 × 250 g a.s./ha to spring cereals at BBCH 65 considering EVA derived deposition rates and a worst-case interception of 100 %

STEP 4		Max PEC _{sw} (µg/L) considering following mitigation:											
No spray buffer (m)		10	10	10	20	20	20	10	20	10	10	20	20
Vegetative strip (m)		none	none	none	none	none	none	10	20	10	10	20	20
Nozzle reduction		none	75%	90%	none	75%	90%	none	none	75%	90%	75%	90%
D3	Ditch	0.738	0.626	0.604	0.417	0.359	0.348	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
D4	Pond	0.142	0.118	0.113	0.084	0.068	0.065	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
D4	Stream	0.504	0.375	0.353	0.279	0.214	0.203	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.

D5	Pond	0.142	0.118	0.113	0.084	0.068	0.065	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
D5	Stream	0.588	0.464	0.442	0.329	0.266	0.255	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
R4	Stream	0.416	0.281	0.254	0.229	0.159	0.145	0.416	0.229	0.281	0.247	0.159	0.145
No spray buffer (m)		25	25	25	30	30	30	35	35	35	25	30	35
Vegetative strip (m)		none	none	none	none	none	none	none	none	none	10	10	10
Nozzle reduction		none	75%	90%	none	75%	90%	none	75%	90%	none	none	none
D3	Ditch	0.321	0.275	0.266	0.248	0.209	0.201	0.195	0.161	0.155	n.r.	n.r.	n.r.
D4	Pond	0.066	0.053	0.050	0.052	0.040	0.038	0.042	0.031	0.029	n.r.	n.r.	n.r.
D4	Stream	0.216	0.163	0.154	0.172	0.126	0.118	0.137	0.096	0.089	n.r.	n.r.	n.r.
D5	Pond	0.066	0.053	0.050	0.052	0.040	0.038	0.042	0.031	0.029	n.r.	n.r.	n.r.
D5	Stream	0.254	0.202	0.194	0.199	0.155	0.148	0.157	0.119	0.112	n.r.	n.r.	n.r.
R4	Stream	0.178	0.122	0.110	0.142	0.094	0.085	0.113	0.073	0.065	0.178	0.142	0.113
No spray buffer (m)		40	40	40	50	50	50	-	-	-	-	40	50
Vegetative strip (m)		none	none	none	none	none	none	-	-	-	-	10	10
Nozzle reduction		none	75%	90%	none	75%	90%	-	-	-	-	none	none
D3	Ditch	0.154	0.124	0.118	0.096	0.072	0.067	-	-	-	-	n.r.	n.r.
D4	Pond	0.034	0.024	0.022	0.023	0.014	0.013	-	-	-	-	n.r.	n.r.
D4	Stream	0.112	0.075	0.068	0.077	0.045	0.040	-	-	-	-	n.r.	n.r.
D5	Pond	0.034	0.024	0.022	0.023	0.014	0.013	-	-	-	-	n.r.	n.r.
D5	Stream	0.127	0.091	0.086	0.085	0.054	0.049	-	-	-	-	n.r.	n.r.
R4	Stream	0.092	0.057	0.057	0.064	0.057	0.057	-	-	-	-	0.092	0.064

PECsw values above the RAC of 0.130 µg/L are shown in **bold**.

1. Spring cereals at BBCH 30:

• Fenpropidin:

- D3 scenario: risk acceptable: 40 m NBZ+75% DRN
- D4 scenario (pond): risk acceptable: 10 m NBZ+75% DRN or 20 m NBZ
- D4 scenario (stream): risk acceptable: 20 m NBZ+75% DRN or 40 m NBZ
- D5 scenario (pond): risk acceptable: 10 m NBZ+75% DRN or 20 m NBZ
- D5 scenario (stream): risk acceptable: 10 m NBZ+90% DRN or 20 m NBZ+75% DRN or 40 m DRN
- R4 scenario (stream): risk acceptable: 40 m NBZ with 10 m VFS

2. Spring cereals at BBCH 65:

• Fenpropidin:

- D3 scenarios: risk acceptable: 40 m NBZ+75% DRN
- D4 scenario (pond): risk acceptable: 10 m NBZ+75% DRN or 20 m NBZ
- D4 scenario (stream): risk acceptable: 30 m NBZ +75% DRN or 40 m
- D5 scenario (pond): risk acceptable: 10 m NBZ+75% DRN or 20 m NBZ
- D5 scenario (stream): risk acceptable: 35 m NBZ+75% DRN or 40 m NBZ
- R4 scenario (stream): risk acceptable: 25 m +75% DRN or 35 m

Table 9.5-17: Fenpropidin: 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.03502.F.1.A: 1× 250 g a.s./ha post-emergence to winter cereals at BBCH 30-65

Group		Tier-1 assessment (based on laboratory data)						Tier-3 assessment	
		Fish acute	Fish chronic	Invertebrates acute	Invertebrates chronic	Sediment-dweller chronic		Algae sub-chronic	Evaluation of all three available mesocosm studies with fenpropidin
Test species		<i>L. macrochirus</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>C. riparius</i>		<i>S. subspicatus</i>	
Endpoint (µg/L)		LC ₅₀	NOEC	EC ₅₀	NOEC	NOEC	NOEC	E _r C ₅₀	RAC
AF		1900	320	540	320	1000	40000	0.33	1.0
RAC (µg/L)		100	10	100	10	10	10	10	2
		19	32	5.4	32	100	4000	0.033	0.5
FOCUS Scenario	PEC _{SW} max (µg/L)								
Step 1									
µg/L	16.01	0.8	0.5	3.0	0.5	0.2	---	485.2	32.0

Group		Tier-1 assessment (based on laboratory data)						Tier-3 assessment	
		Fish acute	Fish chronic	Invertebrates acute	Invertebrates chronic	Sediment-dweller chronic		Algae sub-chronic	Evaluation of all three available mesocosm studies with fenpropidin
Test species		<i>L. macrochirus</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>C. riparius</i>		<i>S. subspicatus</i>	
Endpoint (µg/L)		LC ₅₀ 1900	NOEC 320	EC ₅₀ 540	NOEC 320	NOEC 1000	NOEC 40000	E _r C ₅₀ 0.33	RAC 1.0
AF		100	10	100	10	10	10	10	2
RAC (µg/L)		19	32	5.4	32	100	4000	0.033	0.5
FOCUS Scenario	PEC _{sw} max (µg/L)								
D4, stream	0.219	—	—	—	—	—	—	6.6	0.4
+ 90 % DRT	0.021	—	—	—	—	—	—	0.6	—
D5, pond	0.032	—	—	—	—	—	—	0.97	0.1
D5, stream	0.237	—	—	—	—	—	—	7.2	0.5
+ 90 % DRT	0.023	—	—	—	—	—	—	0.7	—
R1, pond	0.032	—	—	—	—	—	—	0.97	0.1
R1, stream	0.194	—	—	—	—	—	—	5.9	0.4
+ VS	0.194	—	—	—	—	—	—	5.9	—
R3, stream	0.275	—	—	—	—	—	—	8.3	0.6
+ VS	0.275	—	—	—	—	—	—	8.3	—
R4, stream	0.218	—	—	—	—	—	—	6.6	0.4
+ VS	0.196	—	—	—	—	—	—	5.9	—
Step 4, 20-m NSB (+VS)									
R1, stream	0.147	—	—	—	—	—	—	4.5	—
+ VS	0.100	—	—	—	—	—	—	3.0	—
R3, stream	0.142	—	—	—	—	—	—	4.3	—
+ VS	0.142	—	—	—	—	—	—	4.3	—

Group		Tier-1 assessment (based on laboratory data)						Tier-3 assessment
		Fish acute	Fish chronic	Invertebrates acute	Invertebrates chronic	Sediment-dweller chronic		Algae sub-chronic
Test species		<i>L. macrochirus</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>C. riparius</i>		<i>S. subspicatus</i>
Endpoint (µg/L)		LC ₅₀	NOEC	EC ₅₀	NOEC	NOEC	NOEC	E _r C ₅₀
AF		1900	320	540	320	1000	40000	0.33
RAC (µg/L)		100	10	100	10	10	10	10
		19	32	5.4	32	100	4000	0.033
FOCUS Scenario	PEC _{sw} max (µg/L)							
R4, stream	0.218	—	—	—	—	—	—	6.6
+VS	0.101	—	—	—	—	—	—	3.1

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**; NSB = Non-spraying buffer; VS = Vegetative strip; DRT = Drift-reducing techniques

Table 9.5-18-1: Fenpropidin: 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.03502.F.1.A: 1× 250 g a.s./ha post-emergence to winter cereals at BBCH 65.

Group		Tier-1 assessment (based on laboratory data)						
		Fish acute	Fish chronic	Invertebrates acute	Invertebrates chronic	Sediment-dweller chronic		Algae sub-chronic
Test species		<i>L. macrochirus</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>C. riparius</i>		<i>S. subspicatus</i>
Endpoint (µg/L)		LC ₅₀	NOEC	EC ₅₀	NOEC	NOEC	NOEC	E _r C ₅₀
AF		1900	320	540	320	1000	40000	0.33
RAC (µg/L)		100	10	100	10	10	10	10
		19	32	5.4	32	100	4000	0.033
FOCUS Scenario	PEC _{sw} max (µg/L)							
Step 1								
µg/L	16.01	0.8	0.5	3.0	0.5	0.2	---	485.2
µg/kg sed.	526.61	---	---	---	---	---	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		<i>L. macrochirus</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>C. riparius</i>		<i>S. subspicatus</i>
Endpoint (µg/L)		LC ₅₀ 1900	NOEC 320	EC ₅₀ 540	NOEC 320	NOEC 1000	NOEC 40000	ErC ₅₀ 0.33
AF		100	10	100	10	10	10	10
RAC (µg/L)		19	32	5.4	32	100	4000	0.033
FOCUS Scenario	PEC _{SW max} (µg/L)							
Step 2								
Oct-Feb	2.60 5.75	—	0.08	0.48 1.1	—	—	—	78.8 174.2
Step 3								
D3, ditch	1.567	---	---		---	---	---	47.5
D4, pond	0.053	---	---		---	---	---	1.60
D4, stream	1.350	---	---		---	---	---	40.90
D5, pond	0.053	---	---		---	---	---	1.60
D5, stream	1.456	---	---		---	---	---	44.12
R1, pond	0.059	---	---		---	---	---	1.78
R1, stream	1.031	---	---		---	---	---	31.24
R3, stream	1.446	---	---		---	---	---	43.81
R4, stream	1.031	---	---		---	---	---	31.24
Step 4, 10-m NSB (+VS)								
D3, ditch	0.253	—	—	—	—	—	—	6.75
+90% DRT	0.022	—	—	—	—	—	—	1.66
D4, pond	0.033	—	—	—	—	—	—	1
D4, stream	0.250	—	—	—	—	—	—	7.84

Group		Tier-1 assessment (based on laboratory data)						
		Fish acute	Fish chronic	Invertebrates acute	Invertebrates chronic	Sediment-dweller chronic		Algae sub-chronic
Test species		<i>L. macrochirus</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>C. riparius</i>		<i>S. subspicatus</i>
Endpoint		LC ₅₀	NOEC	EC ₅₀	NOEC	NOEC	NOEC	ErC ₅₀
(µg/L)		1900	320	540	320	1000	40000	0.33
AF		100	10	100	10	10	10	10
RAC (µg/L)		19	32	5.4	32	100	4000	0.033
FOCUS Scenario	PEC _{SW max} (µg/L)							
+90 % DRT	0.025	—	—	—	—	—	—	0.75
D5, pond	0.033	—	—	—	—	—	—	1
D5, stream	0.280	—	—	—	—	—	—	
+90 % DRT	0.027	—	—	—	—	—	—	0.81
R1, pond	0.051	—	—	—	—	—	—	
R1, stream	0.108	—	—	—	—	—	—	6
+VS	0.108	—	—	—	—	—	—	6
R3, stream	0.278	—	—	—	—	—	—	3.42
+VS	0.278	—	—	—	—	—	—	3.42
R4, stream	0.108	—	—	—	—	—	—	6
+VS	0.108	—	—	—	—	—	—	6
Step 4, 20-m NSB (+VS)								
R1, stream	0.153	—	—	—	—	—	—	4.63
+VS	0.102	—	—	—	—	—	—	3.00
R3, stream	0.174	—	—	—	—	—	—	4.36
+VS	0.174	—	—	—	—	—	—	4.36
R4, stream	0.102	—	—	—	—	—	—	3.00
+VS	0.102	—	—	—	—	—	—	3.00

Group		Tier-1 assessment (based on laboratory data)						
		Fish acute	Fish chronic	Invertebrates acute	Invertebrates chronic	Sediment-dweller chronic		Algae sub-chronic
Test species		<i>L. macrochirus</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>C. riparius</i>		<i>S. subspicatus</i>
Endpoint		LC ₅₀	NOEC	EC ₅₀	NOEC	NOEC	NOEC	E _r C ₅₀
(µg/L)		1900	320	540	320	1000	40000	0.33
AF		100	10	100	10	10	10	10
RAC (µg/L)		19	32	5.4	32	100	4000	0.033
FOCUS Scenario	PEC _{SW} max (µg/L)							
R3, stream	0.275							8.33
+75%	0.126							3.81

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**; NSB = Non-spraying buffer; VS = Vegetative strip; DRT = Drift reducing techniques

zRMS comments:

Based on the calculation in Table above, for the maximum application to winters cereals at BBCH 30-65, all Tier 1 PEC/RAC ratios for fenpropidin for algae are below the relevant trigger of 1 at FOCUS Step 4. Further refinement with Tier 3 data relevant for algae with RAC of 0.13 µg a.s./L based on NOEC = 0.39 µg a.s./L with AF = 3 has been considered by zRMS and PEC_{sw} FOCUS scenarios relevant for proposed uses in the GAP.

Fenpropidin: Acceptability of risk (PEC/RAC < 1): 1× 250 g a.s./ha post-emergence to winter cereals at BBCH 30.

Group	Tier-3	
Test species	Mesocosmos	
Endpoint (µg/L)	NOEC=0.39	
AF	3	
RAC (µg/L)	0.13	
	PEC _{sw} (µg/L)	PEC/RAC
STEP 1	16.01	123.15
STEP 2	2.60	20
STEP 3		
FOCUS scenario		
D3, ditch	1.554	11.95
D4, pond	0.052	0.4
D4, stream	1.147	8.82
D5, pond	0.052	0.4

D5, stream	1.239	9.53
R1, pond	0.052	0.4
R1, stream	1.017	7.82
R3, stream	1.437	11.05
R4, stream	1.026	7.90
STEP 4, 10-m NSB (+VS), STEP 4 10-m NSB +DRT		
D3, ditch	0.220	1.69
+ 75 % DRT	0.054	0.41
D4, pond	0.032	0.24
D4, stream	0.219	1.68
+ 75 % DRT	0.054	0.41
D5, pond	0.032	0.24
D5, stream	0.237	1.82
+ 75 % DRT	0.058	0.44
R1, pond	0.032	0.24
R1, stream	0.194	1.49
+ VS	0.194	1.49
R3, stream	0.275	2.11
+ VS	0.275	2.11
R4, stream	0.218	1.67
+ VS	0.196	1.50
R3, stream	0.275	2.11
+ 75 %	0.100	0.76
R4, stream	0.218	1.67
+ 75%	0.218	1.67
Step 4, 20-m NSB (+VS)		
R1, stream	0.194	1.49
+ VS	0.100	0.76
R4, stream	0.218	1.67
+ VS	0.101	0.77
Fenpropidin: Acceptability of risk (PEC/RAC < 1): 1× 250 g a.s./ha post-emergence to winter cereals at BBCH 65.		
Group		Tier 3
Test-species		Mesocosmos
Endpoint		NOEC= 0.39
AF		3
RAC (µg/L)		0.13
	PEC _{sw} (µg/L)	PEC/RAC
STEP 1	16.01	123.15
STEP 2	2.60	20

STEP 3		
D3, ditch	1,567	12,05
D4, pond	0,053	0,40
D4, stream	1,350	10,38
D5, pond	0,053	0,40
D5, stream	1,456	11,2
R1, pond	0,059	0,45
R1, stream	1,031	7,93
R3, stream	1,446	11,12
R4, stream	1,031	7,93
STEP 4, 10-m NSB (+VS), STEP 4 10-m NSB +DRT		
D3, ditch	0,223	1,71
+ 75 % DRT	0,055	0,42
D4, pond	0,033	0,25
D4, stream	0,259	1,99
+ 75% DRT	0,064	0,49
D5, pond	0,033	0,25
D5, stream	0,280	2,15
+ 75 % DRT	0,069	0,53
R1, pond	0,051	0,39
R1, stream	0,198	1,52
+ VS	0,153	1,17
R3, stream	0,278	2,13
+ VS	0,278	2,13
R4, stream	0,198	1,67
+ VS	0,198	1,67
R3, stream	0,278	2,13
+75%	0,126	0,96
R4, stream	0,198	1,52
+75%	0,075	0,57
Step 4, 20-m NSB (+VS)		
R1, stream	0,198	1,67
+ VS	0,102	0,78
R4, stream	0,198	1,67
+ VS	0,102	0,78
Based on the performed calculations following conclusions may be derived:		
1. Winter cereals at BBCH 30:		
• Fenpropidin acceptable risk with 10-m unsprayed buffer zone with 75% DRT in scenarios (D3, D4, D5 and R3)		

• Fenpropidin acceptable risk with 20 m VFS in scenarios R1 and R4

2. Winter cereals at BBCH 65:

- Fenpropidin acceptable risk with 10 m unsprayed buffer zone with 75% DRT in scenarios (D3, D4, D5, R3, R4).
- Fenpropidin acceptable risk with 20 m VFS in scenario R1 and R4 scenarios

Based on the calculations of the risk assessment based on at STEP 3 PEC_{sw} values for the maximum application to winters cereals at BBCH 30-65, all Tier-1 PEC/RAC ratios for fenpropidin for algae are below the relevant trigger of 1 at FOCUS Step 4.

Further refinement with Tier-3 data relevant for algae with RAC of 0.13 µg a.s./L based on NOEC = 0.39 µg a.s./L with AF = 3 has been considered by zRMS with consideration PEC_{sw} FOCUS STEP 4 scenarios relevant for proposed uses in the GAP.

Commenting period process (April 2023)

The PEC_{sw} STEP 4 value considered at the Tables above were questioned in Section 8 during Commenting process and for this reason zRMS crossed out previously accepted risk assessment.

During commenting process, the new PEC_{sw} STEP 4 calculations were submitted in Section 8 for spring and winter cereals. Accordingly, risk assessment for aquatic organisms was updated by the the Applicant based on new Step 4 PEC_{sw} values. The new calculations are provided by zRMS in the Tables below:

It should be indicated that PEC_{sw} values which are above the RAC of 0.130 µg/L are shown in **bold** which indicate an unacceptable risk.

Winter cereals

FOCUS STEP 4 PEC_{sw} for fenpropidin following 1 × 250 g a.s./ha to winter cereals at BBCH 30 considering EVA derived deposition rates and a worst-case interception of 100%

STEP 4		Max PEC _{sw} (µg/L) considering following mitigation:											
No spray buffer (m)		10	10	10	20	20	20	10	20	10	10	20	20
Vegetative strip (m)		none	none	none	none	none	none	10	20	10	10	20	20
Nozzle reduction		none	75%	90%	none	75%	90%	none	none	75%	90%	75%	90%
D3	Ditch	0.656	0.545	0.526	0.370	0.313	0.303	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
D4	Pond	0.141	0.117	0.112	0.084	0.068	0.065	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
D4	Stream	0.281	0.116	0.082	0.149	0.063	0.046	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
D5	Pond	0.141	0.117	0.113	0.084	0.068	0.065	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
D5	Stream	0.303	0.123	0.088	0.160	0.067	0.049	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
R1	Pond	0.142	0.118	0.113	0.084	0.068	0.065	0.141	0.084	0.117	0.113	0.068	0.065
R1	Stream	0.390	0.248	0.220	0.214	0.147	0.147	0.390	0.214	0.248	0.220	0.140	0.126

R3	Stream	0.592	0.421	0.392	0.327	0.240	0.225	0.592	0.327	0.421	0.392	0.240	0.225
R4	Stream	0.416	0.281	0.254	0.229	0.218	0.218	0.416	0.229	0.281	0.254	0.159	0.145
No spray buffer (m)		25	25	25	30	30	30	35	35	35	25	30	35
Vegetative strip (m)		none	none	none	none	none	none	none	none	none	10	10	10
Nozzle reduction		none	75%	90%	none	75%	90%	none	75%	90%	none	none	none
D3	Ditch	0.285	0.239	0.231	0.221	0.182	0.175	0.174	0.140	0.135	n.r.	n.r.	n.r.
D4	Pond	0.066	0.052	0.050	0.052	0.040	0.038	0.042	0.031	0.029	n.r.	n.r.	n.r.
D4	Stream	0.118	0.049	0.035	0.097	0.039	0.028	0.081	0.032	0.022	n.r.	n.r.	n.r.
D5	Pond	0.066	0.053	0.050	0.052	0.040	0.038	0.042	0.031	0.029	n.r.	n.r.	n.r.
D5	Stream	0.127	0.052	0.038	0.104	0.042	0.029	0.088	0.034	0.023	n.r.	n.r.	n.r.
R1	Pond	0.066	0.053	0.050	0.052	0.040	0.038	0.042	0.031	0.030	0.066	0.052	0.042
R1	Stream	0.167	0.147	0.147	0.147	0.147	0.147	0.147	0.147	0.147	0.167	0.133	0.107
R3	Stream	0.254	0.183	0.171	0.202	0.141	0.131	0.161	0.108	0.100	0.254	0.202	0.161
R4	Stream	0.218	0.218	0.218	0.218	0.218	0.218	0.218	0.218	0.218	0.178	0.142	0.113
No spray buffer (m)		40	40	40	50	50	50	-	-	-	-	40	50
Vegetative strip (m)		none	none	none	none	none	none	-	-	-	-	10	10
Nozzle reduction		none	75%	90%	none	75%	90%	-	-	-	-	none	none
D3	Ditch	0.138	0.108	0.103	0.089	0.063	0.059	-	-	-	-	n.r.	n.r.
D4	Pond	0.034	0.024	0.022	0.023	0.014	0.013	-	-	-	-	n.r.	n.r.
D4	Stream	0.070	0.026	0.017	0.053	0.018	0.011	-	-	-	-	n.r.	n.r.
D5	Pond	0.034	0.024	0.022	0.023	0.014	0.013	-	-	-	-	n.r.	n.r.
D5	Stream	0.075	0.028	0.018	0.057	0.019	0.012	-	-	-	-	n.r.	n.r.
R1	Pond	0.034	0.027	0.026	0.027	0.023	0.022	-	-	-	-	0.034	0.023
R1	Stream	0.147	0.147	0.147	0.147	0.147	0.147	-	-	-	-	0.088	0.066
R3	Stream	0.130	0.100	0.100	0.100	0.100	0.100	-	-	-	-	0.130	0.089
R4	Stream	0.218	0.218	0.218	0.218	0.218	0.218	-	-	-	-	0.098	0.098

PECsw values above the RAC of 0.130 µg/L are shown in **bold**.

Based on the performed calculations following conclusions may be derived:

FOCUS STEP 4 PEC_{sw} for fenpropidin following 1 × 250 g a.s./ha to winter cereals at BBCH 65 considering EVA derived deposition rates and a worst-case interception of 100 %

STEP 4		Max PEC _{sw} (µg/L) considering following mitigation:											
No spray buffer (m)		10	10	10	20	20	20	10	20	10	10	20	20
Vegetative strip (m)		none	none	none	none	none	none	10	20	10	10	20	20
Nozzle reduction		none	75%	90%	none	75%	90%	none	none	75%	90%	75%	90%
D3	Ditch	0.768	0.648	0.624	0.433	0.371	0.359	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
D4	Pond	0.142	0.118	0.113	0.084	0.068	0.065	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
D4	Stream	0.522	0.394	0.372	0.290	0.225	0.214	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
D5	Pond	0.142	0.118	0.113	0.084	0.068	0.065	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
D5	Stream	0.596	0.472	0.454	0.333	0.271	0.261	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
R1	Pond	0.141	0.117	0.113	0.084	0.068	0.065	0.141	0.084	-*	-*	-*	-*
R1	Stream	0.416	0.281	0.254	0.229	0.159	0.146	0.416	0.229	-*	-*	-*	-*
R3	Stream	0.595	0.429	0.398	0.329	0.244	0.229	0.595	0.329	-*	-*	-*	-*
R4	Stream	0.416	0.281	0.254	0.229	0.159	0.145	0.416	0.229	-*	-*	-*	-*
No spray buffer (m)		25	25	25	30	30	30	35	35	35	25	30	35
Vegetative strip (m)		none	none	none	none	none	none	none	none	none	10	10	10
Nozzle reduction		none	75%	90%	none	75%	90%	none	75%	90%	none	none	none
D3	Ditch	0.334	0.284	0.274	0.258	0.216	0.207	0.203	0.167	0.160	n.r.	n.r.	n.r.
D4	Pond	0.066	0.053	0.050	0.052	0.040	0.038	0.042	0.031	0.029	n.r.	n.r.	n.r.
D4	Stream	0.224	0.172	0.162	0.177	0.132	0.124	0.141	0.101	0.094	n.r.	n.r.	n.r.
D5	Pond	0.066	0.053	0.050	0.052	0.040	0.038	0.042	0.031	0.029	n.r.	n.r.	n.r.
D5	Stream	0.257	0.206	0.199	0.202	0.158	0.151	0.160	0.121	0.115	n.r.	n.r.	n.r.
R1	Pond	0.066	0.053	0.051	0.053	0.044	0.043	0.045	0.040	0.040	0.066	0.052	0.042
R1	Stream	0.178	0.122	0.115	0.142	0.115	0.115	0.115	0.115	0.115	0.178	0.142	0.113
R3	Stream	0.255	0.186	0.174	0.203	0.144	0.133	0.162	0.110	0.101	0.255	0.203	0.162

R4	Stream	0.178	0.122	0.110	0.142	0.094	0.085	0.113	0.073	0.065	0.178	0.142	0.113
No spray buffer (m)		40	40	40	50	50	50	-	-	-	-	40	50
Vegetative strip (m)		none	none	none	none	none	none	-	-	-	-	10	10
Nozzle reduction		none	75%	90%	none	75%	90%	-	-	-	-	none	none
D3	Ditch	0.160	0.129	0.122	0.100	0.075	0.070	-	-	-	-	n.r.	n.r.
D4	Pond	0.034	0.024	0.022	0.023	0.014	0.013	-	-	-	-	n.r.	n.r.
D4	Stream	0.114	0.078	0.072	0.078	0.046	0.042	-	-	-	-	n.r.	n.r.
D5	Pond	0.034	0.024	0.022	0.023	0.014	0.013	-	-	-	-	n.r.	n.r.
D5	Stream	0.128	0.093	0.088	0.086	0.055	0.051	-	-	-	-	n.r.	n.r.
R1	Pond	0.041	0.038	0.037	0.037	0.035	0.034	-	-	-	-	0.034	0.023
R1	Stream	0.115	0.115	0.115	0.115	0.115	0.115	-	-	-	-	0.093	0.064
R3	Stream	0.132	0.090	0.090	0.090	0.090	0.090	-	-	-	-	0.132	0.090
R4	Stream	0.092	0.057	0.053	0.064	0.053	0.053	-	-	-	-	0.092	0.063

* not calculated, as PEC_{sw} values are expected to be above the trigger value of 0.130 µg/L (RAC)

PEC_{sw} values above the RAC of 0.130 µg/L are shown in **bold**.

Winter cereals at BBCH 30:

• Fenpropidin:

- D3 scenario: risk acceptable: 40 m NBZ+75% DRN
- D4 scenario (pond): risk acceptable: 10 m NBZ+75% DRN or 20 m NBZ
- D4 scenario (stream): risk acceptable: 20 m NBZ+75% DRN or 40 m NBZ
- D5 scenario (pond): risk acceptable: 10 m NBZ+75% DRN or 20 m NBZ
- D5 scenario (stream): risk acceptable: 10 m NBZ+75% DRN or 25 m NBZ
- R1 scenario (pond): risk acceptable: 10 m NBZ+75% DRN or 20 m NBZ
- R1 scenario (stream): risk acceptable: 20 m NBZ+20 m VFS with 90% DRN or 40 m NBZ+10 m VFS
- R3 scenario (stream): risk acceptable: 35 m NBZ+ 75% DRN or 40 m NBZ
- R4 scenario (stream): risk acceptable: 35 m NBZ with 10 m VFS

2. Winter cereals at BBCH 65:

• Fenpropidin:

- D3 scenarios: risk acceptable: 40 m NBZ+75% DRN
- D4 scenario (pond): risk acceptable: 10 m NBZ+75% DRN or 20 m NBZ
- D4 scenario (stream): risk acceptable: 30 m NBZ +90% DRN or 40 m NBZ

- D5 scenario (pond): risk acceptable: 10 m NBZ+75% DRN or 20 m NBZ
- D5 scenario (stream): risk acceptable: 35 m NBZ+75% DRN or 40 m NBZ
- R1 scenario (pond): risk acceptable: 10 m NBZ+75% DRN or 20 m NBZ
- R1 scenario (stream): risk acceptable: 25 m NBZ+75% DRN or 35 m NBZ or 40 m NBZ+10 VFS
- R3 (stream): risk acceptable: 35 m NBZ+75% DRN or 50 m NBZ
- R4 scenario (stream) risk acceptable: 25 m +75% DRN or 35 m

As outlined in the table above, for the maximum application to winter cereals at BBCH 30-65, all Tier 1 PEC/RAC ratios for fenpropidin are below the relevant trigger of 1 at FOCUS Step 4, 10 m NSB + 90% DRT at the latest, except for the R1, stream, R3, stream and R4, stream scenario. Nevertheless, if considering Tier 3 data relevant for algae (i.e., a RAC of 0.5 µg a.s./L), an acceptable risk for all FOCUS scenarios and aquatic groups can be concluded with a 10 m NSB.

zRMS comment:

Based on the performed calculations following conclusions may be derived:

1. Spring cereals at BBCH 30:

- 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.
- ~~• Fenpropidin: acceptable risk with 10 m unsprayed buffer zone with 75% DRT in scenarios (D3, D4, D5).~~
- ~~Fenpropidin acceptable risk~~
- Fenpropidin:
 - D3 scenario: risk acceptable: 40 m NBZ+75% DRN
 - D4 scenario (pond): risk acceptable: 10 m NBZ+75% DRN or 20 m NBZ
 - D4 scenario (stream): risk acceptable: 20 m NBZ+75% DRN or 40 m NBZ
 - D5 scenario (pond): risk acceptable: 10 m NBZ+75% DRN or 20 m NBZ
 - D5 scenario (stream): risk acceptable: 10 m NBZ+90% DRN or 20 m NBZ+75% DRN or 40 m DRN
 - R4 scenario (stream): risk acceptable: 40 m NBZ with 10 m VFS

2. Spring cereals at BBCH 65:

- Prothioconazole: acceptable risk with no need for risk mitigation measures
- JAU 6476-desthio:
 - D scenarios and scenario R4: risk acceptable with no need for risk mitigation measures
- ~~• Fenpropidin acceptable risk with 10 m unsprayed buffer zone with 75% DRT in scenarios (D3, D4, D5, R4).~~
- Fenpropidin:
 - D3 scenarios: risk acceptable: 40 m NBZ+75% DRN
 - D4 scenario (pond): risk acceptable: 10 m NBZ+75% DRN or 20 m NBZ
 - D4 scenario (stream): risk acceptable: 30 m NBZ +75% DRN or 40 m
 - D5 scenario (pond): risk acceptable: 10 m NBZ+75% DRN or 20 m NBZ
 - D5 scenario (stream): risk acceptable: 35 m NBZ+75% DRN or 40 m NBZ
 - R4 scenario (stream): risk acceptable: 25 m +75% DRN or 35 m

It should be noted that 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 at BBCH 30:

- 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.
- ~~• Fenpropidin acceptable risk with 10 m unsprayed buffer zone with 75% DRT in scenarios (D3, D4, D5 and R3).~~
- ~~• Fenpropidin acceptable risk with 20 m VFS in scenarios R1 and R4~~
- Fenpropidin:
 - D3 scenario: risk acceptable: 40 m NBZ+75% DRN
 - D4 scenario (pond): risk acceptable: 10 m NBZ+75% DRN or 20 m NBZ
 - D4 scenario (stream): risk acceptable: 20 m NBZ+75% DRN or 40 m NBZ
 - D5 scenario (pond): risk acceptable: 10 m NBZ+75% DRN or 20 m NBZ
 - D5 scenario (stream): risk acceptable: 10 m NBZ+75% DRN or 25 m NBZ
 - R1 scenario (pond): risk acceptable: 10 m NBZ+75% DRN or 20 m NBZ
 - R1 scenario (stream): risk acceptable: 20 m NBZ+20 m VFS with 90% DRN or

- 40 m NBZ+10 m VFS
- o R3 scenario (stream): risk acceptable: 35 m NBZ+ 75% DRN or 40 m NBZ
- o R4 scenario (stream): risk acceptable: 35 m NBZ with 10 m VFS

4. Winter cereals at BBCH 65:

- Prothioconazole: acceptable risk with no need for risk mitigation measures
- JAU 6476-desthio:
 - o D scenarios: risk acceptable with no need for risk mitigation measures
 - o Scenario R3: risk acceptable with 10 m VFS.
- Fenpropidin acceptable risk with 10-m unsprayed buffer zone with 75% DRT in scenarios (D3, D4, D5, R3, R4).
- Fenpropidin acceptable risk with 20-m VFS in scenario R1 and R4 scenarios
- Fenpropidin:
 - o D3 scenarios: risk acceptable: 40 m NBZ+75% DRN
 - o D4 scenario (pond): risk acceptable: 10 m NBZ+75% DRN or 20 m NBZ
 - o D4 scenario (stream): risk acceptable: 30 m NBZ +90% DRN or 40 m NBZ
 - o D5 scenario (pond): risk acceptable: 10 m NBZ+75% DRN or 20 m NBZ
 - o D5 scenario (stream): risk acceptable: 35 m NBZ+75% DRN or 40 m NBZ
 - o R1 scenario (pond): risk acceptable: 10 m NBZ+75% DRN or 20 m NBZ
 - o R1 scenario (stream): risk acceptable: 25 m NBZ+75% DRN or 35 m NBZ or 40 m NBZ+10 VFS
 - o R3 (stream): risk acceptable: 35 m NBZ+75% DRN or 50 m NBZ
 - o R4 scenario (stream) risk acceptable: 25 m +75% DRN or 35 m

Based on the performed calculations for the worst-case scenario acceptable risk following application of ADM.03500.F.2.B according to the Central Zone GAP may be concluded.

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.

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

Mixture toxicity

For a mixture RA based on measured mixture toxicity, the ETR is calculated by division of the PEC_{mix} divided by the measured mixture toxicity. measured toxicity (EC_{xPPP}) is similar to the mixture composition at the PEC_{mix} in terms of the relative proportions of the individual a.s. As a direct comparison is not informative, as such, the comparison is done based on calculated mixture toxicity (assuming CA) for both mixture compositions, that is, a calculation of $EC_{xmix-CA}$ for the mixture composition of the a.s. at the PEC_{mix} and comparison with the respective estimate calculated for the formulation.

The relative proportion of a.s. is considered sufficiently similar if the outcome of these calculations deviates less than 20 %. Hence, if EC_{xPPP} (proportion of a.s. as contained in PPP) divided by $EC_{xmix-CA}$ (proportion of a.s. at PEC_{mix}) yields a value between 0.8 and 1.2, a direct comparison of PEC_{mix} with the EC_{xPPP} is feasible. If the mixture composition differs more profoundly, the measured data cannot be used directly for calculating ETR. Instead, the calculated approach was used to perform the mixture RA. For details, please refer to Appendix 3 where the $EC_{xmix-CA}$ for the mixture composition of the a.s. at the PEC_{mix} were calculated and compared with the estimate calculated for the formulation.

As it is obvious that PEC_{sw} values at least FOCUS Step 3 level is required to conclude an acceptable risk for fish, Daphnia and aquatic macrophytes (algae risk assessment is based on individual active substance data, for details, see above), the risk assessments were started at FOCUS Step 3 and Step 1/2 values were not taken into account. To further simplify the risk assessments for mixture toxicity, pond scenarios were not considered due to the low PEC_{sw} values.

zRMS comments:

Mixture toxicity assessment

Combination effects of fenpropidin + prothioconazole and fenpropidin + metabolite JAU-S-desthio in ADM.03502.F.1.A.

For a mixture RA based on measured mixture toxicity, the ETR is calculated by division of the PEC_{mix} divided by the measured mixture toxicity. measured toxicity (EC_{xPPP}) is similar to the mixture composition at the PEC_{mix} in terms of the relative proportions of the individual a.s. As a direct comparison is not informative, as such, the comparison is done based on calculated mixture toxicity (assuming CA) for both mixture compositions, that is, a calculation of EC_{xmix-CA} for the mixture composition of the a.s. at the PEC_{mix} and comparison with the respective estimate calculated for the formulation.

The relative proportion of a.s. is considered sufficiently similar if the outcome of these calculations deviates less than 20 %. Hence, if EC_{xPPP} (proportion of a.s. as contained in PPP) divided by EC_{xmix-CA} (proportion of a.s. at PEC_{mix}) yields a value between 0.8 and 1.2, a direct comparison of PEC_{mix} with the EC_{xPPP} is feasible. If the mixture composition differs more profoundly, the measured data cannot be used directly for calculating ETR. Instead, the calculated approach was used to perform the mixture RA.

zRMS provided files considering either fenpropidin + prothioconazole or fenpropidin + prothioconazole-desthio based on the already provided information that prothioconazole is quickly and fully degraded to prothioconazole-desthio.

For the mixture toxicity risk assessment of fish (acute), aquatic invertebrates (acute) and algae, where both product data as well as active substances data are available, the new excel based Aquatic Mixtox calculation tool (Aquatic mixtox assessment (v.1.15) recommended by the Central Zone was used.

For mixture toxicity risk assessment of macrophytes where only product data are available the tool is not suitable and thus were performed with own calculation sheets.

According to the EFSA Aquatic Guidance (EFSA, 2013) measured and calculated mixture toxicity should be compared to determine synergistic, additive or antagonistic effects of the formulation. In the following text, the concentration addition (CA) model is used as proposed by EFSA. To determine the respective formulation effect, EFSA proposed to calculate the model deviation ratio (MDR), which divides the calculated mixture toxicity (LC₅₀/EC₅₀ mix-CA) by the measured mixture toxicity (LC₅₀/EC₅₀ PPP).

Ecotoxicity studies are biological test systems which underlie a certain natural biological variability when repeating a study. Hence, a threshold has to be defined when an increased/decreased mixture toxicity effect cannot be seen as only additive any longer. EFSA proposes a factor of 5, i.e. if the MDR is between 0.2 and 5 the observed and calculated mixture toxicities are considered in agreement.

Considering the lowest EC₅₀ values determined for fenpropidin, prothioconazole and prothioconazole-desthio their nominal concentrations in ADM.03502.F.1.A the resulting EC₅₀, mix-CA value ADM.03502.F.1.A were calculated and shown below.

A surrogate endpoint for CA is calculated using the following equation.

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

With:

EC _{x mix-CA}	surrogate endpoint for additive mixture toxicity
n	number of mixture components
i	index from 1...n mixture components
p _i	the ith component as a relative fraction of the mixture composition (Σ p _i = 1)
EC _{xi}	concentration of component I provoking X % effect (or NOEC _i)

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

$$p_i = c_i / c_1 + \dots + c_n$$

Based on active substance concentrations of 250 g/L for fenpropidin and 175 g/L for prothioconazole (or prothioconazole-desthio, since transformed from parent to 100%, see argumentation provided above), fractions (pi) of 0.59 and 0.41 g/L are calculated respectively for fenpropidin and prothioconazole or prothioconazole-desthio.

The surrogate endpoint is related to the measured ECX or NOEC (ECX PPP) from product studies, where available, building the Model Deviation Ratio (MDR).

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

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

The EFSA guidance further requests to evaluate the relevance of formulation toxicity data for the active substance composition at PECmix.

$$PEC_{mix} = \sum_{i=1}^n PEC_i$$

Measured toxicity data for the product, in principle are considered relevant for mixture toxicity assessments only in case the mixture composition in the formulation is similar to the mixture composition at PECmix; i.e. if the ratio of calculated mixture toxicity (based on CA) for both mixture compositions does not deviate by more than 20%, respectively if:

$$\frac{EC_{X\text{ mix-CA for PPP}}}{EC_{X\text{ mix-CA for PECmix}}} = 0.8 - 1.2$$

The ECX mix-CA for PECmix is calculated based on relative proportions of individual actives at PECmix. In the following table, the mixture toxicity evaluation is summarized.

The assessment below followed the decision scheme as presented in the guidance document, and the excel file. PEC_{sw} calculated for each substance are used in the risk assessment. Screenshots of the Excel file are presented below.

Composition and toxicity of ADM.03502.F.1.A and its active substances.

Product data	
Product name	ADM.03502.F.1.A
Density of product [g/cm ³]	1,04
LC ₅₀ fish [mg prod./L]	6,23
LC ₅₀ fish a.s. based [mg sum of a.s./L]	2,5459
EC ₅₀ invertrebrates [mg prod./L]	5,57
LC ₅₀ invertrebrates a.s. based [mg sum of a.s./L]	2,2762
EC ₅₀ algae [mg prod./L]	0,000895
EC ₅₀ algae a.s. based [mg sum of a.s./L]	0,0004
EC ₅₀ macrophytes [mg prod./L]	0,596
EC ₅₀ macrophytes a.s. based [mg sum of a.s./L]	0,2436

Active substances data for aquatic organism.

Active Substance (a.s.) standard data (Tier 1 EP)				
Active substance names	Fenpropidin	Prothioconazole		
Concentration in Product [g a.s./L or g a.s./kg]	250	175		
p(X) (fraction in product)	0,59	0,41		
LC ₅₀ fish [mg a.s./L]	1,9	1,83		
LC ₅₀ invertebrates [mg a.s./L]	0,54	1,3		
EC ₅₀ algae [mg a.s./L]	0,00033	0,25		
EC ₅₀ macrophytes [mg a.s./L]				
Additional a.s. data (i.e. most sensitive species tested as Tier 1 data or refinements Tier 2A/B EP)				
LC ₅₀ fish [mg a.s./L]				
LC ₅₀ invertebrates [mg a.s./L]				
EC ₅₀ algae [mg a.s./L]				
EC ₅₀ macrophytes [mg a.s./L]				
AF for RAC				
Fish	100	100	100	100
Invertebrates	100	100	100	100
Algae	10	10	10	10
Macrophytes	10	10	10	10
RAC				
Fish	0,019	0,0183		
Invertebrates	0,0054	0,013		
Algae	0,000033	0,025		
Macrophytes				
Data used for calculation (after Step 3)				
Active substances	Fenpropidin	Prothioconazole		
Concentration in Product [g a.s./L]	250	175		
p(X) (fraction in product)	0,59	0,41		
LC ₅₀ fish [mg a.s./L]	1,9	1,83		
LC ₅₀ invertebrates [mg a.s./L]	0,54	1,3		
EC ₅₀ algae [mg a.s./L]	0,00033	0,25		
EC ₅₀ macrophytes [mg a.s./L]				

Active substances and JAU-desthio metabolite data for aquatic organism.

Active Substance (a.s.) standard data (Tier 1 EP)				
Active substance names	Fenpropidin	JAU destio		
Concentration in Product [g a.s./L or g a.s./kg]	250	175		
p(X) (fraction in product)	0,59	0,41		
LC ₅₀ fish [mg a.s./L]	1,9	6,63		
LC ₅₀ invertebrates [mg a.s./L]	0,54	10		
EC ₅₀ algae [mg a.s./L]	0,00033	0,55		
EC ₅₀ macrophytes [mg a.s./L]				
Additional a.s. data (i.e. most sensitive species tested as Tier 1 data or refinements Tier 2A/B EP)				
LC ₅₀ fish [mg a.s./L]				
LC ₅₀ invertebrates [mg a.s./L]				
EC ₅₀ algae [mg a.s./L]				
EC ₅₀ macrophytes [mg a.s./L]				
AF for RAC				
Fish	100	100	100	100
Invertebrates	100	100	100	100
Algae	10	10	10	10
Macrophytes	10	10	10	10
RAC				
Fish	0,019	0,0663		
Invertebrates	0,0054	0,1		
Algae	0,000033	0,055		
Macrophytes				
Data used for calculation (after Step 3)				
Active substances	Fenpropidin	JAU destio		
Concentration in Product [g a.s./L]	250	175		
p(X) (fraction in product)	0,59	0,41		
LC ₅₀ fish [mg a.s./L]	1,9	6,63		
LC ₅₀ invertebrates [mg a.s./L]	0,54	10		
EC ₅₀ algae [mg a.s./L]	0,00033	0,55		
EC ₅₀ macrophytes [mg a.s./L]				

Model Deviation Ratio (MDR) - fenpropidin + prothioconazole based on Tier 1 data.

Species	Substance	Concentration (C) in formulation (g a.s./L)	P _i	EC _{xi} (mg a.s./L)	EC _{mix-CA} (mg sum a.s. /L)	EC _{ppp} (mg sum a.s. /L)	MDR
Fish, acute toxicity							
<i>O. mykiss</i>	Fenpropidin	250	0,59	1,9	1,87	2,545913462	0,73
<i>O. mykiss</i>	Prothioconazole	175	0,41	1,83			
Invertebrates, acute toxicity							
<i>Daphnia magna</i>	Fenpropidin	250	0,59	0,54	0,71	2,276201923	0,31
<i>Daphnia magna</i>	Prothioconazole	175	0,41	1,3			
<i>P. subcapitata</i>	Fenpropidin	250	0,59	0,00033	0,001	0,000365745	1,53
	Prothioconazole	175	0,41	0,25			

**Model Deviation Ratio (MDR) - fenpropidin + prothioconazole-desthio (JAU-desthio)
Based on Tier 1 data.**

Species	Substance	Concentration (C) in formulation (g a.s./L)	P _i	ECxi (mg a.s./L)	ECx _{mix-CA} (mg sum a.s. /L)	ECx _{ppp} (mg sum a.s. /L)	MDR
Fish, acute toxicity							
species sp.	Fenpropidin	250	0,59	1,9	2,6903	2,545913462	1,06
	JAU destio	175	0,41	6,63			
Invertebrates, acute toxicity							
species sp.	Fenpropidin	250	0,59	0,54	0,88	2,276201923	0,39
	JAU destio	175	0,41	10			
Invertebrates, acute toxicity							
species sp.	Fenpropidin	250	0,59	0,00033	0,00	0,000365745	1,53
	JAU destio	175	0,41	0,55			

For fish and aquatic invertebrates, algae the observed and calculated mixture toxicities are considered in agreement, since the MDR is between 0.2 and 5. In such a case, measured mixture toxicity should be used in the risk assessment, at least if mixture compositions in the study and at PEC_{SW}(mix) are compatible.

Based on the tier 1 data calculations provided in Aquatic Tool agd_aquamix_v115 below fenpropidin is considered be a driver active substance for algae. Since it is obvious that algae are by far the most sensitive species for fenpropidin and higher tier data is required anyway to conclude an acceptable risk for algae.

Thus, it is deemed acceptable to perform the risk assessment individually on the basis of the data of the active substances which is in line with the recommendations of the EFSA guidance document.

Since for aquatic macrophytes only data for the formulated product are available which could be used for mixture toxicity, the product endpoint corrected for active substance content (i.e., 243.6 µg a.s.sum/L, based on product density of 1.04 g/cm³) was used in the risk assessment.

The consecutive steps of the mixture toxicity are not shown here in detail but instead the Excel calculation sheets for both combinations (fenpropidin + prothio and fenpropidin + prothio-desthio) for all uses in winter and spring cereals.

The assessment is shown separately for the mixture of fenpropidin + prothioconazole and fenpropidin + prothioconazole-desthio for the relevant uses in winter cereals and spring cereals.

However, in a comprehensive approach, some notes are provided here to clarify the steps taken in the Excel calculation sheets.

Mixture toxicity of Fenpropidin + Prothioconazole

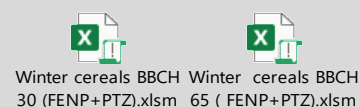
- It is noted that for algae for the mixture assessment of fenpropidin +prothioconazole, different species (*S. subspicatus* vs *P. subcapitata*) have to be compared due to the restricted data base. However, since both are green algae, this is considered acceptable.
- The required information on toxicity data for the product and the active substances and metabolite JAU dethio was entered into the “Input Tox” tab. For fenpropidin, a Tier 3 NOEC = 0.39 µg/L with AF 3 from mesocosms studies was not included as is stated in the aquatic guidance document (2013).
- The PEC_{SW} values were entered into the “Input PEC” tab for steps 1-3 of relevant FOCUS scenarios.

4. The “In-between Calc” table then provided automatically the MDR and ECx, mix-CA and ECxPPP values as provided in the tables above.
5. In the “Step 1” table the data availability is checked. Option 1 was chosen, since endpoints are available for the a.s. and the product and consequently, the Excel calculation sheet is directing to Step 2.
6. In the “Step 2” table MDR calculations are checked. The MDRs were between 0.2 and 5 and therefore Option 1 was chosen and consequently the Excel calculation sheet is directing to Step 3.
7. In the “Step 3” tab, the mixture composition in the product and at PEC_{mix} is compared. For fish, aquatic invertebrates and algae FOCUS STEP 2 and FOCUS STEP 3 scenarios that show agreement between mixture compositions, the Excel calculation sheet leads to Step 4.
8. In the “Step 4” ETRmix-PPP for fish and aquatic invertebrates was below than trigger which indicated low risk but in case of algae ETRmix-PPP was higher than trigger, low risk was not indicated for all STEP 3 scenarios. In this case the Excel calculation sheet leads to Step 5/8.
- 9.. In the “STEP 5” option 1 was chosen as fenpropidin contribute to more than 90% of the toxicity for all STEP3 scenarios and consequently the Excel calculation sheet is directing to Step 6.
10. According to STEP 6 the risk assessment should be conducted with individual a.s. toxicity data for the identified driver of mixture toxicity. In this case for a.s. fenpropidin.

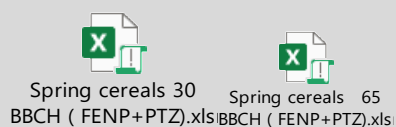
For fenpropidin mesocosm study was available and the risk assessment was based on RAC of 0.13 microgram fenpropidin /L with FOCUS STEP 4 PEC_{sw} calculations. Therefore, the risk for algae was provided in the Tables above.

Nested EXCEL files:

Aquatic mixture toxicity for fenpropidin + prothioconazole for application in winter cereals at BBCH 30 and BBCH 65:



Aquatic mixture toxicity for fenpropidin + prothioconazole for application in spring cereals at BBCH 30 and BBCH 65:



Mixture toxicity of Fenpropidin and metabolite prothioconazole-desthio (JAU desthio)

1. The PEC_{sw} values were entered into the “Input PEC” tab for steps 1-3 of relevant FOCUS scenarios.
2. The required information on toxicity data for the product and the active substances and metabolite JAU desthio was entered into the “Input Tox” tab. For fenpropidin, a Tier 3 NOEC= 0.39 µg/L with AF 3 from mesocosms studies was not provided for the mixture toxicity data as is stated in the aquatic guidance document (2013).
3. The PEC_{sw} values were entered into the “Input PEC” tab for steps 1-3 of relevant FOCUS scenarios.

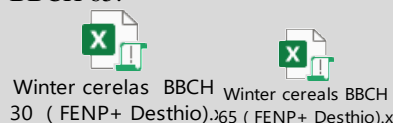
4. The “In-between Calc” table then provided automatically the MDR and ECx, mix-CA and ECxPPP values as provided in the tables above.
5. In the “Step 1” table the data availability is checked. Option 1 was chosen, since endpoints are available for the a.s. and the product and consequently, the Excel calculation sheet is directing to Step 2.

6. In the “Step 2” table MDR calculations are checked. The MDRs were between 0.2 and 5 and therefore Option 1 was chosen and consequently the Excel calculation sheet is directing to Step 3.
7. In the “Step 3” tab, the mixture composition in the product and at PEC_{mix} is compared. For fish, aquatic invertebrates and algae FOCUS STEP 3 scenarios that show not agreement between mixture compositions the Excel calculation sheet leads to Step 5 and to “STEP 4”.
8. In the “Step 4” ETRmix-PPP for scenarios for which the values were below the trigger, the low risk was identified.
- 9.. In the “STEP 5” option 1 was chosen as fenpropidin contribute to more than 90% of the toxicity for STEP 3 scenarios for fish, aquatic invertebrates and for algae and consequently the Excel calculation sheet is directing to Step 6.
10. According to STEP 6 the risk assessment should be conducted with individual a.s. toxicity data for the identified driver of mixture toxicity. In this case for a.s. fenpropidin.

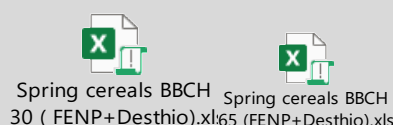
For fenpropidin – driver active substance mesocosm study was available and the risk assessment was based on RAC of 0.13 $\mu\text{g a.s./L}$ with STEP 4 PEC_{sw} calculations.

Nested EXCEL files:

Aquatic mixture toxicity for fenpropidin + prothioconazole-desthio for application in winter cereals at BBCH 30 and BBCH 65.



Aquatic mixture toxicity for fenpropidin + prothioconazole-desthio for application in spring cereals at BBCH 30 and BBCH 65:



Commenting period process:

Mixture toxicity assessment:

According to information provided in Section 8 PEC_{sw} values calculated in FOCUS Step 3-4 for the active substance fenpropidin were re-calculated by the Applicant and was considered valid by e-fate expert. In case of PEC_{sw} values at BBCH 65 at STEP 3 a slight difference were noted in comparison to previously presented in Section 8. There, where necessary zRMS amended the calculations for these scenarios in exe files.

Based on the Tier-1 data calculations provided in Aquatic Tool agd_aquamix_v115, fenpropidin is considered be a driver active substance for algae. Since it is obvious that algae are by far the most sensitive species for fenpropidin and higher tier data is required anyway to conclude an acceptable risk for algae, it is deemed acceptable to perform the risk assessment individually on the basis of the data of the active substances which is in line with the recommendations of the EFSA guidance document and with the conclusions of the zRMS Thus, for algae also no additional mixture toxicity calculations need to be provided here.

Finally, for fish (chronic), the only aquatic group where no acceptable risk could be concluded at FOCUS Step 3, the zRMS considered only Step 4 PEC_{sw} values for the metabolite JAU-desthio, while for the active substance fenpropidin only Step 2 PEC_{sw} values were used in the mixture toxicity assessment. Since Step 4 values for the metabolite were not recalculated, also this assessment does not need to be updated.

Table 9.5-19: **Mixture toxicity: Acceptability of risk for aquatic macrophytes** ~~each organism group~~
based on FOCUS Step 3 calculations for the maximum application rate of
ADM.03502.F.1.A to spring cereals at BBCH 30-65

Intended use	Spring cereals, BBCH 30
Product	ADM.03502.F.1.A
Organisms	<i>Daphnia</i> , acute

Organisms	Aquatic macrophytes								
Measured endpoint [µg/L]	243.6 (also covering fish, chronic [NOEC _{mix} = 304 µg/L] as well as invertebrates, chronic [NOEC _{mix} = 388.6 µg/L])								
Calculated endpoint [µg/L]	Not available								
	Spring cereals BBCH 30-65								
FOCUS scenario	Substance	FOCUS step	PEC_{sw} (µg/L)	PEC_{mix}	EC_xPPP / EC_xmix-CA ratio ¹⁾	≥ 90% contribution of a.s. to mixture toxicity	ETR_{mix}	Trigger	Acceptable risk?
D3 ditch	Prothioconazole	Step 3	1.107 1.109	2.662 2.664	Not relevant	Not relevant	0.0109 0.010	0.1	Yes
	Fenpropidin		1.555						
D4 stream	Prothioconazole	Step 3	0.905 0.954	2.175 2.32	Not relevant	Not relevant	0.0089 0.0095	0.1	Yes
	Fenpropidin		1.270 1.366						
D5 stream	Prothioconazole	Step 3	0.929 1.031	2.234 2.486	Not relevant	Not relevant	0.0092 0.010	0.1	Yes
	Fenpropidin		1.305 1.455						
R4 stream	Prothioconazole	Step 3	0.732	1.758 1.763	Not relevant	Not relevant	0.0072	0.1	Yes
	Fenpropidin		1.026 1.031						

⁺ If EC_xPPP (proportion of a.s. as contained in PPP) divided by EC_xmix-CA (proportion of a.s. at PEC_{mix}) yields a value between 0.8 and 1.2, a direct comparison of PEC_{mix} with the EC_xPPP is feasible. Otherwise, the EC_xmix-CA was used, unless ²⁾ one of the active substance contributes ≥ 90 % to mixture toxicity. In such cases, no further calculations are considered to be required and risk assessments are performed on individual active substance data (according to recommendations of EFSA Journal 2013;11(7):3290. Data for EC_xPPP (proportion of a.s. as contained in PPP) / EC_xmix-CA (proportion of a.s. at PEC_{mix}) were provided in Appendix 3

*Values at BBCH 65

Table 9.5-20:

Intended-use	Winter cereals, BBCH 30								
Product	ADM.03502.F1.A								
Organisms	<i>Daphnia</i> , acute								
Measured endpoint [µg/L]	2276 (also covering fish, acute [LC _{50-mix} = 2546 µg/L]))								
Calculated endpoint [µg/L]	711.2								
FOCUS-scenario	Substance	FOCUS step	PEC _{sw} (µg/L)	PEC _{mix}	EC ₅₀ PPP / EC ₅₀ mix- CA ratio ¹⁾	≥90%-con- tribution-of a.s. to mix- ture toxicity ²⁾	ETR _{mix}	Trigger	Accept- able risk?
D3-ditch	Prothioconazole	Step 3	1.106	2.66	1.0	Not relevant	0.0012	0.01	Yes
	Fenpropidin		1.554						
D4-stream	Prothioconazole	Step 3	0.817	1.964	1.0	Not relevant	0.0009	0.01	Yes
	Fenpropidin		1.147						
D5-stream	Prothioconazole	Step 3	0.883	2.122	1.0	Not relevant	0.0009	0.01	Yes
	Fenpropidin		1.239						
R1-stream	Prothioconazole	Step 3	0.726	1.743	1.0	Not relevant	0.0008	0.01	Yes
	Fenpropidin		1.017						
R3-stream	Prothioconazole	Step 3	1.023	2.46	1.0	Not relevant	0.0011	0.01	Yes
	Fenpropidin		1.437						
R4-stream	Prothioconazole	Step 3	0.732	1.758	1.0	Not relevant	0.0008	0.01	Yes
			1.026						

Organisms	Aquatic macrophytes								
Measured endpoint [µg/L]	243.6 (also covering fish, chronic [NOEC _{mix} = 304 µg/L] as well as invertebrates, chronic [NOEC _{mix} = 388.6 µg/L])								
Calculated endpoint [µg/L]	Not available								
	Winter cereals BBCH 30-65								
FOCUS scenario	Substance	FOCUS step	PEC _{sw} (µg/L)	PEC _{mix}	EC _x PPP / EC _x mix-CA ratio ¹⁾	≥ 90% contribution of a.s. to mixture toxicity	ETR _{mix}	Trigger	Acceptable risk?
D3 ditch	Prothioconazole	Step 3	1.106 1.110	2.66 2.677	Not relevant	Not relevant	0.0109	0.1	Yes
	Fenpropidin		1.554 1.567						
D4 stream	Prothioconazole	Step 3	0.817 0.957	1.964 2.307	Not relevant	Not relevant	0.0081 0.0094	0.1	Yes
	Fenpropidin		1.147 1.350						
D5 stream	Prothioconazole	Step 3	0.883 1.032	2.122 2.488	Not relevant	Not relevant	0.0087 0.0090	0.1	Yes
	Fenpropidin		1.239 1.456						
R1 stream	Prothioconazole	Step 3	0.726 0.732	1.743 1.763	Not relevant	Not relevant	0.0072	0.1	Yes
	Fenpropidin		1.017 1.031						
R3 stream	Prothioconazole	Step 3	1.023 1.025	2.46 2.471	Not relevant	Not relevant	0.0101	0.1	Yes
	Fenpropidin		1.437 1.446						
R4 stream	Prothioconazole	Step 3	0.732	1.758 1.763	Not relevant	Not relevant	0.0072	0.1	Yes
	Fenpropidin		1.026 1.031						

¹⁾ If EC_xPPP (proportion of a.s. as contained in PPP) divided by EC_xmix-CA (proportion of a.s. at PEC_{mix}) yields a value between 0.8 and 1.2, a direct comparison of PEC_{mix} with the EC_xPPP is feasible. Otherwise, the EC_xmix-CA was used, unless ²⁾ one of the active substance contributes ≥ 90 % to mixture toxicity. In such cases, no further calculations are considered to be required and risk assessments are performed on individual active substance data (according to recommendations of EFSA Journal 2013;11(7):3290. Data for EC_xPPP (proportion of a.s. as contained in PPP) / EC_xmix-CA (proportion of a.s. at PEC_{mix}) were provided in Appendix 3 values at BBCH 65

zRMS comments:

Chronic mixture toxicity:

It should be noted that the risk assessment for the mixture should also include the prothioconazole metabolite JAU 6476-desthio, whose chronic toxicity to fish is significantly higher compared to the parent substance. Therefore, a combined toxicity assessment for the chronic fish is presented in the following:
The evaluation of potential mixture toxicity is performed under consideration of the current EFSA guidance (2013).

1. Are measured toxicity data (EC_x) available for the given endpoint (typically chronic data available only for a.s.)?

Only for the a.s. (EC_x_{a.s.}): Go to 7

For both formulation (EC_xPPP) and a.s. (EC_x_{a.s.}): Go to 2

Measured toxicity data (NOEC) for chronic fish is available only for the active substances.

➔ **Go to 7**

7. Is there evidence that synergistic interactions between mixture components might occur (e.g. based on toxicological knowledge from literature or from counter-checking measured and calculated mixture toxicity in other species) which cannot be ruled out for the given species with sufficient certainty?

Yes (mixture toxicity calculation not feasible): Measured mixture toxicity data required for RA (if becoming available: Go to 2)

No (mixture toxicity calculation feasible): Go to 8

There is no evidence for synergistic interactions between mixture components.

➔ **Go to 8**

8. Conduct a mixture RA based on calculated mixture toxicity according to 10.3.8:

$$ETR_{mix-CA} = \frac{PEC_{mix}}{ECx_{mix-CA}}$$

If $ETR_{mix-CA} < \text{trigger}$: Low risk

If $ETR_{mix-CA} > \text{trigger}$: Low risk not demonstrated, check single-substance refinement options

If the endpoints to be used for the RA refer to the same taxonomic group but are associated with different AFs (e.g. single species test, Geomean or SSD), the calculation of the mixture risk is assessed by:

$$RQ_{mix} = \sum_{i=1}^n \frac{PEC_i}{RAC_i}$$

If $RQ < 1$: Low risk

If $RQ > 1$: Low risk not demonstrated/check exposure refinement options (see 10.3.10)

A mixture long term risk assessment for fish based on calculated mixture toxicity according to 10.3.8. (RQ_{mix} approach) has been conducted by zRMS, considering the biologically active metabolite of prothioconazole JAU 6476-desthio and a.s. fenpropidin. The RQ values for each compound are taken from the relevant Tables.

The RQ_{mix} calculations are presented below:

Scenario specific combined toxicity assessment – Long-term fish Spring cereals BBCH 30.

	RQ values	RQ values	RQ_{mix}	Trigger
Focus scenario	JAU desthio (Step 3)	Fenpropidin (Step 2)		
D3 Ditch	0.1	0.08	0.18	<1
D4 Pond	0.02	0.08	0.1	
D4 Stream	0.1	0.08	0.18	
D5 Pond	0.02	0.08	0.1	
D5 Stream	0.1	0.08	0.18	
R4 Stream	1.40	0.08	1.48	
Focus scenario (Step 4) Step 4, 10-m NSB (+ VS)		Fenpropidin (Step 2)	RQ_{mix}	
R4 Stream	0.7	0.08	0.78	

Scenario specific combined toxicity assessment – Long-term fish, Spring cereals BBCH 65.

	RQ values	RQ values	RQ_{mix}	Trigger
Focus scenario	JAU desthio (Step 3)	Fenpropidin (Step 2)		
D3 Ditch	0.11	0.08	0.19	<1
D4 Pond	0.020	0.08	0.10	
D4 Stream	0.075	0.08	0.155	
D5 Pond	0.020	0.08	0.1	
D5 Stream	0.11	0.08	0.19	
R4 Stream	0.06	0.08	0.14	

Scenario specific combined toxicity assessment – Long-term fish, Winter cereals BBCH 30.

	RQ values	RQ values	RQ_{mix}	Trigger
Focus scenario	JAU desthio (Step 3)	Fenpropidin (Step 2)		
D3 Ditch	0.1	0.08	0.19	<1
D4 Pond	0.02	0.08	0.1	
D4 Stream	0.1	0.08	0.19	
D5 Pond	0.02	0.08	0.1	
D5 Stream	0.1	0.08	0.19	
R1 Pond	0.1	0.08	0.19	
R1 Stream	1.3	0.08	1.38	
R3 Stream	1.1	0.08	1.18	
R4 Stream	1.7	0.08	1.78	
Focus scenario (Step 4) 10-m NSB (+ VS)		Fenpropidin (Step 2)	RQ_{mix}	
R1, stream	0.6	0.08	0.82	
R3, stream	0.5	0.08	0.58	
R4, stream	0.8	0.08	0.88	

Scenario specific combined toxicity assessment – Long-term fish, Winter cereals BBCH 65.

Focus scenario (Step 3)	RQ values		RQmix	Trigger
	JAU desthio (Step 3)	Fenpropidin (Step 2)		
D3 Ditch	0.146	0.08	0.226	<1
D4 Pond	0.020	0.08	0.1	
D4 Stream	0.078	0.08	0.158	
D5 Pond	0.02	0.08	0.1	
D5 Stream	0.11	0.08	0.19	
R1 Pond	0.20	0.08	0.28	
R1 Stream	0.784	0.08	0.864	
R3 Stream	1.158	0.08	1.238	
R4 Stream	0.06	0.08	0.14	
Focus scenario (Step 4) 10-m NSB (+ VS)		Fenpropidin (Step 2)		
R3 Stream	0.51	0.08	0.59	

Based on calculations above the combined chronic risk assessment is considered acceptable.

9.5.3 Overall conclusions

Based on PEC/RAC calculations for the active substances prothioconazole, fenpropidin as well as 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.

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.03502.F.1.A, 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 the active substances. 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.03502.F.1.A, 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	Fenpropidin technical	contact	48-h LD ₅₀ = 46 > 10 µg a.s./bee	EFSA Scientific Report (2007) 124, 1-84
		oral	48-h LD ₅₀ > 10 46.0 µg a.s./bee	
Apis mellifera	ADM.03502.F.1.A (250 g fenpropidin + 175 g prothioconazole/L)	contact	48-h LD ₅₀ = 470 µg prod./bee	KCP 10.3.1.1/01 Franke, M., 2020, report no.: 2048BAA0028
		oral	48-h LD ₅₀ = 505 µg prod./bee	
Chronic toxicity				
Apis mellifera	ADM.03502.F.1.A (250 g fenpropidin + 175 g prothioconazole/L)	oral, adults	10-d LDD ₅₀ = 56.6 µg prod./bee/d NOEDD = 31.9 µg prod./bee/d	KCP 10.3.1.2/01: Dreßler, K., 2021, report no.: 2048BAC0011
Apis mellifera	ADM.03502.F.1.A (250 g fenpropidin + 175 g prothioconazole/L)	oral, larvae	22-d NOED = 0.02 µg prod./larva	KCP 10.3.1.3/01: Hänsel, M., 2021 report no.: 2048BLC0013
Semi-field tests				
Apis mellifera	ADM.03500.F.2.B (Prothioconazole EC 250)	No effects on adult honey bees and developmental stages from the application of 1.0 L ADM.03500.F.2.B/ha (equivalent to 250 g prothioconazole/ha) could be detected in a semi-field test conducted in Germany.		KCP 10.3.1.5/01: Persigehl <i>et al.</i> , 2021, report no.: B19010-3

Species	Substance	Exposure System	Results	Reference
<i>Apis mellifera</i>	ADM.1351.F.1.A (371 g fenpropidin/L + 109 g difenoco-nazole/L)	No effects on adult honey bees and developmental stages from the application of 1.0 L ADM.1351.F.1.A/ha (equivalent to 371 g fenpropidin/ha) could be detected in a semi-field test conducted in Germany.		KCP 10.3.1.5/02; Hecht-Rost, S., 2020; report no.: R1940026

zRMS comments:

Acute bee toxicity data for fenpropidin and prothioconazole provided in Table 9.6-1 are in line with EU agreed endpoints reported in EFSA Journal (2007) 124, 1-84 and EFSA Scientific Report (2007) 106, respectively.

To fulfil the data requirements as set by Commission Regulation (EU) No 284/2013, studies on acute toxicity to adult bees and chronic and larvae toxicity to bees were submitted with the formulated product.

Studies on effects of the formulated product to bees listed in Table above 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.

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.03502.F.1.A to adult honeybees as well as for honeybee larval toxicity. These studies are considered to be required according to Regulation (EC) No. 284/2013.

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 (SAN-CO/10329/2002 rev 2 (final), October 17, 2002). The 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.03502.F.1.A is an emulsifiable concentrate (EC) containing 175 g/L of the active substance prothioconazole and 250 g/L of the active substance fenpropidin. It is a fungicide applied as spray to infested foliage of cereals. The timing of application is post-emergence. The worst-case application scenario leading to maximum contamination of the environment is a single spray application at a rate of 1.0 L prod./ha (corresponding to 175 g prothioconazole/ha and 250 g fenpropidin/ha). For a detailed summary of the GAP uses of ADM.03502.F.1.A, 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 1.0 L prod./ha [equivalent to 175 g prothioconazole/ha + 250 g fenpropidin/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) / LD₅₀ (in µg/bee)] confirming an acceptable acute contact risk for bees were calculated considering the lowest contact LD₅₀ values and the maximum single application rate of 1.0 L prod./ha [equivalent to 175 g prothioconazole/ha + 250 g fenpropidin/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.03502.F.1.A

Intended use	Cereals, 1 × 1.0 L prod./ha, BBCH ≥ 10		
Active substance 1	Prothioconazole		
Application rate (g/ha)	1 × 175 g a.s./ha		
Test design	LD₅₀ (lab.) (µg a.s./bee)	Single application rate (g a.s./ha)	HQ_{contact} criterion: HQ ≤ 50
Contact toxicity	> 200	175	< 0.9
Active substance 2	Fenpropidin		
Application rate (g/ha)	1 × 250 g a.s./ha		
Test design	LD₅₀ (lab.) (µg a.s./bee)	Single application rate (g a.s./ha)	HQ_{contact} criterion: HQ ≤ 50
Contact toxicity	46 > 10	250	5.43 < 25
Product	ADM.03502.F.1.A		
Application rate (L prod./ha)	1 × 1.0 L prod./ha		
Test design	LD₅₀ (lab.) (µg prod./bee)	Single application rate (g prod./ha)	HQ_{contact} criterion: HQ ≤ 50
Contact toxicity	470	1040*	2.2

HQ: Hazard quotients for contact exposure

* Calculated on the basis of a density of 1.04 g/mL

As outlined in the table above, HQ_{contact} values for the active substances 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) / LD₅₀ (in µg/bee)] confirming an acceptable acute oral risk for bees were calculated considering the lowest oral LD₅₀ values and the maximum single application rate of 1.0 L prod./ha [equivalent to 175 g prothioconazole/ha + 250 g fenpropidin/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.03502.F.1.A

Intended use	Cereals, 1 × 1.0 L prod./ha, BBCH ≥ 10		
Active substance 1	Prothioconazole		
Application rate (g/ha)	1 × 175 g a.s./ha		
Test design	LD₅₀ (lab.) (µg a.s./bee)	Single application rate (g a.s./ha)	HQ_{contact} criterion: HQ ≤ 50
Oral toxicity	> 71	175	< 2.5

Intended use	Cereals, 1 × 1.0 L prod./ha, BBCH ≥ 10		
Active substance 2	Fenpropidin		
Application rate (g/ha)	1 × 250 g a.s./ha		
Test design	LD₅₀ (lab.) (µg a.s./bee)	Single application rate (g a.s./ha)	HQ_{contact} criterion: HQ ≤ 50
Oral toxicity	> 10 46	250	< 25 5.4
Product	ADM.03502.F.1.A		
Application rate (g/ha)	1 × 1.0 L prod./ha		
Test design	LD₅₀ (lab.) (µg prod a.s./bee)	Single application rate (µg prod a.s./ha)	HQ_{contact} criterion: HQ ≤ 50
Oral toxicity	505	1040*	2.1

HQ: Hazard quotients for coral exposure * Calculated on the basis of a density of 1.04 g/mL

As outlined in the table above, HQ_{oral} values for the active substances 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. HQ_{oral, contact} values for the active substances and the formulated product are below the trigger of 50, indicating a low acute risk for bees.

Please note that the evaluation has been performed in line with SANCO/10329/2002 rev 2 final.

Overall, acceptable risk to bees may be concluded from the intended uses of ADM.03502.F.1.A.

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. However, no deterministic risk assessment was conducted for chronic exposure, as there is currently no approved assessment scheme. Additionally, a chronic risk to bees might be not expected for the following reasons:

(1) Exposure to treated crops

Cereals are not mentioned as being attractive to bees in common handbooks on honeybee foraging plants (e.g. Maurizio & Schaper, 1994; Pritsch, 2007). In conclusion, a potential risk arising from the consumption of pollen and nectar from the treated crops can be reasonably excluded and thus no chronic risk assessment for the exposure scenario “treated crops” needs to be provided.

(2) Exposure to weeds in the treated field

Additionally, as part of an industry led initiative, the European Crop Protection Association (ECPA) performed a data analysis to check the relevance of the ‘weeds in the treated field’ exposure scenario (Last *et al.*, 2019). Background for this is the following statement in the EFSA GD, that if < 10 % of the area of use contains attractive flowering weeds then the exposure route is not relevant:

“If the first step results in an unacceptable risk, it may be checked whether it is likely that a significant fraction of the surface area of the treated fields is covered by weeds at the application time. If this is likely in less than 10 % of the area of use of the substance, no weeds will occur in a 90th percentile case and thus their exposure can be ignored (box 2). For example, weeds are usually not abundant in annual crops - abundant weed growth is more likely to occur in, for example, orchards. However, at this moment no guidance for the assessment of the abundance of weeds is available for most crops”.

For this, herbicide efficacy trial control data from a range of arable crops (sunflower, maize, oilseed rape,

cereals, sugar beet, potatoes, peas and beans) as well as some permanent crops (orchards, citrus and grapes) were supplied by industry and form a large data set of information on the presence of weed species within trial plots (consisting of over 8500 efficacy trials, conducted throughout Europe, comprising 45000 individual data recordings where weed BBCH growth stage data are available). Relevant information has been extracted from the efficacy data with the intention of demonstrating that, for some crops, the occurrence of attractive flowering weeds in treated fields is relatively rare and constitutes less than 10 % of the area of use, thereby highlighting that the weeds in the treated field scenario is not applicable for many typical commercially grown crops. The data were analysed and assessments made specifically on the presence of weed species during each trial, the growth stage of the weed species present, the attractiveness to bees of the weed species present, the ground coverage of the weed species present, the trial location, dates of the trial and the crop growth stage used in the trials.

The analysis of the herbicide efficacy trial data has demonstrated that the incidence of attractive flowering weeds in arable fields is low (less than 4 %). Due to the large volume of trials considered and the wide distribution of these trials throughout Europe, it is considered that these findings are representative of what would occur throughout Europe for these particular arable crops. It is further noted that the incidence of attractive flowering weeds with > 10 % ground cover is even lower (less than 0.4 %) in the arable fields assessed. The total ground cover of attractive flowering weeds was also very low for the arable fields assessed; mean values ranged from 0 % to 1.6 % and 90th percentile values were 0 % for each of the arable crop species.

Taking all of this into account, it is considered that attractive flowering weeds will not be present at a significant frequency or ground coverage in arable fields according to the intended GAP uses of ADM.03502.F.1.A in cereals.

Nevertheless, additionally semi-field tests with formulated prothioconazole as well as formulated fenpropidin (combi product with difenoconazole) are available, indicating that no chronic risk must be expected for bees exposed to the active substances.

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

Semi-field study with formulated prothioconazole

A study with ADM.3500.F.2.B (250 g prothioconazole/L) is available (Persigehl *et al.*, 2021; KCP 10.3.1.5/01) which determined possible side effects of ADM.03500.F.2.B after spray application on honey bees (*Apis mellifera* L.) in tunnel tents under confined semi-field conditions. The methods of investigating the development of the honey bees is based on the Guideline OEPP/EPPO No. 170 (4) (2010).

The study was conducted in North Rhine-Westphalia, Germany. The study field was sown with *Phacelia*, which served as a surrogate crop. For each treatment group (control, test item and reference item) four tunnels were set up for the measurements of effects (assessment tunnels), three additional tunnels treated with the test item were set up for the collection of residue samples (sampling tunnels), resulting in 15 tunnels in total. Honey bee colonies were placed in the tunnels with *Phacelia* (BBCH 65) three days before application. Applications of the test item (ADM.3500.F.2.B), reference item (dimethoate) and control were conducted by spraying the whole area of *Phacelia* plants within the tunnels during full bee flight and at full flowering of the crop. The crop height was approximately 80 cm in all tunnels. Plants in the control group were sprayed with tap water (400 L/ha). The application rate in the test item treatment group was 0.8 L prod./ha, corresponding to nominal 200 g prothioconazole/ha. The reference item tunnels were sprayed with 1.2 L product/ha (corresponding to nominal 480 g dimethoate/ha).

During the pre-exposure and the exposure phase mortality was assessed using dead-bee traps and non-woven sheets. Also, the foraging activity and any behavioural symptoms of intoxication were recorded in each replicate. Residues of prothioconazole and prothioconazole-desthio on flowers, pollen and nectar were assessed, in order to proof the exposure of honey bees to the test item. The limit of quantification (LOQ) was 0.01 mg/kg with a limit of detection (LOD) of 0.003 mg/kg.

No effects on mortality of adult honey bees and colony strength could be detected after application of the product ADM.3500.F.2.B (prothioconazole 250 g/L) in this semi-field test. Additionally, results for the reference item (dimethoate) treatment group together with additionally recorded parameters such as foraging activity and the analytical results show that the test system provided adequate exposure and sensitivity.

Semi-field study with formulated fenpropidin

Another study with ADM.1351.F.1.A (371 g fenpropidin/L + 109 g difenoconazole/L) is available (Hecht-Rost, S., 2020; KCP 10.3.1.5/02) which determined possible adverse effects of ADM.1351.F.1.A (Spyrale) on colonies of honeybees (*Apis mellifera* L.) under semi-field conditions in *Phacelia tanacetifolia* in Germany in accordance with the OEPP/EPPO Guideline 1/170 (4) (2010). Since the amount of fenpropidin considered in the semi-field test is much higher than the maximum application rate of ADM.03502.F.1.A (i.e. 250 g fenpropidin/ha) in cereals, the results of this study can be seen as absolute worst-case approach.

The study included one test item treatment, one tap water treated control and one reference item treatment (Danadim® Progress; a.s. dimethoate). The treatment groups comprised four replicates. For collection of certain specimens for residue analysis three additional tunnels were assembled, one for the control and two for the test item group.

The nominal application rate of the test item was 1.0 L ADM.1351.F.1.A F/ha (a.s. analysed: 371 g fenpropidin/ha, 109 g difenoconazole/ha) for the test item group and in the additional tunnels for residue samplings of pollen and nectar. A second group treated with tap water served as control and in the additional tunnel for residue samplings of pollen and nectar. As reference item Danadim® Progress (a.s. dimethoate) was applied at a rate of 1.2 L product/ha (480 g a.s./ha). All applications were carried out during full flowering and honeybee-flight with a spray volume of 400 L water/ha. Colony development and mortality were assessed as well as sublethal parameters like foraging activity and behaviour of honeybees in order to evaluate possible impact of the test item on honeybees. Additionally, flowers, pollen and nectar from forager bees were sampled and analysed for potential residues of the test item. Analytical results demonstrated that honeybees were exposed to ADM.1351.F.1.A inside the tunnels throughout the entire exposure period within the tunnels. A residue decline of both active substances could be observed in nectar and pollen. The limit of quantification (LOQ) of the analytical method for each matrix was 0.01 mg a.s./kg and the LOD was set at 0.003 mg a.s./kg (30% of the LOQ) for both active substances (fenpropidin and difenoconazole).

The application of ADM.1351.F.1.A did not cause adverse effects on the survival of adult worker bees, bee pupae, behaviour, colony strength and colony development. Overall, this study demonstrated that Spyrale applied at a nominal rate of 1.0 L product/ha (371 g a.s. fenpropidin/ha, 109 g a.s. difenoconazole/ha) during honeybee flight did not adversely affect mortality, behaviour, strength, and development of honeybee colonies.

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 acute and chronic risks to adult honey bees and honey bee larvae bees from the use of ADM.03502.F.1.A

were assessed using the maximum single application rates and the respective ‘hazard quotients’ (HQs) and ‘exposure toxicity ratios’ (ETRs).

Test	Endpoint µg prod./bee	Calculation factor	ETR	Trigger	Risk acceptable?
Cereals, BBCH 30-83, maximum application dose 1.04 kg product/ha					
Oral route of exposure					
Honey bee, chronic	56.6	7.6 / 10.6	0.140	0.03	No
Honey bee, larvae	0.02	4.4 / 6.1	228.80	0.2	No

HQ/ETR values in bold are above the trigger value

Considering the proposed uses of ADM.03502.F.1.A at a maximum application rate of 1.04 kg product/ha a potential risk of formulation is indicated following the chronic exposure of adults and for honey bee larvae at this stage of testing. Therefore, 1st tier oral risk assessments were carried out (see Table below).

1st tier, oral 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 ADM.03502.F.1.A. will be applied to cereals.

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

Crop (Crop group according to EFSA tool)	Endpoint	ETR (oral exposure scenario)					Trigger
		Treated crop	Weeds	Field margin	Adjacent crop	Next crop	
Maximum single application rate: 1.04 kg product/ha, BBCH 30-39							
Cereals	adult, chronic	0.012	0.019	0.000	0.000	0.007	0.03
	larvae	6.63	48.62	0.89	0.64	17.68	0.2
Maximum single application rate: 1.04 kg product/ha, BBCH 40-69							
Cereals	adult, chronic	0.012	0.012	0.000	0.000	0.007	0.03
	larvae	6.63	29.17	0.89	0.64	17.68	0.2

Based on provided above calculations for application to cereals an acceptable chronic risk could be concluded only for adult bees. In the same time an unacceptable chronic risk for bee larvae was identified for all scenarios. Risk assessment based on EFSA (2013) is provided above for informative purposes only and is not the basis for derivation of conclusion regarding the risk to bees at the zonal level.

In order to resolve the chronic risk for ADM.03502.F.1.A the Applicant submitted higher tier studies performed with solo formulation of the individual active compounds. It is, however, noted that the combined risk resulting from the exposure to mixture of prothioconazole and fenpropodrin cannot be addressed based on semi-field studies performed with solo formulations of particular compounds and the semi-field studies should be performed with the formulation for which authorisation is sought, at least in case of products containing more than one active substances.

In conclusion, the zRMS is of the opinion that the available data are not sufficient to support chronic risk from application of ADM.03502.F.1.A.

This issue should be further resolved at the product authorisation in Member States considering indications of the not yet noted EFSA guidance in their national assessments.

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.03502.F.1.A 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.03502.F.1.A were not evaluated as part of the EU assessment of the active substances prothioconazole and prothioconazole. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

The effects of ADM.03502.F.1.A on non-target arthropods were evaluated within the framework of standard laboratory tests using artificial substrate. Tests with the standard species *Aphidius rhopalosiph* 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
<i>Aphidius rhopalosiph</i>	ADM.03502.F.1.A (250 g fenpropidin + 175 g prothioconazole/L)	Standard lab test (2-D), glass plates, test rates: 0.125-2.0 L prod./ha	LR ₅₀ > 2.0 L prod./ha ER ₅₀ > 2.0 L prod./ha	KCP 10.3.2/01 Röhlig, U., 2020a, report no. 2048NAL0006
<i>Typhlodromus pyri</i>	ADM.03502.F.1.A (250 g fenpropidin + 175 g prothioconazole/L)	Standard lab test (2-D), glass plates, test rates: 0.125-2.0 L prod./ha	LR ₅₀ = 1.485 L prod./ha ER ₅₀ > 1.0 L prod./ha	KCP 10.3.2/02 Röhlig, U., 2020b, report no. 2048NTL0006

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

zRMS comments:

The studies performed with the formulated product were evaluated and agreed by the zRMS (for details, please refer to respective points in Appendix 2). Endpoints reported in Table 9.7-1 are confirmed to be correct.

9.7.1.1 Justification for new endpoints

New endpoints are provided for the formulated product ADM.03502.F.1.A. Those endpoints are considered to be more relevant in terms of non-target arthropod exposure under field conditions than effects of

the active substances 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.03502.F.1.A is an emulsifiable concentrate (EC) containing 175 g/L of the active substance prothioconazole and 250 g/L of the active substance fenpropidin. It is a fungicide applied as spray to infested foliage of cereals. The timing of application is post-emergence. The worst-case application scenario leading to maximum contamination of the environment is a single spray application at a rate of 1.0 L prod./ha (corresponding to 175 g prothioconazole/ha and 250 g fenpropidin/ha). For a detailed summary of the GAP uses of ADM.03502.F.1.A, 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 1.0 L prod./ha in field crops was considered as worst-case application scenario, covering all intended GAP uses of ADM.03502.F.1.A. The exposure of non-target arthropods to ADM.03502.F.1.A 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 9-4: Calculation of Predicted Environmental Rates in the treated field (PER_{in-field})

PER_{in-field}	= A · MAF	[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

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 LR₅₀ 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.03502.F.1.A

Intended use	Cereals, 1 × 1.0 L prod./ha, BBCH ≥ 10		
Product	ADM.03502.F.1.A		
Application rate (L prod./ha)	1.0		
MAF	1.0		
Test species Tier-1	Rate with ≤ 50 % effect (L prod./ha)	PER_{in-field} (L prod./ha)	HQ_{in-field} < 2?
<i>Aphidius rhopalosiphi</i>	> 2.0	1.0	Yes (HQ < 0.5)
<i>Typhlodromus pyri</i>	1.485	1.0	Yes (HQ = 0.7)

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.03502.F.1.A (*worst-case approach*).

zRMS comments:

The risk assessment presented in Table 9.7-2 is agreed by the zRMS.

Based on calculations performed with consideration of the Tier I laboratory data acceptable in-field risk to non-target arthropods from all intended uses of ADM.03502.F.1.A may be concluded.

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.03502.F.1.A to cereals, the drift scenario „field crops“ was considered as most relevant. Since the maximum seasonal application rate of ADM.03502.F.1.A was considered in the risk assessment, the 90th percentile drift values were implemented in the calculations (MAF = 1).

Equation 9-5: Calculation of the Predicted Environmental Rates in the off-field (PER_{off-field}) for non-target arthropods

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

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-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.03502.F.1.A

Intended use	Cereals, 1× 1.0 L prod./ha, BBCH ≥ 10			
Product	ADM.03502.F.1.A			
Application rate (L prod./ha)	1.0			
MAF	1.0			
vdf	10 (2-D), 5 (2-D), CF=10			
Test species Tier-1	LR₅₀ (lab.) (L prod./ha)	Drift rate (Field crops, 1 m)	PER_{off-field}* (L prod./ha)	HQ_{off-field} < 2?
<i>Aphidius rhopalosiphi</i> (2-D)	> 2.0	0.0277 (90 th)	0.0554 0.0277	Yes (HQ < 0.0277) Yes (HQ < 0.014)
<i>Typhlodromus pyri</i> (2-D)	1.485	0.0277 (90 th)	0.0554 0.0277	Yes (HQ < 0.037) Yes (HQ < 0.019)

MAF: Multiple application factor; vdf: Vegetation distribution factor; PER: Predicted environmental rate; CF: Correction factor
* including a vdf of 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 risk assessment presented in Table 9.7-3 is not validated 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. For this reason, zRMS amended the calculations in the Table 9.7-3.

Based on calculations performed with consideration of the Tier I laboratory data acceptable off-field risk to non-target arthropods from all intended uses of ADM.03502.F.1.A may be concluded with no need for risk mitigation measures.

9.7.2.3 Additional higher-tier risk assessment

Not considered to be relevant.

9.7.2.4 Risk mitigation measures

No risk mitigation is 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.03502.F.1.A in cereals. 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 earthworms and other non-target soil organisms (meso- and macrofauna) have been carried out with the active substance prothioconazole as well as relevant metabolites in soil. 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.03502.F.1.A, 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.

Since in the first earthworm reproductive toxicity study (Friedrich, S., 2020a, report no. 2048TEC0035) the NOEC was higher than the highest tested concentration as no effects were observed, a second study (Friedrich, S., 2021, report no. 2148TEC0034) was conducted considering higher test concentrations. Again, no effects were observed up to the highest test concentration. For the risk assessment the higher NOEC derived from the second study was considered most appropriate.

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
<i>Eisenia fetida</i>	ADM.03502.F.1.A (250 g fenpropidin + 175 g prothio- conazole/L)	56 d, chronic 10 % peat	NOEC _{reproduction} ≥ 1.4 mg prod./kg soil _{dw} NOEC _{corr} ≥ 0.7 mg prod./kg soil _{dw} ¹⁾ [equivalent to 0.29 mg a.s.sum/kg soil _{dw} ^{2) 3)} EC ₁₀ reproduction n.d	KCP 10.4.1.1/01 Friedrich, S., 2020a, report no. 2048TEC0035
<i>Eisenia fetida</i>	ADM.03502.F.1.A (250 g fenpropidin + 175 g prothio- conazole/L)	56 d, chronic 10 % peat	NOEC _{reproduction} ≥ 5.46 mg prod./kg soil _{dw} NOEC _{corr} ≥ 2.73 mg prod./kg soil _{dw} ¹⁾ [equivalent to 1.12 mg a.s.sum/kg soil _{dw} ^{2) 3)} EC ₁₀ reproduction n.d	KCP 10.4.1.1/02 Friedrich, S., 2021, report no. 2148TEC0034
<i>Eisenia fetida</i>	Fenpropidin (applied as 750 EC formulation)	56 d, chronic 10 % peat	NOEC _{reproduction} = 26.7 mg prod./kg soil _{dw} NOEC _{corr} = 13.4 mg prod./kg soil _{dw} ¹⁾ [equivalent to 10 mg a.s./kg soil _{dw}]	EFSA Scientific Report (2007) 124, 1-84
<i>Eisenia fetida</i>	CGA 289267	Acute	LC ₅₀ >100 mg/kg dws	EFSA Scientific Report (2007) 124, 1-84
<i>Eisenia fetida</i>	JAU- desthio (M4) (metabolite of prothioconazole)	56 d, chronic 10 % peat	NOEC = 1.0 mg met./kg soil _{dw} NOEC _{corr} = 0.5 mg met./kg soil _{dw} ¹⁾ mg a.s./kg dws	EFSA Scientific Report (2007) 106, 1-98
Prothioconazole (applied as 250 EC formulation)	Prothioconazole applied as	56 d, chronic	NOEC=1.33 mg a.s./kg dws NOEC _{corr} = 0.665 mg a.s./kg dws	EFSA Scientific Report (2007) 106, 1-98

Species	Substance	Exposure System	Results	Reference
<i>Eisenia fetida</i>	JAU-S-methyl (M1) (metabolite of prothioconazole)	56 d, chronic 10 % peat	NOEC = 100 mg met./kg soil _{dw} NOEC _{corr} = 50 mg met./kg soil _{dw} ¹⁾	EFSA Scientific Report (2007) 106, 1-98
<i>Folsomia candida</i>	Prothioconazole technical	28 d, chronic 5 % peat content	NOEC = 64 mg a.s./kg soil _{dw} NOEC _{corr} = 32 mg a.s./kg soil _{dw} ¹⁾	EFSA Scientific Report (2007) 106, 1-98
<i>Folsomia candida</i>	Fenpropidin (applied as 750 EC formulation)	28 d, chronic 5 % peat content	NOEC = 124 mg prod./kg soil _{dw} NOEC _{corr} = 62 mg prod./kg soil _{dw} ¹⁾ [equivalent to 46.5 mg a.s./kg soil _{dw}]	EFSA Scientific Report (2007) 124, 1-84
<i>Folsomia candida</i>	ADM.03502.F.1.A (250 g fenpropidin + 175 g prothioconazole/L)	28 d, chronic 5 % peat content	NOEC _{reproduction} = 308.6 mg prod./kg soil _{dw} NOEC _{corr} = 154.3 mg/kg soil _{dw} ¹⁾ [equivalent to 63.1 mg a.s.sum/kg soil _{dw} ²⁾ EC ₁₀ = 318.1 mg prod./kg soil _{dw} EC _{10corr} = 159.05 mg prod./kg soil _{dw} ¹⁾	KCP 10.4.2.1/01: Friedrich, S., 2020b, report no. 2048TCC0025
<i>Folsomia candida</i>	JAU-desthio (M4) (metabolite of prothioconazole)	28 d, chronic 5 % peat content	NOEC > 62.5 mg met. /kg soil _{dw} NOEC _{corr} > 31.25 mg met./kg soil _{dw}	EFSA Scientific Report (2007) 106, 1-98
<i>Folsomia candida</i>	JAU-S-methyl (M1) (metabolite of prothioconazole)	28 d, chronic 5 % peat content	NOEC > 31.6 mg met./kg soil _{dw} NOEC _{corr} > 15.8 mg met./kg soil _{dw}	EFSA Scientific Report (2007) 106, 1-98
<i>Hypoaspis aculeifer</i>	Prothioconazole technical	14 d, chronic LUF 2.1 soil with 0.9% organic carbon	NOEC = 100 mg a.s./kg soil _{dw} NOEC = 50 mg a.s./kg soil _{dw} (no correction required)	EFSA Scientific Report (2007) 106, 1-98
<i>Hypoaspis aculeifer</i>	ADM.03502.F.1.A (250 g fenpropidin + 175 g prothioconazole/L)	14 d, chronic 5 % peat content	NOEC _{reproduction} = 93 mg prod./kg soil _{dw} NOEC _{corr} = 46.5 mg/kg soil _{dw} ¹⁾ [equivalent to 19.0 mg a.s.sum/kg soil _{dw} ²⁾ EC _{10 reproduction} = 110.42 mg prod./kg soil _{dw} EC _{10corr} = 55.21 mg prod./kg soil _{dw} ¹⁾	KCP 10.4.2.1/02: Schulz, L., 2020a, report no.: 2048THC0021

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

²⁾ Based on the content of the active substances in the formulation and a product density of 1.04 g/mL

³⁾ NOEC is based on the highest test concentration in the study

zRMS comments:

Data for earthworms for fenpropidin and prothioconazole provided in Table 9.8-1 are in line with EU agreed endpoints reported in EFSA Journal (2007) 124, 1-84 and EFSA Scientific Report (2007) 106, respectively. Information regarding toxicity of prothioconazole and its metabolites to *Folsomia candida* are also in line with EU agreed values.

No toxicity data for other soil organisms (*H.aculeifer*) was available from the EU review of fenpropidin and prothioconazole soil metabolite.

Nevertheless, studies on toxicity of ADM.03502.F.1.A to *Hypoaspis aculeifer* cover effects of fenpropidin and prothioconazole metabolite in the product and are considered sufficient at until endpoints from renewal are available.

Studies on effects of the formulated product to earthworm and other soil macro-organism listed in Table 9.8-1 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.

According to the current guidance document SANCO/10239, EC 2002, endpoints (LC₅₀, NOEC or EC₁₀) considered in the risk assessment for soil macro- and mesofauna should be divided by a factor of 2, if the log P_{ow} 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 and EFSA Scientific Report (2007) 124, the log P_{ow} values for the active substances prothioconazole and fenpropidin as well as the prothioconazole soil metabolites JAU-S-methyl and JAU-desthio were determined > 2 and thus, a correction factor has to be taken into account 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.03502.F.1.A. 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.03502.F.1.A is an emulsifiable concentrate (EC) containing 175 g/L of the active substance prothioconazole and 250 g/L of the active substance fenpropidin. It is a fungicide applied as spray to infested foliage of cereals. The timing of application is post-emergence. The worst-case application scenario leading to maximum contamination of the environment is a single spray application at a rate of 1.0 L prod./ha (corresponding to 175 g prothioconazole/ha and 250 g fenpropidin/ha). For a detailed summary of the GAP uses of ADM.03502.F.1.A, please refer to Table 9.1-1.

Exposure levels were calculated based on a worst-case application scenario for ADM.03502.F.1.A resulting in the maximum PEC_{soil} i.e. 1 × 1.0 L prod./ha (BBCH 30-65, 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.03502.F.1.A in cereals

Intended use	Cereals, 1 × 1.0 L prod./ha at BBCH 30-65		
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{LT} (criterion TER ≥ 5)
JAU-desthio (M4) (metabolite of prothioconazole)	0.5 (corr) ¹⁾	0.024 0.021	20.83 23.8
JAU-S-methyl (M1) (metabolite of prothioconazole)	50 (corr) ¹⁾	0.007	7142.9
Fenpropidin (applied as 750 EC formulation)	10 (corr) ¹⁾	0.069	144.9
Prothioconazole (applied as 250 EC formulation)	0.665 (corr) ¹⁾	0.047	14.1
CGA 289267 (metabolite of fenpropidin)	1.0 ³⁾	0.008	125.0
ADM.03502.F.1.A	≥ 2.73 (prod., corr) ¹⁾⁴⁾	0.277 (prod.) ⁵⁾	≥ 9.9
ADM.03502.F.1.A	≥ 1.12 (a.s.sum., corr) ¹⁾	0.1164 (a.s.sum) ²⁾	9.65 ≥ 9.7

Bold: below the trigger, indicating an unacceptable risk at Tier-1

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

²⁾ Based on PEC_{soil, ini} of 0.047 mg/kg soil_{dw} for prothioconazole + PEC_{soil, accum} of 0.069 mg/kg soil_{dw} for fenpropidin

³⁾ Since no measured toxicity data are available, it was assumed that the metabolite is 10 x more toxic than the parent compound fenpropidin (*unrealistic worst-case approach*)

⁴⁾ NOEC is based on the highest test concentration in the study

⁵⁾ PEC_{prod} value taken from Section 8

As outlined above, all TER_{LT} values for prothioconazole, fenpropidin as well as their metabolites 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. Thus, no further considerations have to be taken into account.

zRMS comments:

The risk assessment for earthworms is agreed by the zRMS. All TER_{LT} values for earthworms for prothioconazole, fenpropidin as well as their metabolites potentially relevant in soil are greater than the trigger of 5, indicating an overall acceptable risk.

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.03502.F.1.A in cereals

Intended use	Cereals, 1 × 1.0 L prod./ha at BBCH 30-65		
Product/active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{LT} (criterion TER ≥ 5)
Chronic effects on <i>Folsomia candida</i>			
Prothioconazole technical	32 (corr) ¹⁾	0.047	680.9
JAU-desthio (M4) (metabolite of prothioconazole)	3.2 ²⁾ 31.25	0.024 0.021	133.3 152.4 1302.08
JAU-S-methyl (M1) (metabolite of prothioconazole)	3.2 ²⁾ >15.8	0.007	457.1 2257.14
Fenpropidin (applied as 750 EC formulation)	46.5 (a.s., corr) ¹⁾	0.069	673.9
CGA 289267 (metabolite of fenpropidin)	4.65 ²⁾	0.008	581.3

Intended use	Cereals, 1 × 1.0 L prod./ha at BBCH 30-65		
Product/active substance	NOEC (mg/kg dw)	PEC_{soil} (mg/kg dw)	TER_{LT} (criterion TER ≥ 5)
ADM.03502.F.1.A	154.3 (prod., corr) ¹⁾	0.277 (prod.) ⁵⁾	557.0
ADM.03502.F.1.A	63.1 (a.s.sum, corr) ^{1) 3)}	0.116 (a.s.sum) ⁴⁾	544.0
Chronic effects on <i>Hypoaspis aculeifer</i>			
Prothioconazole technical	100 50	0.047	2128 1063.83
JAU-desthio (M4) (metabolite of prothioconazole)	10 ²⁾	0.024 0.021	416.66 476.2
JAU-S-methyl (M1) (metabolite of prothioconazole)	10 ²⁾	0.007	1429
ADM.03502.F.1.A	46.5 (prod., corr) 1)	0.277 (prod.) ⁵⁾	167.9
ADM.03502.F.1.A	19.0 (a.s.sum, corr) ^{1) 3)}	0.11 ⁴⁾ (a.s.sum) ⁴⁾	163.8

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 compounds prothioconazole or fenpropidin (*unrealistic worst-case approach*)

³⁾ Based on the content of the active substances in the formulation and a product density of 1.04 g/mL

⁴⁾ Based on PEC_{soil, ini} of 0.047 mg/kg soil_{dw} for prothioconazole + PEC_{soil, accum} of 0.069 mg/kg soil_{dw} for fenpropidin

⁵⁾ PEC_{prod} value taken from Section 8

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:

The risk assessment for soil macro- and meso-fauna is agreed by the zRMS.

We agree with the assumption that the prothioconazole metabolites are 10 x more toxic than the parent compounds prothioconazole in case of *H. aculeifer* since no measured toxicity data are available for them.

In case of fenpropidin metabolite we agree with the assumption that the metabolite is 10 x more toxic than the parent compounds for *Folsomia candida* since no measured toxicity data are available for it.

No toxicity data for other soil organisms (*H. aculeifer*) was available from the EU review of fenpropidin.

Nevertheless, studies on toxicity of ADM.03502.F.1.A to *Hypoaspis aculeifer* cover effects of fenpropidin metabolite in the product and are considered sufficient until endpoints from renewal are available.

All TER_{LT} values for soil meso- and macrofauna (other than earthworms) are greater than the trigger of 5, indicating an overall acceptable risk.

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 substances and 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, fenpropidin and metabolites potentially relevant in soil. 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	Prothioconazole technical	28 d, aerobic soil type	No detrimental effects ($E < \pm 25\%$ 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.03502.F.1.A (250 g fenpropidin + 175 g prothioconazole/L)	28 d, aerobic soil type	No detrimental effects ($E < \pm 25\%$ of the control) on N-transformation (28 d) up to 10.0 L prod./ha (= 13.87 mg prod./kg soil _{dw} ; equivalent to 5.67 mg a.s. _{sum} /kg soil _{dw} ¹⁾)	KCP 10.5/01 Schulz, L., 2020b, report no.: 2048SMN0022
N-transformation	JAU-desthio (M4) (metabolite of prothioconazole)	28 d, aerobic soil type	No detrimental effects ($E < \pm 25\%$ of the control) on N-transformation (28 d) up to 1.0 kg/ha (= 1.33 mg met./kg soil _{dw})	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 ($E < \pm 25\%$ of the control) on C-/N-transformation (28 d) up to 2 kg/ha (= 2.67 mg met./kg soil _{dw})	EFSA Scientific Report (2007) 106, 1-98
Nitrogen mineralisation	Fenpropidin applied as TERN 750 EC (A-7516 A)	73 days	no stat. sign. effects at 1.1 to 6.0 mg a.s./kg	EFSA Scientific Report (2007) 124, 1-84
C -N-transformation	CGA 289267 (fenpropidin metabolite)	28 d, aerobic soil type	No detrimental effects ($E < \pm 25\%$ of the control) on C -N-transformation (28 d) up to 10 mg met./kg soil _{dw}	EFSA Scientific Report (2007) 124, 1-84

1) Based on the content of the active substances in the formulation and a product density of 1.04 g/mL

zRMS comments:

Data for soil microorganism for fenpropidin and prothioconazole and their metabolites provided in Table 9.9-1 are in line with EU agreed endpoints reported in EFSA Journal (2007) 124, 1-84 and EFSA Scientific Report (2007) 106, respectively.

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

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.03502.F.1.A 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.03502.F.1.A resulting in the maximum PEC_{soil} i.e. 1×1.0 L prod./ha (BBCH 30-65, 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.03502.F.1.A in cereals

Intended use	Cereals, 1 × 1.0 L prod./ha at BBCH 30-65		
Product/active substance	Max. conc. with effects ≤ 25 % (mg/kg dw)	PEC _{soil} (mg/kg dw)	Risk acceptable?
Prothioconazole technical	2.67 (a.s.)	0.047	Yes
JAU-desthio (M4) (prothioconazole metabolite)	1.33 (met.)	0.024 0.021	Yes
JAU-S-methyl (M1) (prothioconazole metabolite)	2.67 (met.)	0.007	Yes
Fenpropidin applied as TERN 750 EC (A-7516 A)	1.1 -6 mg a.s.	0.069	Yes
CGA 289267 (fenpropidin metabolite)	10 (met.)	0.008	Yes
ADM.03502.F.1.A	13.87 (prod.)	0.277	Yes
ADM.03502.F.1.A	5.67 (a.s.sum) ¹⁾	0.116 ²⁾	Yes

¹⁾ Based on the content of the active substances in the formulation and a product density of 1.04 g/mL

²⁾ Based on PEC_{soil, ini} of 0.047 mg/kg soil_{dw} for prothioconazole + PEC_{soil, accum} of 0.069 mg/kg soil_{dw} for fenpropidin

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.

The effects on the nitrogen transformations are acceptable (<25%) at concentration which is higher than the maximum relevant PECs for the maximum application rate of active substances and the product ADM.03502.F.1.A.

Overall, no unacceptable effects on soil microbial activity are expected following application of ADM.03502.F.1.A.

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 × 1.0 L prod./ha (BBCH 30-65, considering 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 the intended GAP uses of ADM.03502.F.1.A in cereals.

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 substances. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on non-target terrestrial plants of ADM.03502.F.1.A, the formulation for which authorisation is sought, were not evaluated as part of the EU assessment of active substances. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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.03502.F.1.A. The dose-response tests were performed with 6 ~~10~~ representative plant species: sugar beet, rape, tomato, soybean, ryegrass, onion. Endpoints are summarised in Table 9.10-1 below. All ER₅₀ values were above the highest concentration tested in the vegetative vigour test and a seedling emergence test and is therefore set at > 1.0 L prod./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
Prothionazole technical	Seedling emergence	Pigweed	Lowest ER ₅₀ > 200 g a.s./ha	EFSA Scientific Report (2007) 106, 1-98
Prothionazole technical	Vegetative vigour	Pigweed, sugar beet	Lowest ER ₅₀ > 250 g a.s./ha	EFSA Scientific Report (2007) 106, 1-98
ADM.03502.F.1.A (250 g fenpropidin + 175 g prothio-cozole/L)	Seedling emergence	--- (NOER of all tested plants is 1.0 L prod./ha)	Lowest ER₅₀ > 1.0 L prod./ha	KCP 10.6.1/01 Kästner, K., 2020a report no. 2046PSE0007
ADM.03502.F.1.A (250 g fenpropidin + 175 g prothio-cozole/L)	Vegetative vigour	--- (NOER of all tested plants is 1.0 L prod./ha)	Lowest ER₅₀ > 1.0 L prod./ha	KCP 10.6.1/02 Kästner, K., 2020b report no. 2046PVV0009

Bold: Endpoint considered most relevant with respect to risk assessment for non-target terrestrial plants; n.a. = not applicable

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 substances 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: Prothioconazole - screening risk assessment for terrestrial non-target plants based on the results of the vegetative vigour and seedling emergence tests

Intended use	Cereals, 1× 1.0 L prod./ha, BBCH ≥ 10		
Active substance 1	Prothioconazole		
Application rate (g a.s./ha)	1× 175		
Test system	Lowest ER ₅₀ [g prothioconazole/ha]	Max. single application rate [g prothioconazole/ha]	Risk for fungicides according to SANCO/10329/2002 recommendations
Vegetative vigour test	> 250	175	Acceptable risk is indicated since the lowest ER ₅₀ exceeds the maximum single application rate
Seedling emergence test	> 200	175	Acceptable risk is indicated since the lowest ER ₅₀ exceeds the maximum single application rate

Table 9.10-3: ADM.03502.F.1.A - screening risk assessment for terrestrial non-target plants based on the results of the vegetative vigour and seedling emergence tests

Intended use	Cereals, 1 × 1.0 L prod./ha, BBCH ≥ 10		
Product	ADM.03502.F.1.A		
Application rate (L prod./ha)	1 × 1.0		
Test system	Lowest ER₅₀ [L prod./ha]	Max. single application rate [L prod./ha]	Risk for fungicides according to SANCO/10329/2002 recommenda- tions
Vegetative vigour test	> 1.0	1.0	Acceptable risk is indicated since the lowest ER ₅₀ exceeds the maximum single application rate
Seedling emergence test	> 1.0	1.0	Acceptable risk is indicated since the lowest ER ₅₀ exceeds the maximum single application rate

As outlined in the table above, the ER₅₀ values of the two test systems are determinable above the maximum test rates (seedling emergence tests and vegetative vigour tests), covering the maximum single application rate of ADM.03502.F.1.A in cereals. On this account, an acceptable risk for terrestrial non-target plants exposed to applications of the fungicide ADM.03502.F.1.A is indicated. No mitigation measures need to be applied.

zRMS comments:

zRMS agrees with approach provided by the Applicant.

In accordance with SANCO/10329 (17 October 2002), the risk to non-target terrestrial plants can be considered acceptable at the screening level if there were no effects on any species >50% at the maximum intended application rate.

The ER₅₀ values > 1 L/ha from seedling emergence and vegetative vigour tests are above the maximum test rates, covering the maximum single application rate of ADM.03502.F.1.A in cereals (1 L/ha) indicating an acceptable risk to non-target crops.

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.03502.F.1.A. 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.03502.F.1.A. 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.03502.F.1.A is classified as ‘very toxic to aquatic life with long lasting effects’ (H410).

zRMS comments:

zRMS agrees with the classification H410 for formulation ADM.03502.F.1.A.

The following justification are provided below.

Acute aquatic hazard:

Valid test data for all the three trophic levels are available for the mixture as a whole, therefore no need to consider bridging principles or classification of individual components for acute hazard classification of the mixture. Test data showed that ErC_{50} for primary producers is < 1 mg /L with 72h $ErC_{50} = 0.595$ mg/L. Consequently, classification “Acute 1” (H400) for acute aquatic hazard is required.

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.

Long-term aquatic hazard:

In absence of chronic toxicity data for product the classification for the chronic aquatic hazard should be based on summation method.

Information on classification including associated M factors and the % of the components in the mixture are as follows:

Compound	Acute aquatic hazard	M	Long-term aquatic hazard	M	C (%)
Prothioconazole	Acute 1 (H400)	10	Chronic 1 (H410)	10	16.8
Fenpropidin	Acute 1 (H400)	100	Chronic 1 (H410)	100	24.0

Step 1: Classify as Chronic 1 if:

$$\sum(\text{Chronic 1} \times M) \geq 25 \%$$

$$= (16.8 \times 10) + (24.0 \times 100) > 25$$

Test data showed that $L(E)C_{50} < 1$ mg/L for primary producers. Consequently, classification “Acute 1” (H400) for acute aquatic hazard is required. According to the summation method, $\sum(\text{Chronic 1} \times M) \geq 25 \%$, and the product should thus be classified as chronic 1 (**H410**).


Following phrases must be included in the label:

Hazard statement: H410

Signal word: Warning

Pictogram: GHS09

Safety phrases: P391, P501

Hazard pictograms:	GHS09 
Signal word:	Warning
Hazard statement(s):	H410 - Very toxic to aquatic life with long lasting effects
Precautionary statement(s):	P391: Collect spillage 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 Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.2.1/01	...	2020a	Acute toxicity of ADM.03502.F.1.A to <i>Oncorhynchus mykiss</i> in a 96-hour semi-static test Report no GLP Unpublished	Y	ADM
KCP 10.2.1/02	Renner, P.	2020b	Acute toxicity of ADM.03502.F.1.A to <i>Daphnia magna</i> in a 48-hour semi-static test Report no 20 48 ADL 0008, Sponsor no.: 000104840 BioChem agrar, Machern/Gerichshain, Germany GLP Unpublished	N	ADM
KCP 10.2.1/03	Scheerbaum, D.	2021	ADM.03502.F.1.A - Alga, Growth Inhibition Test with <i>Desmodesmus subspicatus</i> , 72 hours Report no. SO21519 / SSO19707, Sponsor no.: 000108687 Noack Laboratorien GmbH, Sarstedt, Germany GLP Unpublished	N	ADM
KCP 10.2.1/04	Renner, P.	2021	Effects of ADM.03502.F.1.A on <i>Lemna gibba</i> in a growth inhibition test under semi-static test conditions Report no 2048ALE0006, Sponsor no.: 000104842 BioChem agrar, Machern/Gerichshain, Germany GLP Unpublished	N	ADM

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.2.3/01	Wellmann, P., Hommen, P., Böhmer, W.,	2006	Community level study with Fenpropidin in outdoor aquatic mesocosm ponds Fraunhofer-Institute Molecular Biology and Applied Ecology (IME), Schmallenberg, Germany Report No: FEI-010/4-52 GLP Unpublished	N	ADM
KCP 10.2.3/02	Arts, G.H.P and Brock, T.C.M.	2009	Evaluation of the reports: Neumann Ch. (1997): CGA 114900 EC 750 (A-7516 A): Outdoor aquatic mesocosm study of the environmental fate and ecological effects. Novartis Crop Protection AG, Sector of Unit R&D, Ecotoxicology Department, Switzerland. Project No 95N001. (Syngenta file No. CGA 114900/0500) including Ashwell J., Hamer M. And Coulson M., 2007. Fenpropidin: Syngenta response to Evaluation Table rev. 0-0 (19.02.2007). Data requirement 5.2 – statistical analysis of mesocosms study by Neumann 1997.and Huber, W. (1995): Effects of A-7503 C in aquatic outdoor microcosms. Technical University Munich-Weihenstephan. Institute for Landscape and Botany, Germany. Report No. (Syngenta file No. CGA 64250/2997) and Wellmann P. (2006): Community level study with Fenpropidin in outdoor aquatic mesocosm ponds, Fraunhofer-Institute Schmallenberg, Germany & Gaiax, Aachen, Germany Alterra, Wageningen University and Research Centre, Centre for Water and Climate P.O. Box 47, 6700 AA Wageningen, The Netherlands Report no: n.a. non-GLP Unpublished	N	ADM
KCP 10.3.1.1/01	Franke, M.	2020	Acute toxicity of ADM.03502.F.1.A to the honeybee <i>Apis mellifera</i> L. under laboratory conditions Report no.: 2048BAA0028, Sponsor no.: 000104843 BioChem agrar, Machern/Gerichshain, Germany GLP Unpublished	N	ADM

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.3.1.2/01	Dreßler, K.	2021	Chronic oral toxicity of ADM.03502.F.1.A to the honey bee <i>Apis mellifera</i> L. under laboratory conditions Report no.: 2048BAC0011, Sponsor no.: 000104844 BioChem agrar, Machern/Gerichshain, Germany GLP Unpublished	N	ADM
KCP 10.3.1.3/01	Hänsel, M.	2021	ADM.03502.F.1.A – Repeated exposure of honey bee larvae (<i>Apis mellifera</i> L.) under laboratory conditions Report no.: 2048BLC0013, Sponsor no.: 000104845 BioChem agrar, Machern/Gerichshain, Germany GLP Unpublished	N	ADM
KCP 10.3.1.5/01	Persigehl, M., Beinert, M., Hotopp, I., Zumkier, U.	2021	Study on the Effect of ADM.3500.F.2.B on Honey bee Colonies (<i>Apis mellifera</i> L.) under Semi-Field Conditions in Germany report no.: B19010-3, sponsor no.: 000102470 tier3 solutions GmbH, Leverkusen, Germany GLP Unpublished	N	ADAMA
KCP 10.3.1.5/02	Hecht-Rost, S.	2020	Semi-field study to evaluate potential effects of ADM.1351.F.1.A (Spirale) on the development of honeybee colonies (<i>Apis mellifera</i> L.), Germany report no.: R1940026, sponsor no.: 000102476 RIFCON GmbH, Hirschberg, Germany GLP Unpublished	N	ADAMA

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.3.2.2/01	Röhlig, U.	2020a	Effects of ADM.03502.F.1.A on the parasitic wasp <i>Aphidius rhopalosiphi</i> (DESTEFANI-PEREZ) in a laboratory test Report no.: 2048NAL0006, Sponsor no.: 000104847 BioChem agrar, Machern/Gerichshain, Germany GLP Unpublished	N	ADM
KCP 10.3.2.2/02	Röhlig, U.	2020b	Effects of ADM.03502.F.1.A on the predatory mite <i>Typhlodromus pyri</i> SCHEUTEN in a laboratory test Report no.: 2048NTL0006, Sponsor no.: 000104846 BioChem agrar, Machern/Gerichshain, Germany GLP Unpublished	N	ADM
KCP 10.4.1.1/01	Friedrich, S.	2020a	Effects of ADM.03502.F.1.A on the mortality, growth and reproduction of the earthworm <i>Eisenia fetida</i> in artificial soil Report no.: 2048TEC0035, Sponsor no.: 000104848 BioChem agrar, Machern/Gerichshain, Germany GLP Unpublished	N	ADM
KCP 10.4.1.1/02	Friedrich, S.	2021	Effects of ADM.03502.F.1.A on the mortality, growth and reproduction of the earthworm <i>Eisenia fetida</i> in artificial soil Report no.: 2148TEC0034, Sponsor no.: 000108316 BioChem agrar, Machern/Gerichshain, Germany GLP Unpublished	N	ADM

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.4.2.1/01	Friedrich, S.	2020b	Effects of ADM.03502.F.1.A on the mortality and reproduction of the collembolan <i>Folsomia candida</i> Report no.: 2048TCC0025, Sponsor no.: 000104849 BioChem agrar, Machern/Gerichshain, Germany GLP Unpublished	N	ADM
KCP 10.4.2.1/02	Schulz, L.	2020a	Effects of ADM.03502.F.1.A on the reproduction of the predatory mite <i>Hypoaspis aculeifer</i> Report no.: 2048THC0021, Sponsor no.: 000104850 BioChem agrar, Machern/Gerichshain, Germany GLP Unpublished	N	ADM
KCP 10.5/01	Schulz, L.	2020b	Effects of ADM.03502.F.1.A on the activity of soil microflora (Nitrogen transformation test) Report no.: 2048SMN0022, Sponsor no.: 000104851 BioChem agrar, Machern/Gerichshain, Germany GLP Unpublished	N	ADM
KCP 10.6.1/01	Kästner, K.	2020a	Effects of ADM.03502.F.1.A on seedling emergence and seedling growth of six non-target terrestrial plant species under greenhouse conditions Report no.: 2046PSE0007, Sponsor no.: 000104852 BioChem agrar, Machern/Gerichshain, Germany GLP Unpublished	N	ADM
KCP 10.6.1/02	Kästner, K.	2020b	Effects of ADM.03502.F.1.A on vegetative vigour of six non-target terrestrial plant species under greenhouse conditions Report no.: 2046PVV0009, Sponsor no.: 000104853 BioChem agrar, Machern/Gerichshain, Germany GLP Unpublished	N	ADM

List of data submitted by the applicant and not relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.3.1.5/01	Persigehl, M., Beinert, M., Hotopp, I., Zumkier, U.	2021	Study on the Effect of ADM.3500.F.2.B on Honey bee Colonies (Apis mellifera L.) under Semi-Field Conditions in Germany report no.: B19010-3, sponsor no.: 000102470 tier3 solutions GmbH, Leverkusen, Germany GLP Unpublished	N	ADAMA
KCP 10.3.1.5/02	Hecht-Rost, S.	2020	Semi-field study to evaluate potential effects of ADM.1351.F.1.A (Spyrale) on the development of honeybee colonies (Apis mellifera L.), Germany report no.: R1940026, sponsor no.: 000102476 RIFCON GmbH, Hirschberg, Germany GLP Unpublished	N	ADAMA
-	EBRC Consulting GmbH	2023	Updated exposure and risk assessment for aquatic organisms considering volatilisation and deposition of fenpropidin in Step 4 PECsw modelling. Sponsor: Adama-Makteshim Ltd., Isreal, 17 April 2023. EBRC no.: ADM-230417-01	N	ADAMA

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

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status 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.03502.F.1.A 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 substances are 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 and fenpropidin technical and the formulated product (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.03502.F.1.A 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.03502.F.1.A, 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, fenpropidin and metabolites, 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

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

Comments of zRMS:	<p>The study was conducted in line with OECD 203+ with no minor deviations.</p> <p>The test concentration of both active substances was verified in ‘fresh’ and ‘aged’ test solutions at test start, at test solution renewals and at test end.</p> <p>The test concentrations of both active substances were verified at the beginning and at the end of the exposure and on every renewal day (0, 24, 48 and 72 hours) of all tested concentration levels. The measured concentrations of Prothioconazol were in the range of 93% to 118.9% of the nominal values in freshly prepared medium (0, 24, 48 and 72 hours) and from 92.9 to 105.9% of the nominal values in old medium (24, 48 and 72 hours).</p> <p>The measured concentrations of Fenpropidin were in the range of 103.4 % to 119.8% of the nominal values in freshly prepared medium (0, 24, 48, 72 and 96 hours) and from 98.7 to 108.6% of the nominal values in old medium (24, 48, 72 and 96 hours).</p> <p>Therefore, the endpoints of items are based on nominal test concentration.</p> <p>All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>96 h LC₅₀ = 6.23 mg/L (based on nominal concentration)</p>
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Reference:	KCP 10.2.1/01
Report:	Acute toxicity of ADM.03502.F.1.A to <i>Oncorhynchus mykiss</i> in a 96-hour semi-static test,, sponsor no.:
Guideline(s):	OECD 203 (2019)
Deviations:	None
GLP:	Yes
Acceptability/Reliability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

In this study with rainbow trout (*Oncorhynchus mykiss*), the acute toxicity of ADM.03502.F.1.A (250 g fenpropidin/L, 175 g prothioconazole/L, nominal) was analysed for 96 hours under semi-static conditions. Fish were kept in test medium, to which the test item had been added. The aim of the test was to evaluate possible toxic effects within 96 hours under semi-static conditions. Fish were exposed to serial dilutions of the test item. Mortality was recorded at 3, 6, 24, 48, 72 and 96 hours of exposure. Animals were considered dead if no visible movement (e.g. gill movements) was apparent and if touching of the caudal peduncle did not provoke any reaction. Dead fish were removed upon observance. Sub-lethal effects were monitored at 3, 6, 24, 30, 48, 54, 72, 78 and 96 hours of exposure. Measurements of pH and dissolved oxygen were carried out in 24-hour intervals in ‘fresh’ and ‘aged’ test solutions. The total organic content (TOC) of fresh test medium was measured once. Test solutions were exchanged daily. The temperature was recorded continuously and in individual test vessels. Recoveries of prothioconazole and fenpropidin were within 80 to 120 % of nominal concentrations in fresh and aged test solutions. Therefore, results were expressed as nominal test item concentration.

After 24 h, all fish were dead at the two highest test item concentrations of 9.23 and 12.0 mg/L and partial mortality of 28.6 % was found at 7.10 mg/L test item. After 48 hours, all fish were dead at ≥ 7.10 mg/L. No mortality and no sub-lethal effects were found at 4.20 and 5.46 mg/L test item at any of the observations. After 96 hours, a LC₅₀ of 6.23 mg/L test item nominal was determined.

I. Materials and methods

A. Materials

1. Test material:
Lot/Batch no.:
Content:
Density:
Control:
Toxic reference:
ADM.03502.F.1.A
1191-101219-01
250 g fenpropidin/L, 175 g prothioconazole/L (nominal)
253.7 g fenpropidin/L, 175.9 g prothioconazole/L (analysed)
1.04 g/mL
untreated medium control
None
2. Test organisms -
Species:
Age:
Mean body length (test start):
Mean body weight (test start):
Mean loading:
Source:
Acclimatisation period:
No of fish:
Feeding during test:
rainbow trout (*Oncorhynchus mykiss*)
not stated
5.20 ± 0.0577 cm
1.50 ± 0.140
0.53 g fish/L
purchased from a local fish farm supplier's information:
„Forellenzucht Trostadt GbR“Reurieth, Germany
74 days
7 fish per replicate; 1 replicate per treatment
none
3. Test units -
Type and size:
Test procedure:
Test duration:
stainless steel container (approx. 22 L volume) with 20 L test solution
Semi static test, daily test solution renewal
96 hours
4. Test conditions -
Test medium:
Water hardness:
Water temperature:
Photoperiod:
Dissolved oxygen:
pH value:
reconstituted water according to OECD Guideline 203
230 mg CaCO₃/L
13.0 – 13.8°C
16 hours light/8 hours dark
8.73 – 9.00
7.70 – 7.91

B. Study design and method

1. In life dates:
May 18 to May 27, 2020 (experimental dates)
2. Test design:

Fish were kept in test medium, to which the test item had been added. The aim of the test was to evaluate possible toxic effects within 96 hours under semi-static conditions. Fish were exposed to serial dilutions of the test item. Mortality was recorded at 3, 6, 24, 48, 72 and 96 hours of exposure. Animals were considered dead if no visible movement (e.g. gill movements) was apparent and if touching of the caudal peduncle did not provoke any reaction. Dead fish were removed upon observance. Sub-lethal effects were monitored at 3, 6, 24, 30, 48, 54, 72, 78 and 96 hours of exposure. Measurements of pH and dissolved oxygen were carried out in 24-hour intervals in 'fresh' and 'aged' test solutions.

The total organic content (TOC) of fresh test medium was measured once. Test solutions were exchanged daily. The temperature was recorded continuously and in individual test vessels.

3. Analytical verification:

Standard analytical methods were used to determine concentrations of prothioconazole and fenpropidin in the test solutions at test start, at medium renewal and at test end. Storage stability samples were analysed in addition

4. Statistics:

The determination of lowest observed effects concentration (LOEC) was carried out by hypothesis testing for binomial distributed data. Prior to final statistical testing, the monotonicity of the dataset was investigated (qualitative trend analysis by contrasts). The linear trend could not be computed and statistically checked due to mathematical issues. Step-down Cochran-Armitage Test Procedure (96 h; $p \leq 0.05$, one-sided greater) was applied. As the study resulted in no concentration with partial mortality, classical maximum likelihood methods could not be used to estimate the LC_{10} , LC_{20} and LC_{50} . According to the recommendations of OECD Guideline 203 (2019), estimates of the LC_{50} were made using the binomial method. $LC_{10/20}$ values cannot be determined by this method. Statistical evaluation was carried out using ToxRat Professional (RATTE, 2018, version 3.3.0).

II. Results and discussion

A. Analytical data

Recoveries of Prothioconazole and Fenpropidin were within 80 to 120 % of nominal concentrations in fresh and aged test solutions. Storage stability samples were within 80 to 120 % of nominal a.s. concentrations.

Summary of analytical results, recoveries of Prothioconazole in tested samples.

Nominal test item [mg/L]	Nominal Prothioconazole [mg/L]	Analysed Prothioconazole [mg/L]	Recovery [% of nominal]	Analysed Prothioconazole [mg/L]	Recovery [% of nominal]
		0 h fresh		24 h aged	
Control	0	< LOD	-	< LOD	-
4.20	0.7107	0.7413	104.3	0.6603	92.9
5.46	0.9238	0.9592	103.8	0.9	97.4
7.10	1.201	1.318	109.8	1.161	96.7
9.23	1.561	1.856	118.9	1.569	100.5
12.00	2.03	2.316	114.1	2.112	104.1
		24 h fresh		48 h aged	
Control	0	< LOD	-	< LOD	-
4.20	0.7106	0.7054	99.3	0.6699	94.3
5.46	0.9238	0.9440	102.2	0.8680	94.0
7.10	1.201	1.298	108.1	1.268	105.6
*	-	-	-	-	-
*	-	-	-	-	-
		48 h fresh		72 h aged	
Control	0	< LOD	-	< LOD	-
4.20	0.7106	0.7506	105.6	0.6819	96.0
5.46	0.9238	0.9872	106.9	0.9085	98.3
*	-	-	-	-	-
*	-	-	-	-	-
*	-	-	-	-	-
		72 h fresh		96 h aged	
Control	0	< LOD	-	< LOD	-
4.20	0.7106	0.7869	110.7	0.7077	99.6
5.46	0.9238	0.9994	108.2	0.9516	103.0
*	-	-	-	-	-
*	-	-	-	-	-
*	-	-	-	-	-

* not prepared (100 % mortality); LOD = 0.1054 mg/L (defined as 30% of the LOQ)

Summary of analytical results, recoveries of Fenpropidin in tested samples.

Nominal test item [mg/L]	Nominal Fenpropidin [mg/L]	Analysed Fenpropidin [mg/L]	Recovery [% of nominal]	Analysed Fenpropidin [mg/L]	Recovery [% of nominal]
		0 h fresh		24 h aged	
Control	0	< LOD	-	< LOD	-
4.20	1.025	1.06	103.4	1.012	98.7
5.46	1.332	1.381	103.6	1.358	101.9
7.10	1.732	1.946	112.4	1.757	101.4
9.23	2.252	2.699	119.8	2.333	103.6
12.00	2.927	3.414	116.6	3.148	107.5
		24 h fresh		48 h aged	
Control	0	< LOD	-	< LOD	-
4.20	1.025	1.065	103.9	1.059	103.4
5.46	1.332	1.391	104.4	1.385	104.0
7.10	1.732	1.882	108.7	1.881	108.6
*	-	-	-	-	-
*	-	-	-	-	-
		48 h fresh		72 h aged	
Control	0	< LOD	-	< LOD	-
4.20	1.025	1.076	105.0	1.043	101.8
5.46	1.332	1.423	106.8	1.375	103.2
*	-	-	-	-	-
*	-	-	-	-	-
*	-	-	-	-	-
		72 h fresh		96 h aged	
Control	0	< LOD	-	< LOD	-
4.20	1.025	1.095	106.9	1.067	104.2
5.46	1.332	1.416	106.3	1.421	106.6
*	-	-	-	-	-
*	-	-	-	-	-
*	-	-	-	-	-

* not prepared (100 % mortality); LOD = 0.1517 mg/L (defined as 30% of the LOQ)

B. Mortality

Control fish showed no mortality during the course of the test. The lowest observed effects concentration (LOEC) was determined using Step-down Cochran-Armitage Test Procedure ($p \leq 0.05$, one-sided greater) for binomial distributed data. Lethal concentrations LC_{50} were determined by interpolation (binomial method). The results are presented in the table below.

Table A 1: Mortality (%) of fish in the test

Nominal test item concentration (mg/L)	Control	4.20	5.46	7.10	9.23	12.00
Time (hour)	Mortality (%)					
3	0.0	0.0	0.0	0.0	0.0	0.0
6	0.0	0.0	0.0	0.0	0.0	100.0
24	0.0	0.0	0.0	28.6	100.0	100.0
30	0.0	0.0	0.0	100.0	100.0	100.0
48	0.0	0.0	0.0	100.0	100.0	100.0
54	0.0	0.0	0.0	100.0	100.0	100.0
72	0.0	0.0	0.0	100.0	100.0	100.0
78	0.0	0.0	0.0	100.0	100.0	100.0
96	0.0	0.0	0.0	100.0	100.0	100.0

C. Toxicological symptoms

Control fish showed did not show any unusual behaviour during the course of the test.

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 $\geq 78.5\%$ of the air saturation throughout the test.
Analytical measurement of test concentrations is compulsory.	Recoveries of prothioconazole and fenpropidin were within 80 to 120 % of nominal concentrations in fresh and aged test

	solutions. Therefore, results were expressed as nominal test item concentration.
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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 this study with rainbow trout (*Oncorhynchus mykiss*), the acute toxicity of ADM.03502.F.1.A (250 g fenpropidin/L, 175 g prothioconazole/L, nominal) was analysed for 96 hours under semi-static conditions. After 96 hours, a LC₅₀ of 6.23 mg/L test item nominal was determined. The study is considered valid (see: “D. Validity of the test” above).

Comments of zRMS:	<p>The study was conducted in line with OECD 202 with no minor deviations.</p> <p>The test concentration of both active substances was verified in ‘fresh’ and ‘aged’ test solutions at test start, at test solution renewals and at test end.</p> <p>The test concentrations of both active substances were verified at the beginning and at the end of the exposure and on every renewal day (0, 24, 48 hours) of all tested concentration levels. The measured concentrations of Prothioconazol were in the range of 95.6% to 117.4% of the nominal values in freshly prepared medium (0, 24, 48 hours) and from 85.2 to 115.9% of the nominal values in old medium (24, 48 hours).</p> <p>The measured concentrations of Fenpropidin were in the range of 108.8 % to 112.5% of the nominal values in freshly prepared medium (0, 24, 48 hours) and from 110.7 to 116.4% of the nominal values in old medium (24, 48, hours).</p> <p>Therefore, the endpoints of items are based on nominal test concentration.</p> <p>48 h EC₅₀ = 5.57 mg/L (based on nominal concentration)</p>
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Reference:	KCP 10.2.1/02
Report:	Acute toxicity of ADM.03502.F.1.A to <i>Daphnia magna</i> in a 48-hour semi-static test, Renner, P., 2020b, report no 20 48 ADL 0008, sponsor no.: 000104840
Guideline(s):	OECD 202 (2004)
Deviations:	None
GLP:	Yes
Acceptability/Reliability:	Yes
Duplication (if vertebrate study)	Not applicable

Executive summary

Purpose of this study was the effect assessment of ADM.03502.F.1.A related to immobilisation of *Daphnia magna* under semi-static conditions. Young *D. magna*, less than 24 hours old at test start, were exposed to test solutions containing ADM.03502.F.1.A for a period of 48 hours. Immobilisation of *D. magna* was recorded at 24 and 48 hours after test start and was compared to control level. Results were analysed in order to obtain the effect concentrations EC₁₀, EC₂₀, EC₅₀ and LOECs at 24 and 48 hours after test start.

NOECs were derived from the LOEC. Test solutions were renewed after 24 hours. At test start and test end, as well as at the renewal step in aged and aged samples the pH and content of dissolved oxygen in

test solutions were measured. The temperature was measured continuously. After 48 hours, a EC_{50} of 5.57 mg prod./L was determined.

I. Materials and methods

A. Materials

1. Test material:
Lot/Batch no.:
Content:
Density:
Control:
Toxic reference:
ADM.03502.F.1.A
1191-101219-01
250 g fenpropidin/L, 175 g prothioconazole/L (nominal)
253.7 g fenpropidin/L, 175.9 g prothioconazole/L (analysed)
1.04 g/mL
untreated medium control
None
2. Test organisms -
Species:
Age:
Source:
Acclimatisation:
Feeding:
No of organisms:
Daphnia magna Straus
max. 24 hours old
In house culture in the test facility under standardised laboratory conditions
brood *Daphnia magna* were maintained in 100 % test medium at test temperature for at least 48 hours prior to test start
none (during the study)
20 per treatment, divided in 5 test organisms per replicate
3. Test units and exposure –
Type and size:
Test procedure:
Test duration:
glass beaker (25 ml), were filled up with 10 ml test solution
semi-static test, medium renewal after 24 h
48 hours
4. Test conditions -
Test medium:
Water temperature:
Aeration:
Photoperiod:
Light intensity:
Dissolved oxygen:
pH value:
reconstituted water according to OECD 202 and ISO
20.5 – 20.7°C
no aeration of the test vessels during the test
16 hours light/8 hours dark
660 lux
7.86 – 8.51 mg O₂/L
6.02* - 8.04 (* assumed to be a measuring mistake due to overall data context)

B. Study design and method

1. In life dates:
July 02 to November 16, 2020 (experimental phase)
2. Test design:

Purpose of this study was the effect assessment of ADM.03502.F.1.A related to immobilisation of *Daphnia magna* under semi-static conditions. Young *D. magna*, less than 24 hours old at test start, were exposed to test solutions containing ADM.03502.F.1.A for a period of 48 hours. Immobilisation of *D. magna* was recorded at 24 and 48 hours after test start and was compared to control level. Results were analysed in order to obtain the effect concentrations EC_{10} , EC_{20} , EC_{50} and LOECs at 24 and 48 hours after test start.

NOECs were derived from the LOEC. Test solutions were renewed after 24 hours. At test start and test end, as well as at the renewal step in aged and aged samples the pH and content of dissolved oxygen in test solutions were measured. The temperature was measured continuously. Recoveries of prothiocona-

zole and fenpropidin were within 80 to 120 % of nominal a.s. concentrations in ‘fresh’ and ‘aged’ test solutions After 48 hours, a EC₅₀ of 5.57 mg prod./L was determined.

3. Analytical verification:

Standard analytical methods (LC-MS/MS) were used to determine concentrations of prothioconazole and fenpropidin in test solutions at test start and test end in ‘fresh’ and ‘aged’ test solutions.

4. Statistics:

Effect concentrations reported throughout this report refer to the endpoint immobility. By default, the statistical software provides the LC_x for some procedures (e.g. binominal procedures). However, this has to be consequently read as EC_x values. Effect concentrations of EC₁₀, EC₂₀ and EC₅₀ values were calculated by Weibull analysis using maximum likelihood regression as this procedure provided the best fit. Fit criteria were the goodness of fit (p(Chi²)) and significance of the slope being different from zero. LOEC-determinations were carried out using the Step-down Cochran-Armitage Test (p ≤ 0.05, one-sided great). NOECs were derived from LOECs. To justify the usage of the Step-down Cochran-Armitage Test, the binominal distributed data was checked for monotonicity (linear trend, p ≤ 0.01) and variance homogeneity (extra binomial variances). Both of these pre-tests were passed.

II. Results and discussion

A. Analytical data

Recoveries of prothioconazole and fenpropidin were within 80 to 120 % of nominal a.s. concentrations in ‘fresh’ and ‘aged’ test solutions. Recoveries of storage stability samples were also within 80 to 120 % of nominal a.s. concentrations. Hence endpoints were based on nominal test item concentrations.

Summary of analytical results, recoveries Prothioconazole in tested samples

Nominal test item [mg/L]	Nominal Prothioconazole [mg/L]	Analysed Prothioconazole [mg/L]	Recovery [% of nominal]	Analysed Prothioconazole [mg/L]	Recovery [% of nominal]
		0 h fresh		24 h aged	
Control	0	< LOD	-	< LOD	-
2.34	0.3965	0.3788	95.6	0.3378	85.2
3.05	0.5157	0.5026	97.5	0.4798	93.0
3.97	0.6713	0.6480	96.5	0.7043	104.9
5.15	0.8708	1.022	117.4	0.9436	108.4
6.70	1.133	1.225	108.0	1.313	115.9
		24 h fresh		48 h aged	
Control	0	< LOD	-	< LOD	-
2.34	0.3957	0.3913	98.9	0.3977	100.5
3.04	0.5149	0.5056	98.2	0.5290	102.7
3.96	0.6699	0.6696	100.0	0.6506	97.1
5.16	0.8725	0.8679	99.5	0.9316	106.8
6.70	1.132	1.105	97.6	1.205	106.4

LOD = 0.05614 mg/L

Summary of analytical results, recoveries Fenpropidin in tested samples

Nominal test item [mg/L]	Nominal Fenpropidin [mg/L]	Analysed Fenpropidin [mg/L]	Recovery [% of nominal]	Analysed Fenpropidin [mg/L]	Recovery [% of nominal]
		0 h fresh		24 h aged	
Control	0	< LOD	-	< LOD	-
2.34	0.5718	0.6297	110.1	0.6342	110.9
3.05	0.7437	0.8368	112.5	0.8347	112.2
3.97	0.9682	1.086	112.2	1.086	112.1
5.15	1.256	1.378	109.7	1.391	110.7
6.70	1.635	1.832	112.1	1.840	112.6
		24 h fresh		48 h aged	
Control	0	< LOD	-	< LOD	-
2.34	0.5708	0.6301	110.4	0.6587	115.4
3.04	0.7427	0.8116	109.3	0.8582	115.6
3.96	0.9662	1.055	109.2	1.113	115.2
5.16	1.258	1.370	108.8	1.464	116.4
6.70	1.633	1.780	109.0	1.881	115.2

LOD = 0.08097 mg/L

B. Immobilisation

No visible signs of abnormalities of behaviour or appearance of surviving *D. magna* were observed at any assessment. No unusual behaviour was observed for any surviving daphnid. Immobility after 3, 24 and 48 hours after application are shown in the table below.

Table A 2: Number of immobilised *Daphnia magna* and percentage immobility

ADM.03502.F.1.A (mg/L)	Immobilised <i>D. magna</i> (number)			Immobilised <i>D. magna</i> (%)		
	3 h	24 h	48 h	3 h	24 h	48 h
Control	0	0	0	0.0	0.0	0.0
2.34	0	0	0	0.0	0.0	0.0
3.05	0	0	0	0.0	0.0	0.0
3.96	0	0	0	0.0	0.0	0.0
5.15	0	0	4	0.0	0.0	20.0+
6.70	0	0	20	0.0	0.0	100.0+
Effect concentration ADM.03502.F.1.A (mg/L)						
LOEC	≥ 6.7			5.15		
NOEC	≥ 6.7			3.96		
EC ₁₀	n.d.			4.92 (4.09-5.23)		
EC ₂₀	n.d.			5.17 (4.66-5.57)		
EC ₅₀	n.d.			5.57 (5.23-6.63)		

+ significantly different from the control, Step-down Cochran-Armitage Test, $p \leq 0.05$, one-sided greater)

Reference item

The reference item potassium dichromate was tested in a separate study at concentration of 0.88, 1.14, 1.48, 1.92 and 2.50 mg/L to control the sensitivity of the test system. The EC₅₀ of the reference item value was within the expected range of toxicity for reference toxicity tests performed at the test facility (range of 1.0 – 2.0 mg /L at 48 hours).

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 ≥ 7.86 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.03502.F.1.A (250 g fenpropidin/L, 175 g prothioconazole/L, nominal) under semi-static conditions. After 48 hours, a EC₅₀ of 5.57 mg prod./L was determined. The study is considered valid (see: “C. Validity of the test” above).

Comments of zRMS:	<p>The study was conducted in line with OECD 201-2.</p> <p>The test concentrations of both active substances were verified in the fresh media (0 hours) and old media (72 hours) of all tested concentration levels.</p> <p>The measured concentrations of Prothioconazole were in the range of 94 to 119 % of the nominal values at the start of the exposure intervals (0 hours) and 52 to 105 % at the end of the exposure (72 hours).</p> <p>The measured concentration of prothioconazole at the lowest concentration tested</p>
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	<p>after 24 h ,48 and 72 hours was below LOQ. In addition, after 72 hours measured concentration of prothiconazole below LOQ at concentration of 0.420 µg prod/L. In these cases, the test concentration was calculated as LOQ/2.</p> <p>The measured concentrations of Fenpropidin were in the range of 100 to 111 % of the nominal values at the start of the exposure intervals (0 hours) and 80 to 103 % at the end of the exposure (72 hours).</p> <p>Therefore, the endpoints are based on the geometric mean measured test item concentrations calculated from the geometric mean measured concentration of the active substance Prothioconazole and Fenpropidin.</p> <p>All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>Growth rate:</p> <p>72 h E_rC_{50} = 0.895 µg product/L (geometric mean measured concentration) 72 h E_rC_{20} = 0.478 µg product/L (geometric mean measured concentration) 72 h E_rC_{10} = 0.331 µg product/L (geometric mean measured concentration) NOE_rC = 0.164 µg product/L (geometric mean measured concentration)</p> <p>Yield</p> <p>72 h E_yC_{50} = 0.472 µg product/L (geometric mean measured concentration) 72 h E_yC_{20} = 0.184 µg product/L (geometric mean measured concentration) 72 h E_yC_{10} = 0.0987 µg product/L (geometric mean measured concentration) NOE_yC = 0.128 µg product/L (geometric mean measured concentration)</p>
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Reference:	KCP 10.2.1/03
Report:	ADM.03502.F.1.A - Alga, Growth Inhibition Test with <i>Desmodesmus subspicatus</i> , 72 hours, Scheerbaum, D., 2021, report no.: SO21519 / SSO19707, sponsor no.: 000108687
Guideline(s):	OECD 201 (2006, corrected 2011)
Deviations:	None
GLP:	Yes (certified laboratory)
Acceptability/Reliability:	Yes
Duplication (if vertebrate study)	Not applicable

Executive summary

The toxicity of ADM.03502.F.1.A to the unicellular freshwater green alga *Desmodesmus subspicatus* was determined according to the principles of OECD 201. The aim of the study was the determination of the effects on growth rate and yield over a period of 72 hours. The study was conducted under static conditions with an initial cell density of 4520 cells/mL. A geometrical series with a dilution factor of 2.1 was tested: 0.200 - 0.420 - 0.882 - 1.85 - 3.89 - 8.17 - 17.2 µg prod./L. Three replicates were tested for each test item concentration and six replicates for the control. The environmental conditions were within the acceptable limits.

The active substances prothioconazole and fenpropidin of ADM.03502.F.1.A were analytically verified via LC-MS/MS at the start (0 hours), after 24 and 48 hours and at the end of the exposure (72 hours) with algae.

At the start of the exposure the measured concentrations for both analytes were in the range of 94 to 119 % of the nominal concentrations. During the exposure the concentrations of fenpropidin remained in the range of 80 to 112 % of the nominal concentrations. As expected, the concentrations of prothioconazole decreased after 72 hours. All effect values given are based on the nominal concentrations of the product ADM.03502.F.1.A as well as on the calculated product concentrations which are based on the measured geometric mean concentrations of both active substances. After 72 hours, an E_rC_{50} value of 0.965 µg

prod./L and an E_yC_{50} value of 0.551 (nominal) were determined; equivalent to an E_rC_{50} of 0.895 µg prod./L and an E_yC_{50} of 0.472 µg prod./L (geomean).

I. Materials and methods

A. Materials

1. Test material: ADM.03502.F.1.A
Lot/Batch no.: 1191-101219-01
Content: 250 g fenpropidin/L, 175 g prothioconazole/L (nominal)
253.7 g fenpropidin/L, 175.9 g prothioconazole/L (analysed)
Density: 1.04 g/mL
Control: untreated medium control
Toxic reference: Potassium dichromate, 100 % purity (tested in a separate study)
2. Test organisms -
Species: *Desmodesmus subspicatus*, strain: 86.81 SAG
Source: SAG Culture Collection of Algae, Goettingen, Germany
Cell density: 4520 cells/mL
3. Test units and exposure –
Type and size: 250 mL Erlenmeyer flask containing 100 mL test volume.
Test procedure: static
Test duration: 72 hours
4. Test conditions –
Test medium: OECD medium (OECD 201)
Water temperature: 22.5°C mean (22 - 23°C)
Photoperiod: constant light
Light intensity: 5515 lux (mean)
pH value: 8.06 (start) – 7.96 (end)

B. Study design and method

1. In life dates: September 06 to September 10, 2021 (experimental phase)
2. Test design:

The aim of the study was the determination of the effects on growth rate and yield over a period of 72 hours. The static study was conducted under static conditions with an initial cell density of 4520 cells/mL. A stock solution with a nominal concentration of 10.0 mg test item/L was freshly prepared with dilution water and diluted in two steps to a stock solution of 17.2 µg test item/L. The stock solutions were agitated until the solutions were visually clear.

From the 17.2 µg/L stock solution, seven concentrations were prepared and tested in a geometrical series with a dilution factor of 2.1: 0.200 - 0.420 - 0.882 - 1.85 - 3.89 - 8.17 - 17.2 µg test item/L. Three replicates were tested for each test item concentration and six replicates for the control. The environmental conditions were within the acceptable limits. The cell density was measured daily via Chlorophyll a fluorescence, excitation at 436 nm, emission at 685 nm. Dilution water was used as a background signal. No self-fluorescence was found in the preliminary range finding test at the concentration of 10.0 mg/L. The algae cells were evaluated microscopically at the start and the end of the incubation period. The cells were checked for unusual cell shapes, colour differences, differences in chloroplast morphology, flocculation, adherence of algae to test containers and agglutination of algae cells. The pH-value at the start of the exposure was measured in one additional replicate of each test item concentration and the control. At the end of the exposure, it was measured in a pooled sample of the test item concentrations and the control.

The room temperature was measured continuously. Light intensity was measured prior to the start of the test.

3. Analytical verification:

The active substances prothioconazole 175 g/L + fenpropidin 250 g/L of ADM.03502.F.1.A were analytically verified via LC-MS/MS at the start (0 hours), after 24 and 48 hours and at the end of the exposure (72 hours) with algae.

4. Statistics

EC-values and statistical analyses: EC_{10} -, EC_{20} - and EC_{50} - values of growth rate inhibition and yield inhibition after 72 hours were estimated using the following tests:

Growth rate: 4-param. logistic. cumulative distribution function

Yield: Weibull analysis using linear max. likelihood regression

NOEC, LOEC and statistical analyses: The NOEC / LOEC was determined by calculation of statistically significant differences of growth rate and yield using the following tests:

Growth rate

Normality: Shapiro-Wilk Normality test, P-value 0.05, α -value 0.01

Variance Homogeneity: Levene's Test, P-value 0.01

Monotonicity of Concentration/Response: Trend analysis by contrasts

Significance: Williams Multiple Sequential t-test, α -value 0.05

Yield

Normality: Shapiro-Wilk Normality test, P-value 0.05, α -value 0.01

Variance Homogeneity: Levene's Test, P-value 0.01

Significance: multiple sequentially-rejective Welch-t-test after Bonferroni-Holm, α -value 0.05

II. Results and discussion

A. Analytical data

At the start of the exposure the measured concentrations for both analytes were in the range of 94 to 119 % of the nominal concentrations. During the exposure the concentrations of Fenpropidin remained in the range of 80 to 112 % of the nominal concentrations. As expected, the concentrations of prothioconazole decreased after 72 hours (for details, see study report). All effect values given are based on the nominal concentrations of the product ADM.03502.F.1.A as well as on the calculated product concentrations which are based on the measured geometric mean concentrations of both active substances

Measured Concentrations of the Active Substance Prothioconazole of the Test Item ADM.03502.F.1.A during the Definitive Test (0, 24, 48, 72 hours).

Sampling date		Fresh medium, 0 hours		Aged medium, 24 hours		Aged medium, 48 hours		Aged medium, 72 hours	
Nominal concentration of the		ADM.03502.F.1.A Prothioconazole							
test item [µg/L]	active substance [µg a.s./L]	Meas. conc. [µg a.s./L]	%	Meas. conc. [µg a.s./L]	%	Meas. conc. [µg a.s./L]	%	Meas. conc. [µg a.s./L]	%
17.2	2.91	2.85	98	2.94	101	3.25	112	3.05	105
8.17	1.38	1.30	94	1.60	116	1.61	116	1.09	79
3.89	0.658	0.624	95	0.624	95	0.600	91	0.426	65
1.85	0.313	0.309	99	0.250	80	0.235	75	0.164	52
0.882	0.149	0.144	97	0.138	92	0.121	81	0.0857	57
0.420	0.0710	0.0848	119	0.0711	100	0.0805	113	< LOQ	
0.200	0.0338	0.0371	110	< LOQ		< LOQ		< LOD	
Control		< LOD		< LOD		< LOD		< LOD	

Meas. conc. = measured concentration of the active substance, dilution factors taken into account
 % = percent of the nominal concentration of the active substance
 a.s. = active substance
 LOQ = limit of quantification (0.200 µg/L of the test item, corresponding to 0.0338 µg Prothioconazole/L)
 LOD = limit of detection (0.01 µg Prothioconazole/L)

Measured Concentrations of the Active Substance Fenpropidin of the Test Item ADM.03502.F.1.A during the Definitive Test (0, 24, 48, 72 hours)

Sampling date		Fresh medium, 0 hours		Aged medium, 24 hours		Aged medium, 48 hours		Aged medium, 72 hours	
Nominal concentration of the		ADM.03502.F.1.A Fenpropidin							
test item [µg/L]	active substance [µg a.s./L]	Meas. conc. [µg a.s./L]	%	Meas. conc. [µg a.s./L]	%	Meas. conc. [µg a.s./L]	%	Meas. conc. [µg a.s./L]	%
17.2	4.20	4.65	111	4.54	108	4.66	111	4.31	103
8.17	1.99	2.19	110	2.24	112	2.10	106	1.94	98
3.89	0.949	1.02	108	1.02	107	0.988	104	0.934	98
1.85	0.451	0.494	109	0.486	108	0.475	105	0.432	96
0.882	0.215	0.233	108	0.229	106	0.224	104	0.193	90
0.420	0.102	0.110	107	0.108	106	0.107	104	0.0819	80
0.200	0.0488	0.0486	100	0.0501	103	0.0496	102	0.0415	85
Control		< LOD		< LOD		< LOD		< LOD	

Meas. conc. = measured concentration of the active substance, dilution factors taken into account
 % = percent of the nominal concentration of the active substance
 a.s. = active substance
 LOQ = limit of quantification (0.150 µg/L of the test item, corresponding to 0.0366 µg Fenpropidin/L)
 LOD = limit of detection (0.005 µg Fenpropidin/L)

Geometric Mean of the Sum of the Active Substances and the corrected Nominal Product Concentration of the Test item ADM.03502.F.1.A (0, 24, 48, 72 hours)

Sampling date		Fresh medium, 0 hours	Aged medium, 24 hours	Aged medium, 48 hours	Aged medium, 72 hours
Nominal concentration of the test item [µg/L]		ADM.03502.F.1.A Prothioconazole and Fenpropidin			
total active substances [µg a.s./L]		Total conc. [µg a.s./L]	Total conc. [µg a.s./L]	Total conc. [µg a.s./L]	Total conc. [µg a.s./L]
17.2	7.10	7.50	7.48	7.91	7.36
8.17	3.37	3.49	3.84	3.71	3.03
3.89	1.61	1.64	1.64	1.59	1.36
1.85	0.764	0.803	0.736	0.710	0.596
0.882	0.364	0.377	0.367	0.345	0.279
0.420	0.173	0.195	0.179	0.188	0.0988 ¹⁾
0.200	0.0826	0.0857	0.0670 ¹⁾	0.0665 ¹⁾	0.0416 ²⁾
Nominal concentration of the test item [µg/L]		ADM.03502.F.1.A Prothioconazole and Fenpropidin			
total active substances [µg a.s./L]		Geometric mean of the sum of the I active substances [µg a.s./L]		Measured / calculated product concentration [µg/L]	
17.2	7.10	7.56	106	18.3	
8.17	3.37	3.50	104	8.47	
3.89	1.61	1.55	97	3.76	
1.85	0.764	0.705	92	1.71	
0.882	0.364	0.339	93	0.821	
0.420	0.173	0.155	89	0.374	
0.200	0.0826	0.0530	64	0.128	

Total conc: = sum of the measured concentrations of the active substances Prothioconazole and Fenpropidin
a.s. = active substance

¹⁾ = Prothioconazol < LOQ, therefore the value of 1/2x LOQ was taken into account

²⁾ = Prothioconazol < LOD, therefore the value of 1% LOD was taken into account

B. Biological data

All effect values given are based on the nominal concentrations of the product ADM.03502.F.1.A as well as on the calculated product concentrations which are based on the measured geometric mean concentrations of both active substances. Results determined at test start, at 24, 48 and 72 hours after test start are summarized in the tables below.

Table A 3: Cell densities of algal cells

Concentration		Replicate No.	Cell density [cells/mL]			
Nominal product [µg/L]	Measured /calculated product [µg/L]		0 hours	24 hours	48 hours	72 hours
17.2	18.3	1	4520	12276	28694	9124
		2	4520	19384	25432	19878
		3	4520	14963	17961	9831
		Mean	4520	15541	24029	12944
8.17	8.47	1	4520	14556	18596	20480
		2	4520	12500	24581	12159
		3	4520	13387	16268	27140
		Mean	4520	13481	19815	19926
3.89	3.76	1	4520	13112	36329	16895
		2	4520	19831	34255	21271
		3	4520	14761	40031	29896

		Mean	4520	15901	36872	22687
1.85	1.71	1	4520	26630	49547	22895
		2	4520	16741	56416	32466
		3	4520	18321	65807	51761
		Mean	4520	20564	57257	35707
0.882	0.821	1	4520	21114	102667	160920
		2	4520	16055	82668	121713
		3	4520	25648	107990	174467
		Mean	4520	20939	97775	152367
0.420	0.374	1	4520	32913	165216	780125
		2	4520	25083	147797	572621
		3	4520	32257	165435	629840
		Mean	4520	30084	159483	660862
0.200	0.128	1	4520	24039	177312	875135
		2	4520	20403	156096	1015340
		3	4520	25036	186754	959331
		Mean	4520	23159	173387	949935
Control		1	4520	24057	121142	913843
		2	4520	25443	160494	838187
		3	4520	31366	209421	1260012
		4	4520	31491	201387	1153896
		5	4520	38463	228379	1111596
		6	4520	28522	165040	925755
		Mean	4520	29890	180977	1033882

Table A 4: Evaluation of growth rate and yield (statistically significant differences of growth rates and yield compared to control values are marked (s), not significant differences are marked (ns))

Concentration		Replicate No.	Growth rate [d ⁻¹]	Inhibition of growth rate [%]	Yield [cells/mL]	Inhibition of yield [%]
Nominal product [µg/L]	Measured / calculated product [µg/L]					
17.2	18.3	1	0.23	87	4604	100
		2	0.49	73	15358	99
		3	0.26	86	5311	99
		Mean	(s) 0.33	82	(s) 8424	99
8.17	8.47	1	0.50	72	15960	98
		2	0.33	82	7639	99
		3	0.60	67	22620	98
		Mean	(s) 0.48	74	(s) 15406	99
3.89	3.76	1	0.44	76	12375	99
		2	0.52	71	16751	98
		3	0.63	65	25376	98
		Mean	(s) 0.53	71	(s) 18167	98
1.85	1.71	1	0.54	70	18375	98
		2	0.66	64	27946	97
		3	0.81	55	47241	95
		Mean	(s) 0.67	63	(s) 31187	97
0.882	0.821	1	1.19	34	156400	85
		2	1.10	39	117193	89
		3	1.22	33	169947	83
		Mean	(s) 1.17	35	(s) 147847	86
0.420	0.374	1	1.72	5	775605	25
		2	1.61	11	568101	45
		3	1.65	9	625320	39
		Mean	(s) 1.66	8	(s) 656342	36
0.200	0.128	1	1.76	3	870615	15
		2	1.81	0	1010820	2

		3	1.79	1	954811	7
		Mean	(ns) 1.78	1	(ns) 945415	8
Control	1	1.77		909323		
	2	1.74		833667		
	3	1.88		1255492		
	4	1.85		1149376		
	5	1.84		1107076		
	6	1.77		921235		
	Mean	1.81		1029362		

E. Endpoints

Table A 5: NOEC, LOEC and EC_x-values of ADM.03502.F.1.A (0 - 72 hours) based on the nominal concentrations of the product and the geometric mean measured concentrations of the active substances [µg/L]

	ADM.03502.F.1.A	
	Nominal product concentration [µg/L]	Measured / calculated product concentration [µg/L]
	Inhibition of Growth Rate	Inhibition of Growth Rate
NOEC	0.200	0.164
LOEC	0.420	0.374
ErC ₁₀	0.363 (95% CI: 0.248 – 0.460)	0.331 (95% CI: 0.224 – 0.422)
ErC ₂₀	0.521 (95% CI: 0.396 – 0.621)	0.478 (95% CI: 0.360 – 0.571)
ErC ₅₀	0.965 (95% CI: 0.829 – 1.11)	0.895 (95% CI: 0.768 – 1.03)
	Inhibition of Yield	Inhibition of Yield
	NOEC	0.200
	LOEC	0.420
EyC ₁₀	0.134 (95% CI: 0.0918 – 0.176)	0.0987 (95% CI: 0.0770 – 0.148)
EyC ₂₀	0.235 (95% CI: 0.179 – 0.289)	0.184 (95% CI: 0.154 – 0.249)
EyC ₅₀	0.551 (95% CI: 0.474 – 0.627)	0.472 (95% CI: 0.425 – 0.562)

F. Reference item

The toxicity of potassium dichromate (purity 100.0%) to the unicellular freshwater green alga *Desmodesmus subspicatus* was determined over a period of 72 hours in a separate test. The reference item toxicity is in the valid range which was established by calculation of the average of the historic reference data since 2006, and the limits were set using the threefold standard deviation of these values

	Current Study	Valid Range (average ± 3 x SD)
	Growth Rate inhibition	
E _r C ₅₀	0.629	0.664 ± 0.361
95% confidence interval	0.576 – 0.697	
	Yield inhibition	
E _y C ₅₀	0.276	0.314 ± 0.130

95% confidence interval	0.240 – 0.327	
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G. Validity of the test:

Validity Criterion	Required	This study
Increase of the cell growth in the control cultures	Exponentially, ≥ 16 -fold corresponding to a specific growth rate of 0.92 day^{-1}	229-fold (specific growth rate 1.81 day^{-1})
Mean coefficients of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3) in the control cultures	$\leq 35\%$	7.66%
Coefficient of variation of average specific growth rates during the whole test period in replicate control cultures	$\leq 7\%$	2.94%

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 algae growth inhibition test, *Desmodesmus subspicatus* was exposed to a nominal concentration of 0.200, 0.420, 0.882, 1.85, 3.89, 8.17 and 17.2 $\mu\text{g ADM.03501.F.1.A/L}$. After 72 hours, an E_rC_{50} value of 0.965 $\mu\text{g prod./L}$ and an E_yC_{50} value of 0.551 (nominal) were determined; equivalent to an E_rC_{50} of 0.895 $\mu\text{g prod./L}$ and an E_yC_{50} of 0.472 $\mu\text{g prod./L}$ (geomean). The study is considered valid (see: “G. Validity of the test” above).

Comments of zRMS:	<p>The study was conducted in line with OECD 221 with no deviations.</p> <p>The test concentrations of both active substances were verified at the beginning and at the end of the exposure and on every renewal day of all tested concentration levels.</p> <p>The measured concentrations of Prothioconazole were in the range of 51.6 % to 108.8% of the nominal values in freshly prepared medium (Day 0, 2 and 5) and from LOQ/2 for TWA (for the lowest tested concentration) to 100.2% of the nominal values in old medium (Day 3, 5 and 7).</p> <p>The measured concentrations of Fenpropidin were in the range 96.7% to 113.0% of the nominal values in freshly prepared medium (Day 0, 3 and 5) and 52.4% to 107% of the nominal values in old medium (Day 3, 5 and 7).</p> <p>The endpoints are based on time weighed average test item concentration calculated based on the sum of the two active substances time weighted average concentrations.</p> <p>All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>Growth rate based on frond number: $E_rC_{50} = 0.596 \text{ mg product/L}$ (based on time weighed average test item concentration) $E_rC_{20} = 0.073 \text{ mg product/L}$ (based on time weighed average test item concentration) $E_rC_{10} = 0.024 \text{ mg product/L}$ (based on time weighed average test item concentration) $NOE_rC = 0.0135 \text{ mg product/L}$ (based on time weighed average test item concentration)</p> <p>Yield based on frond number: $E_yC_{50} = 0.148 \text{ mg product/L}$ (based on time weighed average test item concentration) $E_yC_{20} = 0.026 \text{ mg product/L}$ (based on time weighed average test item concentration) $E_yC_{10} = 0.010 \text{ mg product/L}$ (based on time weighed average test item concentration) $NOE_yC = 0.0135 \text{ mg product/L}$ (based on time weighed average test item concentration)</p> <p>Growth rate based on biomass:</p>
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	<p>7 d E_rC_{50} = 1.242 mg product/L (based on time weighed average test item concentration) 7 d E_rC_{20} = 0.127 mg product/L (based on time weighed average test item concentration) 7 d E_rC_{10} = 0.038 mg product/L (based on time weighed average test item concentration) NOE_rC = 0.0449 mg product/L based on time weighed average test item concentration)</p> <p>Yield based on biomass: 7 d E_yC_{50} = 0.0192 mg product/L (based on time weighed average test item concentration) 7 d E_yC_{20} = 0.017 mg product/L (based on time weighed average test item concentration) 7 d E_yC_{10} = 0.005 mg product/L (based on time weighed average test item concentration) NOE_yC = 0.0135 mg product/L (based on time weighed average test item concentration)</p>
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Reference:	KCP 10.2.1/04
Report:	Effects of ADM.03502.F.1.A on <i>Lemna gibba</i> in a growth inhibition test under semi-static test conditions, Renner, P., 2021, report no.: 2048ALE0006, sponsor no.: 000104842
Guideline(s):	OECD 221 (2006)
Deviations:	None
GLP:	Yes
Acceptability/Reliability:	Yes
Duplication (if vertebrate study)	Not applicable

Executive summary

The *Lemna* growth inhibition test determines effects on vegetative growth based on the assessment of frond number and dry weight as an indication of the toxicity of the test item. For this purpose, the test organism was exposed to aqueous test solutions of different concentrations for a period of 7 days (0.0186, 0.0596, 0.191, 0.610, 1.95, 6.25, 20.0 mg prod./L nominal). Test vessels were kept in a temperature-controlled water bath with constant illumination, were set up randomly at test start and at test solution renewals and were covered with clear glass lids to minimize test solution evaporation and contamination.

Each test vessel was filled with 100 mL test solution and contained 9 fronds. At test start, the weight of the fronds was determined. Untreated representative plants (not used in the test) were dried at 60 °C to constant weight to determine mean initial dry weight per replicate. Frond number and the appearance of colonies (phytotoxic effects) were recorded at days 0, 3, 5 and 7 after exposure start. Following a semi-static test regime, test solutions were renewed at day 3 and 5. Test solutions were freshly prepared prior renewals. Samples for chemical analysis were taken at test start, at test solution renewals and at test end as 'fresh' and 'aged'. The pH of test solutions was measured at these occasions. Measurement of temperature was carried out continuously.

The content of prothioconazole and fenpropidin was analysed by LC-MS/MS in 'fresh' and 'aged' test solutions at test start, at test solution renewals and at test end. The doubling time of fronds in controls was calculated to monitor test validity. Average specific growth rate was calculated based on changes in frond number determined during the 7-day exposure period. Furthermore, the final dry weight per treatment was determined.

Recoveries of prothioconazole and fenpropidin were within 80 to 120 % of nominal concentrations in 'fresh' test solutions. Recoveries in 'aged' samples (two or three days old, respectively) were within 23 to 100 % of nominal for prothioconazole and within 52 to 107 % of nominal for fenpropidin, respectively. Recoveries of the storage stability samples were within 80 to 120 % of nominal concentrations. Hence endpoints were based on nominal and time weighted average test item concentrations.

Based on nominal test item concentrations, the most sensitive E_rC_{50} was 0.649 mg/L. The most sensitive E_yC_{50} was 0.176 mg/L. Based on time weighted average test item concentrations, the E_rC_{50} was 0.596 mg/L and the E_yC_{50} was 0.148 mg/L. Biomass (based on dry weight) was the most sensitive parameter.

I. Materials and methods

A. Materials

1. Test material: ADM.03502.F.1.A
Lot/Batch no.: 1191-101219-01
Content: 250 g fenpropidin/L, 175 g prothioconazole/L (nominal)
253.7 g fenpropidin/L, 175.9 g prothioconazole/L (analysed)
Density: 1.04 g/mL
Control: untreated medium control
Toxic reference: 3,5-Dichlorophenol (tested in a separate study)
2. Test organisms -
Species: *Lemna gibba*
Source: Purchased from “Institut für Allgemeine Botanik”, University of Jena, Germany, in June 2007.
No of plants: 3 replicates per treatment, 6 replicates for the control, each with 9 fronds per vessel
Acclimatisation: 7 days
3. Test units and exposure –
Type and size: 150 ml glass beakers each containing 100 ml test solution
Test procedure: semi-static dose-response test (test solution renewal at day 3 and day 5)
Test duration: 7 days
4. Test conditions –
Test medium: 20X AAP growth medium was used for culturing and during the test medium
Temperature: 22.6 – 23.1°C
Photoperiod: constant light
Light intensity: 8000 lx (mean)
pH value: 7.56 - 7.46

B. Study design and method

1. In life dates: 10.07.2020 to 17.07.2020 (experimental phase)
2. Test design:

The *Lemna* growth inhibition test determines effects of ADM.03502.F.1.A on vegetative growth based on the assessment of frond number and dry weight as an indication of the toxicity of the test item. For this purpose, the test organism was exposed to aqueous test solutions of different concentrations for a period of 7 days (0.0186, 0.0596, 0.191, 0.610, 1.95, 6.25, 20.0 mg prod./L nominal). Comparisons were performed against an untreated control. Test vessels were kept in a temperature-controlled water bath with constant illumination, were set up randomly at test start and at test solution renewals and were covered with clear glass lids to minimize test solution evaporation and contamination.

Each test vessel was filled with 100 mL test solution and contained 9 fronds. At test start, the weight of the fronds was determined. Untreated representative plants (not used in the test) were dried at 60 °C to constant weight to determine mean initial dry weight per replicate. Frond number and the appearance of colonies (phytotoxic effects) were recorded at days 0, 3, 5 and 7 after exposure start. Following a semi-static test regime, test solutions were renewed at day 3 and 5. Test solutions were freshly prepared prior renewals. Samples for chemical analysis were taken at test start, at test solution renewals and at test end as ‘fresh’ and ‘aged’. The pH of test solutions was measured at these occasions. Measurement of temperature was carried out continuously.

The doubling time of fronds in controls was calculated to monitor test validity. Average specific growth rate was calculated based on changes in frond number determined during the 7-day exposure period. Furthermore, the final dry weight per treatment was determined.

Effect concentrations of E_rC_x , E_yC_x (i.e., EC_{10} , EC_{20} and EC_{50}) were determined by concentrations-response modelling. LOECs were determined employing suitable statistical tests. Endpoints were based on the inhibition of *Lemna* growth (growth rate and yield inhibition based in frond number and dry weight) over a period of 7 days. Effects on roots were assessed qualitatively.

3,5-dichlorophenol was routinely tested in a separate *Lemna* growth inhibition reference toxicity test at concentrations of 0.18, 0.39, 0.81, 1.70, 3.57, 7.50 mg/L to verify the sensitivity of the test system.

3. Analytical verification:

The content of prothioconazole and fenpropidin was analysed by LC-MS/MS in ‘fresh’ and ‘aged’ test solutions at test start, at test solution renewals and at test end. Storage stability samples were analysed in addition.

4. Statistics

To determine the most suitable statistical procedure a sequence of pretesting was performed on each dataset of frond number and biomass considering growth rate as well as yield. The criteria of normal distribution (Shapiro-Wilks test, $p \leq 0.01$) and variance homogeneity (Levene’s test, $p \leq 0.01$) were fulfilled. The criteria of monotonicity (trend analysis by contrasts, $p \leq 0.05$) was not fulfilled for one dataset; growth rate based on biomass (dry weight). Based on these findings, the usage of the Williams t-test and for growth rate based on biomass (dry weight) the Welch’s t-test ($p \leq 0.05$, one-sided smaller) was justified. Estimates of E_rC_x and E_yC_x values were calculated by Probit analysis using maximum likelihood regression. The goodness of fit was based on the $p(\chi^2)$ and $p(F)$ statistics. The best fit was found using the Probit function.

Data were analysed using ToxRat Professional (version 3.3.0; RATTE, 2018).

II. Results and discussion

A. Analytical data

Recoveries of prothioconazole and fenpropidin were within 80 to 120 % of nominal concentrations in ‘fresh’ test solutions. Recoveries in ‘aged’ samples (two or three days old, respectively) were within 23 to 100 % of nominal for prothioconazole and within 52 to 107 % of nominal for fenpropidin, respectively. Recoveries of the storage stability samples were within 80 to 120 % of nominal concentrations. Results were expressed as nominal test item and time weighted average (TWA) test item concentration (calculated based on the sum of the two active ingredients time weighted average concentrations).

Summary of analytical results, recoveries Prothioconazole in tested samples

Nominal test item [mg/L]	Nominal Prothioconazole [mg/L]	Analysed Prothioconazole [mg/L]	Recovery [% of nominal]	Analysed Prothioconazole [mg/L]	Recovery [% of nominal]
		fresh		3 d aged	
Control	0	< 30% LOQ	-	< LOD	-
0.0186	0.003153	0.003218	102.1	(0.0001881)*	6.0
0.0596	0.01008	0.01004	99.6	(0.0001009)*	1.0
0.191	0.03226	0.03442	106.7	0.009767	30.3
0.610	0.1032	0.1123	108.8	0.05923	57.4
1.95	0.3305	0.3457	104.6	0.2867	86.8
6.25	1.057	1.112	105.2	0.9828	93.0
20.00	3.383	3.326	98.3	3.190	94.3
-	-	3 d fresh		5 d aged	
Control	0	< LOD	-	< LOD	-
0.0186	0.003151	0.002997	95.1	(0.0007350)*	23.3
0.0596	0.01008	0.009473	93.9	0.003046	30.2
0.191	0.03226	0.01665	51.6	0.02011	62.3
0.611	0.1033	0.1037	100.4	0.07473	72.4
1.95	0.3305	0.3554	107.5	0.2962	89.6
6.26	1.058	1.067	100.8	0.9955	94.1
20.02	3.386	3.196	94.4	2.942	86.9
-	-	5 d fresh		7 d aged	
Control	0	< LOD	-	< LOD	-
0.0186	0.003145	0.002799	89.0	(0.0003511)*	-*
0.0596	0.01007	0.009363	93.0	0.004025	40.0
0.190	0.03221	0.03112	96.6	0.01274	39.6
0.610	0.1032	0.1105	107.0	0.07855	76.1
1.95	0.3305	0.3445	104.2	0.3026	91.6
6.25	1.057	1.126	106.5	1.059	100.2
20.00	3.383	3.416	101.0	3.253	96.2

LOQ = 0.001561 mg/L; LOD = 0.00004401 mg/L; * considered as LOQ/2 for TWA

Summary of analytical results, recoveries Fenpropidin in tested samples

Nominal test item [mg/L]	Nominal Fenpropidin [mg/L]	Analysed Fenpropidin [mg/L]	Recovery [% of nominal]	Analysed Fenpropidin [mg/L]	Recovery [% of nominal]
		fresh		3 d aged	
Control	0	< 30% LOQ	-	< 30% LOQ	-
0.0186	0.004547	0.004767	104.8	0.004031	88.7
0.0596	0.01454	0.01510	103.3	0.01352	92.4
0.191	0.04652	0.05038	108.3	0.04660	100.2
0.610	0.1488	0.1629	109.5	0.1496	100.5
1.95	0.4767	0.5284	110.9	0.4834	101.4
6.25	1.525	1.605	105.3	1.580	103.6
20.0	4.879	5.058	103.7	5.220	107.0
-	-	3 d fresh		5 d aged	
Control	0	< 30% LOQ	-	< LOD	-
0.0186	0.004545	0.004495	99.0	0.003222	71.0
0.0596	0.01454	0.01424	98.0	0.01372	94.5
0.191	0.04653	0.04692	100.9	0.04436	95.4
0.611	0.1490	0.1576	105.9	0.1466	98.5
1.95	0.4767	0.5380	113.0	0.4739	99.5
6.25	1.526	1.599	104.9	1.555	102.0
20.0	4.883	5.027	103.0	4.857	99.5
-	-	5 d fresh		7 d aged	
Control	0	< LOD	-	< LOD	-
0.0186	0.004536	0.004385	96.7	0.002378	52.4
0.0596	0.01453	0.01434	98.7	0.01002	69.0
0.191	0.04646	0.04828	103.9	0.03903	84.0
0.610	0.1489	0.1603	107.7	0.1466	98.5
1.95	0.4766	0.4988	104.7	0.4797	100.6
6.25	1.525	1.702	111.6	1.605	105.3
20.0	4.879	5.028	103.0	4.940	101.2

LOQ = 0.002252 mg/L; LOD = 0.000003599 mg/L

Determination of time weighted average test item concentrations

Test item nominal (mg/L)	Prothioconazole*		Fenpropidin		Sum both a.i. nominal	Sum both a.i. analysed	Recovery	Test item TWA measured (mg/L)
	Nominal (mg/L)	TWA (mg/L)	Nominal (mg/L)	TWA (mg/L)	(mg/L)	(mg/L)	%	
0.0186	0.0031	0.001649	0.0045	0.003910	0.0077	0.0056	72.3%	0.0135
0.0596	0.0101	0.004978	0.0145	0.013564	0.0246	0.0185	75.3%	0.0449
0.191	0.0322	0.019504	0.0465	0.046234	0.0787	0.0657	83.5%	0.159
0.610	0.1032	0.087565	0.1489	0.154176	0.2521	0.2417	95.9%	0.585
1.95	0.3305	0.320263	0.4767	0.500803	0.8071	0.8211	101.7%	1.99
6.25	1.0574	1.054886	1.5251	1.605321	2.5826	2.6602	103.0%	6.44
20.0	3.3838	3.224964	4.8805	5.038070	8.2643	8.2630	100.0%	20.0

* where measured concentrations were < LOQ and > LOD, LOQ/2 were used for calculation

B. Biological findings

Table A 6: Frond number and biomass

ADM.03502.F.1.A (mg/L)	-	Number of fronds				Biomass (mg)	
		0 d	3 d	5 d	7 d	0 d	7 d
Control	mean	9.00	15.83	41.33	70.83	0.78	8.50
	SD	0.00	1.17	1.75	2.64	0.00	1.72
0.0186	mean	9.00	16.33	43.33	70.33	0.78	8.60
	SD	0.00	0.58	1.53	1.53	0.00	2.04
0.0603	mean	9.00	12.33	29.67	51.33	0.78	5.20
	SD	0.00	0.58	1.53	2.52	0.00	2.35
0.191	mean	9.00	11.00	15.67	35.00	0.78	5.00
	SD	0.00	1.00	1.15	2.65	0.00	0.78
0.610	mean	9.00	9.67	12.67	24.33	0.78	3.63
	SD	0.00	0.58	0.58	2.52	0.00	0.78
1.95	mean	9.00	9.33	10.33	20.33	0.78	2.67
	SD	0.00	0.58	0.58	4.04	0.00	0.68
6.25	mean	9.00	9.00	10.67	12.67	0.78	1.63
	SD	0.00	0.00	0.58	0.58	0.00	0.40
20.0	mean	9.00	9.00	9.33	9.67	0.78	0.57
	SD	0.00	0.00	0.58	0.58	0.00	0.42

SD = standard deviation

Table A 7: Inhibition of growth rate based on frond number and biomass after 7 days

ADM.03502.F.1.A (mg/L)		Growth rate frond number		Growth rate biomass	
		growth rate μ_{0-7}	growth inhibition % I_r	growth rate μ_{0-7}	growth inhibition % I_r
control	mean	0.295	-	0.338	-
	SD	0.005		0.028	
0.0186	mean	0.294	0.3	0.340	-0.4
	SD	0.003		0.033	
0.0603	mean	0.249	15.6+	0.260	23.2+
	SD	0.007		0.069	
0.191	mean	0.194	34.2+	0.264	22.1+
	SD	0.011		0.022	
0.610	mean	0.142	51.9+	0.217	35.8+
	SD	0.015		0.030	
1.95	mean	0.114	61.2+	0.172	49.3+

	SD	0.030		0.040	
6.25	mean	0.049	83.5+	0.102	69.9+
	SD	0.007		0.037	
20.0	mean	0.010	96.6+	-0.097 ¹	128.6+
	SD	0.009		0.172	

SD = standard deviation; I_r = inhibition of the average specific growth rate; + significantly different to untreated control (growth rate frond number: Williams t-test, $p \leq 0.05$, one-sided smaller; growth rate biomass: Welch's t-test, $p \leq 0.05$, one-sided smaller); ¹ indicating a weight loss compared to control level (representative samples taken at test start)

Table A 8: Yield evaluation of frond number and biomass

ADM.03502.F.1.A (mg/L)	yield frond number			yield biomass		
	-	yield	% Inhibition		yield	% Inhibition
control	mean	61.83	-	mean	7.717	-
	SD	2.64		SD	1.721	
0.0186	mean	61.33	0.8	mean	7.817	-1.3
	SD	1.53		SD	2.042	
0.0603	mean	42.33	31.5+	mean	4.417	42.8+
	SD	2.52		SD	2.352	
0.191	mean	26.00	58.0+	mean	4.217	45.4+
	SD	2.65		SD	0.781	
0.610	mean	15.33	75.2+	mean	2.850	63.1+
	SD	2.52		SD	0.777	
1.95	mean	11.33	81.7+	mean	1.883	75.6+
	SD	4.04		SD	0.681	
6.25	mean	3.67	94.1+	mean	0.850	89.0+
	SD	0.58		SD	0.404	
20.0	mean	0.67	98.9+	mean	-0.217 ¹	102.8+
	SD	0.58		SD	0.416	

SD = standard deviation; I_r = inhibition of the average specific growth rate; + significantly different to untreated control (Williams t-test; $p \leq 0.05$, one-sided smaller); ¹ indicating a weight loss compared to control level (representative samples taken at test start)

Table A 9: Chlorosis and effects on root development

ADM.03502.F.1.A (mg/L)	Chlorosis and effects on root length days after start of exposure					
	% chlorosis			Inhibition of root length (%)		
	day 3	day 5	day 7	day 3	day 5	day 7
control	0	0	0	0	0	0
0.0186	0	0	0	0	2.7	0
0.0603	0	0	0	0	8.1	5.7
0.191	0	0	0	0	18.9	17.1
0.610	0	0	0	0	18.9	42.9
1.95	0	13.2	11.5	33.3	45.9	57.1
6.25	0	29.0	36.8	33.3	56.8	65.7
20.0	0	16.1	37.9	55.6	67.6	91.4

SD=standard deviation

Chlorotic effects were observed 5 and 7 days after test start at concentrations ≥ 1.95 mg/L. Effects on root length were found after 3 days at concentrations ≥ 1.95 mg/L. At test end, effects on roots were found at concentrations ≥ 0.191 mg/L with an inhibition of 18.9 %. Evidently, concentration and time-dependent effects were found

Table A 10: Results of the reference item (toxic standard)

Effect concentration	3,5-dichlorophenol (mg/L)			
	Growth rate inhibition		Yield inhibition	
	Frond number	Biomass	Frond number	Biomass
EC ₅₀				
test item nominal	2.96	4.87	1.90	2.62
(CI)	(2.61 – 3.34)	(4.74 – 5.00)	(1.53 – 2.34)	(1.83 – 3.72)

CI – 95 % confidence intervals, upper – lower

The results of the most recent reference study with 3,5-dichlorophenol are summarised in the table above. The recommended range of toxicity is 2.2 – 3.8 mg/L 3,5-dichlorophenol based on growth rate frond number and yield frond number.

C. Endpoints

Table A 11: Effects of ADM.03502.F.1.A on *Lemna gibba* applied under semi-static test conditions (day 7)

Effect concentrations after 7 days ADM.03502.F.1.A (mg/L)				
	Growth rate inhibition		Yield inhibition	
	Frond number	Biomass	Frond number	Biomass
LOEC				
test item based on nominal concentration	0.060	0.191*	0.060	0.060
test item based on measured concentration (TWA)	0.0449	0.159*	0.0449	0.0449
NOEC				
test item based on nominal	0.019	0.060*	0.019	0.019
test item based on measured concentration (TWA)	0.0135	0.0449*	0.0135	0.0135
EC ₁₀				
test item based on nominal concentration	0.032	0.048	0.015	0.007
(CI)	(0.018 – 0.049)	(0.012 – 0.110)	(0.008 – 0.024)	(0.001 – 0.020)
test item based on measured concentration (TWA)	0.024	0.038	0.010	0.005
(CI)	(0.013 – 0.039)	(0.008 – 0.095)	(0.005 – 0.017)	(0.001 – 0.014)
EC ₂₀				
test item based on nominal concentration	0.090	0.149	0.035	0.023
(CI)	(0.060 – 0.125)	(0.055 – 0.280)	(0.023 – 0.049)	(0.006 – 0.051)
test item based on measured concentration (TWA)	0.073	0.127	0.026	0.017
(CI)	(0.046 – 0.104)	(0.041 – 0.252)	(0.016 – 0.037)	(0.004 – 0.039)

Calculations performed using unrounded values; CI – 95 % confidence intervals, upper – lower; TWA = Time weighted average; * expert judgement (a LOEC could not be determined by the software due to mathematical issues. However, a significant difference between the control treatment and 0.191 mg/L test item was found)

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 number in controls was 2.4 days. The biomass increased 7.9-fold over 7 days. The mean growth rate was 0.295 d ⁻¹ .

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.03502.F.1.A under semi-static conditions. An untreated control was also run in parallel. Based on nominal test item concentrations, the most sensitive E_rC₅₀ was 0.649 mg/L. The most sensitive E_yC₅₀ was 0.176 mg/L. Based on time weighted average test item concentrations, the E_rC₅₀ was 0.596 mg/L and the E_yC₅₀ was 0.148 mg/L. Biomass (based on dry weight) was the most sensitive parameter. 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.03502.F.1.A were conducted, as the results of the performed studies indicate no undue toxicity of the formulated product in comparison with the active substances.

A 2.2.3 KCP 10.2.3 Further testing on aquatic organisms

Comments of zRMS:	<p>Two fenpropidin mesocosm studies are EU-approved, and to support product risk assessment, this additional mesocosm study was performed with formulated fenpropidin (Wellmann et al, 2006).</p> <p>This study by Wellmann et al (2006) was submitted after the Annex I inclusion of fenpropidin, but has been evaluated by the RMS Sweden. The results of that evaluation can be found in “Addendum following the evaluation of new Annex II data Post-Annex I inclusion, Fenpropidin, Volume 3, Annex B Ecotoxicology (Sept 2011).” The RMS concluded that a NOEC could not be established. The NOEAEC (considering 8 weeks recovery) could be set to 1.0 µg a.s./L.</p> <p>The results from this mesocosm are not relied upon in this risk assessment.</p>
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Reference: KCP 10.2.3/01

Report Community level study with Fenpropidin in outdoor aquatic mesocosm ponds, Wellmann, P., Hommen, P., Böhmer, W., 2006, Report No: FEI-010/4-52

Guideline(s): OECD Guidance document “Freshwater Lentic Field Tests” (2004, Draft); SANCO/3268/2001 rev 4 (final), 17 October 2002.
The recommendations of the most recent expert workshop CLASSIC (Giddings et al., 2002, SETAC) were accounted as far as possible.

Deviations: No

GLP: Yes

Acceptability: Yes

Duplication
(if vertebrate study) No

Executive summary

A community level study with MCW-273 750 EC (761.8 g fenpropidin/L) was performed in outdoor aquatic mesocosm ponds (cylindrical basins made of polyethylene, volume: approx. 5000 L of water). The test item was sprayed twice onto the water surface at nominal initial water concentrations of 0.3, 1.0, 3.0, 10, 30 and 100 µg a.s./L with two replicate basins per concentration. The application interval was 14 days. Three untreated basins were used as controls. Physical and chemical properties of the water phase were monitored. Phytoplankton, periphyton (including floating filamentous algae), macrophytes, zooplankton and macroinvertebrates were monitored in regular intervals. The analysis of water samples from treated basins showed that fenpropidin dissipated considerably from the water column within the first weeks after treatment with a mean DT50 of 3.6 days and a mean DT90 of 12 days. The analysis of sediment samples, which were collected and analysed for the highest treatment level only, showed that fenpropidin concentrations in the sediment increased up to approx. 3 mg/kg at test end, with no significant increase from day 28 to 70. Generally, a high number of phytoplankton, zooplankton and macroinvertebrates species was observed during the study. Significant differences to the control were observed at the highest test concentration for several populations.

At test concentrations ≤ 30 µg a.s./L only slight and/or transient direct or indirect effects were observed with recovery within eight weeks after the 2nd application. The only exception was found for submerged macrophytes which were significantly more abundant in all treated ponds than in the controls. However, this effect was not considered as ecologically adverse. The authors concluded that it is likely that the missing growth of macrophytes in the controls was caused by the fact that the macrophytes were introduced into the mesocosms just before test start while the filamentous algae were present in (and adapted to) the system before. In the controls, the introduced macrophytes seemed to be overgrown by the algae. In the treated systems, fenpropidin inhibited the blooms of the filamentous algae, which allowed the growth of the less sensitive but more slowly growing macrophytes. Thus, the higher abundance of macrophytes is likely a result of experimental design but not representative for the field situation. Therefore, the NOEAEC was established at 30 µg a.s./L.

I. Materials and methods

A. Materials

1. Test material: LEANDER (MCW-273 750 EC)
Description: liquid
Lot/Batch no.: 030405
Active ingredient content: 761.8 g/L fenpropidin
CAS no.: 67306-00-7
Stability of test compound: date of expiry: April 2007
Density: 0.9342 g/mL (20°C)
2. Negative control: untreated ponds
Solvent: water
3. Test organisms
Species (indigenous): phytoplankton and zooplankton organisms from field collections from three local non-polluted ponds

Species (introduced):	macrophytes (<i>Myriophyllum sp.</i> , <i>Potamogeton sp.</i> and <i>Chara globularis</i>)
Source:	not stated

4. Test units

Type and location:	a total of 15 cylindrical ponds made of polyethylene located in Aachen, Germany
Size of each basin:	2.5 m diameter, 1.5 m total depth, 1 m water depth above sediment surface, 4.91 m ² surface area, volume: approximately 5000 L of water
Source of sediment:	natural non-polluted shallow local pond
Characterisation of sediment:	middle silty clay, 31.2% clay, 50.6% silt, 18.2% sand, Cation exchange capacity: 20.4 meq/100 g, 6% organic carbon, 3278 mg/kg total phosphorus, 0.5 mg/kg total nitrogen
Source of water:	tap water
Replicates:	two replicate enclosures per concentration, three control enclosures
Test procedure:	lentic (static)
Observation period:	21 days before until 84 days after 1 st application

5. Test conditions

Environmental conditions	
Water hardness (day 0):	0.7 to 0.8 mmol/L
Dissolved organic carbon:	ca. 3.5 mg/L before 1 st application, 10.3 mg/L two weeks after 2 nd application
Climatic conditions:	typical spring and summer conditions (records of air temperature, sunshine duration and precipitation from the nearby weather station are given in the original report)
Water temperature:	11 – 22° C

B. Study design and method

1. In life dates: May 02 to August 20, 2005

2. Test system and application

The test item was applied twice onto the water surface with an interval of 14 days by means of a hand held spray boom with a conventional hydraulic nozzle. The following nominal initial water concentrations were chosen for both applications 0.3, 1.0, 3.0, 10, 30 and 100 µg a.s./L with two replicate basins per concentration. Three untreated basins were used as controls. Three species of submerged macrophytes were introduced into the mesocosms at the beginning of the study. The macrophytes were planted in plastic pots with sediment (one pot per species and plant) and the pots were fixed at a water depth of about 20 – 80 cm below the water surface.

3. Observations

Water samples for analysis of the population dynamics of the pelagial communities (phytoplankton and zooplankton) were collected on day -14, -7, 0, 1, 2, 4, 7, 14, 15, 16, 18, 21, 28, 35, 42, 49, 56 and 70. The investigation on the phytoplankton included chlorophyll *a* measurements. Glass slides were used as artificial substrate for the development of the periphyton community. The slides were introduced 21 days before the 1st application and collected on day 0, 7, 14, 21, 28, 42, 56, 70 and 84. The periphyton biomass was determined by dry weight and chlorophyll-measurements. Macroinvertebrate communities settling on the walls of the basins were sampled by Plexiglas plates which were introduced 21 days before the 1st application. Artificial substrate samplers for macroinvertebrates were placed on the top of the sediment of each pond 14 days before the 1st application. Samples were collected every 14 days. The development of macrophytes was photographically documented and quantitatively assessed every 14 days. Biomass was determined at the end of the study.

Dissolved oxygen, water temperature, pH and conductivity were measured on day -7, 0, 1, 2, 4, 7, 14, 15, 16, 18, 21, 28, 35, 42, 49, 56, 70 and 84. Total phosphorus, dissolved *ortho*-phosphate, total nitrogen, dissolved nitrate, nitrite and ammonium, and turbidity were determined on day -7, 0, 7, 14, 21, 28, 42, 56 and 70. Water samples for residue analysis were taken 2 and 6 hours after each application and on day 1, 2, 4, 7, 14, 15, 16, 18, 28, 49 and 70. Sediment samples for residue analysis were only taken in basins treated at the highest concentration (on day 2, 4, 14, 16, 18, 28, 49 and 70).

4. Analysis of test item concentrations in water and sediment samples

Fenpropidin in water samples was determined by GC/EI-MS/MS using fenpropimorph as internal standard following acidification of the water sample. Fenpropidin in sediment samples was extracted with internal standard solution, acetone and methanol followed by extraction with purified water and cyclohexane and solid phase extraction thereafter. Fenpropidin was measured by Triple Quad GC-MS/MS using Des-tBu-fenpropidin as internal standard.

5. Statistics

Community level:

- Species presence and dominance:	Evaluation of sum over all samples
- NOEC for total abundance over time:	Williams-test applied to log-transformed abundance
- NOEC for diversity indices over time:	Diversity indices (species number, Shannon index, evenness for each sampling date)
- Similarity between treatment and control:	Similarity analysis using Steinhaus' and Stander's index
- Effect on community structure over time:	Principal response curves (for the whole data set)
- Community NOEC:	Williams-test applied to PCA (principal component analysis) sample scores (for each sampling date)

Recovery was assumed if an endpoint (population abundance or a community related measure) after a direct effect showed a clear increase and reached the level of the controls again. The estimated recovery potential was used to determine the NOEAEC for the whole study according to the EU Technical Guidance Document (SANCO/3268/2001 rev 4 (final), 17 October 2002).

II. Results and discussion

A. Analytical data

Method validation

Analytical methods for the determination of fenpropidin in water and sediment were validated according to SANCO 825/00 rev. 7 (2004) and SANCO 3029/99 rev.4 (2000) with satisfactory results with regard to accuracy (recovery), specificity, linearity, and repeatability (precision) for both matrices. Overall mean recovery \pm RSD was $96.9\% \pm 6.2\%$ in water fortified at 0.027, 0.274 and 90.3 $\mu\text{g a.s./L}$, and $121\% \pm 12.6\%$ in sediment fortified at 0.05 and 0.5 mg a.s./kg. The response in blank samples of both matrices was lower than 30% of LOQ. The LOQ was 0.03 $\mu\text{g a.s./L}$ for water samples and 0.05 mg a.s./kg for sediment samples.

The routine phase of the water analysis was checked by the analysis of tap water fortified at 100 $\mu\text{g a.s./L}$. The mean recovery \pm SD was $101.5\% \pm 5.01\%$ (RSD = 4.93%). The sediment analysis was not checked by the analysis of recovery samples, because all samples were worked up and measured in one sample set.

Fenpropidin concentrations in the water phase

Initial concentrations measured 2 hours after each application were in the range of 84 – 133% of nominal with a mean of 106% and 112% for the 1st and 2nd application, respectively. A considerably rapid decrease of fenpropidin concentrations in the water phase was observed in all mesocosms. The mean half

life time (DT₅₀) was 3.6 days for both applications. 90% dissipation was approached after 12 days per average.

Fenpropidin concentrations in the sediment

Fenpropidin concentrations measured in the sediment layer of the two ponds treated at 100 µg a.s./L increased up to approx. 3 mg/kg at test end. No significant increase was observed from day 28 to day 70.

B. Water quality monitoring

Oxygen concentration, pH and conductivity of the water as indicators of total primary production showed a clear inhibition of photosynthesis at all tested concentrations. Recovery was observed within eight weeks.

Analysed ammonium concentrations in the water phase remained low in the control ponds. In treated mesocosms the ammonium level increased in direct relation to the test concentration up to day 28 and decreased thereafter with exception of the highest treatment level. Nitrite and nitrate concentrations were low and decreased during the study. The concentrations of dissolved *ortho*-phosphate and total phosphate were low in the first weeks of the study and increased after the second application more pronounced in the treated mesocosm than in the controls. No treatment-related effect was observed from day 42 onwards.

C. Effects on community and population dynamics

Observed effects including time of recovery were classified according to the Guidance Document on Aquatic Ecotoxicology (SANCO/3268/2001 rev 4 (final), 17 October 2002). Short-term effects on phytoplankton (community and population level) were observed at all test concentrations. However, recovery within eight weeks after the 2nd application was observed at concentrations ≤ 30 µg a.s./L.

Filamentous algae showed a remarkable growth especially in the controls starting at the walls of the basins and resulting in mats of algae floating at the surface. The periphyton (including floating filamentous algae) was affected at all test concentrations. Recovery was observed at ≤ 30 µg a.s./L within eight weeks after the last application.

The submerged macrophytes, which were introduced into the mesocosms, generally showed better growth in the treated systems than in the controls. Indications of a possible direct effect were only found for one species (*Chara*) exposed to 100 µg a.s./L, two and three weeks after the 2nd application (decrease of growth). The NOEC for an increase of shoot length, surface coverage and biomass at the end of the study was < 0.3 µg a.s./L. The total macrophyte biomass at the end of the study was significantly higher for all test concentrations compared to the controls.

The increased growth of macrophytes observed at all test concentrations compared to the controls may be due to the direct competition with the faster growing algae in the controls. In the controls, the introduced macrophytes seemed to be overgrown by the algae. In the treated systems, fenpropidin inhibited the blooms of the filamentous algae, which allowed the growth of the less sensitive but more slowly growing macrophytes.

The largest effects on the zooplankton were observed around 3 weeks after the 2nd application, when reduced densities of some crustaceans and rotifer populations were found at all treatment levels and also on the community level.

The effects were considered to be likely indirect effects due to shifts in the community or primary producers. Recovery of the zooplankton was demonstrated up to at least 30 µg a.s./L within eight weeks after application.

Macroinvertebrates living in or on the sediment showed no adverse effects at concentrations up to 30 µg a.s./L. At 100 µg a.s./L delayed or long-term effects could not be excluded for snails, beetles and phan-

tom midges. No adverse long-term effects were observed for macroinvertebrates living on the mesocosm walls. However, upon study termination snails were more abundant for the highest test concentration.

Table A 12: Overview on effects and recovery observed after two applications of MCW-273 750 EC

Endpoint	Test concentration (µg a.s./L)					
	0.3	1.0	3.0	10	30	100
Phytoplankton:						
Total abundance	2	2	2	3	3	3
Number of taxa	1	1	2	3	3	5
Diversity	1	1	1	1	1	5
Similarity	1	1	3	3	3	3
PRCs, PCAs	3	3	3	3	3	3
<i>Chroococcus</i>	1	1	2	2	2	3
<i>Ochromonas</i>	3	3	3	3	3	3
<i>Characium</i>	3	3	3	3	3	5
<i>Carteria</i>	1	1	2	2	2	2
<i>Cryptomonas</i> (> 25 µm)	3	3	3	3	3	3
<i>Achnantes</i>	2	2	2	2	2	2
<i>Oedogonium</i>	2	2	3	3	3	5
<i>Cyclotella</i>	1	1	1	1	1	5 +
<i>Chroomonas</i>	3 +	3 +	3 +	3 +	3 +	3 +
<i>Cryptomonas</i> (< 25 cm)	1	1	3 +	3 +	3 +	3 +
Periphyton:						
Chlorophyll a on plates	2 +	2 +	2 +	2 +	2 +	2 / 2 +
Dry weight on plates	2	2	2	2	2	2
Filamentous algae	3	3	3	3	3	5
Epiphytic algae	3	3	3	3	3	5
Submerged macrophytes						
Total biomass at the end	5 +	5 +	5 +	5 +	5 +	5 +
<i>Myriophyllum</i>	2 +	5 +	5 +	5 +	5 +	5 +
<i>Potamogeton</i>	5 +	5 +	5 +	5 +	5 +	5 +
<i>Chara</i>	3 +	5 +	5 +	5 +	5 +	3 / 5 +
Indicators of total primary production						
pH	3	3	3	3	3	3
Oxygen	3	3	3	3	3	3
Conductivity	3 +	3 +	3 +	3 +	3 +	3 +
Zooplankton						
Total abundance	2	2	2	2	2	3
Number of taxa	1	2	2	2	3	3
Diversity	1	2	2	2	3	3
Similarity	1	2	2	2	2	5
PRCs including PCAs	2	3	3	3	3	5
Phyllopoda	1	2	2	3	3	5
Copepoda	1	1	1	1	1	3
Ostracoda	2	2	2	3	3	5
Rotatoria	1	2	2	2	2	3
Chaoborus	1	1	1	1	1	2

Endpoint	Test concentration (µg a.s./L)					
	0.3	1.0	3.0	10	30	100
<i>Simocephalus</i>	1	2	3	3	3	3
<i>Chydorus</i>	1	2	3	3	3	5
Nauplii	1	1	1	1	1	5
Cyclopoid copepods	1	2	2	2	3	5
<i>Keratella quadrata</i>	1	1	1	1	1	2
<i>Keratella cochlearis</i>	1	1	1	1	1	2
<i>Synchaeta spec.</i>	2	3	3	3	3	3
<i>Polyarthra spec.</i>	1	1	1	1	3	3
<i>Lepadella spec.</i>	2	2	2	2	2	2
<i>Lecane spec.</i>	1	2	2	2	2	2
<i>Mytilina spec.</i>	1	1	1	1	1	5
<i>Trichocerca spec.</i>	1	2	2	3	3	3
Macroinvertebrates in Artificial substrate samplers						
Total abundance	1	1	1	1	1	1
Number of taxa	1	1	1	1	1	2
Diversity	1	1	1	1	1	1
Similarity	1	1	1	1	1	1
PRCs	1	1	1	1	1	1
<i>Chironomus pulmosus thummi</i>	1	1	1	1	1	1
Chironomidae indet	2	2	2	2	2	2
<i>Physa</i>	1	1	1	1	1	5
<i>Hyphydrus</i>	1	1	1	1	1	5 +
<i>Chaoborus</i>	1	1	1	1	1	5
Macroinvertebrates on glass plates						
Total abundance	2 +	2 +	2 +	2 +	2 +	5 +
Number of taxa	1	1	1	1	1	1
Diversity	1	1	1	1	1	2
Similarity	1	1	1	1	1	1
PRCs, PCAs	2 +	2 +	2 +	2 +	2 +	5 +
<i>Radix ovata</i>	2 +	2 +	2 +	2 +	2 +	5 +
<i>Gyraulus albus</i>	1	1	2 +	2 +	2 +	2 +
<i>Physa fontinalis</i>	1	1	1	1	1	1
<i>Helopdella stagnalis</i>	1	1	1	1	1	1
Chironomidae	1	1	1	2 +	2 +	2 +

+ indicates an increase of abundance

1 = Effect could not be demonstrated; 2 = slight temporary effect; 3 = pronounced short-term effect with recovery within 8 weeks

5 = pronounced effect until 8 weeks after the last application

PRC = Principal Response Curves (multivariate approach to analyse effects on the community level)

PCA = Principal Component Analysis (multivariate technique to analyze ecological data sets with regard to differences in species composition between samples.)

D. No observed ecologically adverse effect concentration (NOEAEC)

The NOEAEC is defined as the concentration at or below which no long-lasting adverse effects were observed in a higher tier study, i.e. those effects on individuals that have no or only transient effects on populations and communities and are considered of minor ecological relevance (generally class 1, 2 and 3). Upon termination of this study, significant differences to the control were observed at the highest test

concentration for several populations (class 5). At test concentrations $\leq 30 \mu\text{g a.s./L}$ only slight and/or transient direct or indirect effects were observed with recovery within 8 weeks after the 2nd application. The only exception was found for submerged macrophytes which were significantly more abundant in all treated ponds than in the controls. However, this effect was not considered as ecologically adverse.

The authors concluded that it is likely that the missing growth of macrophytes in the controls was caused by the fact that the macrophytes were introduced into the mesocosms just before test start while the filamentous algae were present in (and adapted to) the system before. In the controls, the introduced macrophytes seemed to be overgrown by the algae. In the treated systems, fenpropidin inhibited the blooms of the filamentous alga, which allowed the growth of the less sensitive but more slowly growing macrophytes. Thus, the higher abundance of macrophytes is likely a result of experimental design but not representative for the field situation. Therefore, the NOEAEC was established at $30 \mu\text{g a.s./L}$.

E. Deficiencies

No unusual circumstances were reported that might have affected the integrity and quality of the study. The study focussed on endpoints of organisms that are potentially at risk based on the results of lower-tier studies. NOEC values were established for all determined parameters. The amount of test material applied and the exposure concentration in the water column was determined analytically at start of exposure. The duration of the study was appropriate to the life-cycle of the organisms of interest (e.g. algae). Thus, the test was considered to be valid without restrictions.

III. Conclusions

A community level study with MCW-273 750 EC (761.8 g fenpropidin/L) was performed in outdoor aquatic mesocosm ponds (cylindrical basins made of polyethylene, volume: approx. 5000 L of water). The test item was sprayed twice onto the water surface at nominal initial water concentrations of 0.3, 1.0, 3.0, 10, 30 and $100 \mu\text{g a.s./L}$ with two replicate basins per concentration. The application interval was 14 days. Three untreated basins were used as controls. Physical and chemical properties of the water phase were monitored. Phytoplankton, periphyton (including floating filamentous algae), macrophytes, zooplankton and macroinvertebrates were monitored in regular intervals.

The analysis of water samples from treated basins showed that fenpropidin dissipated considerably from the water column within the first weeks after treatment with a mean DT_{50} of 3.6 days and a mean DT_{90} of 12 days. The analysis of sediment samples, which were collected and analysed for the highest treatment level only, showed that fenpropidin concentrations in the sediment increased up to approx. 3 mg/kg at test end, with no significant increase from day 28 to 70.

Generally, a high number of phytoplankton, zooplankton and macroinvertebrates species was observed during the study. Significant differences to the control were observed at the highest test concentration for several populations. At test concentrations $\leq 30 \mu\text{g a.s./L}$ only slight and/or transient direct or indirect effects were observed with recovery within eight weeks after the 2nd application. The only exception was found for submerged macrophytes which were significantly more abundant in all treated ponds than in the controls. However, this effect was not considered as ecologically adverse. The authors concluded that the higher abundance of macrophytes is likely a result of experimental design but not representative for the field situation. Therefore, the NOEAEC was established at $30 \mu\text{g a.s./L}$.

The endpoints are summarised in the table below.

Table A 13: Summarised endpoints

Test system: outdoor mesocosm study
Exposure: 2 applications of the test item, test concentrations: 0.3, 1.0, 3.0, 10, 30 and $100 \mu\text{g a.s./L}$
Test item: MCW-273 750 EC, active ingredient content: 761.8 g fenpropidin/L
Endpoints: based on initial nominal concentrations

Parameter	Endpoint ¹	Concentration (µg a.s./L)
Indicators of total primary production	NOEC NOAEC	< 0.3 100
Phytoplankton, community level	NOEC NOAEC	< 0.3 30
Phytoplankton, population level	NOEC NOAEC	< 0.3 30
Periphyton (including floating filamentous algae)	NOEC NOAEC	< 0.3 30
Macrophytes (growth)	NOEC	< 0.3
Zooplankton, community level	NOEC NOAEC	< 0.3 30
Zooplankton, population level	NOEC NOAEC	< 0.3 30
Macroinvertebrates, AAS	NOEC NOAEC	< 0.3 30
Macroinvertebrates, Plexiglas plates	NOEC NOAEC	30 30
No observed ecologically adverse effect concentration	NOEAEC	30

¹ Effects rated as class 1, 2 or 3 were considered as not ecologically adverse in compliance with SANCO/3268/2001 rev 4 (2002). (Class 1 = no effect, class 2 = temporary slight effect, class 3 = clear short-term effect with recovery)

Recently, three aquatic micro- and mesocosm studies with fenpropidin or fenpropidin formulations (see EFSA Scientific Report 124, 2007) were evaluated by external academic experts with the aim of deriving an overall NOEAEC. The results of the evaluation are summarised below.

Comments of zRMS:	<p>In: “Addendum following the evaluation of new Annex II data Post-Annex I inclusion, Fenpropidin, Volume 3, Annex B Ecotoxicology (Sept 2011)” the following is stated regarding this expert evaluation:</p> <p>” The RMS (SE) agrees with the conclusion by Arts and Brock (2009) that no NOEC could be demonstrated in the study by Wellman (2006) submitted after the Annex I inclusion and therefore cannot support a change of the NOEC agreed in the List of End Point (i.e. NOEC of 0.39 µg a.s./L).</p> <p>If, however, Member States would like to take 8 weeks recovery into account the Wellman study gives support for a NOEAEC of 1 µg a.s./L.”</p> <p>The mesocosm NOEC 0.39 µg/L from the LoEP remains as a relevant endpoint for fenpropidin.</p>
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Reference: KCP 10.2.3/02

Report Evaluation of the reports: Neumann Ch. (1997): CGA 114900 EC 750 (A-7516 A): Outdoor aquatic mesocosm study of the environmental fate and ecological effects. Novartis Crop Protection AG, Sector of Unit R&D, Ecotoxicology Department, Switzerland. Project No 95N001. (Syngenta file No. CGA 114900/0500) including Ashwell J., Hamer M. And Coulson M., 2007. Fenpropidin: Syngenta response to Evaluation Table rev. 0-0 (19.02.2007). Data requirement 5.2 – statistical analysis of mesocosms study by Neumann 1997. and Huber, W. (1995): Effects of A-7503 C in aquatic outdoor microcosms. Technical University Munich-Weihenstephan. Institute for Landscape and Botany, Germany. Report No. (Syngenta file No. CGA 64250/2997) and Wellmann P. (2006): Community level study with Fenpropidin in outdoor aquatic mesocosm ponds, Fraunhofer-Institute Schmallerberg, Germany & Gaiac, Aachen, Germany, Arts, G.H.P and Brock, T.C.M., 2009

Guideline(s):	not applicable
Deviations:	not applicable
GLP:	not applicable
Acceptability:	Yes
Duplication (if vertebrate study)	not applicable

Conclusion

NOEC

From the study of Neumann only, an overall NOEC of 0.13 µg a.s./L (in DAR 0.11 µg a.s./L) could be derived, at least when considering an Effect class 2 response on the endpoint ‘phytoplankton similarity index’ of minor importance (see summary table below) This concentration of this NOEC is expressed in terms of mean measured peak concentrations after the two applications. Note that in this study a relatively worst-case approach was adopted for assessing the peak concentration, since mean measured concentrations 6 h post first and second applications were used.

NOEAEC

If an Effect class 3A response on sensitive endpoints (e.g. algae) is considered acceptable, while also considering the long-term increase in macrophytes in the Wellmann-study as not unacceptable, the No Observed Ecologically Adverse Effect Concentration (NOEAEC) values presented in Table 4.1, and expressed in terms of mean peak concentrations, can be derived from the three experimental pond studies evaluated. The NOEAEC of the study of Huber can be used as an indicative value only and is therefore presented in parentheses (see table below).

In addition, the test concentrations above the NOEAEC of each study, termed here the LOEAEC (Lowest Observed Ecologically Adverse Effect Concentration), are presented as well in order to help derive an overall NOEAEC.

Table A 14: NOEAEC values from outdoor aquatic micro- or mesocosm studies with fenpropidin

Study	Endpoint [µg a.s./L]	Remark
Neumann (1997)	0.39	NOEAEC expressed in terms of mean measured concentrations 6 h post treatments LOEAEC = 1.4 µg a.s./L
Wellmann (2006)	1.00	NOEAEC expressed in terms of nominal concentration (similar to mean measured concentrations 2 h post applications) LOEAEC = 3.0 µg a.s./L
Huber (1995) ^a	0.55	NOEAEC expressed in terms of nominal fenpropidin concentration. Verification of exposure by post-treatment measurement was not performed. Formulation also contained propiconazole. LOEAEC = (2.20 µg a.s./L)

^a Study has some quality deficiencies, therefore value should be used only indicatively

The range in derived NOEAEC values for fenpropidin from the three experimental pond studies is relatively small (0.39 to 1.0 µg a.s./L) and the difference between the lowest and highest value is less than a factor of 3. In this context it should be noted that the lowest available NOEAEC is expressed in terms on measured concentrations 6 h post the first and second application, 28 and consequently can be considered a worst-case estimate since the dissipation of fenpropidin from water is relatively fast (dissipation DT₅₀ approximately 3.6 days). In addition, the highest NOEAEC of 1.0 µg a.s./L (in the study of Wellmann 2006) is lower than the lowest LOEAEC of 1.4 µg a.s./L (in the study of Neumann 2006). Based on these observations, we derive an overall NOEAEC of 1.0 µg a.s./L.

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

Comments of zRMS:	<p>The study was conducted in line with OECD 213 with minor deviations. The humidity was in range from 49-65% (recommended value 50-70%) Short-term deviations (≥ 2 hours) from the recommended ranges is noted. As control performance met the guideline validity criteria, these short-term deviations are considered to have no impact on the validity of the stud All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>The contact LD₅₀ (48 h) = 470 µg ADM.03502.F.1.A/bee. The oral LD₅₀ (48 h) =505 µg ADM.03502.F.1.A/bee.</p>
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Reference:	KCP 10.3.1.1/01
Report:	Acute toxicity of ADM.03502.F.1.A to the honeybee <i>Apis mellifera</i> L. under laboratory conditions, Franke, M., 2020, report no.: 2048BAA0028, sponsor no.: 000104843
Guideline(s):	OECD 213 and 214 (1998)
Deviations:	None
GLP:	Yes
Acceptability/Reliability:	Yes
Duplication (if vertebrate study)	Not applicable

Executive summary

In a 48 hour acute oral and contact toxicity study, adult worker honeybees (*Apis mellifera* L.) were exposed to ADM.03502.F.1.A at nominal doses of 800, 400, 200, 100, 50.0 µg prod./bee in the contact test and 1600, 800, 400, 200, 100 µg prod./bee in the oral test. Mortality and unusual behaviour were recorded after 4, 24 and 48 hours. LD₅₀-values were determined after 24 and 48 hours. The 48 h LD₅₀ for contact toxicity was calculated to be 470 µg ADM.03502.F.1.A/bee. Based on the effective food consumption the 48 h LD₅₀ for oral toxicity was calculated to be 505 µg ADM.03502.F.1.A/bee. No behavioural abnormalities were observed after 48 hours.

I. Materials and methods

A. Materials

- Test material: ADM.03502.F.1.A
 Lot/Batch no.: 1191-101219-01
 Content: 250 g fenpropidin/L, 175 g prothioconazole/L (nominal)
 253.7 g fenpropidin/L, 175.9 g prothioconazole/L (analysed)
 Density: 1.04 g/cm³
 Control: oral: 50 % w/v sucrose solution. Contact: deionised water /
 1 % v/v tween solution (Tween®80 as wetting agent)
 Toxic reference: Danadim® Progress (Dimethoate: 400 g/L, nominal)
- Test organisms -
 Species: *Apis mellifera* L.

Sex and age:	Female, adult worker bees (forager bees)
Source	own breeding
No. of organisms:	3 replicates, each consisting of 10 bees per cage per treatment
Feeding:	<i>ad libitum</i> with 50 % (w/v) sucrose solution
Acclimatisation	bees acclimatised to the test room conditions for about 1 h
3. Test units and exposure –	
Type and size:	Disposable cardboard cages with holes in the bottom side for ventilation and a glass plate in front (95 mm x 50 mm x 65 mm (length x width x height)).
Test procedure:	oral and contact exposure, dose-response test
Test duration:	48 h
4. Test conditions –	
Temperature:	24.2 – 25.2 °C
Relative humidity:	49 - 60 %
Photoperiod:	constant darkness

B. Study design and method

1. In-life dates: July 16 to July 18, 2020 (experimental phase)

2. Test design:

Contact test: bees in each test cage were anaesthetised with CO₂, removed from the cages and applied with a single drop on the bee thorax; droplet with 4 µL/bee in the controls and 2 µL/bee in test and reference item groups, respectively; bee were continuously fed with 50 % (w/v) sucrose solution *ad libitum*

Oral test: administration of 20 µL 50% (w/v) sucrose solution/bee (as group feeding with 200 µL/replicate); bees starved for approximately 1 h before food administration; after ingestion of the spiked feeding solution was completed bees were fed with 50 % (w/v) sucrose solution *ad libitum*.

The mortality and the behaviour were assessed 4, 24, 48 hours after application for the contact and oral test Controls and Reference: tested in parallel to the test item

3. Statistics:

The statistical calculations were performed with the computer program ToxRat Professional 3.3.0 (2018) Calculation of LD₅₀ values: Test item, contact test: Spearman-Kärber computation. Test item, oral test: Probit analysis (linear maximum likelihood regression). Reference item, contact test: Probit analysis (linear maximum likelihood regression). Reference item, oral test: Probit analysis (linear maximum likelihood regression). Statistical significance of mortality values: Test item: Fisher's Exact Binomial Test with Bonferroni-Holm Correction ($p < 0.05$), one-sided greater. Reference item: Fisher's Exact Binomial Test with Bonferroni-Holm Correction ($p < 0.05$), one-sided greater.

II. Results and discussion

A. Mortality

Contact toxicity

In both control groups, either treated with deionised water or 1 % v/v tween solution no mortality was observed after 48 hours. In the test item treatment groups, statistically significant increased mortalities of 96.7 and 20.0 % were observed after thoracic application of 800 and 400 µg ADM.03502.F.1.A/bee, respectively. Mortality of 3.3 % occurred at the dose rates of 200 and 100 µg ADM.03502.F.1.A/bee. No mortality was observed at the lowest dose rate of 50.0 µg ADM.03502.F.1.A/bee after 48 hours. The LD₅₀ (48 h) was determined to be 470 µg ADM.03502.F.1.A/bee.

Effects on the behaviour were observed at the two highest dose rates of ≥ 400 µg ADM.03502.F.1.A./bee at the 4-hour and 24-hour assessments. After 4 hours, 30 out of 30 bees and 8 out of 30 bees showed behavioural impairments (impaired locomotion, moribund symptoms) compared to the control at dose rates of 800 and 400 µg ADM.03502.F.1.A./bee, respectively. After 24 hours, the same dose rates revealed 4 out of 4 bees and 7 out of 27 bees with behavioural abnormalities, respectively. No behavioural abnormalities were observed after 48 hours in these dose rates. No effects on behaviour were observed at the lower dose rates of up to 200 µg ADM.03502.F.1.A/bee throughout the entire contact toxicity test during 48 hours.

Table A 15: Mortality and behavioural abnormalities of the bees in the contact toxicity test

Dose µg prod./bee	4 hours		24 hours		48 hours	
	Mean mortality		Mean mortality %		Mean mortality %	
	total	corr	total	corr	total	corr
Control						
Deionised water	0.0	---	0.0	---	0.0	---
Tween solution	0.0	---	0.00	---	0.0	---
ADM.03502.F.1.A						
1535	0.0	---	86.7*	---	96.7*	---
776	0.0	---	10.0	---	20.0*	---
380	0.0	---	0.0	---	6.7	---
195	0.0	---	3.3	---	3.3	---
98.3	0.0	---	0.0	---	0.0	---
Reference item µg a.s./bee						
0.250	0.0	---	90.0*	---	96.7*	---
0.175	0.0	---	76.7*	---	83.3*	---
0.123	0.0	---	50.0*	---	56.7*	---
0.086	0.0	---	13.3	---	20.0*	---

Mortality results are mean based on 3 replicates consisting of 10 bees each; corr.: corrected mortality (according to SCHNEIDERORELLI 1947), “-“ = in case of no control mortality no corrected mortality was calculated; * Significant difference in pair-wise comparison between treatment and wetting agent control (tween solution) by Fisher’s Exact Binominal Test with Bonferro-ni-Holm Correction for mortality data; $\alpha=0.05$; one sided greater); Calculations were performed with non-rounded values

Oral toxicity

In the control no mortality was observed after 48 hours. In the test item treatment group, statistically significant increased mortalities of 90.0, 63.3 and 40.0 % were observed after oral consumption of 1535, 776 and 380 µg ADM.03502.F.1.A/bee, respectively. The dose rates of 195 and 98.3 µg consumed ADM.03502.F.1.A/bee revealed 16.7 and 3.3 % mortality without any statistical significance compared to the control. The LD₅₀ (48 h) was determined to be 505 µg ADM.03502.F.1.A/bee.

Effects on the behaviour were predominantly observed at the early assessment after 4 hours, whereas no behavioural impairments occurred at the 24 hour and 48 hour assessments, except one affected bee after 24 hours. After 4 hours, 30 out of 30 bees, 17 out of 30 bees, 7 out of 30 bees and 1 out of 30 bees were recognised with behavioural impairments (impaired locomotion, moribund symptoms) compared to the control at effective dose rates of 1535, 776, 380, 195 µg ADM.03502.F.1.A/ bee, respectively. After 24 hours, 1 out of 19 bees was affected at the dose rate of 380 µg ADM.03502.F.1.A/bee, whereas the other dose rates revealed no behavioural effects on honeybees up to 1535 µg ADM.03502.F.1.A/bee.

Furthermore, no effects on behaviour were observed at any dose after 48 hours. At the lowest dose rate of 98.3 µg ADM.03502.F.1.A/bee no behavioural abnormalities were observed during the entire course of the study.

Table A 16: Mortality and behavioural abnormalities of the bees in the oral toxicity test

Dose µg prod./bee	4 hours		24 hours		48 hours	
	Mean mortality		Mean mortality %		Mean mortality %	
	total	corr	total	corr	total	corr
Control						
Sucrose solution	0.0	---	0.0	---	0.0	---
ADM.03502.F.1.A						
1535	0.0	---	90.0*	---	90.0*	---
776	0.0	---	63.3*	---	63.3*	---
380	0.0	---	36.7*	---	40.0*	---
195	0.0	---	16.7	---	16.7	---
98.3	0.0	---	3.3	---	3.3	---
Reference item µg a.s./bee						
0.250	0.0	---	96.7*	---	100.0*	---
0.175	0.0	---	83.3*	---	90.0*	---
0.123	0.0	---	63.3*	---	73.3*	---
0.086	0.0	---	20.0*	---	26.7*	---

Mortality results are averages based on 3 replicates consisting of 10 bees each; corr.: corrected mortality (according to SCHNEIDERORELLI 1947), “-“ = in case of no control mortality no corrected mortality was calculated; * Significant difference in pair-wise comparison between treatment and sucrose solution by Fisher’s Exact Binominal Test after Bonferroni-Holm Correction for mortality data; α=0.05; one sided greater); Calculations were performed with non-rounded values

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 0 % (contact and 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 24 h contact LD ₅₀ of the toxic standard was 0.148 µg a.s./bee and the 24 h oral LD ₅₀ of the toxic standard was 0.115 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

The acute contact and oral toxicity of ADM.03502.F.1.A on honeybees was investigated under laboratory

conditions over a period of 48 hours. The contact LD₅₀ (48 h) was determined to be 470 µg ADM.03502.F.1.A/bee. And the oral LD₅₀ (48 h) was determined to be 505 µg ADM.03502.F.1.A/bee. No behavioural abnormalities were observed after 48 hours. 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:	<p>The study was conducted in line with OECD 245 with no deviation.</p> <p>The concentrations of the active ingredients in the applied test item feeding solutions were within the required range of $\pm 20\%$ of the nominal concentrations.</p> <p>All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>LDD50 = 56.6 µg product/bee/day NOEDD = 31.9 µg product/bee/day</p> <p>LC50 = 2.401 g product/kg food NOEC = 0.941 g product/kg food</p>
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Reference:	KCP 10.3.1.2/01
Report:	Chronic oral toxicity of ADM.03502.F.1.A to the honey bee <i>Apis mellifera</i> L. under laboratory conditions, Dreßler, K., 2021, report no.: 2048BAC0011, sponsor no.: 000104844
Guideline(s):	OECD 245 (2017)
Deviations:	None
GLP:	Yes
Acceptability/Reliability:	Yes
Duplication (if vertebrate study)	Not applicable

Executive summary

In a 10-day chronic toxicity feeding test, max. 2 days old worker honey bees (*Apis mellifera* L. subspecies iberiensis (Engel)) were exposed to a daily application of ADM.03502.F.1.A diluted in the bee food (50% (w/v) aqueous sucrose solution). The chronic oral toxicity of the test item was determined at nominal doses of 242, 151, 94.6, 59.1 and 37.0 µg prod./bee/day. The corresponding test item concentrations in the feeding solutions were 6.168, 3.855, 2.409, 1.506 and 0.941 g prod./kg food. Taking into account the actual food uptake and evaporated amount of feeding solution, the bees effectively consumed doses of 81.5, 80.2, 54.8, 46.1 and 31.9 µg prod./bee/day. An additional group of honey bees was exposed to a daily application of dimethoate diluted in the bee food (50% (w/v) aqueous sucrose solution) as a reference item at a nominal dose of 27.3 ng a.s./bee/day. Untreated 50% (w/v) aqueous sucrose solution served as control.

In the analytical phase of the study, the concentration of both active ingredients in the highest and lowest test item feeding solution applied on each day of application was determined.

After 10 days of continuous exposure, a mortality of 0.0% was observed in the control group. Taking into account the actual food uptake and evaporated amount of feeding solution, the bees effectively consumed doses of 81.5, 80.2, 54.8, 46.1 and 31.9 µg prod./bee/day which resulted in mortalities of 100, 100, 43.3, 13.3 and 3.3% after 10 days, respectively. Mortalities in all test item doses but the lowest (81.5, 80.2, 54.8, and 46.1 µg prod./bee/day) were statistically significantly increased compared to the control group.

The LDD₅₀ was calculated to be 56.6 µg prod./bee/day and the LC₅₀ to be 2.401 g prod./kg food, respectively. The LDD₂₀ was calculated to be 47.7 µg prod./bee/day and the LC₂₀ to be 1.791 g prod./kg food, respectively. The LDD₁₀ was calculated to be 42.5 µg prod./bee/day and the LC₁₀ to be 1.476 g prod./kg food, respectively. The NOEDD was determined to be 31.9 µg prod./bee/day and the NOEC to be 0.941 g prod./kg food, respectively.

I. Materials and methods

A. Materials

1. Test material:
Lot/Batch no.:
Content:
Density
Toxic reference:
ADM.03502.F.1.A
1191-101219-01
250 g fenpropidin/L, 175 g prothioconazole/L (nominal)
253.7 g fenpropidin/L, 175.9 g prothioconazole/L (analysed)
1.04 g/cm³
Danadim® Progress ; Dimethoate: 400 g/L (nominal); 411.20 g/L (analysed)
2. Test organisms -
Species:
Age:
Source
No. of organisms:
Feeding:
Acclimatisation
Apis mellifera L.
young adult worker bees (2 days old)
own breeding
3 replicates per concentration, each consisting of 10 bees per cage per treatment
Young worker bees were provided continuously with treated or untreated test solution via plastic syringes (tips removed) through a hole in the lateral wall
For the following 24 ± 2 hours (until D 0), the bees were held in the test cages at 33 ± 2 °C and 50 – 70 % relative humidity and provided with 50 % (w/v) aqueous sucrose solution for acclimatisation to the test conditions. Moribund and dead bees were rejected and replaced by healthy bees that were held in spare cages under acclimatisation conditions before starting the test.
3. Test units and exposure –
Type and size:
Test procedure:
Test duration:
Aluminium cages with the dimensions: 95 mm (width) x 70 mm (height) x 60 mm (depth) with holes in the lateral walls for ventilation and sufficient air supply and two glass plates (one in front and one in the back) for observation of the bees
chronic oral exposure
10 days
4. Test conditions –
Temperature:
Relative humidity:
Photoperiod:
32.1 – 33.8 °C
60.2 – 66.9 %
constant darkness

B. Study design and method

1. In-life dates: June 16 to June 26, 2020 (experimental phase)

2. Test design:

Exposure took place over a period of 10 days. Test item feeding solutions were freshly prepared every day by serial dilution just before administration of food (glass equipment was used, i.e., beakers and volumetric flasks made of glass). The bees were fed with 50% (w/v) aqueous sucrose solution including the test item or the reference item. The control treatment was fed with untreated 50% (w/v) aqueous sucrose solution.

The treated/untreated food was provided *ad libitum* in a plastic syringe which was weighed before application. The syringes with treated/untreated food remained in the cages for about 24 hours (± 2 hours). The actual consumption was determined by re-weighing the syringe containing the remaining test solution each day after removal from the test units. Any unconsumed food was rejected. Old syringes were replaced by new feeders. The difference of the syringe weight at the start and end of each feeding period represents the food consumed by the bees in one cage during 24 hours.

To consider the evaporation of feeding solutions from the syringes, three additional test units with untreated 50% (w/v) aqueous sucrose solution and no bees present were set up alongside the actual test units. At the daily feeder exchange, the syringes were re-weighed and replaced by new feeders. The mean evaporation figure was then subtracted from the calculated uptake to give the real uptake accounting the loss by evaporation. This amount of food was divided by the number of living bees at the start of the corresponding exposure interval. In case the subtraction of the mean evaporation figure from the calculated food consumption led to a negative value, the food consumption of the respective day was considered to be “0”.

Due to their social feeding behaviour, the honey bees of a distinct group are assumed to share the applied feeding solution (trophallaxis) and thus receive similar doses of the applied respective item. The syringes were introduced through a hole in the side of the cage. In order to reduce stress to the bees, the process of retrieving old syringes and replacing them with fresh food was conducted daily at about the same time and as fast as possible. To avoid any adsorption of the test item to the surface of syringes, the first drawing up was discarded and the second drawing up was used for the test.

The chronic oral toxicity of the test item was determined at nominal doses of 242, 151, 94.6, 59.1 and 37.0 $\mu\text{g prod./bee/day}$. The corresponding test item concentrations in the feeding solutions were 6.168, 3.855, 2.409, 1.506 and 0.941 g prod./kg food.

Mortality and behavioural abnormalities were recorded daily at about the same time of the day (every 24 hours ± 2 hours), starting 24 hours ± 2 hours after start of the test period (initial feeding). Behavioural abnormalities were recorded according to the following categories: healthy/normal, moribund, affected in terms of uncoordinated movements, cramping, apathetic, vomiting. Any other behavioural abnormalities were noted and clearly described if observed.

3. Analytical verification:

For verification of the exposure concentrations, samples of test item feeding solutions with the highest and lowest applied concentration as well as of control feeding solution were sampled in duplicate to provide analysis and retained samples directly after preparation on each day of application (D 0 to D 9).

4. Statistics:

Statistical software used: ToxRat Professional 3.3.0 (2018). Step-down Cochran-Armitage Test Procedure for mortality data and determination of NOEDD/NOEC (one-sided greater, $\alpha = 0.05$). Weibull analysis

using linear maximum likelihood regression for the calculation of LDD_x and LC_x values along with their 95 % confidence limits. The following endpoints were determined:

- mean daily uptake per bee and mean total uptake during 10 days (or until death) per bee
- NOEDD/NOEC (no observed effect dietary dose/concentration)
- LDD_{50/20/10}/LC_{50/20/10} (lethal dietary dose/concentration)

II. Results and discussion

A. Analytical data

The recovery rates of prothioconazole ranged between 89.6 % and 98.3 % in the highest test item concentration and between 90.8 % and 98.6 % in the lowest test item concentration. The recovery rates of fenpropidin ranged between 89.6 % and 98.6 % in the highest test item concentration and between 91.6 % and 98.8 % in the lowest test item concentration. Hence, the concentrations of the active ingredients in the applied test item feeding solutions were within the required range of ± 20 % of the nominal concentrations, and therefore, verified. No residues of prothioconazole or fenpropidin were found in the control samples.

B. Mortality & behaviour

After 10 days of continuous exposure, a mortality of 0.0 % was observed in the control group. Taking into account the actual food uptake and evaporated amount of feeding solution, the bees effectively consumed doses of 81.5, 80.2, 54.8, 46.1 and 31.9 $\mu\text{g prod./bee/day}$ which resulted in mortalities of 100, 100, 43.3, 13.3 and 3.3 % after 10 days, respectively. Mortalities in all test item doses but the lowest (81.5, 80.2, 54.8, and 46.1 $\mu\text{g prod./ bee/day}$) were statistically significantly increased compared to the control group. The reference item group tested in the study was fed with 27.3 ng dimethoate/bee/day. The effective reference dosage was 12.4 ng dimethoate/bee/day which resulted in a mortality of 100%. The results are listed in the table below.

Table A 17: Mortality and behavioural abnormalities of the bees in the chronic oral toxicity test

Mortality and behavioural abnormalities of the bees in the chronic oral toxicity test							
Treatment group	Treatment group	Daily dose		Concentration	After 10 days		
		nominal	consumed		Mean mortality		Number of bees showing behavioural abnormalities ²
		µg prod./bee/day		g prod./kg food	absolute [%]	corrected [%]	
Control	1	---	---	---	0.0	---	0 out of 30
Test item	1	242	81.5	6.168	100*	---	---
	2	151	80.2	3.855	100*	---	---
	3	94.6	54.8	2.409	43.3*	---	5 out of 17
	4				13.3*	---	0 out of 26
	5				3.3	---	1 out of 29
Reference item		ng a.s./bee/day		mg a.s./kg food			
	1	27.3	12.4		100	---	---

Results are averages based on 3 replicates, containing 10 bees each; Calculations are performed with non-rounded values.

corrected: corrected mortality (according to SCHNEIDER-ORELLI 1947); Due to 0 % mortality in the control group, no correction is needed. * Statistically significant difference in pairwise comparison between treatment and untreated control group AC

(Step-down Cochran-Armitage Test Procedure; $\alpha = 0.05$; one-sided greater)

1 Taking into account the actual food uptake and evaporation

2 Number of bees showing behavioural abnormalities referring to the number of remaining bees

The LDD₅₀ was calculated to be 56.6 $\mu\text{g prod./bee/day}$ and the LC₅₀ to be 2.401 g prod./kg food, respectively. The LDD₂₀ was calculated to be 47.7 $\mu\text{g prod./bee/day}$ and the LC₂₀ to be 1.791 g prod./kg food, respectively. The LDD₁₀ was calculated to be 42.5 $\mu\text{g prod./bee/day}$ and the LC₁₀ to be 1.476 g prod./kg food, respectively. The NOEDD was determined to be 31.9 $\mu\text{g prod./bee/day}$ and the NOEC to be 0.941 g prod./kg food, respectively.

During the course of the test, behavioural abnormalities were observed at effective doses of 81.5, 80.2, 54.8 and 31.9 µg prod./ bee/day. Single bees were observed as being affected (uncoordinated movements) on days 5, 6, 7 and 8. On the final assessment day (day 10), five bees out of 17 remaining bees were observed as being affected (uncoordinated movements) at an effective dose 54.8 µg prod./bee/day. Moreover, one bee out of 29 remaining bees was observed as being affected (uncoordinated movements) at an effective dose of 31.9 µg prod./bee/day. No other behavioural abnormalities were observed in any test item treatment group on any other assessment day.

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 0.0 % 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 % on day 10.

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 chronic oral toxicity of ADM.03502.F.1.A to young adult honey bees (*Apis mellifera* L.) was investigated in a 10-day chronic, dose-response feeding study under laboratory conditions. Correct dosing was verified by the analysis of prothioconazole and fenpropidin in the highest and lowest test item feeding concentration, which displayed to be in the required range of ± 20% of the nominal concentrations. The LDD₅₀ was calculated to be 56.6 µg prod./bee/day and the LC₅₀ to be 2.401 g prod./kg food, respectively. The LDD₂₀ was calculated to be 47.7 µg prod./bee/day and the LC₂₀ to be 1.791 g prod./kg food, respectively. The LDD₁₀ was calculated to be 42.5 µg prod./bee/day and the LC₁₀ to be 1.476 g prod./kg food, respectively. The NOEDD was determined to be 31.9 µg prod./bee/day and the NOEC to be 0.941 g prod./kg food, respectively. On the final assessment day (day 10), five bees out of 17 remaining bees were observed as being affected (uncoordinated movements) at an effective dose 54.8 µg consumed product/bee/day. Moreover, one bee out of 29 remaining bees was observed as being affected (uncoordinated movements) at an effective dose of 31.9 µg prod./bee/day. 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:	<p>The study was conducted in line with OECD 239 with no deviations.</p> <p>The chemical analysis of the two active substances prothioconazole and fenpropidin in the aqueous sugar stock solutions of all test item concentration at all feeding days, was provided, resulting in recovery of 80.4 %-115 % for prothioconazole and 80.0 %-114 % for fenpropidin.</p> <p>All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>EC₅₀ (D22) >10.26 mg test item/kg food NOEC = 0.13 mg test item/kg food ED₅₀ >1.62 µg test item/larva NOED (D22) = 0.02 µg test item/larva</p>
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Reference:	KCP 10.3.1.3/01
Report:	ADM.03502.F.1.A – Repeated exposure of honey bee larvae (<i>Apis mellifera</i> L.) under laboratory conditions, Hänsel, M., 2021, report no.: 2048BLC0013, sponsor no.: 000104845
Guideline(s):	OECD GD 239 (2016)
Deviations:	None
GLP:	Yes
Acceptability/Reliability:	Yes
Duplication (if vertebrate study)	Not applicable

Executive summary

In a chronic toxicity test, honey bee (*Apis mellifera* L. subspecies *iberiensis* (Engel)) larvae were repeatedly exposed to the test item ADM.03502.F.1.A diluted in the larval food according to OECD GD 239. The toxicity of the test item was determined at cumulative doses of 1.62, 0.54, 0.18, 0.06 and 0.02 µg prod./larva (total amount fed on D3 to D6). The respective concentrations of the test item in the diet were 10.26, 3.42, 1.14, 0.38 and 0.13 mg prod./kg food.

Additionally, honey bee larvae were exposed to dimethoate tech. spiked diet as reference item at a cumulative dose of 7.6 µg dimethoate/larva (concentration: 48 mg a.s./kg) and to an untreated diet as control. Mortality of the larvae was finally assessed on D8 and on D15. The emergence rate of the adult bees was determined on D22. Other observations such as abnormal behaviour or small body size were assessed at each mortality assessment. Unconsumed food was noted on D8.

On D8 of the test, no mortality was observed in the untreated control. In the test item groups, the mean cumulative mortalities ranged between 0.0% and 2.8%. The mean mortality in the reference group was above 50 %, i.e. being 66.7%.

The mean mortality between D8 and D15 (based on 36 introduced larvae) was 8.3% in the untreated control and ranged between 16.7% and 44.4% in the test item group (corrected for control: 9.1% and 39.4%). The mean mortality between D8 and D15 in the reference item group was 13.9% (corrected for control: 6.1%).

On D22, the mean adult emergence rate in the untreated control was 80.6% (total mortality 19.4%). In the test item treatment group, the adult emergence rate was 47.2%, 52.8%, 52.8%, 61.1% and 77.8% (from the highest to the lowest dose/concentration). The respective mean total mortality was 52.8%, 47.2%, 47.2%, 38.9% and 22.2% (corrected for control: 41.4%, 34.5%, 34.5%, 24.1% and 3.4%). The mean adult emergence in the reference item group was 2.8% (total mortality was 97.2%; corrected for control: 96.6%). There were statistically significant differences of the adult emergence rates in all test item treatment groups, except for the lowest test item dose on D22 compared to the control (Step-down Cochran-Armitage Test procedure, $\alpha = 0.05$, one-sided greater). No remaining food was observed at any of the remaining larvae at the end of the feeding phase and no other sublethal effects such as abnormal behaviour or small body size occurred in any of the treatments on the respective mortality assessments. Correct dosing of the test item was verified by chemical analysis of the two active ingredients prothioconazole and fenpropidin in the aqueous sugar stock solutions of all test item concentration at all feeding days (D3 to D6), resulting in recovery rates per sample of 80.4%-115% for prothioconazole and 80.0%-114% for fenpropidin. No active ingredients have been detected in the control samples.

Based on the obtained results, the ED₅₀ of the test item was > 1.62 µg prod./larva, which corresponds to an EC₅₀ (D22) of > 10.26 mg prod./kg food, respectively. The ED₂₀ was determined to be 0.116 µg prod./larva corresponding to an EC₂₀ of 0.738 mg prod./kg food. The ED₁₀ (D22) was calculated to be < 0.02 µg prod./larva, which corresponds to an EC₁₀ (D22) of < 0.13 mg prod./kg food, respectively. The NOED (D22) was determined to be 0.02 µg prod./larva which corresponds to a NOEC (D22) of 0.13 mg prod./kg food.

I. Materials and methods

A. Materials

1. Test material: ADM.03502.F.1.A
Lot/Batch no.: 1191-101219-01
Content: 250 g fenpropidin/L, 175 g prothioconazole/L (nominal)
253.7 g fenpropidin/L, 175.9 g prothioconazole/L (analysed)
Density: 1.04 g/cm³
Control: water mixed to the diet
Toxic reference: Dimethoate tech. (analysed purity: 98.8 ± 0.5%)
2. Test organisms -
Species: *Apis mellifera* L. subspecies *iberiensis* (Engel)
Age: one day old (first instar larvae, L1) at the time of grafting
Source: The colonies were provided by BioChem AGROLOGÍA S.L.U., Spain (Test Site for biological phase). All larvae used in the test derived from three healthy (free of clinical symptoms of any disease) and queen-right bee colonies, each representing one replicate. The larvae were taken from colonies that had not received treatments with chemical substances for at least one month

No. of organisms: 3 replicates per concentration, each consisting of 12 larvae per treatment
Feeding: Three different diets were used to feed the larvae. Due to larval growth the food amount was daily increased during the exposure period (D3 –D6). Sterile filtered aqueous sugar/yeast solutions (ASS-A, ASS-B and ASS-C) as one component of the artificial diets were prepared prior to the test and stored in a freezer until use. Every day before each feeding occasion the sugar/yeast solution (ASS-B or ASS-C) was mixed with the test item, diluted and mixed with royal jelly and the final diets were obtained. The reference item was mixed with ASS only on D3 (ASS-B) and D4 (ASS-C) and the stock solution from D4 was then stored refrigerated for usage on the following application days. Each larva was fed separately using a sterile pipette. Whereas on the day of grafting (D1) the larvae were placed on the food, on the subsequent feedings the food drop was placed next to the larvae to avoid drowning. Before feeding, the final diets were warmed to 34.5°C. During the process, the culture plate in operation was placed on a warming plate
Pre-treatment culturing conditions: The bee colonies producing the larvae were held under field conditions in hives including a healthy queen. Brood in egg, larval and pupal stages as well as filled food combs (containing nectar and pollen) were present. A sufficient amount of food was present in the bee hives
3. Test units and exposure –
Type and size: 36 Crystal polystyrene grafting cells (CNE Nicotplast, internal diameter 9 mm) were placed in three groups (= replicates, each representing larvae of one colony) of 12 cells on each 48 well plate. Well plates were placed on an adjustable warming plate. On day 1 (D1), untreated artificial diet A was pipetted into the grafting cells, followed by the transfer of one larva per cell.
Test procedure: larval toxicity test, repeated exposure

Test duration:	22 days
4. Test conditions –	
Temperature:	34.0 °C – 34.9 °C
Relative humidity:	from D1 to D8 = 97 – 100%, from D8 to D15: 75 – 85%, from D15 to D22: 60– 70%
Ventilation:	By the air-conditioning equipment of the climatic chamber
Photoperiod:	constant darkness

B. Study design and method

1. In-life dates: June 08 to June 29, 2020 (experimental phase)

2. Test design:

One-day-old honey bee larvae (D1) of *Apis mellifera* L. were transferred from brood combs to polystyrene grafting cells to 48-well cell culture plates 2 days before the first administration of spiked food. On four successive days (D3 to D6), the larvae were repeatedly exposed to ADM.03502.F.1.A diluted in the larval food (aqueous sugar/yeast solution mixed with royal jelly).

The toxicity of the test item was determined at cumulative doses of 1.62, 0.54, 0.18, 0.06 and 0.02 µg prod./larva (total amount fed on D3 to D6). The respective concentrations of the test item in the diet were 10.26, 3.42, 1.14, 0.38 and 0.13 mg prod./kg food. Additionally, honey bee larvae were exposed to dimethoate tech. spiked diet as reference item at a cumulative dose of 7.6 µg dimethoate/larva (concentration: 48 mg a.s./kg) and to an untreated diet as control. In total, three treatment groups with 3 replicates per dose and 12 larvae per replicate were set up: one group with 5 doses of the test item, one untreated control group (control) and one dose of the reference item (toxic standard). After the applications, no additional feedings of the larvae took place but the subsequent development was followed. Assessments of larval mortality were done on D4, D5, D6, D7 and D8. A further mortality assessment was done on D15 and adult emergence was evaluated on D22. Additionally, other observations such as abnormal behaviour or small body size were assessed at each mortality assessment. Any remaining food was noted on D8.

3. Analytical verification:

The determination of the active ingredients prothioconazole and fenpropidin in stock solutions was conducted by an in-house developed method using reversed phase high performance liquid chromatography (RP-HPLC) coupled with tandem mass-spectrometric detection (LC-MS/MS). The analytical method was validated according to SANCO/3029/99 rev. 4 (11/07/2000).

4. Statistics:

The NOEC/NOED and ED₅₀/EC₅₀, ED₂₀/EC₂₀ and ED₁₀/EC₁₀ were determined for D22 based on adult emergence. In order to correct the adult emergence rate of the respective test item treatment groups for the control mortality the statistical evaluation was done using all absolute mortality data of the final assessment on D22. For the determination of the NOEC/NOED, the Step-down Cochran-Armitage Test procedure was used. The accepted significance level was alpha = 0.05 (one-sided greater). Prior, descriptive statistics were performed for justification of the test procedure (Qualitative Trend Analysis by contrasts to check for monotonicity of dose/response and the Tarone's Test procedure to check for extra-binominal variance). As the corrected mortality on D22 was increased by less than 50 % in all test item doses/concentrations compared to the control (i.e. increase was between 3.4 to 41.4%) the respective ED₅₀/EC₅₀ were higher than the highest dose/concentration tested. For determination of the ED₂₀/EC₂₀ and ED₁₀/EC₁₀ values a Probit analysis using linear weighted regression was used. The statistical calculations were performed with the computer program ToxRat Professional 3.3.0 (Ratte, 2018).

II. Results and discussion

A. Analytical data

Correct dosing of the test item was verified by chemical analysis of the two active ingredients prothioconazole and fenpropidin in the aqueous sugar stock solutions of all test item concentration at all feeding days (D3 to D6), resulting in recovery rates per sample of 80.4 %-115 % for prothioconazole and 80.0 %-114 % for fenpropidin. No active ingredient has been detected in the control samples.

B. Mortality

On D8 of the test, no mortality was observed in the untreated control. In the test item groups, the mean cumulative mortalities ranged between 0.0% and 2.8%. The mean mortality in the reference group was above 50 %, i.e. being 66.7 %. The mean mortality between D8 and D15 (based on 36 introduced larvae) was 8.3% in the untreated control and ranged between 16.7 % and 44.4 % in the test item group (corrected for control: 9.1 % and 39.4 %). The mean mortality between D8 and D15 in the reference item group was 13.9 % (corrected for control: 6.1 %).

On D22, the mean adult emergence rate in the untreated control was 80.6% (total mortality 19.4%). In the test item treatment group, the adult emergence rate was 47.2%, 52.8%, 52.8%, 61.1% and 77.8% (from the highest to the lowest dose/concentration). The respective mean total mortality was 52.8%, 47.2%, 47.2%, 38.9% and 22.2% (corrected for control: 41.4%, 34.5%, 34.5%, 24.1% and 3.4%). The mean adult emergence in the reference item group was 2.8% (total mortality was 97.2%; corrected for control: 96.6%). There were statistically significant differences of the adult emergence rates in all test item treatment groups, except for the lowest test item dose on D22 compared to the control (Step-down Cochran-Armitage Test procedure, $\alpha = 0.05$, one-sided greater).

No remaining food was observed at any of the remaining larvae at the end of the feeding phase and no other sublethal effects such as abnormal behaviour or small body size occurred in any of the treatments on the respective mortality assessments.

Table A 18: Effects of ADM.03502.F.1.A to larvae and adult emergence of *Apis mellifera* L. after repeated exposure

Treatment group	Cumulative Dose	Concentration	On D8			D8 – D15*		On D22*		
			Mean mortality D3-D8 [%]		Mean OO [%]	Mean mortality D8-D15 [%]		Mean mortality D3-D22 [%]		Adult emergence rate [%]
	µg prod./ larva	mg prod./kg food	abs.	corr.		abs.	corr.	abs.	corr.	abs.
Control	---	---	0.0	---	0.0	8.3	---	19.4	---	80.6
Test item	1.62	10.26	2.8	2.8	0.0	44.4	39.4	52.8	41.4	47.2**
	0.54	3.42	2.8	2.8	0.0	44.4	39.4	47.2	34.5	52.8**
	0.18	1.14	2.8	2.8	0.0	30.6	24.2	47.2	34.5	52.8**
	0.06	0.38	0.0	0.0	0.0	27.8	21.2	38.9	24.1	61.1**
	0.02	0.13	0.0	0.0	0.0	16.7	9.1	22.2	3.4	77.8
	µg a.s./ larva	mg a.s./ kg food								
Reference item	7.6	48	66.7	66.7	0.0	13.9	6.1	97.2	96.6	2.8

Results are averages based on 3 replicates, containing 12 larvae each; see appendix 3 for details

abs.: mortality as derived from the results of a treatment group; corr.: corrected mortality (according to SCHNEIDER-ORELLI 1947); test/reference item treatment groups corrected for control mortality; negative values were set to "0"; Calculations were performed with non-rounded values. OO: Other observations (e.g., remaining food, small body size)

Mortality on D8-D15: Sum of dead larvae between D8 and D15/ Number of introduced larvae (n = 12) x 100% (replicate wise)

* No Other observations were made

** Statistically significant in comparison to untreated control (Step-down Cochran-Armitage Test procedure; $\alpha=0.05$; one sided greater)

A summary of the results for all endpoints (as amount of formulated product) are listed in the table below.

Table A 19: Statistical outcome of the honey bee larvae test with repeated exposure

Treatment	Endpoint: Successful adult emergence	On D22
Test item doses	ED ₅₀ [µg prod./larva]	> 1.62
	ED ₂₀ [µg prod./larva] (CL) ³	0.116 (0.027 – 0.314)
	ED ₁₀ [µg prod./larva] ³	< 0.02
	NOED [µg prod./larva] ¹	0.02
Test item concentrations	EC ₅₀ [mg prod./kg food]	> 10.26
	EC ₂₀ [mg prod./kg food] (CL) ³	0.738 (0.173 – 1.995)
	EC ₁₀ [mg prod./kg food] ³	< 0.13
	NOEC [mg prod./kg food] ¹	0.13

¹ Step-down Cochran-Armitage Test procedure; $\alpha = 0.05$; one sided greater

² As the corrected mortality on D22 was increased by less than 50 % in all test item doses/concentrations compared to the control (i.e., between 3.4 to 41.4%) the corresponding ED₅₀/EC₅₀ were assumed to be higher than the highest dose/concentration tested

³ Probit analysis using linear weighted regression (CL.: Confidence Limit)

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 0.0 % 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 80.6 %.
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 66.7 % on D8.

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 of ADM.03502.F.1.A on adult emergence of honey bees (*Apis mellifera* L.) after repeated exposure of bee larvae were investigated under laboratory conditions. Correct dosing of the test item was verified by the analysis of prothioconazole and fenpropidin in each test item stock solution of each feeding day, which displayed to be in the required range of ± 20 % of the nominal concentrations. Moreover, no active ingredients were found in the control food. Based on the obtained results, the ED₅₀ of the test item was > 1.62 µg prod./larva, which corresponds to an EC₅₀ (D22) of > 10.26 mg prod./kg food, respectively. The ED₂₀ was determined to be 0.116 µg prod./larva corresponding to an EC₂₀ of 0.738 mg prod./kg food. The ED₁₀ (D22) was calculated to be < 0.02 µg prod./larva, which corresponds to an EC₁₀ (D22) of < 0.13 mg prod./kg food, respectively. The NOED (D22) was determined to be 0.02 µg prod./larva which corresponds to a NOEC (D22) of 0.13 mg prod./kg food. 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

Comments of zRMS:	The study was not evaluated by zRMS in the current dossier as it is not appropriate for use for formulation ADM.03502.F.1.A
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Reference:

KCP 10.3.1.5/01

Report:

Study on the Effect of ADM.3500.F.2.B on Honey bee Colonies (*Apis*

	<i>mellifera</i> L.) under Semi Field Conditions in Germany, Persigehl, M., Beinert, M., Hotopp, I., Zumkier, U. 2021, report no.: B19010-3, sponsor no.: 000102470
Guideline(s):	OEPP/EPPO No. 170(4) (2010)
Deviations:	None relevant (for details see point F. "Study plan deviations")
GLP:	Yes (certified laboratory)
Acceptability/Reliability:	Yes
Duplication (if vertebrate study)	Not applicable

Executive summary

The aim of the study was to determine possible side effects of ADM.3500.F.2.B (250 g/L prothioconazole) after spray application on honey bees (*Apis mellifera* L.) in tunnel tents under confined semi-field conditions in Germany. The methods of investigating the development of the honey bees is based on the Guideline OEPP/EPPO No. 170 (4) (2010). Applications of the test item (ADM.3500.F.2.B), reference item (dimethoate) and control were conducted by spraying the whole area of *Phacelia* plants within the tunnels during full bee flight and at full flowering of the crop (BBCH 65). The crop height was approximately 80 cm in all tunnels. Plants in the control group were sprayed with tap water (400 L/ha). The application rate in the test item treatment group was 0.8 L product/ha, corresponding to nominal 200 g prothioconazole/ha. The reference item tunnels were sprayed with 1.2 L product/ha (corresponding to nominal 480 g dimethoate/ha). During the pre-exposure and the exposure phase mortality was assessed using dead bee traps and non woven sheets. Also, the foraging activity and any behavioural symptoms of intoxication were recorded in each replicate. Residues of prothioconazole and prothioconazole-desethio on flowers, pollen and nectar were assessed, in order to proof the exposure of honey bees to the test item. The limit of quantification (LOQ) was 0.01 mg/kg with a limit of detection (LOD) of 0.003 mg/kg. No effects on mortality of adult honey bees and colony strength could be detected after application of the product ADM.3500.F.2.B (prothioconazole 250 g/L) in this semi-field test. Additionally, results for the reference item (dimethoate) treatment group together with additionally recorded parameters such as foraging activity and the analytical results show that the test system provided adequate exposure and sensitivity.

I. Materials and methods

A. Materials

1. Test material:
 - Lot/Batch no.: ADM.3500.F.2.B 3178-010519-01
 - Content/Purity: 250 g prothioconazole/L (nominal)
252.8 g prothioconazole/L (analysed)
 - Control: water mixed to the diet
 - Toxic reference: Danadim Progress (400 g dimethoate/L)
2. Test organisms
 - Species: *Apis mellifera* L.
 - Source: colonies provided by the apiary of Dr. P. Aumeier, Bochum, Germany
 - No. of colonies: 15 colonies were used for this study. For each treatment group (control, test item and reference) four colonies were used as replicates. Three additional colonies treated with the test item were used for the sampling of residues in pollen and nectar.
 - Colonies: Each colony contained 10 combs including 4 to 8 brood combs that accommodated brood of all stages and a minimum necessary amount of nectar and pollen. Colonies consisted of approximately 5000 adult bees. They were prepared on 30 May 2020, shortly be-

fore instalment within the tunnels and care was taken to ensure that the composition of the colonies was similar, in order to guarantee uniform bee material in all treatment groups. To this end, colonies with sister queens not older than 2 years were chosen. A final assignment of colonies to different treatment groups and replicates was done on DAT 3 after the first condition check of colonies.

— Acclimatisation ————— none

B. Study design and method

1. In life dates: ————— May 28 to July 22, 2020 (field data phase)

2. Study fields:

Location of the study fields

The study site was located in the district of Langenfeld/Leverkusen, North Rhine-Westphalia, Germany. The pre-exposure and exposure phase took place at a study field in Reusrath and the subsequent post-exposure phase on a remote location in Leverkusen, about 3.1 km apart.

Description of the study field

The pre-exposure and the exposure phase was conducted in 15 tunnels located on one study field cultivated with *Phacelia tanacetifolia* as a flowering crop suitable for honey bees (GPS coordinate in the middle of the field: 32 U 358329 E, 5660053 N; study field centre). The tunnels were set up a few days before experimental start in the study field as presented (Figure 1). For each of the three treatment groups (control, test item and reference item) four tunnels were set (assessment tunnels). Three additional tunnels were used for the residue sampling of pollen and nectar during the exposure phase (sampling tunnels).

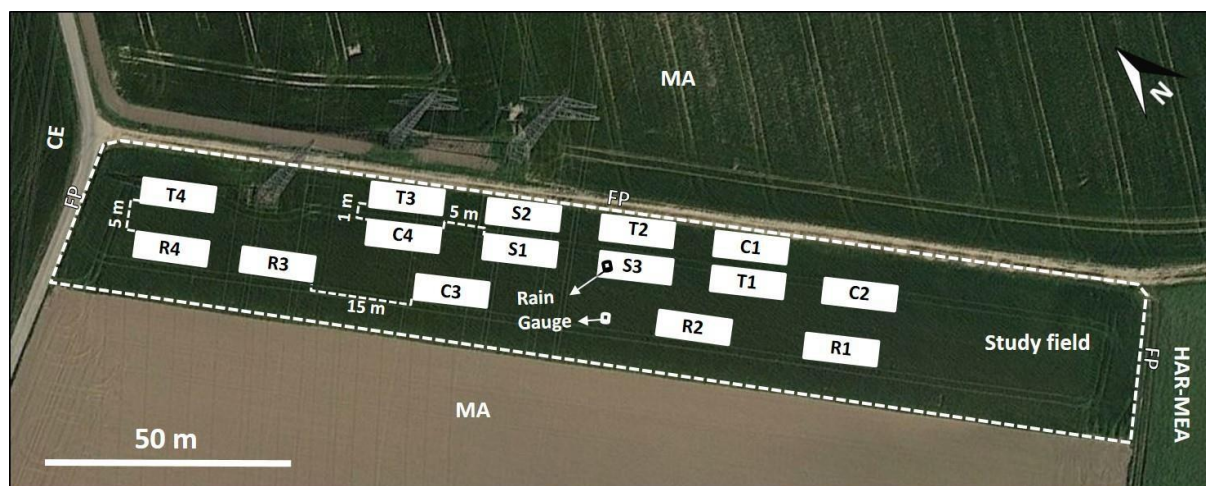


Figure 1 Study field and tunnels C1 – C4, T1 – T4, R1 – R4 and S1 – S3

Picture source: Google Earth Pro (21.07.2020)

Assessment tunnels: Control (C1 – C4), Test item (T1 – T4), Reference item (R1 – R4) and sampling tunnels (S1 – S3, test item treated); Habitats: FP: Field path, HAR-MEA: Harvested meadow, MA: Maize field

Setup of the tunnels

Each tunnel with an effective *Phacelia* crop area of 90 m² (18 m x 5 m) corresponded to one replicate. The cross-section of the tunnels was semi-circular and tunnels were constructed with a tubular steel frame (21 m long, 5.5 m wide and 2.5 m high) that was covered with synthetic gauze (mesh size was approximately 2 mm).

The crop area inside each tunnel was split down the middle by a path (approximately 50 cm wide), which served as a walkway being necessary for performing the application. Additionally, at the front and back ends of each tunnel, plants were cleared from an area of about 5.5 m² to facilitate the placement of bee hives and a water supply as well as to enable work procedures. The outermost 50 cm of the front and back ends as well as the path were covered with non-woven sheets for collection of dead bees during mortality assessments. Tunnels were labelled with consecutive numbers and additionally allocated to treatment groups.

During the post-exposure phase, the 12 assessment colonies were placed at the area of the test facility.

Application of the test item, control and reference item

The whole area of plants in the tunnels was sprayed evenly with a hand-held portable boom sprayer. The sprayer was calibrated before application in order to ensure the exact amount of 400 L/ha \pm 10 % spray solution per tunnel. Applications were conducted on 02 June 2020 in the daytime, during full bee flight and at full flowering of the crop (BBCH 65). The mean crop height in the tunnel was about 80 cm. Applications were carried out between 09:29 and 11:46. First, the control tunnels were sprayed with tap water (400 L/ha), followed by test item treatments with target dose rate of 0.8 L/ha ADM.3500.F.2.B (corresponding to nominal 200 g prothioconazole/ha). One retain sample was taken directly before start of the test item treatments. Finally, the reference item tunnels were sprayed with a target dose rate of 1.2 L Danadim Progress/ha (corresponding to nominal 480 g dimethoate/ha).

Working schedule

The field phase was subdivided in three consecutive phases: pre-exposure phase (until treatments on DAT 0), exposure phase (starting with the treatments on DAT 0) and post-exposure phase.

In-hive mortality assessment

In each assessment tunnel, the in-hive mortality will be recorded by use of dead bee traps (Illies et al., 2002). During each assessment, dead bees will be counted distinguishing adult female bees (workers), drones, pupae and larvae. The assessments of the in-hive mortality will start 3 days (in case of the colony setup on DAT 4) or 2 days (in case of colony setup on DAT 3) before the exposure phase and will be conducted until the end of the post-exposure phase (DAT 22 \pm 1 day). The mortality assessment will be conducted once a day, preferably in the morning before bee flight, except for DAT 0 and DAT 10 \pm 1 day. On DAT 0, the in-hive mortality will be assessed directly before the application and during the first 2 (\pm 1) hours after the spray application. On DAT 10 \pm 1 day, the in-hive mortality will be assessed preferably in the morning before bee flight and in the evening after bee flight directly before the transfer of the colonies from the tunnels to the post-exposure location. If dead bees are visible on the bottom of the hive during the 2nd and 3rd colony condition assessments, they will be counted, as well.

Mortality assessment of forager bees

To assess the mortality of foraging bees, dead bees will be collected from non-woven sheets spread in the middle, at the front and back side of each assessment tunnel, and will be counted distinguishing in workers, drones, pupae or larvae. The mortality assessment of forager bees will start 3 days (in case of the colony setup on DAT 4) or 2 days (in case of colony setup on DAT 3) before the exposure phase and will be conducted until the end of the exposure phase (DAT 10 \pm 1 day). The assessment will be conducted once a day, preferably in the morning before bee flight. However, on DAT 0 the bee forager mortality will be assessed directly before the application and during the first 2 (\pm 1) hours after the spray application.

Foraging activity and behavioural assessments

The assessments will be conducted once on DAT 3 (in case of the colony setup on DAT 4), 2, 1, 1, 2, 3, 5, 7 and 10. Assessments can be shifted to the previous or the next day in case of adverse weather conditions. On DAT 0 the foraging activity and behaviour will be assessed 5 times: directly before the application and within the 1st, 2nd, 4th and 6th hour after spray application. The assessment of foraging activity

ty will be conducted at 10 randomly selected observation areas of approx. 1 m². These observation areas should be evenly distributed over the whole crop area with 5 on each crop side. At each observation area, the number of bees that are present or foraging on flowering plants will be counted for a short time period (snap-shot method, i.e. approx. 15 seconds). If possible, the assessments of the foraging activity should be conducted simultaneously in all three treatment groups to generate comparable results. Behavioural abnormalities of adult bees (e.g. flightlessness, cramping, discoordination or disorientation) will be conducted twice: once along the assessments of the foraging activity and once directly at the beehive. Bees with behavioural abnormalities will be recorded as percentage of present bees per observation area or at the hive (frequency classes: 0%, 1 < 25 %, 25 < 50 %, 50 < 75 %, 75–100 %).

Condition of colonies

Assessments of colony condition checks will be conducted three times: on DAT -4 ±1 day (but before the colonies will be placed in the tunnels), at the end of the exposure phase (DAT 10 ±1 day), and at the end of the post-exposure phase (DAT 22 ±1 day.). The condition checks of the colonies at the end of the exposure and post-exposure phase will be conducted only on the 12 assessment colonies.

To assess the condition of the bee colonies the following parameters will be recorded:

Strength of the colony:

- by estimation of comb area, inner sides of the hive supers and bottom board of the hive covered with bees under consideration of bee density
- by estimation of the number of bees outside the hive at the moment of assessment; the number of incoming bees will be counted for 60 seconds immediately before opening the hive
- Presence and vitality of the queen by observation of the queen and/or eggs
- Comb area with pollen and nectar storage
- Comb area containing brood in different stages (eggs, open and capped worker brood cells as well as open and capped drone brood cells)

All assessments, except of the counting of the incoming bees, will be conducted using an estimation method based on the “Liebefelder Schätzmethode“(Gehrig, 1983; Imdorf et al., 1987). If possible, the same technician should perform all estimations.

With ‘Zander’ hives, that have an area of 8 dm², one comb side that was fully covered with a single layer of bees was deemed to be equivalent to 1000 bees (corresponding to 125 bees/dm²). However, when the frames were covered with multiple layers of bees, the estimated area and thus the number of bees was higher. If only parts of the comb were covered with bees, those were conceptually pushed together into a cluster and their numbers were estimated based on the occupied area. The numbers of bees that were localised on the bottom board and the inner sides of the hive bodies were also estimated based on the area they occupied and their density. The presence of the queen and eggs were also recorded as indicators of the queen’s health and hence, the colonies’ vitality. Numbers of worker brood cells and cells used for food storage were calculated under the assumption that 1 dm² contains 400 cells. Drone brood cells were calculated under the assumption that there are 260 of these cells per dm².

Additionally, the number of incoming bees (during 60 s) were multiplied by 25 to estimate the total number of bees which are foraging outside the hive during the condition check, assuming that one single bee needs 25 minutes for foraging activities until returning to the hive (method by Dr. Pia Aumeier, Bochum, Germany). The number of forager bees was added to the data of the strength of the colony to obtain a realistic value of bees.

Honey stomach preparation

Nectar was sampled from captured honey bee nectar foragers whose honey stomachs were later dissected in the laboratories of the test facility. To this end, honey bee forager were sampled from the 3 sampling tunnels after test item application on DAT 0 after application, DAT 4, DAT 7 and DAT 10. On each sampling day, the hive entrance was sealed before sampling and the forager bees returned to the bee hives

were collected by using a modified portable vacuum collector with a container filled with dry ice. To ensure the collection of the targeted amount of nectar, approximately 150 honey bees should be sampled per duplicate ('A' and 'B'), replicate and sampling event. Collected frozen honey bees were roughly counted and transferred to labelled 250 mL polypropylene bottles. After each sampling event, collected honey bee samples were transferred on dry ice to the test facility, where they were stored deep frozen.

The dissection and extraction of nectar from honey bee stomachs from 'A' samples were carried out in the test facility from 30 June 2020 to 01 July 2020 at room temperature. The 'B' samples will be discarded after the finalisation of the study. For each extraction session, a small number of honey bees (approximately 50) were removed from the freezer for defrosting. After defrosting, the honey stomachs were extracted from the abdomen with tweezers. Immediately after dissection, the nectar was extracted from the honey stomach into a 2.0 ml centrifugation tube. The targeted minimum amount of nectar was 500 mg per duplicate.

Sampling of Phacelia flowers

Phacelia flowers were sampled before the applications (DAT -1) and on DAT 0 after the applications in the 4 control and 4 test item tunnels intended for the bee assessments and in the 3 sampling tunnels. Additionally, in the 3 sampling tunnels flowers were sampled on DAT 2, 7 and 10. Flowers were taken in 2 duplicates ('A' and 'B') for each sampling event.

Flowers were cut at the base of each inflorescence from different plants randomly chosen in each tunnel tent. Target minimum weight of each duplicate was 5.0 g fresh weight. *Phacelia* flowers were collected per sampling day and tunnel tent and transferred into labelled 100 mL polyethylene bottles. Samples were transported on dry ice to the test facility, where they were stored deep frozen.

Pollen sampling

The 3 sampling colonies in the sampling tunnels were fitted with pollen traps in front of the bee hive entrance for the duration of the sampling period. Pollen was sampled on DAT 0 (after application), DAT 4, DAT 7 and DAT 10. On the sampling days, a pollen grid was inserted into the pollen traps for the duration of pollen sampling. To ensure that the pollen was collected on the actual day of sampling, the pollen-trap drawers were emptied and cleaned directly before the grid was inserted. Pollen samples were subdivided in 2 ('A' and 'B' sample) appropriate and labelled 15 mL sampling tubes. The target minimum amount per sampling event was 600 mg pollen for each duplicate. After each sampling event, pollen samples were transported on dry ice to the test facility, where they were stored deep frozen.

Weather conditions during application

During spray applications, air temperature ranged from 23.5°C to 32.7°C and relative air humidity from 20 % to 46 %. Over the whole application period soil temperature was 21°C and wind velocity was between 0.2 m/s and 1.9 m/s. The wind direction changed during the application period from southeast at the beginning to southwest at the end. Cloud cover on DAT 0 was 1 % and no rainfall occurred.

3. Analytical verification:

Analysis of residues was conducted in the test facility of the analytical phase (Eurofins Agroscience Services GmbH, Hamburg). A samples (flowers, pollen and nectar) were analysed for their content of prothioconazole and its metabolite prothioconazole-desthio (via LC MS/MS). Residues are reported in terms of mg active substance/kg for flowers, pollen or nectar. The Limit of Quantification (LOQ) value was 0.01 mg/kg and the Limit of Detection (LOD) 0.003 mg/kg in flowers, pollen and nectar.

4. Statistics:

The evaluation of the data was performed using Generalized Linear Mixed Models (GLMMs) using *R* (version 4.0.2). For further details, please refer to the study report.

H. Results and discussion

A. Analytical data

Residues of prothioconazole and prothioconazole-desthio on flowers, pollen and nectar were assessed, in order to proof the exposure of honey bees to the test item (see tables below). The limit of quantification (LOQ) was 0.01 mg/kg with a limit of detection (LOD) of 0.003 mg/kg.

Flowers

For the verification of the exposure to the test item prothioconazole and its metabolite prothioconazole-desthio flowers were sampled before application (on DAT –1) and within 2 hours after application in the four control (C1 – C4) and test item treatment replicates (T1 – T4) established for the effect monitoring, as well as in the three test item treatment replicates (S1 – S3) used for residue sampling. In flower samples, sampled in all replicates before application, no prothioconazole was detected, with the exception of one sample (sample from control group, replicate C1 on DAT –1; < 0.01 mg/kg). The residues of the metabolite prothioconazole-desthio were always below the limit of quantification (LOQ; 10 flower samples) or the limit of detection (LOD; 5 samples). After test item applications in the test item tunnel (T1–T4) and the sampling tunnel (S1 and S2), prothioconazole and its metabolite prothioconazole-desthio was found in all samples at the same concentration level.

Therefore, it can be concluded that the test item was applied correctly in all replicates of test item treatment group and the sampling tunnels with a comparable application rate and honey bees were exposed to the test item.

Pollen

Pollen was collected by pollen loads from returning forager bees in three additional test item treatment tunnels (sampling tunnel S1, S2 and S3) on DAT 0 after application, on DAT 4, 7 and 10. Maximum concentrations of prothioconazole and its metabolite prothioconazole-desthio in pollen were detected on DAT 0 (Replicate: S2). At the end of the exposure phase (DAT 10) prothioconazole and its metabolite prothioconazole-desthio was still detectable in all replicates.

Therefore, it can be concluded, that honey bees in the test item (T1–T4) tunnels were exposed to the test item throughout the complete exposure phase.

Nectar

Nectar foraging bees were collected in the three additional test item treatment tunnels (sampling tunnel S1, S2 and S3) on DAT 0 after application, on DAT 4, 7 and 10. Prothioconazole was detected in nectar samples only on DAT 0 with maximum concentration of 0.11 mg/kg nectar. Residues of the metabolite prothioconazole-desthio were detected after application on DAT 0 and DAT 4. On DAT 7, residues of all samples were below LOQ and not even detectable (< LOD) on DAT 10. As the nectar samples were extracted from the honey stomach of the returning nectar foraging bees, it can be concluded that honey bees in the test item (T1–T4) tunnels were exposed to the test item in comparable concentrations.

Table A 20: Prothioconazole residues in flowers, pollen and nectar

Prothioconazole residues in flowers, pollen and nectar							
Treatment	Sampling date	Prothioconazole residues [mg/kg]					
		Flowers		Pollen		Nectar	
		Mean	±SD	Mean	±SD	Mean	±SD
Before treatment							
C	DAT -1	<LOD < LOQ	-	-			
T	DAT -1	<LOD	-				
S	DAT -1	<LOD	-				

After treatment							
C	DAT0	<LOD	-	-			
T	DAT0	7.15	1.40				
S	DAT0	6.87	0.32	10.03	1.79	0.09	0.03
	DAT2	0.71	0.46	-	-	-	-
	DAT4	-	-	<LOQ-0.01	-	<LOD	-
	DAT7	0.02	0.01	<LOQ-0.01	-	<LOD	-
	DAT10	<LOQ-0.01	-	0.01	-	<LOD	-

LOQ = Limit of Quantification, LOD = Limit of Detection; C = Control group T = Test item group; S = Testitem treated sampling tunnels

Table A 21: Prothioconazole-desthio-residues in flowers, pollen and nectar

Treatment		Sampling date	Prothioconazole-desthio-residues [mg/kg]					
			Flowers		Pollen		Nectar	
			Mean	±SD	Mean	±SD	Mean	±SD
Before treatment								
C	DAT-1	<LOD < LOQ	-	-				
T	DAT-1	<LOD < LOQ	-					
S	DAT-1	<LOD < LOQ	-					
After treatment								
C	DAT0	<LOQ	-	-				
T	DAT0	4.90	1.12					
S	DAT0	4.83	0.32	1.77	0.31	0.03	0.01	
	DAT2	4.27	2.64	-	-	-	-	
	DAT4	-	-	0.29	0.10	0.02	0.01	
	DAT7	0.64	0.05	0.16	0.02	<LOQ	-	
	DAT10	0.44	0.19	0.22	0.03	<LOD	-	

LOQ = Limit of Quantification, LOD = Limit of Detection; C = Control group T = Test item group; S = Testitem treated sampling tunnels

B. Mortality

Forager mortality

In the control and the test item treatment group the mortality of foraging bees was generally low in average (≤ 50 bees/tunnel). All treatment groups showed three peaks throughout the exposure phase: on DAT 1 the day after application, on DAT 7 and at the end of the exposure phase on DAT 9 and 10 (see table below). However, there was no detectable effect of the application on mortality of foraging bees in the test item treatment group; the average mortality was in the same range as for the control on all assessment days following the application. In the reference item group the application of the dimethoate on DAT 0 led to a marked increase in bee forager mortality on DAT 1 and decreased in the following days until it reached on DAT 4 to the level of the control colonies.

Table A 22: Average ± SD daily number of dead forager bees on non-woven sheets per treatment group and throughout the pre-exposure and exposure phases.

On the day of the application (DAT 0) two assessments were done: one shortly before application and one 2 hours after application. Values for DAT 0 refer to mortality recorded before application; the number of dead bees recorded on the same day after application were added to the numbers for the following day (DAT 1).

Phase	DAT	Control		Reference-Item		Test-Item	
		Mean	±SD	Mean	±SD	Mean	±SD
pre-exposure	-2	3.0	2.2	4.0	1.8	3.5	2.9
	-1	4.5	3.1	7.8	3.8	3.8	1.0
	0	10.2	2.6	3.5	5.0	4.5	2.5
Post	1	23.2	5.7	186.8	93.5	11.8	4.6

	2	9.2	6.9	39.2	7.4	11.5	6.6
	3	11.2	5.0	29.2	19.7	9.2	4.6
	4	15.2	2.5	26.5	16.5	9.8	4.8
	5	17.0	17.0	7.2	2.9	11.5	5.8
	6	13.2	11.6	17.8	3.8	12.8	9.9
	7	37.0	27.3	65.2	13.6	30.8	13.9
	8	9.5	5.2	32.8	10.8	11.5	3.3
	9	33.8	17.1	42.0	19.0	24.5	13.2
	10	50.8	28.0	33.0	9.6	50.5	23.2

In hive mortality

Over the complete experimental period the in hive mortality of the control and test item treatment group were low in average (≤ 20 bees per tunnel). On DAT 1, the in hive mortality in the control and the test item group was comparably low (in mean below 6 dead bees per replicate). Whereas on DAT 1 the mean mortality of 637 (± 84.6) dead bees per replicate after application in the dimethoate group was significantly higher (see table below).

Table A 23: Average \pm SD daily number of dead workers in dead bee traps (in-hive mortality) per treatment group and throughout the experimental phases

On the day of the application (DAT 0) two assessments were done: one shortly before application and one 2 hours after application. Values for DAT 0 refer to mortality recorded before application; the number of dead bees recorded on the same day after application were added to the numbers for the following day (DAT 1). The number of dead bees, recorded during a second assessment on DAT 10 directly before transport of hives to the post exposure location, were added to the numbers for the following day (DAT 11).

Phase	DAT	Control		Reference Item		Test Item	
		Mean	\pm SD	Mean	\pm SD	Mean	\pm SD
pre-exposure	-2	7.0	2.6	8.0	8.2	5.2	3.3
	-1	9.2	3.5	6.8	3.1	6.0	4.0
	0	5.2	2.2	5.0	2.2	7.0	3.7
exposure	1	4.0	1.6	637.0	84.6	5.5	1.0
	2	6.0	2.6	33.0	9.2	1.0	1.4
	3	7.8	2.6	22.0	25.4	4.2	4.0
	4	12.0	8.3	15.2	14.2	1.2	0.5
	5	20.0	8.0	7.2	3.2	10.0	4.1
	6	7.5	2.4	18.2	16.3	6.0	2.2
	7	9.2	5.4	33.8	11.0	5.2	4.0
	8	6.8	2.2	7.8	6.9	4.5	3.0
	9	10.0	4.2	17.8	5.4	5.2	1.5
post-exposure	10	6.2	2.8	13.2	5.1	2.5	1.3
	11	10.2	1.0	22.2	24.3	9.2	5.8
	12	0.8	0.5	2.2	1.0	1.2	1.9
	13	3.2	1.9	1.2	1.5	2.8	0.5
	14	1.8	1.7	1.5	2.4	1.5	0.6
	15	0.8	0.5	0.5	0.6	1.0	2.0
	16	3.5	3.0	2.8	1.0	2.8	1.0
	17	0.8	1.0	0.8	1.0	1.5	1.7
	18	2.0	2.3	0.8	1.0	2.0	2.7
	19	2.0	1.4	1.5	0.6	3.0	5.4

	20	5.2	4.1	3.0	1.6	5.8	8.2
	21	2.8	2.2	2.8	1.0	4.2	1.9
	22	2.0	0.8	1.8	1.3	3.5	1.3

Pupae/Larvae (immature stages)

No dead pupae or larvae were found in the control and test item treatment group on the non-woven sheets in the tunnels during the entire confinement period (DAT -2 to DAT 10, pre-exposure and exposure phase). However, the number of dead pupae or larvae in the reference group were very low, too. In total, the mortality of immature life stages occurred sporadically in all treatment groups and in very low numbers. The highest recorded average number was 2.5 (\pm 4.4) dead immature bees in the reference group at DAT 7. There was no indication that any mortality of immature life stages beyond the naturally occurring mortality in honey bee colonies occurred in the test item treatment group.

Drones

The number of dead drones recorded during the whole study period was very low. The daily mean mortality on the non-woven sheets ranged between 0 and 5 in the three treatment groups. And in the dead bee traps 0 to 8 dead drones were recorded. In general, drone mortality always needs to be interpreted very carefully because the presence of drones is highly heterogeneous in different colonies, even when all colonies are exposed to the same conditions and were recently assembled. However, as the mortality of drones in the test item treatment groups was low throughout the experiment and in a similar range as the control group, there was no indication that any mortality of drones beyond the naturally occurring mortality in honey bee colonies occurred.

C. Foraging activity

The assessments of foraging activity were conducted in order to verify the exposure of honey bees to flowers treated with the test item as well as to monitor any short effects on foraging behaviour (e.g. avoidance caused by a repellent effect). Honey bees were actively foraging on the crop in all treatment groups before the start of the applications on DAT 0. Throughout the assessments after the application the foraging activity in the test item treatment group was comparable with the control and within the same range of foraging activity recorded during the pre-exposure phase (see table below). On DAT 0, in the dimethoate reference tunnels the foraging activity was comparable to the control before the application of the product and decreased afterwards. Therefore, it can be concluded that the test item did not alter honey bee foraging activity and that honey bees in this treatment group were exposed to the test item. On DAT 3, rainy and cold weather conditions (mean temperature: 10.9° C) led to a low foraging activity (\leq 1 bee per tunnel) in all three treatment groups.

Table A 24: Average \pm SD number of foraging bees per m² per treatment group and throughout the experimental phases

On the application day (DAT 0) five assessments were done: one shortly before application, one within the first hour, and one after 2, 4 and 6 hours after application.

Phase	DAT	HAT	Control		Reference-item		Test-item	
			Mean	\pm SD	Mean	\pm SD	Mean	\pm SD
pre-exposure	-2	-48	6.2	2.0	5.8	2.0	5.6	2.1
	-1	-24	10.8	3.8	11.8	3.3	9.9	2.8
	0	0	13.3	3.1	16.6	3.3	17.0	2.8
Application								
post-exposure	0	1	20.4	4.5	0.0	0.2	18.8	2.6
		2	21.1	3.4	0.0	0.2	18.8	4.3
		4	19.4	5.1	0.2	0.5	21.0	3.9
		6	16.4	3.8	0.2	0.4	15.4	4.4

1	24	16.2	3.2	0.0	0.2	12.8	3.4
2	48	3.0	1.9	0.0	0.0	6.8	3.2
3	72	0.0	0.2	0.0	0.0	0.0	0.2
5	120	14.8	7.9	0.1	0.3	20.2	12.2
7	168	19.6	4.7	0.8	0.8	23.8	4.1
10	240	20.9	6.9	0.3	0.7	20.8	6.3

D. Behavioural abnormalities

In the control group no abnormal behaviour was observed during the foraging activity assessments throughout the complete exposure phase. The apathy (1 bee) and moribund (3 bees) behaviour observed in the control group during the behavioural abnormality assessment in front of the beehive on DAT 5 most likely due to the rainy and cold weather conditions during this day. In the test item treatment group in one of the replicates (T3) limitation of movement was observed during the behavioural abnormality assessment throughout the foraging activity assessments on DAT 2, whereas no abnormal behaviour was recorded in front of the beehives throughout the complete exposure phase. Generally, the behavioural abnormalities observed in the control and test item treatment group were low and did not occur directly after application on DAT 0.

In the reference item treatment group abnormal behaviour was observed throughout the complete exposure phase. After application on DAT 0 strong treatment effects were recorded during the foraging activity assessments and the behavioural assessments in front of the beehives.

E. Condition of the colonies

All colonies entered the experiment with a comparable amount of adult worker bees. The colony strength (i.e. the estimated number of adult honey bees per colony) of all treatment groups increased until the end of the experiment. However, the colony strength of control and test item group developed comparably during the experiment, while the colony strength of the reference item group was slightly below the two other groups.

Presence and vitality of the queen

In all colonies, the initial bee queens (thoracically colored before the start of the study) were found regularly during colony assessments. If a queen was not found, her presence could always be verified by freshly laid eggs. Also, no queen brood cells were found throughout the colony assessment, which would have been a prerequisite for a queen replacement.

Strength of the colonies

Three colony assessments were conducted during this experiment: one before the application (DAT 3) and one at the end of the exposure phase (DAT 10) and one at the end of the post exposure phase (DAT 22). Colony strength (i.e. the estimated number of adult honey bees per colony) was similar in all treatment groups on DAT 3 so that all colonies entered the experiment with a comparable amount of worker bees. At the end of the exposure phase (DAT 10) the mean colony size was increased in all three treatment groups (see table below). The mean colony strength in the control group was comparable to the colonies in test item group, whereas the colonies of the reference group were in mean significantly smaller. Until DAT 22 colonies of the control group developed up to 12569 (± 3759) worker bees per colony and in the test item group mean colony strength was slightly higher with 13584 (± 1456) worker bees. In mean, colonies of the reference group were slightly smaller (11147 ± 2834 worker bees) in comparison to the control or the test item group. However, throughout the experiment the mean number of worker bees for colonies in the test item treatment group was comparable to colonies of the control group, indicating that there was no visible treatment short term and/or long term effect by the test item on the development of the colonies.

Table A 25: Colony strength determined as the average \pm SD number of worker bees per treatment group and throughout the pre-exposure, exposure and post-exposure phase

DAT	Number of worker bees*					
	Control		Reference-item		Test-item	
	Mean	\pm SD	Mean	\pm SD	Mean	\pm SD
-3	5031.25	80.69	5031.25	62.5	5109.38	106.74
10	9440.62	1429.54	7440.62	1721.23	9996.88	720.41
22	12568.8	3758.9	11146.9	2834.25	13584.4	1455.93

*Number of worker bees corrected by the number of absent foraging bees. Number of foraging bees was calculated by the number of bees arriving within 60 sec (counted immediately before colony assessment) and the assumed foraging duration of 25 minutes.

Brood development

In parallel to the assessments of colony strength the amount of brood in all stages (eggs, open brood (= larvae) and capped brood) was also estimated (see table below). Additionally, sum of all brood stages was calculated (= immature life stages). The sum of all immature life stages summarizes the intra-colony variability between the different brood stages, as the brood development is not synchronized between different colonies. The mean number of immature life stages in the control group was comparable to the colonies in test item treatment group. Already during the first assessment on DAT -3 the mean number of immature life stages in the reference item group was slightly lower compared to the control. This difference became stronger by the end of the exposure phase DAT 10 but decreased again by the end of the study at DAT 22.

However, the mean number of cells with immature life stages in colonies of the test item group were comparable to colonies of the control group throughout the experiment, indicating that there was no treatment short term and/or long term effect by the test item on the development of the colonies visible.

Table A 26: Average \pm SD number of cells containing eggs, open and capped brood per treatment group and throughout the pre-exposure, exposure and post-exposure phase

Brood-stage	DAT	Control		Test-item		Reference-item	
		Mean	\pm SD	Mean	\pm SD	Mean	\pm SD
Number of cells with eggs	-3	700	1000	900	416.3	1000	432
	10	2850	4150	4350	998.3	4150	1100
	22	3250	2350	3650	1676.3	2350	806.2
Number of cells with open worker brood (larvae)	-3	2800	2700	3050	1181.8	2700	529.2
	10	4650	3700	5250	772.4	3700	621.8
	22	8900	7400	9250	1112.1	7400	1557.8
Number of cells with capped worker brood	-3	9350	8850	10500	1465.2	8850	660.8
	10	10000	4350	7200	1540.6	4350	838.6
	22	12150	11450	12550	1135.8	11450	1968.9

Brood-stage	DAT	Control		Test-item		Reference-item	
		Mean	±SD	Mean	±SD	Mean	±SD
Number-of-cells-with brood-of-allstages	3	12850	12550	14450	1927.9	12550	472.6
	10	17500	12200	16800	1616.6	12200	2019.9
	22	24300	21200	25450	2217.4	21200	2280.4

F. Study plan deviations

- On 03 June 2020 the freezing cell temperature rose to 14.6°C between 14:00 and 15:00 because the door was not closed properly after samples were stored. No impact on the study since the temperature deviation was only for a short interval and the samples remained frozen.
- During the exposure phase, the anemometer stopped recording on 11 June 2020 (Time: 07:19) due to electronic problems. However, it was only a short data gap before the colonies were transported to the post exposure location on 12 June 2020.

The deviations have no effects on the outcome of the study.

G. Validity of the test:

The application of the reference item caused a statistically significant effect on the mortality, foraging activity, or behavioural abnormalities of adult honey bees both for the assessments in the tunnel and in front of the hive. This shows that the test system and application technique provided adequate exposure and that the test organisms used were sensitive enough to reveal effects of a plant protection product on the mortality of honey bee colonies.

Moreover, analytical results revealed that the test item was applied correctly in all replicates of test item treatment group and the sampling tunnels with a comparable application rate and honey bees were exposed to the test item.

Thus, the study is considered valid.

III. Assessment and conclusion

No effects on mortality of adult honey bees and colony strength could be detected after application of the product ADM.3500.F.2.B (prothioconazole 250 g/L) in this semi-field test based on OEPP/EPPO No. 170 (4) (2010). Additionally, results for the reference item (dimethoate) treatment group together with additionally recorded parameters such as foraging activity and the analytical results show that the test system provided adequate exposure and sensitivity.

Comments of zRMS:	The study was not evaluated by zRMS in the current dossier as it is not appropriate for use for formulation ADM.03502.F.1.A
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Reference:	KCP-10.3.1.5/02
Report:	Semi-field study to evaluate potential effects of ADM.1351.F.1.A (Spyrale) on the development of honeybee colonies (<i>Apis mellifera</i> L.), Germany, Hecht Rost, S., 2020, report no.: R1940026, sponsor no.: 000102476
Guideline(s):	OEPP/EPPO Guideline 1/170 (2010), EFSA Guidance Document on the risk assessment of plant protection products on bees, SANCO/3029/99 rev.4 (11/07/00)
Deviations:	None relevant (for details see point G. “Study plan deviations”)

GLP:	Yes (certified laboratory)
Acceptability/Reliability:	Yes
Duplication (if vertebrate study)	Not applicable

Executive summary

The aim of this study was to investigate possible adverse effects of ADM.1351.F.1.A (Spyrale) on colonies of honeybees (*Apis mellifera* L.) under semi field conditions in *Phacelia tanacetifolia* in Germany in accordance to the OEPP/EPPO Guideline 1/170 (4) (2010). The study included one test item treatment, one tap water treated control and one reference item treatment (Danadim® Progress; a.s. dimethoate). The treatment groups comprised four replicates. For collection of certain specimens for residue analysis three additional tunnels were assembled, one for the control and two for the test item group.

The nominal application rate of the test item was 1.0 L ADM.1351.F.1.A F/ha (a.s. analysed: 371 g fenpropidin/ha, 109 g difenoconazole/ha) for the test item group and in the additional tunnels for residue samplings of pollen and nectar. A second group treated with tap water served as control and in the additional tunnel for residue samplings of pollen and nectar. As reference item Danadim® Progress (a.s. dimethoate) was applied at a rate of 1.2 L product/ha (480 g a.s./ha). All applications were carried out during full flowering and honeybee flight with a spray volume of 400 L water/ha.

Colony development and mortality were assessed as well as sublethal parameters like foraging activity and behaviour of honeybees in order to evaluate possible impact of the test item on honeybees. Additionally, flowers, pollen and nectar from forager bees were sampled and analysed for potential residues of the test item. Analytical results demonstrated that honeybees were exposed to ADM.1351.F.1.A inside the tunnels throughout the entire exposure period within the tunnels. A residue decline of both active substances could be observed in nectar and pollen. The limit of quantification (LOQ) of the analytical method for each matrix was 0.01 mg a.s./kg and the LOD was set at 0.003 mg a.s./kg (30% of the LOQ) for both active substances (fenpropidin and difenoconazole).

The application of ADM.1351.F.1.A did not cause adverse effects on the survival of adult worker bees, bee pupae, behaviour, colony strength and colony development. Overall, this study demonstrated that Spyrale applied at a nominal rate of 1.0 L product/ha (371 g a.s. fenpropidin/ha, 109 g a.s. difenoconazole/ha) during honeybee flight did not adversely affect mortality, behaviour, strength, and development of honeybee colonies.

I. Materials and methods

A. Materials

- Test material:
 - Lot/Batch no.: HEM7C00017/1
 - Content/Purity: 371 g fenpropidin/L (analysed), 109 g difenoconazole (analysed)
 - Control: water mixed to the diet
 - Toxic reference: Danadim® Progress (400 g dimethoate/L)
- Test organisms
 - Species: *Apis mellifera* L.
 - Source: produced by the company's own apiary under non-GLP conditions
 - No. of colonies: Honeybee (*Apis mellifera* L.) colonies (ten combs) with mean numbers of 5,379, 6,321 and 4,729 worker bees (see Study plan deviations), 17,450, 16,800 and 19,650 brood cells, 16,400,

Colonies:	17,850 and 15,900 food cells on DAA ⁻¹ were used in the control, test item and reference item groups, respectively. Sister queens from the previous year (2018) were used in order to ensure the greatest possible equality of the colonies
Acclimatisation	Honeybees free of visible clinical symptoms of disease (e.g. Varroosis, Nosemosis, Amoebiasis, Chalkbrood, Sacbrood, American or European Foulbrood) or from pests (e.g. Varroa destructor) were used. Colonies were free of unusual occurrences (e.g. presence of dark "bald" bees, "crawlers" or flightless bees, unusual brood patterns or brood age structure). At least four weeks before the start of the test, no medical treatment was carried out. The colonies were as homogeneous as possible, with a natural distribution of all worker ages. All hives were equipped with a dead bee trap at the entrance to count the number of dead honeybees that were carried out of the hives. The colonies used for the residue samplings were equipped with a pollen trap instead of a dead bee trap.

B. Study design and method

1. In life dates: June 28 to July 30, 2019 (field data phase)

2. Study fields:

Location of the study fields

The study was conducted on a field with the bee attractive crop *Phacelia tanacetifolia*. The study field was located near the municipality Ladenburg in the federal state Baden-Württemberg, Southern Germany. The GPS coordinates of the western corner of the study field were N 49°29'6.84", E 8°35'53.60" [dd° mm'ss.ss"].

Description of the study field

The bee attractive plant *P. tanacetifolia* (crop variety: beehappy), was sown on the study field on 02 May 2019 at a drilling rate of 15 kg seeds/ha (Non-GLP). Prior to set up of the test bee hives 15 tunnel tents were assembled on the field site. Each tunnel consisted of semi-circular metal frames and measured 18 m x 6 m x 2.9 m (L x W x H); thus the area covered by one tunnel was approximately 108 m². A light transparent gauze fabric covered the tunnels (mesh size: 2 mm). The distance between each tunnel was 2.50 m.

The colonies were set up in the tunnel tents located at the study site in the evening of 28 June 2019, shortly before full flowering (BBCH growth stage 61-63 according to Meier and Blendholder 2016) of the crop. That is four days before the application of the control, test item and the reference item during bee flight activity. One honeybee colony per tunnel was used for the biological assessments and two colonies per tunnel used for collecting residue specimens. The colonies were exposed in the tunnels for a period of ten days after the application and afterwards transported to the monitoring site in the evening of 12 July 2019. Each tunnel covered an effective crop area in the tunnel of ~84 m² (2 x 42.0 m²), which was divided into two areas of *P. tanacetifolia* with ~2.5 m width and ~16.8 m length, separated by a path of 0.6 m which was cut into the crop. Sheets with a width of 0.6 m were spread out at the inner walls of the short sides and on the path in the middle of the tunnel.

After the ten-day exposure period the colonies were brought to a monitoring site in a forest near Hirschberg in the federal state Baden-Württemberg, Southern Germany for further observations. This location was chosen to avoid any contaminations of the colonies by pesticides. The GPS coordinates of the monitoring site were N 49°29'23.3"; E 8° 41'32.0" [dd° mm'ss.s"]. The distance of the monitoring site to the field site was approximately 6.8 km.

3. Application of the test item, control and reference item:

The nominal application rates of the test item and the reference item are presented in the table below:

Table A 27: Application rates and details

Treatment	Code	Amount of product	Amount of a.s.	Spray volume [L/ha]
Control (tap water)	C	None	None	400
Test item (ADM.1351.F.1.A)	T	1.0 L/ha	371 g fenpropidin/ha ¹⁾ 109 g difenoconazole/ha ¹⁾	
Reference item (Danadim [®] Progress)	R	1.2 L/ha	480 g dimethoate/ha ²⁾	

¹⁾ Calculation based on the analysed content of a.s.

²⁾ Calculation based on the nominal content of a.s.

The application of tap water for the control, the test item and reference item took place at BBCH growth stage 65–67 (see Study plan deviations) on 02 July 2019. For the application, a calibrated boom sprayer was used according to good agricultural practice.

The sprayer was equipped with a calibrated flow meter. During application plastic sheets covered the hives in order to protect them from direct spray residues. The test and the reference item were pre-weighted at the laboratory and added to the respective amount of water shortly before application. Homogeneity of spray solutions was obtained by thorough stirring and mixing immediately before application.

After the application in each tunnel, the applied spray volume was determined by measuring the remaining spray solution. Differences regarding the target and actually spray rates ranged from 0.30 % to 1.79 % for the control, 0.27 % to 0.62 % for the test item and 3.0 % to 2.05 % for the reference item and were thus within the acceptable spray tolerance of ± 5 %.

The following criteria were met for the application:

- Full flowering of *P. tanacetifolia* (BBCH growth stage 65–67, see Study plan deviations)
- Wind speed outside the tunnels was < 2 m/s (maximum: 1.9 m/s)
- Crop was dry
- Mean foraging activity per treatment group was > 10 honeybees/m² (minimum: 11.7 honeybees/m²)
- No rainfall occurred on the day of application

The temperature was between 15°C and 30°C.

4. Recording of the meteorological data:

The weather was recorded during the whole Field Phase. Temperature and humidity (daily minimum (min), maximum (max) and mean) were recorded by a non-GLP weather station 2.04 km distanced from the field site in Ladenburg (49°29'38.91"N, 8°37'24.28"E, see Study plan deviation) and rainfall by a rain gauge in T3 during the pre-exposure and exposure period. During the monitoring period, temperature and humidity were recorded by a data logger (daily min and max were determined and mean was calculated) and rainfall by a rain gauge next to the hives. Cloudiness was recorded during pre-exposure and exposure period during the daily assessments. Wind speed was recorded on the day of application.

5. Biological assessments:

Mortality

The assessments of the number of dead honeybees were carried out in the morning at approximately the same time. Mortality of honeybees was assessed on sheets which were spread out at the front, middle and back of the tunnels (DAA 3 to DAA 10)). Additionally the dead honeybees were counted in the dead bee

traps which were attached to the entrance of the hives. The assessments were done according to the time table presented in Table 6. The number of dead bees was separated into adult bees and pupae and furthermore differentiated into female (worker bee) and male (drone) individuals. At each assessment day dead honeybees were removed. Only the mortality of the female developmental stages is reported.

Foraging activity

Foraging activity was recorded on visual estimated areas of 1 m² at three different places in each tunnel according to the time schedule. During each assessment the numbers of honeybees foraging on flowering *P. tanacetifolia* were counted for approximately 15 seconds per visually estimated area. At each assessment, the area to be observed was chosen randomly.

Behaviour

The behaviour of the honeybees was observed in parallel to the foraging activity assessments as well as during emptying the dead bee traps.

The following parameters were checked:

- Aggressiveness (e. g. honeybees attack the personnel)
- Frequent flower visits without foraging activity (only during the exposure period in the tunnels)
- Landing on the plastic gauze (only during the exposure period in the tunnels)
- Intoxication symptoms (paralyzed honeybees, cramping honeybees)
- Clustering

Colony assessments

To assess potential effects of the test item on the condition of the colonies the following parameters were assessed five times during the Field Phase of the study (except colonies used for the residue samplings, which were checked only once before the first sampling event to assure that the colonies were in good condition):

- Strength of the colonies (number of worker honeybees)
- Presence of a healthy queen (e.g. presence of eggs)
- Comb area containing pollen and nectar
- Comb area containing eggs, larvae and capped cells (pupae)

The colony assessments were conducted according to the Liebefeld method of Imdorf et al. (1987) and Imdorf & Gerig (1999) as well as according to Aumeier (2008). For this purpose the comb was visually divided in areas of 1 dm². This was done for both sides of all combs of each hive. According to Imdorf et al. (1987) and Imdorf & Gerig (1999) one square of 100 cm² covered densely with honeybees represents approximately 130 worker bees, 400 worker bee cells containing brood such as eggs and larvae or food such as pollen and honey, or 230 male brood cells.

Sampling details, sample storage and sample shipments

For residue analysis *P. tanacetifolia* flowers (only blossoms), nectar (from forager bees) and pollen (from pollen traps) were collected. From each matrix two subsamples, one A sample (for residue analysis) and one B sample (backup sample), were taken. Sampling equipment was exchanged or cleaned with ethanol before the next using.

All specimens were packed in at least two vessels (PE bottle and PE bag or two PE bags) and labelled with the following information: Company name, GLP study code, sampling date, matrix, A or B sample, GLP ID. All specimens were kept on dry ice directly after sampling and stored at ≤ -18.0 °C at the Test Facility within 3.75 hours after completion of the last sampling.

After consultation with the Principal Investigator of the Analytical Phase, the A samples were shipped on 31 July 2019 from the Test Facility (RIFCON GmbH) to the Test Site of the Analytical Phase (Eurofins

Agroscience Services Chem GmbH) at $\leq -18.0\text{ }^{\circ}\text{C}$ (on dry ice). The B samples were taken as backup for the residue analysis and kept deep frozen at $\leq -18.0\text{ }^{\circ}\text{C}$ at the Test Facility until delivery of the Final Report to the Sponsor. Storage (see Study plan deviations) and shipment conditions were recorded by use of a data logger.

Sampling of flowers

Flower specimens were taken in the three additional assembled residue tunnels (C residue, T residue 1, T residue 2) and in all control, test item and due to organizational reason also in all reference item tunnels (C1 to C4, T1 to T4 and R1 to R4; see Study plan deviations). Collection of the specimens took place on DAA 1 and DAA 0aa. The treated specimens were taken on the day of application within 4 hours after application of the test item (DAA 0aa). The reference item samples on DAA 0aa tunnels were sampled for potential dimethoate residue analyses. As the reference item showed its expected effect (increased mortality after application), a residue analysis of these samples was not necessary.

P. tanacetifolia flower specimens (only blossoms, as little as possible of the green plant material) were taken inside the tunnels at randomly selected locations by hand. Each subsample (A and B) contained $\geq 5.0\text{ g}$ of flowers. After the sampling the collected flowers were divided into A and B samples, were packed in two vessels (two PE bags) and were labelled.

Sampling of pollen

The pollen samples were only taken in the three additionally assembled residue tunnels (C residue, T residue 1, T residue 2). The specimens were taken one day before the application (DAA 1), on the day of the application after the application (DAA 0aa), four, seven and nine days after the application (DAA 4, DAA 7 and DAA 10).

For the collection of pollen from pollen loads, a pollen trap was attached to the hive on the day of set up. The grid of the pollen traps was only inserted on the days of sampling and was kept in place until the sample amount of $\geq 1.0\text{ g}$ ($2 \times \geq 0.5\text{ g}$ (A and B sample)) was reached (see Study plan deviations).

On DAA 1 and DAA 0aa, no pollen in the control could be collected from pollen traps, therefore the pollen were completely taken from forager preparation. The samples from the two test item tunnels on DAA 1 were also taken from the forager preparation due to no pollen in pollen traps. On DAA 0aa, not enough pollen could be collected from pollen trap in T residue 1 and on DAA 7, the same situation in T residue 2, therefore additional pollen was taken from forager preparation, respectively. Due to no pollen in all pollen traps on DAA 3, the traps were kept closed until DAA 4 to increase the sample size. As on DAA 7, the pollen amounts in the traps were not sufficient, the traps were kept closed until DAA 8 before foraging activity in the morning. Thus, the whole pollen sample amount collected on DAA 8 originated from DAA 7.

Sampling of forager bees for honey stomach preparation

The specimens were taken on DAA 1, DAA 0aa, DAA 3 and DAA 7. On DAA 10 no forager bees could be sampled, due to absence of forager bees (see Study plan deviations). For the collection of nectar from honey stomach, forager bees in front of the hives were caught. For this purpose, on the day of sampling, the hive entrances were sealed before sampling and the returning forager bees were collected by using a modified hand held hoover. After each sampling the hive entrances were reopened allowing the bees to return to and leave the hive. This procedure was repeated until approximately 500 forager bees ($2 \times$ approximately 250 forager bees (A and B sample)) were reached. After the sampling the collected bees were divided into A and B samples, packed in two vessels (PE bottle and PE bag) labelled and stored deep frozen.

At the Test Facility the nectar was dissected from the honeybee stomachs. Therefore, the bees were defrosted and fixed with a pair of tweezers at their thorax and abdomen. Then the bees were stretched and the abdomen removed, so that the stomach was freed and could be removed. The content of the stomach was then collected into a sample vessel until a sample amount of $\geq 0.5\text{ g}$ was reached for the A sample

and it was then stored deep frozen (at $\leq -18.0\text{ }^{\circ}\text{C}$) immediately after preparation. After preparation, the A-samples were packed in two vessels (Eppendorf vessel and PE bag), labelled, and stored at $\leq -18.0\text{ }^{\circ}\text{C}$ at RIFCON GmbH until transport to the Test Site of the Analytical Phase.

6. Statistics:

The test assumptions of normality (ND) and homogeneity of variance (HV) for mortality and foraging activity data were tested with a Shapiro-Wilk test and Bartlett's test, respectively. In cases where assumptions of normality and homogeneity of variances were met, an analysis of variance (ANOVA) followed by Dunnett's test was used to determine the differences among treatment groups. Where these assumptions were violated, a Kruskal-Wallis test followed by U test (Wilcoxon-Mann-Whitney) was conducted. The significance levels of the tests were $\alpha = 0.05$. Statistics were conducted with the statistical and programming environment R (R-Core Team 2019, R version 3.6.1).

H. Results and discussion

A. Analytical data

Samples of flowers, pollen and nectar were collected over a period of 10 days after the application and were analysed for the active substances fenpropidin and difenoconazole. Both analytes were found in all test item flower, pollen and nectar samples after the application, whereas the residue levels decreased with the sampling days. The residue levels of difenoconazole were in general much lower (two to eight-fold) compared to the levels of fenpropidin. Nectar samples taken on DAA 3 and DAA 7 showed almost no residues of fenpropidin and no residues of difenoconazole (DAA 3: 0.02 and $<\text{LOD}$; DAA 7: 0.01 and $<\text{LOD}$). No nectar could be sampled on the last assessment day (DAA 10). Residues of fenpropidin were also found in flower samples of the control group. Due the vapour pressure of fenpropidin ($1.7 \times 10^{-2}\text{ Pa}$ at 25°C), a high volatility of fenpropidin needs to be considered. Therefore a contamination by volatilisation is very likely. The limit of quantification (LOQ) of the analytical method for each matrix was 0.01 mg a.s./kg and the LOD was set at 0.003 mg a.s./kg (30% of the LOQ) for both active substances (fenpropidin and difenoconazole). The method was successfully validated according to the Analytical Phase Report.

Fenpropidin and difenoconazole residues from all samples collected during the Field Phase after application of Spyrale were analysed by the Test Site Eurofins Agroscience Services Chem GmbH. The analytical results are summarised in the tables below:

Table A 28: — Residues of fenpropidin and difenoconazole in flowers

Timing	Tunnel	Treatment	Sample-ID	EAS-Chem-Internal-Code	Residue-of-Fenpropidin {mg/kg}	Residue-of-Difenoconazole {mg/kg}
DAA-1	C1	C	C-FL-001-A	101-W	$<\text{LOD}$	$<\text{LOD}$
DAA-1	C2	C	C-FL-002-A	102-W	$<\text{LOD}$	$<\text{LOD}$
DAA-1	C3	C	C-FL-003-A	103-W	$<\text{LOD}$	$<\text{LOD}$
DAA-1	C4	C	C-FL-004-A	104-W	$<\text{LOD}$	$<\text{LOD}$
DAA-1	C-residue	C	C-FL-005-A	105-W	$<\text{LOD}$	$<\text{LOD}$
DAA-1	T1	T	T-FL-001-A	120-W	$<\text{LOD}$	$<\text{LOD}$
DAA-1	T2	T	T-FL-002-A	121-W	$<\text{LOD}$	$<\text{LOD}$
DAA-1	T3	T	T-FL-003-A	122-W	$<\text{LOD}$	$<\text{LOD}$
DAA-1	T4	T	T-FL-004-A	123-W	$<\text{LOD}$	$<\text{LOD}$
DAA-1	T1-residue	T	T-FL-005-A	124-W	<0.01	$<\text{LOD}$
DAA-1	T2-residue	T	T-FL-006-A	125-W	$<\text{LOD}$	$<\text{LOD}$
DAA0aa	C1	C	C-FL-006-A	106-W	0.01	$<\text{LOD}$
DAA0aa	C2	C	C-FL-007-A	107-W	0.05	$<\text{LOD}$

DAA0aa	C3	C	C-FL-008-A	108-W	0.43	<LOD
DAA0aa	C4	C	C-FL-009-A	109-W	0.01	<LOD
DAA0aa	C-residue	C	C-FL-010-A	110-W	0.52	<LOD
DAA0aa	T1	T	T-FL-007-A	130-W	117	33.5
DAA0aa	T2	T	T-FL-008-A	131-W	118	32.8
DAA0aa	T3	T	T-FL-009-A	132-W	113	31.7
DAA0aa	T4	T	T-FL-010-A	133-W	129	38.5
DAA0aa	T1-residue	T	T-FL-011-A	134-W	147	42.4
DAA0aa	T2-residue	T	T-FL-012-A	135-W	124	35.1

DAA = days after application; T = treated; C = untreated; LOD set at 30 % of the LOQ; Residues are not corrected for procedural recoveries

Table A 29: — Residues of fenpropidin and difenoconazole in pollen

Timing	Tunnel	Treatment	Sample ID	EAS-Chem-Internal-Code		Residue of Fenpropidin [mg/kg]	Residue of Difenoconazole [mg/kg]
DAA-1	C-residue	C	C-PT-001-A	111		<0.01***	<LOD
DAA-0aa	C-residue	C	C-PT-002-A	112		0.16	<LOD
DAA-3/4	C-residue	C	C-PT-003-A	113		0.03	<LOD
DAA-7/8	C-residue	C	C-PT-004-A	114		0.02	<LOD
DAA-10	C-residue	C	C-PT-005-A	115		<0.01	<LOD
DAA-1	T1-residue	T	T-PT-000-A	140		<LOD	<LOD
DAA-0aa	T1-residue	T	T-PT-001-A	141		44	20
DAA-3/4	T1-residue	T	T-PT-005-A	142		2.9	0.86
DAA-7/8	T1-residue	T	T-PT-007-A	143		1.3	0.50
DAA-10	T1-residue	T	T-PT-009-A	144		0.42	0.09
DAA-1	T2-residue	T	T-PT-003-A	145		<0.01***	<LOD
DAA-0aa	T2-residue	T	T-PT-002-A	146		32	10
DAA-3/4	T2-residue	T	T-PT-006-A	147		4.9	1.1
DAA-7/8	T2-residue	T	T-PT-008-A	148		1.7	0.65
DAA-10	T2-residue	T	T-PT-010-A	149		1.0	0.24

DAA = days after application; T = treated; C = untreated; LOD set at 30 % of the LOQ; Residues are not corrected for procedural recoveries; *** sample weight below 100 mg, therefore a different aliquotation was done during work up

Table A 30: — Residues of fenpropidin and difenoconazole in nectar

Timing	Tunnel	Treatment	Sample ID	EAS-Chem-Internal-Code		Residue of Fenpropidin [mg/kg]	Residue of Difenoconazole [mg/kg]
DAA-1	C-residue	C	C-NF-001-A	116		<LOD	<LOD
DAA0aa	C-residue	C	C-NF-002-A	117		<LOD	<LOD
DAA3	C-residue	C	C-NF-003-A	118		<LOD	<LOD
DAA7	C-residue	C	C-NF-004-A	119		<LOD	<LOD
DAA-1	T1-residue	T	T-NF-000-A	150		<LOD	<LOD
DAA0aa	T1-residue	T	T-NF-001-A	151		1.1	0.13
DAA3	T1-residue	T	T-NF-005-A	152		0.03	<LOD
DAA7	T1-residue	T	T-NF-007-A	153		<0.01	<LOD

DAA1	T2-residue	T	T-NF-003-A	154	<LOD	<LOD
DAA0aa	T2-residue	T	T-NF-002-A	155	1.7	0.22
DAA3	T2-residue	T	T-NF-006-A	156	0.02	<LOD
DAA7	T2-residue	T	T-NF-008-A	157	0.01	<LOD

DAA = days after application; T = treated; C = untreated; LOD set at 30 % of the LOQ; Residues are not corrected for procedural recoveries

B. Mortality

During the pre-application period, the daily mean mortality of adult worker bees was slightly increased but on similar levels for all treatment groups. As most of the dead bees were found on the sheets and only on the first two days after set-up of the colonies inside the tunnels, it can be assumed that the colonies had short term problems with acclimatising to the new environmental conditions inside the tunnels.

In comparison to the mean adult bee mortality during the pre-exposure period, the mean mortality during the exposure period of the control group and the test item group was slightly decreased, which led to the assumption that the bees had thus adapted to the new environmental conditions inside the tunnels. However, the mean mortality of the reference item group was increased significantly, which showed that the reference item showed its expected effect (=high adult mortality). Over the entire post-exposure period (DAA 11 to DAA 28), the mean mortality were on similar levels for all treatments groups. The mean honey bee mortalities are summarised in the table below.

Table A 31: Mean worker bee mortality in the different treatment groups

Date [dd.mm.yyyy]	DAA	Control [n]		Test item [n]			Reference item [n] ³⁾		
		Mean	SD	Mean	SD	Statistics	Mean	SD	Statistics
29.06.2019	-3	19.5	9.7	32.8	17.9	n.s. ^D	24.0	8.5	n.s. ^D
30.06.2019	-2	90.0	20.4	156.3	83.5	n.s. ^D	102.0	43.2	n.s. ^D
01.07.2019	-1	74.0	27.0	158.0	68.2	n.s. ^D	150.0	120.7	n.s. ^D
02.07.2019	0ba	24.3	5.3	35.5	19.9	n.s. ^D	28.8	11.8	n.s. ^D
Mean DAA -3 to 0ba ⁴⁾		51.9	35.4	95.6	39.9	n.s. ^D	76.2	79.3	n.s. ^D
02.07.2019	0aa1	7.8	2.5	13.8	4.0	0.029 ^{±U}	289.5	68.3	0.014 ^{±U}
	0aa2	8.3	4.3	19.5	9.7	n.s. ^D	438.8	58.6	<0.001 ^{±D}
03.07.2019	1	8.8	2.9	16.8	8.8	n.s. ^U	91.3	20.8	<0.001 ^{±D}
Mean (Σ0aa +1) ⁴⁾		24.8	4.7	50.0	21.2	0.047 ^{±D}	819.5	65.6	<0.001 ^{±D}
04.07.2019	2	29.3	6.2	47.3	24.4	n.s. ^D	115.3	47.3	0.002 ^{±D}
05.07.2019	3	29.3	5.9	43.3	21.7	n.s. ^D	93.5	38.9	0.004 ^{±D}
06.07.2019	4	33.3	4.2	54.3	23.4	n.s. ^D	66.8	46.2	n.s. ^D
07.07.2019	5	40.5	12.8	57.5	9.0	n.s. ^D	52.3	25.3	n.s. ^D
08.07.2019	6	27.3	14.5	47.0	18.8	n.s. ^D	14.3	4.3	n.s. ^D
09.07.2019	7	35.3	6.6	46.8	21.5	n.s. ^D	19.0	4.1	n.s. ^D
10.07.2019	8	45.8	23.1	47.8	26.4	n.s. ^D	23.8	5.7	n.s. ^D
11.07.2019	9	48.0	23.1	57.5	15.3	n.s. ^D	33.8	11.6	n.s. ^D
12.07.2019	10	33.3	6.8	30.0	5.8	n.s. ^D	18.0	9.8	n.s. ^D

Mean (ΣDAA 0aa +1 to 10)		34.7	13.5	48.1	19.0	n.s. ^D	123.7	238.7	<0.001* ^D
13.07.2019	11	2.0	2.0	1.5	1.7	n.s. ^D	1.3	1.5	n.s. ^D
14.07.2019	12	1.8	1.5	4.3	4.6	n.s. ^D	1.8	2.1	n.s. ^D
15.07.2019	13	2.0	1.0	0.8	1.0	n.s. ^D	1.8	1.3	n.s. ^D
16.07.2019	14	3.0	1.4	1.8	2.2	n.s. ^D	1.0	0.8	n.s. ^D
17.07.2019	15	15.8	2.4	13.8	5.0	n.s. ^D	11.5	11.3	n.s. ^D
18.07.2019	16	7.3	3.0	8.8	7.1	n.s. ^U	3.5	6.4	n.s. ^U
19.07.2019	17	5.8	4.4	10.5	7.9	n.s. ^D	4.0	3.2	n.s. ^D
20.07.2019	18	1.8	2.1	7.8	6.7	n.s. ^D	3.0	3.6	n.s. ^D
21.07.2019	19	2.8	3.6	4.8	6.8	n.s. ^U	3.0	1.8	n.s. ^U
22.07.2019	20	1.8	1.5	3.8	2.5	n.s. ^D	4.8	3.6	n.s. ^D
23.07.2019	21	4.3	6.6	1.0	0.8	n.s. ^D	3.0	6.0	n.s. ^D
24.07.2019	22	14.8	12.5	4.5	5.3	n.s. ^D	3.3	2.6	n.s. ^D
25.07.2019	23	12.8	14.4	4.0	6.1	n.s. ^D	2.8	1.9	n.s. ^D
26.07.2019	24	14.0	15.4	4.8	5.2	n.s. ^D	3.3	2.2	n.s. ^D
27.07.2019	25	16.5	12.0	3.5	2.9	n.s. ^D	1.8	1.7	n.s. ^D
28.07.2019	26	17.3	17.6	3.3	2.8	n.s. ^D	3.0	1.8	n.s. ^D
29.07.2019	27	6.5	6.0	3.8	1.9	n.s. ^D	2.0	2.7	n.s. ^D
30.07.2019	28	2.8	2.9	3.5	2.4	n.s. ^D	3.3	3.8	n.s. ^D
Mean (11 to 28)		7.4	9.3	4.8	5.2	n.s. ^D	n.s. ^D	4.2	n.s. ^D
Mean (Σ0aa + 1 to 28)		17.2	17.1	20.3	24.1	n.s. ^D	n.s. ^D	152.9	<0.001* ^D

No mortality assessment was conducted for colony C4 on DAA 13 (see Study plan deviation). Sum of dead individuals found in dead bee traps and on sheets in the tunnels (DAA 3 to DAA 10) / dead individuals found in dead bee traps, only (DAA 11 to 28); n.s. = not statistically significantly different compared to the control group; * statistically significantly different compared to the control group (p<0.05); D ANOVA / Dunnett's test; U U-Test; DAA = days after application; ba = before application, aa = after application; SD = standard deviation; n number

Pupae/Larvae (immature stages)

The pupae mortality of the test item replicates was not increased at any time and therefore comparable to the pupae mortality of the control group. A test item related adverse effect on the survival of the honeybees after the application of ADM.1351.F.1.A can be excluded. The mean pupal mortalities are summarised in the table below.

Table A 32: Mean pupal mortality in the different treatment groups

Date [dd.mm.yyyy]	DAA	Control [n]		Test item [n]			Reference item [n]		
		Mean	SD	Mean	SD	Statistics	Mean	SD	Statistics
29.06.2019	-3	0.0	0.0	0.0	0.0	-	0.0	0.0	-
30.06.2019	-2	1.5	1.3	0.8	1.0	n.s. ^D	1.3	1.9	n.s. ^D
01.07.2019	-1	0.3	0.5	0.0	0.0	n.s. ^U	0.0	0.0	n.s. ^U
02.07.2019	0ba	0.8	1.5	0.3	0.5	n.s. ^U	0.0	0.0	n.s. ^U
Mean DAA -3 to 0ba		0.6	1.1	0.3	0.6	n.s. ^D	0.3	1.0	n.s. ^D
02.07.2019	0aa1	0.3	0.5	0.0	0.0	n.s. ^U	0.0	0.0	n.s. ^U
	0aa2	0.0	0.0	0.0	0.0	-	0.0	0.0	-
03.07.2019	1	1.0	2.0	0.0	0.0	n.s. ^U	0.0	0.0	n.s. ^U
Mean (Σ0aa + 1)		1.3	2.5	0.0	0.0	n.s. ^U	n.s. ^U	0.0	n.s. ^U
04.07.2019	2	0.3	0.5	0.0	0.0	n.s. ^U	0.0	0.0	n.s. ^U
05.07.2019	3	0.3	0.5	0.3	0.5	n.s. ^U	0.0	0.0	n.s. ^U
06.07.2019	4	0.8	1.5	1.0	1.2	n.s. ^U	0.5	1.0	n.s. ^U
07.07.2019	5	0.5	0.6	0.3	0.5	n.s. ^D	1.3	1.9	n.s. ^D
08.07.2019	6	0.8	1.5	0.3	0.5	n.s. ^U	0.5	0.6	n.s. ^U
09.07.2019	7	0.5	1.0	0.0	0.0	n.s. ^U	1.0	1.4	n.s. ^U

10.07.2019	8	1.0	0.8	1.0	1.4	n.s. ^B	0.5	1.0	n.s. ^B
11.07.2019	9	1.0	1.4	0.3	0.5	n.s. ^U	0.8	1.5	n.s. ^U
12.07.2019	10	0.0	0.0	0.0	0.0	n.s. ^U	0.0	0.0	n.s. ^U
Mean (ΣDAA 0aa + 1 to 10)		0.6	1.1	0.3	0.7	n.s. ^B	0.5	1.0	n.s. ^B
13.07.2019	11	0.0	0.0	0.0	0.0	-	0.0	0.0	-
14.07.2019	12	0.3	0.5	0.0	0.0	n.s. ^U	0.0	0.0	n.s. ^U
15.07.2019	13	0.0	0.0	0.0	0.0	-	0.0	0.0	-
16.07.2019	14	0.0	0.0	0.0	0.0	-	0.0	0.0	-
17.07.2019	15	0.3	0.5	0.0	0.0	n.s. ^U	0.8	1.5	n.s. ^U
18.07.2019	16	0.0	0.0	0.0	0.0	-	0.0	0.0	-
19.07.2019	17	0.0	0.0	0.0	0.0	-	0.0	0.0	-
20.07.2019	18	0.0	0.0	0.3	0.5	n.s. ^U	0.3	0.5	n.s. ^U
21.07.2019	19	0.0	0.0	0.0	0.0	-	0.0	0.0	-
22.07.2019	20	0.0	0.0	0.0	0.0	-	0.0	0.0	-
23.07.2019	21	0.0	0.0	0.0	0.0	n.s. ^U	0.3	0.5	n.s. ^U
24.07.2019	22	0.5	1.0	0.0	0.0	n.s. ^U	0.0	0.0	n.s. ^U
25.07.2019	23	0.0	0.0	0.3	0.5	n.s. ^U	0.0	0.0	n.s. ^U
26.07.2019	24	0.0	0.0	0.0	0.0	n.s. ^U	0.3	0.5	n.s. ^U
27.07.2019	25	0.0	0.0	0.0	0.0	-	0.0	0.0	-
28.07.2019	26	0.3	0.5	0.0	0.0	n.s. ^U	0.0	0.0	n.s. ^U
29.07.2019	27	0.0	0.0	0.3	0.5	n.s. ^U	0.0	0.0	n.s. ^U
30.07.2019	28	0.0	0.0	0.0	0.0	-	0.0	0.0	-
Mean (11 to 28)		0.1	0.3	0.0	0.2	n.s. ^B	0.1	0.4	n.s. ^B
Mean (Σ0aa + 1 to 28)		0.3	0.8	0.1	0.5	n.s. ^B	0.2	0.7	n.s. ^B

Sum of dead individuals found in dead bee traps and on sheets in the tunnels (DAA 3 to DAA 10) / dead individuals found in dead bee traps, only (DAA 11 to 28); — not applicable; n.s. = not statistically significantly different compared to the control group; * statistically significantly different compared to the control group (p<0.05); D-ANOVA / Dunnett's test; U-U Test; DAA = days after application; ba = before application; aa = after application; SD = standard deviation; n = number

C. Foraging activity

The mean foraging activity during the pre-exposure period were comparable for all treatment groups. Apart of a very short lasting reduction of the mean foraging activity in the test item group tunnels (DAA 0aa, 15 minutes after application), no adverse effects of the test item on the mean foraging activity compared to that of the control group was observed (see table below).

The mean foraging activity in the reference item group was on a normal level within the first half hour after the application. Afterwards, a significantly decrease in comparison with the control group could be observed on 9 of 10 assessment days. An obvious test item related adverse effect on the foraging activity can be excluded.

Table A 33: Mean foraging activities in the different treatment groups

Date [dd.mm.yyyy]	DAA	Control [bees/m ² /15s]		Test item [bees/m ² /15s]			Reference item ²⁾ [bees/m ² /15s]		
		mean	±SD	mean	±SD	statistics	mean	±SD	statistics
29.06.2019	-3	10.8	5.5	12.3	7.0	n.s. ^B	9.8	3.8	n.s. ^B
30.06.2019	-2	12.6	2.1	15.3	3.8	n.s. ^B	13.3	2.6	n.s. ^B
01.07.2019	-1	12.6	2.7	13.3	3.3	n.s. ^B	12.1	2.6	n.s. ^B
02.07.2019	0ba	19.0	2.4	19.9	4.2	n.s. ^B	17.8	6.6	n.s. ^B
Mean DAA -3 to 0ba		13.7	4.6	15.2	5.5	n.s. ^B	13.2	5.0	n.s. ^B
03.07.2019	0aa1	19.3	3.0	3.9	2.8	<0.001* ^B	17.6	5.5	n.s. ^B

	0aa2	22.3	2.7	14.8	4.1	n.s. ^D	15.5	4.8	n.s. ^D
	0aa3	22.5	2.0	25.1	6.7	n.s. ^D	7.3	4.7	0.001 ^{*D}
	0aa4	22.3	2.8	24.7	3.4	n.s. ^U	4.9	3.9	0.010 ^{*U}
	0aa5	22.3	2.8	22.2	3.4	n.s. ^U	1.6	1.2	0.010 ^{*U}
	0aa6	27.8	1.8	22.6	3.5	0.009 ^{*U}	0.3	0.5	0.009 ^{*U}
	0aa7	25.3	3.0	22.4	2.9	n.s. ^U	0.5	1.0	0.010 ^{*U}
02.07.2019	0aa-(mean)	23.1	3.6	19.4	8.1	0.014 ^{*U}	6.8	7.5	0.014 ^{*U}
03.07.2019	1	21.9	2.4	22.3	3.1	n.s. ^U	0.0	0.0	0.007 ^{*U}
04.07.2019	2	25.3	2.3	22.3	4.6	n.s. ^U	0.0	0.0	0.007 ^{*U}
05.07.2019	3	23.2	2.3	23.9	4.9	n.s. ^U	0.0	0.0	0.007 ^{*U}
06.07.2019	4	24.0	6.4	21.8	5.3	n.s. ^U	0.4	0.8	0.009 ^{*U}
07.07.2019	5	11.3	4.2	7.9	3.7	n.s. ^D	0.3	0.6	<0.001 ^{*D}
08.07.2019	6	20.0	3.8	19.6	4.5	n.s. ^U	0.0	0.0	0.007 ^{*U}
09.07.2019	7	14.8	2.6	12.8	4.0	n.s. ^D	0.1	0.3	<0.001 ^{*D}
10.07.2019	8	19.0	6.2	16.3	4.1	n.s. ^U	2.5	2.4	0.001 ^{*D}
11.07.2019	9	5.8	5.5	9.8	5.1	n.s. ^D	3.3	2.3	n.s. ^D
12.07.2019	10	7.8	1.9	5.5	2.4	n.s. ^U	0.8	2.1	0.010 ^{*U}
Mean DAA 0aa to 10		19.5	7.0	17.5	8.0	n.s. ^U	3.2	5.8	0.014 ^{*U}

7 assessments on DAA 0aa (0aa1: 15 minutes aa, 0aa2: 30 minutes aa, 0aa3: 45 minutes aa, 0aa4: 1 hour aa, 0aa5: 2 hours aa, 0aa6 4 hours aa, 0aa7: 6 hours aa); DAA = days after application (ba = before application, aa = after application); SD = standard deviation; n.s. = foraging activity not statistically significantly different compared to the control group; * = foraging activity statistically significantly different ($p > 0.05$) compared to the control foraging activity; D = ANOVA / Dunnett's test; U = U Test

D. Behavioural abnormalities

The behaviour of the honeybees before application was generally inconspicuous in the control and test item groups. No unusual behaviour was observed in the control group and in the test item group, where only in one instance (DAA 2 in T1) one single bee was observed while cramping on the linen. Immediately after the application of the reference item, some worker bees showed behavioural changes or cramping while sitting in the dead bee trap. This behaviour was only visible during DAA 0aa and DAA 3. Details on all observations are given in the table below.

Table A 34: Conspicuous observations during the entire study period groups

Date [dd.mm.yyyy]	DAA	Replicate	Observation
02.07.2019	0aa	R1	13 bees cramping in DBT (approx. 1 hour after application);
			50 bees cramping in DBT (approx. 2 hours after application);
			20 bees cramping in DBT (approx. 4 hours after application);
			20 bees cramping in DBT (approx. 6 hours after application);
			30 bees cramping in DBT (approx. 10 hours after application)
		R2	3 bees paralysed on linen and 8 bees cramping in DBT (approx. 1 hour after application);
			8 bees cramping in DBT (approx. 2 hours after application);
			40 bees cramping in DBT (approx. 4 hours after application);
			40 bees cramping in DBT (approx. 6 hours after application);
			25 cramping bees in DBT (approx. 10 hours after application)
		R3	3 bees paralysed and 6 bees cramping in DBT (approx. 1 hour after application);
			20 bees cramping in DBT (approx. 4 hours after application);
			1-20 bees cramping in DBT (approx. 6 hours after application)
		R4	9 bees paralysed, 1 bee cramping in DBT and 1 bee aggressive (approx. 1 hour after application);
			9 bees paralysed in DBT and 2 bees cramping on linen (approx. 2 hours after application);
			50 bees cramping in DBT (approx. 4 hours after application);
			50 bees cramping in DBT (approx. 6 hours after application);

			~50 bees cramping in DBT (approx. 10 hours after application)
03.07.2019	1	R1	11 bees cramping in DBT
		R2	19 bees cramping in DBT
		R3	5 bees cramping in DBT
		R4	3 bees cramping in DBT
04.07.2019	2	T1	1 bee cramping on the linen
		R1	2 bees cramping in DBT
		R2	11 bees cramping in DBT
05.07.2019	3	R4	1 bee cramping in DBT

E. Condition of the colonies

The pre-application colony assessment indicated that honeybee colonies were healthy, all brood stages were present, colony strengths were comparable and a sufficient amount of nectar and pollen was available in all colonies (see tables below).

After the application, the development (colony strength, brood and food) of the control and the test item colonies were in a natural range and comparable during the whole post application period. No indication of a test item related adverse effect on the colony development was given.

Table A 35: Mean colony strength (number of bees) per colony at the assessment dates

Date {dd.mm. yyyy}	DAA	Control-group			Test-item-group			Reference-item-group		
		absolute [n] ¹⁾		Relative develop- ment ²⁾	absolute [n] ¹⁾		Relative develop- ment ²⁾	absolute [n] ¹⁾		Relative develop- ment ²⁾
		mean	±SD		mean	±SD		mean	±SD	
01.07.2019	1	5,379	1,200	100%	6,321	790	100%	4,729	868	100%
09.07.2019	7	7,979	666	148%	8,418	1,341	133%	7,394	2,018	156%
16.07.2019	14	8,206	1,564	153%	8,158	1,285	129%	7,020	827	148%
23.07.2019	21	9,328	1,762	173%	8,141	1,764	129%	6,711	1,855	142%
30.07.2019	28	9,279	2,079	173%	9,214	1,323	146%	7,166	2,717	152%

DAA = days after application; 1) absolute mean strength of the colonies ± standard deviation; 2) relative development of the mean strength of the colonies (mean strength of the colonies at the first assessment was set as basis)

Table A 36: Mean number of brood cells per colony at the assessment dates

Date {dd.mm. yyyy}	DAA	Control-group			Test-item-group			Reference-item-group		
		Absolute [n] ¹⁾		Relative develop- ment ²⁾	Absolute [n] ¹⁾		Relative develop- ment ²⁾	Absolute [n] ¹⁾		Relative develop- ment ²⁾
		Mean	±SD		Mean	±SD		Mean	±SD	
01.07.2019	1	17,450	2,144	100%	16,800	2,123	100%	19,650	5,691	100%
09.07.2019	7	15,050	1,754	86%	15,450	2,087	92%	16,150	3,243	82%
16.07.2019	14	13,950	1,660	80%	14,950	2,778	89%	11,550	4,787	59%
23.07.2019	21	14,750	2,374	85%	11,850	5,308	71%	10,100	3,845	51%
30.07.2019	28	17,250	4,054	99%	14,600	8,052	87%	12,000	5,574	61%

DAA = days after application; 1) absolute mean amount of all brood cells ± standard deviation; 2) relative development of the mean amount of all brood cells (amount of all brood cells at the first assessment was set as basis)

Table A 37: Mean number of brood cells per colony with eggs at the assessment dates

Date {dd.mm. yyyy}	DAA	Control-group			Test-item-group			Reference-item-group		
		Absolute [n] ¹⁾		Relative develop- ment ²⁾	Absolute [n] ¹⁾		Relative develop- ment ²⁾	Absolute [n] ¹⁾		Relative develop- ment ²⁾
		Mean	±SD		Mean	±SD		Mean	±SD	
01.07.2019	1	2,950	1,170	100%	3,300	416	100%	3,350	1,754	100%

09.07.2019	7	2,550	1,269	86%	2,000	1,244	61%	4,350	2,516	130%
16.07.2019	14	2,100	902	71%	2,150	681	65%	2,850	342	85%
23.07.2019	21	3,150	1,792	107%	3,150	2,181	95%	2,600	1,826	78%
30.07.2019	28	2,600	1,781	88%	2,100	2,511	64%	1,650	1,248	49%

DAA = days after application; 1) absolute mean number of eggs \pm standard deviation; 2) relative development of the mean number of eggs (mean number of the eggs at the first assessment was set as basis)

Table A 38: Mean number of brood cells with larvae per colony at the assessment dates

Date {dd.mm. yyyy}	DAA	Control group			Test item group			Reference item group		
		Absolute [n] ¹⁾		Relative develop- ment ²⁾	Absolute [n] ¹⁾		Relative develop- ment ²⁾	Absolute [n] ¹⁾		Relative develop- ment ²⁾
		Mean	\pm SD		Mean	\pm SD		Mean	\pm SD	
01.07.2019	-1	5,050	985	100%	4,750	823	100%	5,750	806	100%
09.07.2019	7	2,950	772	58%	3,650	1,370	77%	2,200	766	38%
16.07.2019	14	3,250	1,215	64%	3,900	346	82%	3,200	1,479	56%
23.07.2019	21	4,050	1,025	80%	2,200	1,558	46%	2,100	841	37%
30.07.2019	28	4,250	1,509	84%	3,050	2,446	64%	3,700	2,676	64%

DAA = days after application; 1) absolute mean number of larvae \pm standard deviation; 2) relative development of the mean number of larvae (mean number of larvae at the first assessment was set as basis)

Table A 39: Mean number of brood cells with pupae (capped brood cells) per colony at the assessment dates

Date {dd.mm. yyyy}	DAA	Control group			Test item group			Reference item group		
		Absolute [n] ¹⁾		Relative develop- ment ²⁾	Absolute [n] ¹⁾		Relative develop- ment ²⁾	Absolute [n] ¹⁾		Relative develop- ment ²⁾
		Mean	\pm SD		Mean	\pm SD		Mean	\pm SD	
01.07.2019	-1	9,450	2,317	100%	8,750	2,521	100%	10,550	3,616	100%
09.07.2019	7	9,550	2,516	101%	9,800	1,071	112%	9,600	1,306	91%
16.07.2019	14	8,600	1,600	91%	8,900	2,324	102%	5,500	4,210	52%
23.07.2019	21	7,550	1,928	80%	6,500	2,802	74%	5,400	4,239	51%
30.07.2019	28	10,400	993	110%	9,450	3,810	108%	6,650	3,284	63%

DAA = days after application; 1) absolute mean number of pupae \pm standard deviation; 2) relative development of the mean number of pupae (mean number of pupae at the first assessment was set as basis)

Food

The supply of food was sufficient in all colonies until the assessment on DAA 14. As the colonies were caged inside the tunnels the food stores, more specifically the pollen stores, decreased due to the reduced crop surface inside the tunnels and the withering plants towards the end of the exposure phase (last day inside the tunnels: DAA 10). Hence, after the assessment conducted on DAA 14, it was deemed necessary to feed the colonies with 0.5 kg bee fondant (Nektapoll) and 1.25 kg food paste (Apifonda) on 17 July 2019 (DAA 15) to avoid starvation. All colonies were fed at the same time and with same amounts. Overall, all treatment groups showed similar fluctuations in food storage cells indicating no test item related effects up to the end of the study. The results are listed in the tables below:

Table A 40: Mean number of food (pollen and nectar) cells per colony at the assessment dates

Date {dd.mm. yyyy}	DAA	Control group			Test item group			Reference item group		
		Absolute [n] ¹⁾		Relative develop- ment ²⁾	Absolute [n] ¹⁾		Relative develop- ment ²⁾	Absolute [n] ¹⁾		Relative develop- ment ²⁾
		Mean	\pm SD		Mean	\pm SD		Mean	\pm SD	
01.07.2019	-1	16,404	3,219	100%	17,850	1,330	100%	15,902	4,741	100%
09.07.2019	7	12,150	1,473	74%	10,950	772	61%	9,800	2,926	62%
16.07.2019	14	9,650	1,914	59%	9,150	1,248	51%	7,650	2,306	48%
23.07.2019	21	13,150	2,106	80%	11,350	1,799	64%	11,850	3,924	75%

30.07.2019	28	11,450	2,715	70%	10,200	2,406	57%	10,950	4,225	69%
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DAA = days after application; 1) absolute mean food storage cells \pm standard deviation; 2) relative development of food storage cells (food storage cells at the first assessment was set as basis)

Table 41: Mean number of honey storage cells per colony at the assessment dates

Date {dd.mm. yyyy}	DAA	Control group			Test item group			Reference item group		
		Absolute [n] ¹⁾		Relative develop- ment ²⁾	Absolute [n] ¹⁾		Relative develop- ment ²⁾	Absolute [n] ¹⁾		Relative develop- ment ²⁾
		Mean	\pm SD		Mean	\pm SD		Mean	\pm SD	
01.07.2019	-1	15,200	3,641	100%	15,150	1,660	100%	13,250	4,190	100%
09.07.2019	7	11,100	1,997	73%	9,550	1,136	63%	8,700	2,543	66%
16.07.2019	14	9,400	1,980	62%	8,550	1,708	56%	7,100	2,151	54%
23.07.2019	21	10,400	2,033	68%	9,750	1,865	64%	9,800	4,056	74%
30.07.2019	28	9,600	2,394	63%	8,700	2,132	57%	9,050	3,890	68%

DAA = days after application; 1) absolute mean honey/nectar stores \pm standard deviation; 2) relative development of the mean honey/nectar stores (mean honey/nectar stores at the first assessment was set as basis)

Table A 42: Mean number of pollen storage cells per colony at the assessment dates

Date {dd.mm. yyyy}	DAA	Control group			Test item group			Reference item group		
		Absolute [n] ¹⁾		Relative develop- ment ²⁾	Absolute [n] ¹⁾		Relative develop- ment ²⁾	Absolute [n] ¹⁾		Relative develop- ment ²⁾
		Mean	\pm SD		Mean	\pm SD		Mean	\pm SD	
01.07.2019	-1	1,200	542	100%	2,700	1,553	100%	2,652	1,857	100%
09.07.2019	7	1,050	640	88%	1,400	589	52%	1,100	1,206	42%
16.07.2019	14	250	191	21%	600	712	22%	550	681	21%
23.07.2019	21	2,750	870	229%	1,600	365	59%	2,050	943	77%
30.07.2019	28	1,850	719	154%	1,500	346	56%	1,900	1,510	72%

DAA = days after application; 1) absolute mean of pollen storage cells \pm standard deviation; 2) relative development of mean pollen storage cells (mean pollen storage cells at the first assessment was set as basis)

F. Weather data

Rainfall at the field site occurred on two out of 14 days (DAA 5 and DAA 10), whereas no rainfall occurred on the other days. It ranged on from 3.5 mm on DAA 5 to 8.0 mm on DAA 10. The minimum temperatures during the time at the field site ranged from 5.8 °C (DAA 8) to 20.3 °C (DAA -1) and the maximum temperatures from 19.2 °C (DAA 9) to 38.1 °C (DAA -2) and provided suitable conditions for a high foraging activity. At the monitoring site, precipitation occurred on five out of 18 recording days (DAA 11, DAA 12, DAA 19, DAA 26, and DAA 27). It ranged from 1.0 mm (DAA 12) to 17.0 mm (DAA 26). The minimum temperatures at the monitoring site ranged from 11.1 °C (DAA 14) to 27.5 °C (DAA 23), the maximum temperatures from 16.7 °C (DAA 12) to 36.9 °C (DAA 23).

G. Study plan deviations

- The mean colony strength of the control item group was 5,379 \pm 1,200 bees/colony at the initial colony assessment, that of the reference item group was 4,729 \pm 868 bees instead of at 6,000 to 8,000 bees/colony.
- The application took place at BBCH growth stage of the plants of 65-67, instead of at 63-65 because the crop was developing very fast due to the heat and dryness during the days after set up.
- Due to organizational reason, a Non-GLP data logger was used to record the rainfall and no data logger was inserted in one of the tunnels. Temperature and humidity were obtained from the Rifcon weather station, ~2 km distance to the field site.
- By mistake, no mortality assessment was conducted for Colony C4 on 15.07.2019 (DAA 13). The mortality was on the same level during the days before and afterwards.
- Due to the weather (low temperature and rain/drizzle), there were no active foragers and no sample

was taken.

- As the colonies were only allocated to the different replicates on DAA 0, pre-application samples on DAA 1 were taken in all tunnels. Therefore, pre-application flower samples were also taken in the reference item tunnels.
- As the freezer, in which the samples were stored, is equipped with a temperature monitoring system, the temperature during storage was recorded only with a data logger, instead of with additionally with a min/max thermometer.
- Due to heavy rain in the morning on DAA 10 (15 mm recorded on DAA 11), the mortality assessment was conducted at noon between 12:55 p.m. and 01:17 p.m. instead of in the morning.
- No B samples were prepared as requested by the study plan. No B samples needed to be prepared as there was no request for further analytics by the analytical test site.
- C residue, DAA 1: only A sample of insufficient sample size was available (0.38 g).
- T1 residue, DAA 1: only A sample of insufficient sample size was available (0.25 g).
- T2 residue, DAA 1: only A sample of insufficient sample size was available (0.15 g).
- T2 residue, DAA 7–8: only A sample of insufficient sample size was available (0.44 g). T2 residues, DAA 9–10: only A sample of insufficient sample size was available: 0.07 g.

The deviations have no effects on the outcome of the study.

H. Validity of the test:

The study is considered to be valid since the brood termination rate of the reference item group was statistically significantly higher compared to the control group. Additionally, the mean foraging activity shortly before the water treatment of the control, the second test item and the reference item application was greater than 10 bees/m².

Moreover, analytical results demonstrated that honeybees were exposed to ADM.1351.F.1.A inside the tunnels throughout the entire exposure period within the tunnels. A residue decline of both active substances could be observed in nectar and pollen.

Thus, the study is considered valid.

III. Assessment and conclusion

To assess the potential effects of ADM.1351.F.1.A (Spyrale) on colony development of honeybees (*Apis mellifera* L.), the test item was applied at a nominal rate of 1.0 L product/ha (371 g fenpropidin/ha, 109 g difenoconazole/ha) on *Phacelia tanacetifolia* during honeybee flight under semi-field conditions based on OEPP/EPPPO No.170 (4) (2010). The application of ADM.1351.F.1.A did not cause adverse effects on the survival of adult worker bees, bee pupae, behaviour, colony strength and colony development. Additionally, results for the reference item (Danadim Progress, 480 g/ha dimethoate) treatment group together with additionally recorded parameters such as foraging activity and the analytical results show that the test system provided adequate exposure and sensitivity.

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:	<p>The study was conducted in line with the respective guideline with no deviations.</p> <p>All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>LR₅₀ > 2.0 L prod./ha ER₅₀ reproduction > 2.0 L prod./ha</p>
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Reference:	KCP 10.3.2.2/01
Report:	Effects of ADM.03502.F.1.A on the parasitic wasp <i>Aphidius rhopalosiphi</i> (DESTEFANI-PEREZ) in a laboratory test, Röhlig, U., 2020a, report no.: 2048NAL0006, sponsor no.: 000104847
Guideline(s):	IOBC (Mead-Briggs <i>et al.</i> , 2000)
Deviations:	None
GLP:	Yes
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.03502.F.1.A (250 g fenpropidin/L, 175 g prothioconazole/L, nominal) after spray application onto glass plates at rates of 0.125, 0.250, 0.5, 1.0 and 2.0 L prod./ha. A control group exposed to tap deionised water without only and a toxic reference (dimethoate) 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 LR₅₀ for estimated to be > 2.0 L prod./ha. The NOER for mortality was considered to be ≥ 2.0 L prod./ha. The ER₅₀ for reproduction was estimated to be > 2.0 L prod./ha. The NOER for reproduction was considered to be ≥ 2.0 L prod./ha.

I. Materials and methods

A. Materials

1. Test material: ADM.03502.F.1.A
 Lot/Batch no.: 1191-101219-01
 Content/Purity: 250 g fenpropidin/L, 175 g prothioconazole/L (nominal)
 253.7 g fenpropidin/L, 175.9 g prothioconazole/L (analysed)
 Control: Deionised water (200 L/ha)
 Toxic reference: Danadim Progress / Dimethoate 400 g/L (nominal), 411.2 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 replicates/group), reproduction phase: 1 female per replicate (15 replicates per group)

Feeding: cotton wool soaked with a 1:3 v/v solution of honey
 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).
 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

Test procedure:	> 100 adult and nymphal aphids (reared in the laboratory of the test facility) and covered at the top of the cylinder with gauze.
Test substrate:	laboratory test under worst-case conditions, rate-response test wheat seedlings (<i>Triticum</i>) planted in potting soil (during parasitisation)
Test duration.	Mortality test: 48 h Reproduction test: 24 h for parasitisation + 11 further days for development of wasps)
4. Test conditions -	
Temperature:	19 - 22°C
Relative humidity:	62 - 74 %
Photoperiod:	16 h light/8 h dark
Light intensity:	1070 lux (exposure phase), 2520 lux (parasitisation phase), 6590 lux (reproduction phase)

B. Study design and method

1. In-life dates: June 29 to July 13, 2020 (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.03502.F.1.A (250 g fenpropiidin/L, 175 g prothioconazole/L, nominal) sprayed on glass plates at application rates of 0.125, 0.250, 0.5, 1.0 and 2.0 L prod./ha. Additional test units were treated with deionised water for the water control and with dimethoate 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:

For statistical analysis 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 FISHER Test after BONFERRONI-HOLM (test item) and Fisher's Exact Binomial Test (reference item) for test as

distribution-free tests which do not require testing for normality or homoscedasticity prior to analysis. The accepted significance level was $\alpha = 0.05$, one-sided greater. Reproductive capacity was analysed for statistical significance using WILLIAMS-t-test $\alpha = 0.05$, one-sided smaller) following SHAPIRO-WILK's test on normal distribution, LEVENE's test on variance homogeneity. Since the mortality and the reduction of reproduction was < 50 % up to the highest test item rates tested, a calculation of the LR₅₀ (median lethal rate) and ER₅₀ (median effect rate) was not possible and were thus assumed to be higher than the maximum test item rate tested.

II. Results and discussions

A. Mortality

After 48 hours, in the water-treated control a mean mortality of 5.0 % was observed. In the test item treatments, the mean mortality ranged between 2.5 and 22.5 %. This resulted in corrected mortality rates between -2.6 % and 18.4 %. No statistically significant increased mortalities were determined in any of the test item rates compared to the control (Multiple Sequentially-rejective FISHER Test after BONFER-RONI-HOLM, $\alpha = 0.05$, one-sided greater). Since the corrected mortality was ≤ 50 % up to the highest test item rate tested, the LR_{50} was assumed to be > 2.0 L/ha prod./ha and the NOER (no observed effect rate) for mortality was determined to be ≥ 2.0 L prod./ha . The reference item caused a mean mortality of 100 %, resulting in a corrected mortality of 100 %.

Table A 43: Mortality of *Aphidius rhopalosiphi*

Treatment group ¹		Dead wasps (number)	Moribund wasps (number)	Surviving wasps (number)	Mortality ² (%)	Corrected mortality (Abbott) [%]
Control	deionised water	2	0	38	5.0	---
ADM.03502.F.1.A (L prod./ha)	0.125	1	0	39	2.5 (n.s.)	-2.6
	0.250	1	0	39	2.5 (n.s.)	-2.6
	0.5	2	0	38	5.0 (n.s.)	0
	1.0	3	2	35	12.5 (n.s.)	7.9
	2.0	7	2	31	22.5 (n.s.)	18.4
Reference item (ml prod./ha)	0.3	40	0	0	100*	100

10 wasps per replicate were introduced (4 replicates per treatment)

1 Application rate in 200 L water/ha

2 mortality including dead and moribund wasps 48 hours after exposure

n.s. not statistically significant different compared to the control: Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm ($\alpha = 0.05$, one-sided greater) for test item * statistically significant different compared to the control: Fisher's Exact Binomial Test ($\alpha = 0.05$, one-sided greater) for reference item

B. Reproduction

The mean number of mummies produced per female in the respective test item treatment groups was between 18.3 and 21.7, compared to the control value of 20.9 mummies/female. No statistically significant different reproduction rates were observed in any of the test item rates compared to the control (WILLIAMS-t-test, $\alpha = 0.05$, one-sided smaller). Since the reduction of the reproduction was ≤ 50 % up to the highest test item rate tested, the ER_{50} was assumed to be > 2.0 L prod./ha and the NOER (no observed effect rate) for reproduction was determined to be ≥ 2.0 L prod./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 44: Reproduction of *Aphidius rhopalosiphi*

Treatment group ¹		Mean number of mummies/female ²	Effect on reproduction (%) ³
Control	deionised water	20.9	---
ADM.03502.F.1.A (L prod./ha)	0.125	21.3 (n.s.)	-1.9
	0.250	20.7 (n.s.)	1.0
	0.5	21.7 (n.s.)	-3.8
	1.0	20.1 (n.s.)	3.8
	2.0	18.3 (n.s.)	12.4

1 Application rate in 200 L water/ha

2 the mean number of mummies/female was calculated from the number of mummies per surviving female

3 Reduction of the parasitisation rate, relative to control. A negative value indicates a higher and a positive value indicates a lower reproduction relative to the control.

n.s. not statistically significantly different compared to the control (WILLIAMS-t-test, $\alpha = 0.05$, one-sided smaller)

C. Validity of the test:

Validity criterion according to Mead-Briggs <i>et al.</i> (2000)	Results of the study
The mortality in the control treatment should not exceed 13 %.	The mean mortality in the control treatment was 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 20.9 per female. No female wasp 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.03502.F.1.A (250 g fenpropidin/L, 175 g prothioconazole/L, nominal) applied to glass plates. After exposure to 0.125, 0.250, 0.5, 1.0 and 2.0 L prod./ha, the LR₅₀ for estimated to be > 2.0 L prod./ha. The NOER for mortality was considered to be ≥ 2.0 L prod./ha. The ER₅₀ for reproduction was estimated to be > 2.0 L prod./ha. The NOER for reproduction was considered to be ≥ 2.0 L prod./ha. The study is considered valid (see: “C. Validity criteria” above).

Comments of zRMS:	<p>The study was conducted in line with the respective guideline with no deviations.</p> <p>All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>LR₅₀ = 1.485 L prod./ha ER₅₀ > 1.0 L prod./ha</p>
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Reference:	KCP 10.3.2.2/02
Report:	Effects of ADM.03502.F.1.A on the predatory mite <i>Typhlodromus pyri</i> SCHEUTEN in a laboratory test, Röhlig, U., 2020b, report no.: 2048NTL0006, sponsor no.: 000104846
Guideline(s):	IOBC (BLÜMEL <i>et al.</i> 2000)
Deviations:	None
GLP:	Yes
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.03502.F.1.A (250 g fenpropidin/L, 175 g prothioconazole/L, nominal) after spray application onto glass plates at rates of 0.125, 0.250, 0.5, 1.0 and 2.0 L prod./ha. 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 LR₅₀ for *Typhlodromus pyri* was estimated to be 1.485 L prod./ha. The NOER for mortality was considered to be 0.5 L prod./ha. The ER₅₀ was estimated to be > 1.0 L prod./ha. The NOER for reproduction was considered to be 0.5 L prod./ha.

I. Materials and methods

A. Materials

1. Test material: ADM.03502.F.1.A

- | | |
|------------------|---|
| Lot/Batch no.: | 1191-101219-01 |
| Content/Purity: | 250 g fenpropidin/L, 175 g prothioconazole/L (nominal)
253.7 g fenpropidin/L, 175.9 g prothioconazole/L (analysed) |
| Control: | Deionised water (200 L/ha) |
| Toxic reference: | Danadim Progress / Dimethoate 400 g/L (nominal), 411.2 g/L (analysed) |
2. Test organisms -
- | | |
|-------------------|---|
| Species: | <i>Typhlodromus pyri</i> (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, <i>Pinus nigra</i>) and birch (<i>Betula pendula</i>), 1:1 |
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
reproduction test: further 7 days |
4. Test conditions -
- | | |
|--------------------|---------------------|
| Temperature: | 23 - 25 °C |
| Relative humidity: | 66 - 73 % |
| Photoperiod: | 16 h light/8 h dark |
| Light intensity: | 1950 lux |

B. Study design and method

1. In-life dates: May 12 to May 26, 2020 (experimental phase)
2. Test design:

Protonymphs were exposed to dried spray residues of different application rates (0.125, 0.250, 0.5, 1.0 and 2.0 L prod./ha) 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 Chi2-2x2 Table test after BONFERRONI-HOLM for the test item and the Chi2-2x2 Table test for reference item as distribution-free tests which does not require testing for normality or homoscedasticity prior to analysis. The accepted significance level was $\alpha = 0.05$, one-sided greater. Reproduction was analysed for statistical significance using WILLIAMS-t-test, $\alpha = 0.05$, one-sided smaller, following SHAPIRO-WILK's test for normal distribution, LEVENE's test procedure for variance homogeneity. For calculation of the LR₅₀ (median lethal rate) SPEARMAN-KARBER procedure was used. The reduction of the reproduction in all test item treatment groups, which were tested, was less than 50 % compared to the control group, hence, a calculation of the ER₅₀ (median effect rate) was not possible and the ER₅₀ was assumed to be higher than the maximum rate tested.

II. Results and discussions

A. Mortality

After 7 days, a mean mortality of 1.0 % was observed in the control. In the test item treatments, mean mortalities ranged between 1.0 and 69.0 %, resulting in corrected mortality rates between 0 and 68.7 %. No statistically significant effects on mortality were determined at treatment rates up to and including 0.5 L prod./ha, whereas at higher rates mortality was statistically significant increased compared to the control (Multiple Sequentially-rejective Chi2-2x2 Table test after BONFERRONI-HOLM, $\alpha = 0.05$, one-sided greater). The LR₅₀ was calculated to be 1.485 L prod./ha. The NOER (no observed effect rate) for mortality was determined to be 0.5 L prod./ha. The reference item caused 76.0 % mortality in exposed mites, resulting in a corrected mortality of 75.8 %.

Table A 45: Mortality in *Typhlodromus pyri* after 7 days of exposure to treated glass plates

Treatment	Rate ¹ (L prod./ha)	Mortality ² (%)	Corrected mortality ³ (%)
Control	---	1.0	---
ADM.03502.F.1.A (L prod./ha)	0.125	2.0 (n.s.)	1.0
	0.250	1.0 (n.s.)	0
	0.5	3.0 (n.s.)	2.0
	1.0	22.0*	21.2
	2.0	69.0*	68.7
Toxic reference (mL prod./ha)	15	76.0*	75.8

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 Chi2 -2x2 Table test after BONFERRONI-HOLM ($\alpha = 0.05$, one-sided greater) (test item) and Chi2-2x2 Table test ($\alpha = 0.05$, one-sided greater) (reference item).

³ mortality corrected according to ABBOTT (1925)

n.s. not statistically significant different compared to the control

* statistically significant different compared to the control

B. Reproduction

The mean reproduction rate in the control was 6.33 eggs/female. The mean reproduction rates in the test item treated groups were between 6.60 and 4.11 eggs/female. Thus, an effect on reproduction between - 4.3 % and 35.1 % was calculated for the test item treated groups compared to the control. No statistically significant effects on reproduction were determined at test item rates up to and including 0.5 L product/ha whereas at a rate of 1.0 L test item/ha reproduction performance was statistically significant lower compared to the control (WILLIAMS-t-test, $\alpha = 0.05$, one-sided smaller). The ER₅₀ was estimated to be > 1.0 L product/ha. The NOER (no observed effect rate) for reproduction was 0.5 L 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 46: Effects on reproduction in *Typhlodromus pyri* after 7 days of exposure to treated glass plates

Treatment	Rate ¹⁾ (L prod./ha)	Mean number eggs per female ²⁾ (7 - 14 day)	Effects on reproduction ³⁾ (%)
Control	---	6.33	---
ADM.03502.F.1.A	0.125	6.40 (n.s.)	-1.1
	0.250	6.60 (n.s.)	-4.3
	0.5	6.53 (n.s.)	-3.2
	1.0	4.11*	35.1

Application rate in 200 L water/ha

2 Results for reproduction compared by WILLIAMS-t-test ($\alpha = 0.05$, one-sided smaller)

3 Reproduction performance relative to control. A positive value indicates a lower and a negative value indicates a higher reproduction performance relative to the control.

n.s. not statistically significantly different compared to the control

* statistically significantly different compared to the control

C. Validity of the test:

Validity criterion according to Blümel <i>et al.</i> (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 1.0 %.
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 was 6.33 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 75.8%.

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

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.03502.F.1.A (250 g fenpropidin/L, 175 g prothioconazole/L, nominal) applied to glass plates. After exposure to 0.125, 0.250, 0.5, 1.0 and 2.0 L prod./ha, the 7-day LR₅₀ for *Typhlodromus pyri* was estimated to be 1.485 L prod./ha. The NOER for mortality was considered to be 0.5 L prod./ha. The ER₅₀ was estimated to be > 1.0 L prod./ha. The NOER for reproduction was considered to be 0.5 L prod./ha. The study is considered valid (see: “C. Validity criteria” above).

A 2.4 CP 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:	<p>The study was conducted in line with OECD 222 with no deviations.</p> <p>All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>56d NOEC \geq 1.400 mg prod./kg dw soil</p> <p>56d EC₁₀ = not determined as the maximum reduction was below 10 %.</p>
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Reference:	KCP 10.4.1.1/01
Report:	Effects of ADM.03502.F.1.A on the mortality, growth and reproduction of the earthworm <i>Eisenia fetida</i> in artificial soil, Friedrich, S., 2020a, report no.: 2048TEC0035, sponsor no.: 000104848
Guideline(s):	OECD 222 (2016)
Deviations:	None
GLP:	Yes
Acceptability/Reliability:	Yes
Duplication (if vertebrate study)	Not applicable

Executive summary

The effects of ADM.03502.F.1.A on mortality, biomass development and reproduction were investigated in an extended laboratory study over 56 days according to OECD Guideline 222 (2016). The test item was mixed into artificial soil at concentrations of 0.023, 0.041, 0.074, 0.133, 0.240, 0.432, 0.778 and 1.400 mg prod./kg soil dry weight. For the control treatment, the soil was left untreated. Four replicates were prepared for the test item treatment groups and 8 replicates were prepared for the control, each containing 10 earthworms. Assessment of worm mortality, body weight and feeding activity was carried out after 28 days, assessment of reproduction (number of juveniles) was carried out after 56 days. The mortality of adult worms ranged between 0 – 2.5 % in the test item treated groups and was 1.3 % in the control group. No statistically significant increased mortality compared to the control was observed at any concentration tested. The test item caused no statistically significant differences on the change in biomass (change in fresh weight after 4 weeks relative to initial fresh weight) compared to the control group at any concentration tested. The mean number of juvenile worms counted on day 56 was 265.6 in the control and between 277.3 and 257.5 in the test item group, meaning a reduction of the reproduction between -4.4 % and 3.1 % compared to the control. No statistically significant differences on the number of juveniles compared to the control group were observed at any concentration tested.

Based on the obtained results, the NOEC for mortality of the earthworm was determined to be \geq 1.400 mg prod./kg soil_{dw}. The LC₅₀ was assumed to be $>$ 1.400 mg prod./kg soil_{dw}, as no mortality \geq 50% was observed up to the highest concentration tested. The NOEC for biomass and reproduction was determined to be \geq 1.400 mg prod./kg soil_{dw}. The EC₁₀, EC₂₀ and EC₅₀ values for reproduction could not have been calculated as the maximum reduction was below 10 %. Thus, it can be concluded that these values were higher than 1.400 mg prod./kg soil_{dw}, the highest concentration tested.

I. Materials and methods

A. Materials

1. Test material: ADM.03502.F.1.A
 Lot/Batch no.: 1191-101219-01
 Content: 250 g fenpropidin/L, 175 g prothioconazole/L (nominal)
 253.7 g fenpropidin/L, 175.9 g prothioconazole/L (analysed)
 Density: 1.04 g/mL
 Control: deionised water mixed into artificial soil
 Toxic reference: Maypon Flow (Carbendazim, SC 500)

2. Test organisms -
 Species: *Eisenia fetida*
 Age: adult worms (with clitellum) approx. 7-month-old
 Source: purchased from Bias Labs Ltd, Unit 19, Enterprise Centre, Myregormie Place, Fife, UK, KY1 3PF

 Weight at test start: 300 - 446 mg
 No. of organisms: 10 worms/replicate, 4 replicates per treatment group, 8 replicates for the control

 Acclimatisation: at least 24 hours in the artificial substrate (with food)
 Feeding: 5 g of finely ground horse manure weekly

3. Test units and exposure –
 Type and size: Plastic vessels (approx. 16.5 cm x 12 cm x 6 cm), filled with 600 g soil_{dw}. 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: 69.5 % industrial sand, 20 % kaolin (kaolinite content > 30 %), 10 % sphagnum peat, 0.5 % calcium carbonate

4. Test conditions –
 pH value: 5.9 - 6.0 (test start), 5.67 - 5.77 (test end)
 Soil moisture: 55.0 - 56.1 % (test start), 54.8 - 55.6 % (test end) of the WHC_{max}
 Temperature: 19.4 - 21.8 °C
 Photoperiod: 16 hours light/8 hours dark
 Light intensity: 640 lux

B. Study design and method

1. In-life dates: June 24 to August 19, 2020 (experimental phase)
2. Test design:

Earthworms were acclimatised in a separate batch of the artificial soil (mixed with horse manure) for approximately 27 hours before test start. On the day of the test start, the test item was introduced by dispersing the quantity of test item required to obtain the desired test concentration in the volume of water required to hydrate the soil to 40-60 % of its water holding capacity (WHC). The control substrate contained the corresponding amount of deionised water only.

Each test vessel was then filled with the treated soil. After a randomising procedure according to the worm fresh eight, selected groups of 10 worms were randomly assigned to each treatment group. The individually weighed worms (10 worms/vessel) were placed on the surface of the soil. After approximately thirty minutes, the test vessels were closed with perforated transparent lids, which allowed gas exchange between substrate and atmosphere and access of light but prevented worms from escaping.

The test vessels were then set up at random in a controlled-environment test room. One day after application, 5 g air-dried and finely ground horse manure was scattered on the soil surface of each test vessel, which was moistened with 5 mL deionised water. The feeding interval was weekly during the first four weeks of the test. The weekly amount of manure (5 g) depended on the feeding activity, which was assessed by visual estimation of the food remaining on the surface before addition of new food.

After four weeks, the adult worms were removed from the test vessels. The number of surviving worms (adult mortality) and their biomass change were determined, behaviour (including feeding activity) and pathological symptoms were recorded. The adult worms were discarded after counting and weighing. Subsequently, the soil of each vessel was mixed carefully with 5 g manure. This was the last feeding occasion of the test. The test was then continued for another four weeks. At the final assessment after 8 weeks, the number of hatched juvenile earthworms in each test vessel was determined. The water content and pH of the artificial soil was also determined at day 56.

3. Statistics:

The endpoints were mortality, change of biomass (difference in fresh weight of surviving worms between test start and four weeks after treatment) and reproduction (the number of juveniles present). The arithmetic mean and the standard deviation per treatment and per control for reproduction, mortality and biomass were calculated. The statistical analysis was performed with the software ToxRat Professional 3.3.0 (2018). Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm was used to investigate the mortality for statistically significant difference. Williams-t-test was used to compare the biomass results (one-sided smaller) and the reproduction performance (one-sided smaller) of the control with the independent test item groups. Since the mortality of adult earthworms was < 50 % up to the highest test item concentration tested and the maximum reduction of reproduction was below 10 %, a calculation of the LC₅₀ as well as EC₁₀, EC₂₀ and EC₅₀ was not possible and were thus assumed to be higher than the maximum test item rate tested.

II. Results and discussions

A. Mortality

Mortality rates of 0 % - 2.5 % were recorded in the test item treatment groups and amounted to be 1.3 % in the control group. No statistically significant increased mortality compared to the control was observed at any concentration tested (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, $\alpha = 0.05$, one-sided greater). No pathological symptoms and no effects on behaviour (including feeding activity) of the worms were observed during the test. Based on the results of the study the LC₅₀ for mortality was assumed to be > 1.400 mg prod./kg soil_{dw} and the NOEC for mortality was determined to be ≥ 1.400 mg prod./kg soil_{dw}.

Table A 47: Effects of ADM.03502.F.1.A on the mortality of adult earthworms

Treatment group	ADM.03502.F.1.A (mg test item/kg soil _{dw})								
	Number of surviving adult worms per replicate (4 weeks after test start)								
Replicate	control	0.023	0.041	0.074	0.133	0.240	0.432	0.778	1.400
1	10	10	10	10	10	10	10	10	10
2	10	10	10	10	10	10	10	10	10
3	10	10	10	10	10	10	10	10	10
4	10	10	10	10	9	10	10	10	10
5	10								
6	9								

7	10								
8	10								
Mean	9.9	10.0	10.0	10.0	9.8	10.0	10.0	10.0	10.0
SD	0.4	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0
CV (%)	3.6	0.0	0.0	0.0	5.1	0.0	0.0	0.0	0.0
	Mortality [%]								
Mean	1.3	0.0	0.0	0.0	2.5	0.0	0.0	0.0	0.0

Mortality at each test item concentration not statistically significantly different compared to the control (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, $\alpha = 0.05$, one-sided greater)
SD: standard deviation, cv %: coefficient of variation, Calculations were performed with unrounded values.

B. Body weight change

At the start of the test, earthworm fresh weight ranged from 300 – 446 mg/worm. The weight change of adult worms ranged between 24.0 % and 29.1 % in the test item groups and was 26.4 % in the control group. Statistical analysis displayed no significant differences compared to the control (Williams-t-test, $\alpha = 0.05$, one-sided smaller) at any test item concentration tested. The NOEC for biomass was determined to be ≥ 1.400 mg prod./kg soil_{dw}.

Table A 48: Effects of ADM.03502.F.1.A on the growth (biomass change during 4 weeks exposure) of adult earthworms

Treatment group	ADM.03502.F.1.A (mg test item/kg soil _{dw})								
	Biomass change (change in fresh weight after 4 weeks relative to initial fresh weight) weight/worm [mg] (mean per replicate)								
Replicate	control	0.023	0.041	0.074	0.133	0.240	0.432	0.778	1.400
1	63.5	75.8	81.5	68.6	88.5	83.9	66.3	72.1	66.9
2	97.0	100.2	86.5	93.6	67.9	105.0	78.0	92.6	94.2
3	73.5	108.1	99.3	87.6	98.9	92.2	86.1	95.4	82.9
4	91.5	94.1	115.1	101.6	100.0	114.4	98.6	113.7	108.8
5	91.1								
6	98.5								
7	96.8								
8	107.7								
Mean	89.9	94.6	95.6	87.9	88.8	98.9	82.3	93.5	88.2
SD	14.4	13.8	15.0	14.1	14.9	13.5	13.6	17.0	17.7

Treatment group	Biomass change (change in fresh weight after 4 weeks relative to initial fresh weight) [%] (mean per replicate)								
Replicate	control	0.023	0.041	0.074	0.133	0.240	0.432	0.778	1.400
1	19.6	22.8	24.4	20.8	26.6	25.1	20.1	21.8	20.4
2	28.8	29.9	25.6	27.9	20.2	31.3	23.3	27.5	27.9
3	21.5	31.5	29.3	25.6	28.8	27.1	25.1	27.8	24.5
4	25.4	26.7	33.3	28.8	28.6	32.7	27.4	32.7	29.8
5	27.3								
6	29.4								
7	28.2								
8	31.3								
Mean	26.4	27.7	28.2	25.8	26.1	29.1	24.0	27.5	25.6

Change in biomass of each test item concentration not statistically significantly different compared to the control (Williams-t-test, $\alpha = 0.05$, one-sided smaller); SD: standard deviation, Calculations were performed with unrounded values

C. Reproduction

The mean number of juvenile earthworms was 265.6 in the control and 257.5, 258.3, 273.3, 260.8, 271.0, 277.3, 274.5 and 259.0 at concentrations of 0.023, 0.041, 0.074, 0.133, 0.240, 0.432, 0.778 and 1.400 mg prod./kg soil_{dw}, respectively. This resulted in a reduction of the reproduction performance compared to the control between -4.4 % and 3.1 %. The statistical analysis displayed no significant differences compared to the control (Williams-t-test, $\alpha = 0.05$, one-sided smaller) at any test item concentration tested. No difference in the number of unhatched cocoons was observed between the control and all test item concentrations tested. The NOEC for reproduction was determined to be ≥ 1.400 mg prod./kg soil_{dw}. The EC₁₀, EC₂₀ and EC₅₀ values for reproduction could not have been calculated as the maximum reduction was below 10 %. Thus, it was concluded that these values were higher than 1.400 mg prod./kg soil_{dw}, the highest concentration tested.

Table A 49: Effects of ADM.03502.F.1.A on the reproduction of adult earthworms

Treatment group	ADM.03502.F.1.A (mg test item/kg soil _{dw})								
Replicate	control	0.023	0.041	0.074	0.133	0.240	0.432	0.778	1.400
1	251	232	235	248	225	263	252	272	231
2	279	219	282	312	284	229	301	250	278
3	291	277	255	230	293	271	245	307	246
4	266	302	261	303	241	321	311	269	281
5	253								
6	228								
7	238								
8	319								
Mean	265.6	257.5	258.3	273.3	260.8	271.0	277.3	274.5	259.0
SD	29.8	38.7	19.3	40.4	32.9	38.0	33.6	23.8	24.5
CV (%)	11.2	15.0	7.5	14.8	12.6	14.0	12.1	8.7	9.5
Reduction of reproduction [%]									
% to control	---	3.1	2.8	-2.9	1.8	-2.0	-4.4	-3.3	2.5

Reproduction at each test item concentration not statistically significantly different compared to the (Williams-t-test, $\alpha = 0.05$, one-sided smaller); SD: standard deviation, cv %: coefficient of variation, Calculations were performed with unrounded values. Negative % values for change of reproduction = increase, relative to the control

Reference item

As a toxic reference, earthworms were exposed in a separate study to Maypon Flow (Carbendazim, SC 500). The results are in line with the OECD requirements (53 and 99 % of reduction in the number of juveniles at concentrations of 5 and 10 mg prod./ kg dry soil respectively).

D. Validity of the test:

Validity criterion according to OECD 222	Results of the study
Each replicate of the controls (containing 10 adults) should	Each replicate (containing 10 adults) produced 228 -319 juve-

produce ≥ 30 juveniles by the end of the test.	niles 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 11.2 %.
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 earthworm reproduction study with ADM.03502.F.1.A, no adverse effects on mortality, biomass development and reproduction of the earthworm *Eisenia fetida* in artificial soil were determined up to and including 1.400 mg prod./kg soil_{dw}, i.e. the highest concentration tested. Therefore, the NOEC for mortality, biomass and reproduction was determined to be ≥ 1.400 mg prod./kg soil_{dw}. The LC₅₀ was assumed to be > 1.400 mg prod./kg soil_{dw}, as no mortality $\geq 50\%$ was observed up to the highest test item concentration tested. As the maximum reduction of the reproduction was below 10 %, the EC₁₀, EC₂₀ and EC₅₀ values for reproduction were assumed to be higher than 1.400 mg prod./kg soil_{dw}, the highest concentration tested. The study is considered valid (see: “D. Validity criteria” above).

Comments of zRMS:	<p>The study was conducted in line with OECD 222 with no deviations.</p> <p>All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>NOEC_{reproduction} ≥ 5.46 mg prod./kg soil dw.</p> <p>EC₁₀= not determined as the maximum reduction was below 10 %</p>
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Reference:	KCP 10.4.1.1/02
Report:	Effects of ADM.03502.F.1.A on the mortality, growth and reproduction of the earthworm <i>Eisenia fetida</i> in artificial soil, Friedrich, S., 2021, report no.: 2148TEC0034, sponsor no.: 000108316
Guideline(s):	OECD 222 (2016)
Deviations:	None
GLP:	Yes
Acceptability/Reliability:	Yes
Duplication (if vertebrate study)	Not applicable

Executive summary

The effects of ADM.03502.F.1.A on mortality, biomass development and reproduction were investigated in an extended laboratory study over 56 days according to OECD Guideline 222 (2016). The test item was mixed into artificial soil at concentrations of 0.870, 1.13, 1.47, 1.91, 2.49, 3.23, 4.20 and 5.46 mg prod./kg soil_{dw}. For the control treatment, the soil was left untreated. Four replicates were prepared for the test item treatment groups and 8 replicates were prepared for the control, each containing 10 earthworms. Assessment of worm mortality, body weight and feeding activity was carried out after 28 days, assessment of reproduction (number of juveniles) was carried out after 56 days. The mortality of adult worms was 0 % in all test item treatment groups and in the control.

No pathological symptoms and no effects on behaviour (including feeding activity) of the worms were observed during the test in any of the test item concentrations. The test item caused no statistically significant differences on the change in biomass (change in fresh weight after 4 weeks relative to initial fresh weight) compared to the control group at any concentration tested. The mean number of juvenile worms counted on day 56 was 291.4 in the control and between 279.3 and 303.8 in the test item group, meaning

a reduction of the reproduction between -4.2% and 4.2% compared to the control. No statistically significant differences on the number of juveniles compared to the control group were observed at any concentration tested. In a separate study the reference item Maypon Flow (Carbendazim, SC 500) had a significant effect on biomass increase and reproduction of earthworms. The reproduction rate was clearly inhibited by 56.5 % and 99.6 % compared to the control at the tested concentrations of 5 and 10 mg prod./kg soil_{dw}. Based on the obtained results, the NOEC for mortality of the earthworm was determined to be ≥ 5.46 mg prod./kg soil_{dw}. The LC₅₀ was assumed to be > 5.46 mg prod./kg soil_{dw}, as no mortality ≥ 50 % was observed up to the highest concentration tested. The NOEC for biomass and reproduction was determined to be ≥ 5.46 mg prod./kg soil_{dw}. The EC₁₀, EC₂₀ and EC₅₀ values for reproduction could not have been calculated as the maximum reduction was below 10 %. Thus, it can be concluded that these values were higher than 5.46 mg prod./kg soil_{dw}, the highest concentration tested.

I. Materials and methods

A. Materials

1. Test material: ADM.03502.F.1.A
 Lot/Batch no.: 1191-101219-01
 Content: 250 g fenpropidin/L, 175 g prothioconazole/L (nominal)
 253.7 g fenpropidin/L, 175.9 g prothioconazole/L (analysed)
 Density: 1.04 g/mL
 Control: deionised water mixed into artificial soil
 Toxic reference: Maypon Flow (Carbendazim, SC 500)

2. Test organisms -
 Species: *Eisenia fetida*
 Age: adult worms (with clitellum) approx. 7-month-old
 Source: purchased from Bias Labs Ltd, Unit 19, Enterprise Centre, Myregormie Place, Fife, UK, KY1 3PF
 Weight at test start: 400 – 599 mg/worm
 No. of organisms: 10 worms/replicate, 4 replicates per treatment group, 8 replicates for the control
 Acclimatisation: at least 24 hours in the artificial substrate (with food)
 Feeding: 5 g of finely ground horse manure weekly

3. Test units and exposure –
 Type and size: Plastic vessels (approx. 16.5 cm x 12 cm x 6 cm), filled with 600 g soil_{dw}. 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
 Composition: 69.5 % industrial sand, 20 % kaolin (kaolinite content > 30 %), 10 % sphagnum peat, 0.5 % calcium carbonate

4. Test conditions –
 pH value: 5.92 - 6.07 (test start), 5.61 - 5.77 (test end)
 Soil moisture: 55.2 - 55.4 % (test start), 54.1 - 54.9 % (test end) of the WHC_{max}
 Temperature: 19.2 - 21.7 °C
 Photoperiod: 16 hours light/8 hours dark
 Light intensity: 590 lux

B. Study design and method

1. In-life dates: April 29 to June 28, 2021 (experimental phase)

2. Test design:

One day before test start, the dry artificial soil was pre-moistened by adding deionised water to obtain approximately half of the final water content. Earthworms were acclimatised in a separate batch of the artificial soil (mixed with horse manure) for approximately 26 hours before test start. On the day of the test start, the test item was introduced by dispersing the quantity of test item required to obtain the desired test concentration in the volume of water required to hydrate the soil to 40-60 % of its water holding capacity (WHC). The control substrate contained the corresponding amount of deionised water only. Each test vessel was then filled with the treated soil. After a randomising procedure according to the worm fresh weight, selected groups of 10 worms were randomly assigned to each treatment group (0.870, 1.13, 1.47, 1.91, 2.49, 3.23, 4.20 and 5.46 mg prod./kg soil_{dw}). The individually weighed worms (10 worms/vessel) were placed on the surface of the soil. After approximately thirty minutes, the test vessels were closed with perforated transparent lids, which allowed gas exchange between substrate and atmosphere and access of light, but prevented worms from escaping. The test vessels were then set up at random in a controlled-environment test room. One day after application, 5 g air-dried and finely ground horse manure was scattered on the soil surface of each test vessel, which was moistened with 5 mL deionised water. The feeding interval was weekly during the first four weeks of the test. The weekly amount of manure (5 g) depended on the feeding activity, which was assessed by visual estimation of the food remaining on the surface before addition of new food. After four weeks, the adult worms were removed from the test vessels. The number of surviving worms (adult mortality) and their biomass change were determined, behaviour (including feeding activity) and pathological symptoms were recorded. The adult worms were discarded after counting and weighing. Subsequently, the soil of each vessel was mixed carefully with 5 g manure. This was the last feeding occasion of the test. The test was then continued for another four weeks. At the final assessment after 8 weeks, the number of hatched juvenile earthworms in each test vessel was determined. The test vessels were placed in a water bath set to 50 - 60 °C and left for a period of approximately 20 minutes which forced the living juvenile earthworms to the soil surface. The juvenile earthworms were removed from the soil surface and counted by hand. Afterwards the soil from each test vessel was carefully checked for any remaining juveniles left in the soil and the number of unhatched cocoons was recorded. The water content and pH of the artificial soil was also determined at day 56.

3. Statistics:

The endpoints were mortality, change of biomass (difference in fresh weight of surviving worms between test start and four weeks after treatment) and reproduction (the number of juveniles present). The arithmetic mean and the standard deviation per treatment and per control for reproduction, mortality and biomass were calculated. The statistical analysis was performed with the software ToxRat Professional 3.3.0 (2018). As no mortality occurred in any test item concentration and in the control, no statistical analysis was performed. Williams-t-test was used to compare the biomass results (one-sided smaller) and the reproduction performance (one-sided smaller) of the control with the independent test item groups. Since the mortality of adult earthworms was 0 % up to the highest test item concentration tested and the maximum reduction of reproduction was below 10 %, a calculation of the LC₅₀ as well as EC₁₀, EC₂₀ and EC₅₀ was not possible and were thus assumed to be higher than the maximum test item rate tested.

II. Results and discussions

A. Mortality

The mortality of adult worms was 0 % in all test item treatment groups and in the control. No pathological symptoms and no effects on behaviour (including feeding activity) of the worms were observed during the test. Based on the results of the study the LC₅₀ for mortality was assumed to be > 5.46 mg prod./kg soil_{dw} and the NOEC for mortality was determined to be ≥ 5.46 mg prod./kg soil_{dw}.

Table A 50: Effects of ADM.03502.F.1.A on the mortality of adult earthworms

Treatment	ADM.03502.F.1.A (mg test item/kg soil _{dw})
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group	Number of surviving adult worms per replicate (4 weeks after test start)								
Replicate	control	0.870	1.13	1.47	1.91	2.49	3.23	4.20	5.46
1	10	10	10	10	10	10	10	10	10
2	10	10	10	10	10	10	10	10	10
3	10	10	10	10	10	10	10	10	10
4	10	10	10	10	9	10	10	10	10
5	10								
6	9								
7	10								
8	10								
Mean	10	10	10	10	10	10	10	10	10
SD	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CV (%)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Mortality [%]								
Mean	0.0	0.0	0.0	0.0	2.5	0.0	0.0	0.0	0.0

SD: standard deviation, cv %: coefficient of variation, dw: dry weight (of artificial soil) Calculations were performed with unrounded values.

B. Body weight change

At the start of the test, earthworm fresh weight ranged from 400 – 599 mg/worm. The weight change of adult worms ranged between 19.6 % and 23.5 % in the test item groups and was 21.4 % in the control group. Statistical analysis displayed no significant differences compared to the control at any test item concentration tested. The NOEC for biomass was determined to be ≥ 5.46 mg prod./kg soil_{dw}.

Table A 51: Effects of ADM.03502.F.1.A on the growth (biomass change during 4 weeks exposure) of adult earthworms

Treatment group	ADM.03502.F.1.A (mg test item/kg soil _{dw})								
	Initial fresh weight/worm [mg] (mean per replicate)								
Replicate	control	0.870	1.13	1.47	1.91	2.49	3.23	4.20	5.46
1	453.7	452.4	457.3	450.1	452.7	463.0	448.9	452.0	437.5
2	473.5	465.5	478.0	470.1	467.8	469.2	463.8	473.3	477.5
3	487.2	492.2	482.8	488.0	488.8	485.3	495.2	490.1	480.9
4	520.2	498.8	498.7	506.8	496.8	495.2	511.7	493.0	527.0
5	458.7								
6	465.9								
7	494.5								
8	498.3								
Mean	481.5	477.2	479.2	478.8	476.5	478.2	479.9	477.1	480.7
SD	22.6	21.9	17.1	24.3	20.0	14.7	28.7	18.9	36.6
cv [%]	4.7	4.6	3.6	5.1	4.2	3.1	6.0	4.0	7.6

Treatment group	Fresh weight/worm [mg] after 4 weeks (mean per replicate)								
Replicate	control	0.870	1.13	1.47	1.91	2.49	3.23	4.20	5.46
1	562.8	544.7	553.6	560.3	562.1	547.1	558.5	539.2	541.2
2	558.8	563.5	588.5	563.6	585.7	572.9	545.9	567.8	566.8
3	587.9	590.1	588.4	600.9	607.3	581.8	598.5	602.0	585.9
4	629.6	609.1	608.4	605.1	598.2	585.9	621.6	586.5	627.7
5	549.7								
6	578.9								
7	611.0								
8	595.9								
Mean	584.3	576.9	584.7	582.5	588.3	571.9	581.1	573.9	580.4
SD	27.4	28.4	22.8	23.8	19.6	17.4	35.1	27.0	36.5
cv [%]	4.7	4.9	3.9	4.1	3.3	3.0	6.0	4.7	6.3
Treatment group	Biomass change (change in fresh weight after 4 weeks relative to initial fresh weight) weight/worm [mg] (mean per replicate)								
Replicate	control	0.870	1.13	1.47	1.91	2.49	3.23	4.20	5.46
1	109.1	92.3	96.3	110.2	109.4	84.1	109.6	87.2	103.7
2	85.3	98.0	110.5	93.5	117.9	103.7	82.1	94.5	89.3
3	100.7	97.9	105.6	112.9	118.5	96.5	103.3	111.9	105.0
4	109.4	110.3	109.7	98.3	101.4	90.7	109.9	93.5	100.7
5	91.0								
6	113.0								
7	116.5								
8	97.6								
Mean	102.8	99.6	105.5	103.7	111.8	93.8	101.2	96.8	99.7
SD	11.0	7.6	6.5	9.3	8.1	8.3	13.1	10.6	7.1
Treatment group	Biomass change (change in fresh weight after 4 weeks relative to initial fresh weight) [%] (mean per replicate)								
Replicate	control	0.870	1.13	1.47	1.91	2.49	3.23	4.20	5.46
1	24.0	20.4	21.1	24.5	24.2	18.2	24.4	19.3	23.7
2	18.0	21.1	23.1	19.9	25.2	22.1	17.7	20.0	18.7
3	20.7	19.9	21.9	23.1	24.2	19.9	20.9	22.8	21.8
4	21.0	22.1	22.0	19.4	20.4	18.3	21.5	19.0	19.1
5	19.8								
6	24.3								
7	23.6								
8	19.6								
Mean	21.4	20.9	22.0	21.7	23.5	19.6	21.1	20.3	20.8

Change in biomass of each test item concentration not statistically significantly different compared to the control (Williams-t-test, $\alpha = 0.05$, one-sided smaller); SD: standard deviation, Calculations were performed with unrounded values

C. Reproduction

The mean number of juvenile earthworms was 291.4 in the control and 283.3, 280.5, 295.3, 286.8, 303.8, 283.8, 279.3 and 283.8 at concentrations of 0.870, 1.13, 1.47, 1.91, 2.49, 3.23, 4.20 and 5.46 mg prod./kg soil_{dw}, respectively. This resulted in a reduction of the reproduction performance compared to the control between -4.2% and 4.2%. The statistical analysis displayed no significant differences compared to the control at any test item concentration tested. No difference in the number of unhatched cocoons was observed between the control and all test item concentrations tested. The NOEC for reproduction was determined to be ≥ 5.46 mg prod./kg soil_{dw}. The EC₁₀, EC₂₀ and EC₅₀ values for reproduction could not be calculated as the maximum reduction was below 10 %. Thus, it was concluded that these values were higher than 5.46 mg prod./kg soil_{dw}, the highest concentration tested

Table A 52: Effects of ADM.03502.F.1.A on the reproduction of adult earthworms

Treatment group	ADM.03502.F.1.A (mg test item/kg soil _{dw})								
Replicate	control	0.870	1.13	1.47	1.91	2.49	3.23	4.20	5.46
1	201	295	299	281	274	280	259	289	285
2	313	273	228	351	262	369	204	271	311
3	345	209	316	240	269	291	351	308	243
4	298	356	279	309	342	275	321	249	296
5	307								

Treatment group	ADM.03502.F.1.A (mg test item/kg soil _{dw})								
	control	0.870	1.13	1.47	1.91	2.49	3.23	4.20	5.46
Replicate									
6	257								
7	322								
8	288								
Mean	291.4	283.4	280.5	295.3	286.8	303.8	283.8	279.3	283.8
SD	44.6	60.7	38.1	46.7	37.2	44.0	65.5	25.2	29.2
CV (%)	15.3	21.4	13.6	15.8	13.0	14.5	23.1	9.0	10.3
Reduction of reproduction [%]									
% to control	---	2.8	3.7	-1.3	1.6	-4.2	2.6	4.2	2.6

Reproduction at each test item concentration not statistically significantly different compared to the (Williams-t-test, $\alpha = 0.05$, one-sided smaller) SD: standard deviation, cv %: coefficient of variation, dw: dry weight (of artificial soil) Calculations were performed with unrounded values. Negative % values for reduction of reproduction = increase, relative to the control

Reference item

As a toxic reference, earthworms were exposed in a separate study to Maypon Flow (Carbendazim, SC 500). The results are in line with the OECD requirements (56.5 and 99.6 % of reduction in the number of juveniles at concentrations of 5 and 10 mg prod./ kg soil_{dw} respectively).

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 201 - 345 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 15.3 %.
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 0.0 %.

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 earthworm reproduction study with ADM.03502.F.1.A, no adverse effects on adult mortality, biomass development of adults and reproduction of the earthworm *Eisenia fetida* in artificial soil were determined up to and including 5.46 mg prod./kg soil_{dw}, i.e. the highest concentration tested. Therefore, the NOEC for mortality, biomass and reproduction was determined to be ≥ 5.46 mg prod./kg soil_{dw}. The LC₅₀ was assumed to be > 5.46 mg prod./kg soil_{dw}, as no mortality $\geq 50\%$ was observed up to the highest test item concentration tested. As the maximum reduction of the reproduction was below 10 %, the EC₁₀, EC₂₀ and EC₅₀ values for reproduction were assumed to be higher than 5.46 mg prod./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 no deviations
	All the validity criteria were met and the study is considered acceptable with the following

	endpoints relevant for the risk assessment: NOEC _{reproduction} = 308.6 mg prod./kg soil _{dw} . EC ₁₀ = 318.1 mg prod./kg soil _{dw}
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Reference:	KCP 10.4.2.1/01
Report:	Effects of ADM.03502.F.1.A on the mortality and reproduction of the collembolan <i>Folsomia candida</i> , Friedrich, S., 2020b, report no.: 2048TCC0025, sponsor no.: 000104849
Guideline(s):	OECD 232 (2016)
Deviations:	None
GLP:	Yes
Acceptability/Reliability:	Yes
Duplication (if vertebrate study)	Not applicable

Executive summary

The effects of ADM.03502.F.1.A on mortality and reproduction of the collembola *Folsomia candida* were investigated in a chronic laboratory experiment over a time period of 28 days according to OECD Guideline 232 (2016). The test item was mixed into artificial soil at concentrations of 16.3, 29.4, 52.9, 95.3, 171.5, 308.6, 555.6 and 1000 mg prod./kg soil_{dw}. For the control treatment, the soil was left untreated. Four replicates were prepared for the test item treatment groups and 8 replicates were prepared for the control, each containing 10 springtails. Assessment of mortality, reproduction and behaviour was carried out 28 days after treatment. Statistically significant effects on mortality compared to the control were observed at concentrations of 555.6 and 1000 mg prod./kg soil_{dw}. Mortality rates of 0.0 % to 32.5 % were recorded in the test item treatment groups. In the control the mortality rate was 2.5 %. Statistically significant effects on the number of juveniles compared to the control group were recorded at concentrations of 555.6 and 1000 mg prod./kg soil_{dw}. The mean number of juveniles counted 28 days after introduction of the parental collembolans into the test vessels was 821 in the control and 812, 806, 803, 819, 798, 778, 467 and 393 at concentrations of 16.3, 29.4, 52.9, 95.3, 171.5, 308.6, 555.6 and 1000 mg prod./kg soil_{dw}, respectively.

In a separate study, the EC₅₀ (reproduction) of the reference item boric acid was determined to be 161 mg/kg soil dry weight, which was close to the value of 100 mg/kg soil_{dw} as stated in OECD 232 (2016) and therefore indicated the sensitivity of the test system. Based on the obtained results, the NOEC for mortality of the parental collembolans was determined to be 308.6 mg prod./kg soil_{dw}. The LC₅₀ was assumed to be > 1000 mg prod./kg soil_{dw}, as no mortality ≥ 50% was observed up to the highest concentration tested. The NOEC for reproduction was determined to be 308.6 mg prod./kg soil_{dw}. The EC₁₀, EC₂₀ and EC₅₀ values for reproduction were determined to be 318.1, 355.5 and 439.5 mg prod./kg soil_{dw}, respectively.

I. Materials and methods

A. Materials

1. Test material:	ADM.03502.F.1.A
Lot/Batch no.:	1191-101219-01
Content:	250 g fenpropidin/L, 175 g prothioconazole/L (nominal) 253.7 g fenpropidin/L, 175.9 g prothioconazole/L (analysed)
Density:	1.04 g/mL
Control:	The control substrate was left untreated using only deionised water
Toxic reference:	Boric Acid (purity: 100.8 %, analysed)

2. Test organisms

Species:	<i>Folsomia candida</i> (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)
Feeding:	granulated dry baker yeast

3. Test units and exposure -

Type and sizes:	Glass container (approximately 150 mL) covered with a glass lid, filled with 30 g soil dry weight per vessel; surface area of the soil 18.9 cm ² ; soil depth approximately 3 cm
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, 20 % kaolin, 5 % sphagnum peat, 0.3 % calcium carbonate

4. Test conditions

pH value:	6.07 – 6.11 (test start), 5.88 – 5.95 (test end)
Soil moisture:	58.2 – 58.4 % (test start), 56.8 - 57.7 % (test end) of WHC
Temperature:	19.0 - 21.8°C
Photoperiod:	16 hours light/8 hours dark
Light intensity:	580 lux

B. Study design and method

1. In life dates: June 30 to July 28, 2020 (experimental phase)

2. Test design:

Two days before the start of the test, the dry artificial soil was pre-moistened by adding deionised water to obtain approximately half of the final water content. On the day of the test start, the test item was introduced by dispersing the quantity of test item required to obtain the desired test concentration in the volume of water required to hydrate the soil to 40-60 % of its maximum water holding capacity (WHC). 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 test vessel, avoiding compression. The test was started using juvenile collembolans of *Folsomia candida*, well-fed and 9 - 12 days old. Ten test organisms were introduced to each test vessel (150 mL), using an aspirator.

After addition of the test organisms, the test vessels were positioned randomly in a controlled-environment test room, and these positions were re-randomised weekly. The test containers were tightly covered with a lid and briefly opened twice a week for aeration. The test organisms were fed twice during the test (at the start of the test and after 14 days) with approximately 2 mg of granulated dry yeast per test vessel. 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. The water loss was compensated weekly.

Four weeks after introducing the test organisms, the parental and juvenile collembolans in the test item and control vessels were counted. The test substrate of each replicate was poured into an individual container (with a volume of about 200 mL) and the test organisms were floated off the substrate by the addition of water. To improve the contrast between the white collembolans and surrounding water surface, the water was stained dark with ink. After gentle stirring, the number of parental and juvenile collembolans floating on the surface was determined. 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, LemnaTec GmbH Aachen), an automated and validated counting technique based

on a video camera connected to a digital image storage and analysis system. The validation of the counting method resulted in a coefficient of variation of 2.6 % for 10 successive runs. The extraction efficiency of the extraction method was determined to be 98 % in a separate extraction run using vessels containing a known number of juveniles kept in untreated test substrate.

3. Statistics:

The statistical analysis was performed with the software ToxRat Professional 3.3.0 (2018). Step-down Cochran-Armitage test and Williams-t-test were used to compare the mortality results (one-sided greater) and the reproduction performance (one-sided smaller) of the control with the independent test item groups. Since the mortality of adult collembolans was < 50 % up to the highest test item concentration tested, a calculation of the LC₅₀ was not possible and the LC₅₀ was thus assumed to be higher than the maximum test item rate tested. The EC₁₀, EC₂₀ and EC₅₀ values for the reproduction were determined by the 4-parametric normal cumulative distribution function (CDF).

II. Results and discussions

A. Mortality

Mean mortalities of the adult collembolans were between 0.0 % and 32.5 % in the respective test item treatment groups. For the control a 2.5 % parental mortality was observed. Statistically significant increased mortalities were recorded at 555.6 and 1000 mg prod./kg soil_{dw} (Stepdown Cochran-Armitage test, $\alpha = 0.05$, one-sided greater). No effects on the behaviour of the adult collembolans were observed during the test. Based on the results of the study the LC₅₀ for mortality was assumed to be > 1000 mg prod./kg soil_{dw} and the NOEC for mortality was determined to be 308.6 mg prod./kg soil_{dw}.

Table A 53: Effects of MCW-2073 on mortality of parental collembolans

Treatment group	mg test item/kg soil _{dw}								
	Control	16.3	29.4	52.9	95.3	171.5	308.6	555.6	1000
Replicate	Number of surviving parental collembolans per replicate (4 weeks after test initiation)								
1	10	10	9	10	10	10	10	8	8
2	10	10	10	10	10	10	10	10	5
3	10	10	10	10	9	10	9	9	8
4	9	10	10	10	10	9	10	8	6
5	10								
6	10								
7	9								
8	10								
Mean	9.8	10.0	9.8	10.0	9.8	9.8	9.8	8.8	6.8
SD	0.5	0.0	0.5	0.0	0.5	0.5	0.5	1.0	1.5
CV (%)	4.7	0.0	5.1	0.0	5.1	5.1	5.1	10.9	22.2
Mortality (%)	2.5	0.0	2.5	0.0	2.5	2.5	2.5	12.5*	32.5*

* 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, dw: dry weight (of artificial soil)

B. Reproduction

The mean number of juvenile collembolans was 821 in the control and 812, 806, 803, 819, 798, 778, 467 and 393 at concentrations of 16.3, 29.4, 52.9, 95.3, 171.5, 308.6, 555.6 and 1000 mg prod./kg soil_{dw}, respectively. This resulted in a reduction of the reproduction performance compared to the control between 0.2% and 52.1%. A statistically significant lower number of juveniles compared to the control group was recorded at 555.6 and 1000 mg prod./kg soil_{dw} (Williams-t-test, $\alpha = 0.05$, one-sided smaller) (Table 4). The NOEC for reproduction was determined to be 308.6 mg prod./kg soil_{dw}. The EC₁₀, EC₂₀ and EC₅₀ values for reproduction were determined to be 318.1, 355.5 and 439.5 mg prod./kg soil_{dw}, respectively.

Table A 54: Effects of MCW-2073 on number of juvenile collembolans

Treatment group	mg test item/kg soil _{dw}								
	Control	16.3	29.4	52.9	95.3	171.5	308.6	555.6	1000
Replicate	Number of juveniles per replicate (4 weeks after test initiation)								
1	877	611	834	709	740	912	814	512	361

2	858	893	741	882	853	672	752	376	356
3	936	876	803	916	801	895	810	464	391
4	733	869	847	705	881	713	735	514	464
5	743								
6	872								
7	653								
8	892								
Mean	821	812	806	803	819	798	778	467*	393*
SD	98.1	134.5	47.3	111.7	62.1	123.2	40.2	64.6	49.8
CV (%)	12.0	16.6	5.9	13.9	7.6	15.4	5.2	13.8	12.7
Reduction of reproduction (% compared to control)	---	1.0	1.7	2.1	0.2	2.7	5.2	43.1	52.1

* 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, dw: dry weight (of artificial soil); Percent reduction: $(1 - R_t/R_c) * 100$; R_t = mean number of juveniles observed in the test item treated groups
 R_c = 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 55: Endpoints derived from the study

	Endpoints (mg test item/kg soil dry weight)
NOEC (mortality)	308.6
NOEC (reproduction)	308.6
LC ₅₀ (mortality) ¹	> 1000
EC ₁₀ (reproduction) ²	318.1 (95 % confidence limits 266.1 – 380.3)
EC ₂₀ (reproduction) ²	355.5 (95 % confidence limits 300.5 – 422.7)
EC ₅₀ (reproduction) ²	439.5 (95 % confidence limits 359.3- 540.8)

1 based on estimation of the data, 2 based on 4-parametric normal CDF

Reference item

In the most recent study with the reference item boric acid (analysed purity: 100.8 %), the EC₅₀ was determined to be 103 mg/kg soil_{dw} and the LC₅₀ 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 EC₅₀ value for the reproduction was close to the value of 100 mg/kg soil_{dw} as stated in OECD 232 (2016). The EC₅₀ therefore showed that the test system is 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 821 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 12.0 % 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 28-day Collembola reproduction study with ADM.03502.F.1.A, the NOEC for mortality of the parental *Folsomia candida* was determined to be 308.6 mg prod./kg soil_{dw}. The LC₅₀ was assumed to be > 1000 mg prod./kg soil_{dw}, as no mortality $\geq 50\%$ was observed up to the highest test item concentration tested.

The NOEC for reproduction was determined to be 308.6 mg prod./kg soil_{dw}. The EC₁₀, EC₂₀ and EC₅₀ values for reproduction were determined to be 318.1, 355.5 and 439.5 mg prod./kg soil_{dw}, respectively.

Comments of zRMS:	<p>The study was conducted in line with OECD 226 239 with no deviations.</p> <p>All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>NOEC=93 mg prod./kg soil dw. EC₁₀=110.42 mg prod./kg soil dw</p>
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Reference:	KCP 10.4.2.1/02
Report:	Effects of ADM.03502.F.1.A on the reproduction of the predatory mite <i>Hypoaspis aculeifer</i> , Schulz, L., 2020a, report no.: 2048THC0021, sponsor no.: 000104850
Guideline(s):	OECD 226 (2016)
Deviations:	None
GLP:	Yes
Acceptability/Reliability:	Yes
Duplication (if vertebrate study)	Not applicable

Executive summary

The effects of ADM.03502.F.1.A on mortality and reproduction of the soil mite *Hypoaspis aculeifer* were investigated in a chronic laboratory experiment over a time period of 14 days according to OECD 226 (2016). The test item was mixed into artificial soil at concentrations of 5, 9, 16, 29, 51, 93, 167 and 300 mg prod./kg soil_{dw}. For the control treatment, the soil was left untreated. 8 replicates and 4 replicates were prepared for the control and test item treatment groups, respectively, each containing 10 adult soil mites (females). Assessment of mortality and reproduction was carried out after the 14-day exposure of the soil mites. Mean mortality rates of 0.0 - 5.0 % were recorded in the test item treatment groups. In the control group the mortality rate was 0.0 %. Thus, the highest corrected mortality was 5.0 %. The test item caused no statistically significantly increased mortality of the adult mites compared to the control at any test item concentration. Differences in the behaviour and the morphology of the mites between the control and the test item treatment groups could not have been observed. Fourteen days after introduction of the parental mites into the test vessels, the mean number of juveniles was 289.0, 289.8, 283.3, 284.8, 274.3, 301.0, 275.3 and 257.3 at concentrations of 5, 9, 16, 29, 51, 93, 167 and 300 mg prod./kg soil_{dw}, respectively. The mean reproduction in the control reached 305.6 juveniles. Thus, the highest reduction of the reproduction performance was 16 % at the highest tested concentration compared to the control. The test item caused statistically significantly lower reproduction at 167 and 300 mg prod./kg soil_{dw}. In a separate study, the EC₅₀ (reproduction) of the reference item dimethoate was determined to be 6.3 mg a.s./kg soil_{dw}, indicating the sensitivity of the test system. In a 14-day *Hypoaspis aculeifer* reproduction study with ADM.03502.F.1.A, the LC₅₀ for mortality and the EC₂₀ and EC₅₀ values for reproduction were determined to be higher than 300 mg prod./kg soil_{dw}, the highest concentration tested. The EC₁₀ value for reproduction was calculated to be 110.42 mg prod./kg soil_{dw}. The NOEC for mortality was determined to be ≥ 300 mg prod./kg soil_{dw}, the corresponding LOEC to be > 300 mg prod./kg soil_{dw}. The NOEC for reproduction was determined to be 93 mg prod./kg soil_{dw}, the corresponding LOEC to be 167 mg prod./kg soil_{dw}.

I. Materials and methods

A. Materials

1. Test material:	ADM.03502.F.1.A
Lot/Batch no.:	1191-101219-01
Content:	250 g fenpropidin/L, 175 g prothioconazole/L (nominal)

Density:	253.7 g fenpropidin/L, 175.9 g prothioconazole/L (analysed)
Control:	1.04 g/mL untreated substrate
Toxic reference:	Dimethoate (98.8 % ± 0.5 %, analysed)
2. Test organisms	
Species:	<i>Hypoaspis aculeifer</i> (Canestrini)
Age:	adult female mites up to 2 days
Source:	obtained synchronised from “Katz Biotech AG”, Baruth, Germany, and kept in the test facility under ambient laboratory conditions until test start
No. of organisms:	10 soil mites per replicate (8 replicates per group, 8 replicates for the control)
Acclimatisation:	none
Feeding:	every 2 - 3 days with <i>Tyrophagus putrescentiae</i> (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:	74.75 % industrial quartz sand, predominantly fine sand with more than 50 % of the particles between 50 and 200 µm, 20 % kaolin clay (kaolinite content > 30 %), 5 % sphagnum peat, 0.25 % calcium carbonate
4. Test conditions	
pH value:	6.3 - 6.5 (test start), 6.2 – 6.4 (test end)
Soil moisture:	46.33 - 48.65 % (test start), 47.24 - 51.07 % (test end) of WHC
Temperature:	19.4 - 21.4°C
Photoperiod:	16 hours light/8 hours dark
Light intensity:	432 lux

B. Study design and method

1. In life dates: July 01 to July 21, 2020 (experimental phase)
2. Test design:

The aim of the test was to evaluate possible effects of the test item on the mortality and reproduction of the soil mites *Hypoaspis aculeifer* during a test period of 14 days. The test item was mixed into artificial soil at concentrations of 5, 9, 16, 29, 51, 93, 167 and 300 mg prod./kg soil_{dw}. For the control treatment, the soil was left untreated. 8 replicates and 4 replicates were prepared for the control and test item treatment groups, respectively, each containing 10 adult soil mites (females). Two weeks after start of exposure, the number of juveniles and surviving parental mites was determined. 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_{dw}) with a water content of 40-60 % of the maximum water holding capacity (WHC). Per test vessel 10 adult females were introduced by means of a moistened brush (= start of exposure).

Afterwards the food mite *Tyrophagus putrescentiae* was added (approximately 20 mg per vessel), the test vessels were closed and randomly set up in a controlled-environment test room. The test was carried out under a controlled light-dark cycle. The water content of the soil substrate in the test vessels was determined at test start (after application) and at day 14 after application and was maintained throughout the test by reweighing the additional test vessels. Water loss was compensated. The vessels were briefly opened every 2 - 3 days for aeration and feeding. Assessment of mortality and reproduction was carried out after the 14-day exposure of the soil mites. On day 14 after application of the test item and introduction of the test organisms, surviving mites and juveniles of *Hypoaspis aculeifer* were extracted from each test replicate using a MacFadyen high-gradient extractor. Following extraction, all juveniles and adults present in the fixing liquid were counted. 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 and amounted to be 91.5 % in a separate extraction run using vessels containing a known number of juveniles and adult mites kept in untreated test substrate.

3. Statistics:

The statistical analysis was performed with the software ToxRat Professional 3.3.0 (RATTE 2018). Multiple Sequentially-rejective Fisher Test for mortality ($\alpha = 0.050$; one-sided greater) and the Williams Multiple

Sequential t-test Procedure for reproduction ($\alpha = 0.05$, one-sided smaller) were used to compare the control

with the independent test item group. Probit analysis using linear maximum likelihood regression was used for EC₁₀ calculation. Since the mortality of adult mites and the difference of the reproduction compared to the control was < 20 % at the highest test item concentration tested, a calculation of the LC₅₀ and EC₂₀ and EC₅₀ was not possible and were thus determined to be higher than the maximum test item concentration tested.

II. Results and discussions

A. Mortality

Mean mortality rates of 0.0 - 5.0 % were recorded in the test item treatment groups. In the control group the mortality rate was 0.0%. Thus, the highest corrected mortality was 5.0 % and the LC₅₀ was thus > 300 mg prod./kg soil_{dw}. The test item caused no statistically significantly increased mortality of the adult mites compared to the control at any test item concentration (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, $\alpha = 0.05$, one-sided greater). Thus, the LOEC was > 300 mg prod./kg soil_{dw} and the corresponding NOEC ≥ 300 mg prod./kg soil_{dw}. Differences in the behaviour and the morphology of the mites between the control and the test item treatment groups could not have been observed.

Table A 56: Effects of the test item on mortality of *Hypoaspis aculeifer* (day 14)

Endpoint	Test item concentration [mg prod./kg soil _{dw}]								
	Control	5	9	16	29	51	93	167	300
Mean adult mortality [%] (day 14)	0.0	5.0	2.5	5.0	0.0	2.5	0.0	5.0	2.5
	Endpoint mg prod./kg soil _{dw}]								
NOEC (mortality)	≥ 300								
LOEC (mortality)	> 300								
LC50 ¹⁾	> 300								

The calculations were performed with unrounded values.

Not statistically significantly different compared to the control (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm for mortality, $\alpha = 0.05$, one-sided greater)

¹⁾ Due to effects < 50% at the highest concentration tested and of lacking concentration-response this value was not possible to calculate and thus above the highest test concentration

B. Reproduction

Fourteen days after introduction of the parental mites into the test vessels, the mean number of juveniles was 289.0, 289.8, 283.3, 284.8, 274.3, 301.0, 275.3 and 257.3 at concentrations of 5, 9, 16, 29, 51, 93, 167 and 300 mg prod./kg soil_{dw}, respectively. The mean reproduction in the control reached 305.6 juveniles. Thus, the highest reduction of the reproduction performance was 16 % at the highest tested concentration compared to the control. The test item caused statistically significantly lower reproduction at 167 and 300 mg prod./kg soil_{dw} (Williams Multiple Sequential t-test Procedure for reproduction, $\alpha = 0.05$, one-sided smaller). Based on the results of the study, the EC₁₀ for reproduction was calculated to be 110.42 mg prod./kg soil_{dw}, whereas the EC₂₀ and EC₅₀ were > 300 mg prod./kg soil_{dw}. The NOEC was determined to be 93 mg prod./kg soil_{dw} and the corresponding LOEC to be 167 mg prod./kg soil_{dw}.

Table A 57: Effects of the test item on reproduction of *Hypoaspis aculeifer* (day 14)

Endpoint	Test item concentration [mg prod./kg soil _{dw}]								
	Control	5	9	16	29	51	93	167	300
Mean number of juveniles [n] (day 14)	305.6	289.0	289.8	283.3	284.8	274.3	301.0	275.3*	257.3*
Reduction of reproduction compared to control [%]	---	5	5	7	7	10	2	10	16
Endpoint mg prod./kg soil _{dw}]									
NOEC (reproduction)	93								
LOEC (reproduction)	167								
EC ₁₀ ²⁾	110.42 (95 % confidence limit 34.89 – 349.41)								
EC ₂₀ ³⁾	> 300								
EC ₅₀ ³⁾	> 300								

The calculations were performed with unrounded values.

Not statistically significantly different compared to the control (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm for mortality, $\alpha = 0.05$, one-sided greater)

* statistically significantly different compared to the control (Williams Multiple Sequential t-test Procedure for reproduction, $\alpha = 0.05$, one-sided smaller)

²⁾ based on Probit analysis

³⁾ Due to effects < 20% at the highest concentration the values were not possible to calculate and thus above the highest test concentration

Reference item

In the most recent study with the reference item dimethoate, the EC₅₀ (reproduction) was determined to be 6.3 mg a.s./kg soil_{dw}. The EC₅₀ value for the reproduction was within the range of 3.0 to 7.0 mg a.s./kg soil_{dw} as stated in OECD 226 (2016). The EC₅₀ therefore showed that the test system is sensitive.

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 0.0 % 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 305.6 at the end of the test.

Validity criterion according to OECD 226	Results of the study
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 5.7 % 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 14-day *Hypoaspis aculeifer* reproduction study with ADM.03502.F.1.A, the LC₅₀ for mortality and the EC₂₀ and EC₅₀ values for reproduction were higher than 300 mg prod./kg soil_{dw}, the highest concentration tested. The EC₁₀ value for reproduction was calculated to be 110.42 mg prod./kg soil_{dw}. The NOEC for mortality was determined to be ≥ 300 mg prod./kg soil_{dw}, the corresponding LOEC to be > 300 mg prod./kg soil_{dw}. The NOEC for reproduction was determined to be 93 mg prod./kg soil_{dw}, the corresponding LOEC to be 167 mg prod./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:	<p>The study was conducted in line with OECD 216 with no deviations.</p> <p>All the validity criteria were met.</p> <p>It may be concluded that the effects of the test item on soil nitrogen formation rates were < 25 % at the end of the study period (28 days) up to 13.87 mg product/kg soil dw.</p>
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Reference	KCP 10.5/01
Report:	Effects of ADM.03502.F.1.A on the activity of soil microflora (Nitrogen transformation test), Schulz, L., 2020b, report no.: 2048SMN0022, sponsor no.: 000104851
Guideline(s):	OECD 216 (2000)
Deviations:	None
GLP:	Yes
Acceptability/Reliability:	Yes
Duplication (if vertebrate study)	Not applicable

Executive summary

The effects of ADM.03502.F.1.A (250 g fenpropidin/L, 175 g prothioconazole/L, nominal) 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 1.0 L /ha and 10.0 L /ha (1.39 mg prod./kg soil_{dw} and 13.87 mg prod./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 NH₄-N-, NO₃-N- and NO₂-N- contents were determined. Under the conditions of this test, ADM.03502.F.1.A 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 13.87 mg prod./kg soil_{dw} (equivalent to 10.0 L prod./ha).

I. Materials and methods

A. Materials

1. Test material: ADM.03502.F.1.A
Lot/Batch no.: 1191-101219-01
Content/Purity: 250 g fenpropidin/L, 175 g prothioconazole/L (nominal)
253.7 g fenpropidin/L, 175.9 g prothioconazole/L (analysed)
Density: 1.04 g/mL
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 (USDA), loamy sand (DIN 4220)
Sand content: 53.0 / 51.6 %
Silt content: 36.2 / 38.3 %
Clay content: 10.8 / 10.1 %
pH-value: 6.1 – 6.3
Organic carbon content: 1.37 %
Microbial biomass (C content): 2.95 % of total organic carbon content
Soil moisture: 44.35 – 45.49 % of WHC
Temperature: 18.8 - 21.5°C
Photoperiod: dark

B. Study design and method

1. In life dates: June 03 to July 01, 2020
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 NH₄-N-content and NO₃-N-content. The NO₃-N-content was 1.53 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 1.0 L /ha and 10.0 L /ha (1.39 mg prod./kg soil_{dw} and 13.87 mg prod./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 NH₄-N-, NO₃-N- and NO₂-N-contents were determined.

3. Statistics:

The mean nitrogen-content (based on NO₃-N), 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:

$$\% \text{ deviation to control} = ((\text{test item rate} - \text{control rate})/\text{control rate}) \times 100$$

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 homogenous two-sided student-t-tests for homogeneous variances were performed (Alpha = 0.050). The student-t-test compares the treatment mean against a single control mean. The statistical analysis was performed with the software Tox-Rat Professional 3.3.0 (Ratte 2018).

II. Results and discussions

A. Nitrogen turnover

No adverse effects (i.e. > 25%) on the nitrogen transformation rate in soil were observed at both test concentrations (1.39 or 13.87 mg prod./kg soil_{dw}) after 28 days (time interval 14 - 28 days) and at all intervals before. Additionally, no statistically significant differences between the nitrogen transformation rates were observed at all time intervals between the respective test item concentrations and the control. The results are summarised in the table below

Table A 58: Effects on nitrogen transformation rate (nitrate/day) after treatment with ADM.03502.F.1.A

Time interval (days)	Control	1.39 mg test item/kg soil _{dw} equivalent to 1 L test item/ha		13.87 mg test item/kg soil _{dw} equivalent to 10 L test item/ha	
	NO ₃ -N/day [mg/kg soil dw]	NO ₃ -N/day [mg/kg soil dw]	% difference to control	NO ₃ -N/day [mg/kg soil dw]	% difference to control
0-7	4.39	4.17 (n.s.)	-5.1	4.35 (n.s.)	-0.9
7-14	1.68	1.73 (n.s.)	2.8	1.59 (n.s.)	-5.4
14-28	1.17	1.18 (n.s.)	1.0	1.15 (n.s.)	-1.2

1) based on NO₃-N-production; - = lower compared to the control; + = higher compared to the control
n.s. = not statistically significantly different to control (Student-t-test for homogeneous variances, 2-sided, p > 0.05).
The calculations were performed with unrounded values

The reference item Dicyandiamide caused a significant reduction of the nitrogen transformation rate of - 62.0 % and -74.3 % at 100 and 200 mg dicyandiamide per kg soil_{dw}, respectively, determined 28 days after application (time interval 14-28), and thus demonstrates the sensitivity of the test system.

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 4.0 %.

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.03502.F.1.A (250 g fenpropidin/L, 175 g prothioconazole/L, nominal) were investigated with agriculturally utilised soil. The

test item was mixed into the soil at concentrations equivalent to application rates of 1.0 L /ha and 10.0 L /ha (1.39 mg prod./kg soil_{dw} and 13.87 mg prod./kg soil_{dw}). Under the conditions of this test, ADM.03502.F.1.A 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 13.87 mg prod./kg soil_{dw} (equivalent to 10.0 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:	<p>The study was conducted in line with OECD 208 with major deviation in environmental conditions.</p> <p>The nominal test concentrations of prothioconazole and fenpropidin were analytically confirmed for the highest test item solution.</p> <p>The recovery of prothioconazole were in the range from 99.2 % to 100.8 %.</p> <p>The recovery of fenpropidin were in the range from 99.7 % to 101.1 %.</p> <p>All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>ER₅₀ seedling emergence, survival of emerged plants, phytotoxicity, plant length and shoot dry weight > 1 L product/ha (Beta vulgaris (sugar beet), Brassica napus (rape), Solanum lycopersicon (tomato), Glycine max (soybean), Lolium perenne (perennial ryegrass), Allium cepa (onion).</p>
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Reference:	KCP 10.6.1/01
Report:	Effects of ADM.03502.F.1.A on seedling emergence and seedling growth of six non-target terrestrial plant species under greenhouse conditions, Kästner, K., 2020a, report no.: 2046PSE0007, sponsor no.: 000104852
Guideline(s):	OECD 208 (2006)
Deviations:	None
GLP:	Yes
Acceptability/Reliability:	Yes
Duplication (if vertebrate study)	Not applicable

Executive summary

The effects of ADM.03502.F.1.A (250 g fenpropidin/L, 175 g prothioconazole/L, nominal) on non-target plants were recorded in a seedling emergence test with 4 dicotyledonous and 2 monocotyledonous species, i.e. sugar beet, rape, tomato, soybean, ryegrass, and onion. ADM.03502.F.1.A was applied to the soil at application rates of 1.0, 0.370, 0.137, 0.051, 0.019 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 BBCH stage, seedling emergence, plant survival of emerged seedlings and visible phytotoxicity compared to untreated control plants on study day 7, 14 and 21. At the end of the test additionally shoot dry weight (biomass of surviving plants) and plant length were recorded. The control and highest test item solution were sampled in duplicate directly after preparation and immediately before application and the samples were analysed via HPLC-with UV-Diode-Array detection. 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 1.0 L prod./ha. The ER₂₅ and ER₅₀ of shoot height, shoot fresh weight and emergence was determined to be > 1.0 L prod./ha.

I. Materials and methods

A. Materials

1. Test material: ADM.03502.F.1.A
Lot/Batch no.: 1191-101219-01
Content/Purity: 250 g fenpropidin/L, 175 g prothioconazole/L (nominal)
253.7 g fenpropidin/L, 175.9 g prothioconazole/L (analysed)
Control: Tap water at 200 L/ha
Solvent/vehicle: water
Toxic reference: none
2. Test organisms -
Dicotyledonous species: *Beta vulgaris* (sugar beet)
Brassica napus (rape)
Solanum lycopersicon (tomato)
Glycine max (soybean)
Monocotyledonous species: *Lolium perenne* (perennial ryegrass)
Allium cepa (onion)
Growth stage at treatment: seeds
No. of plants: 8 -15 replicates per application rate and control, 2 - 6 seeds per pot
3. Test units and exposure –
Test system: Seedling emergence, dose-response test
Type and size: The test vessels (non-porous plastic pots not used before (diameter 15 cm) with holes in the bottom to allow watering)
Test duration: 21 days
4. Test conditions –
Test substrate: Test soil was a natural field soil from site Gerichshain. No pesticides or fertilisers were applied on the origin plot for at least 5 years (the plot was fallow land). Before use, the soil was heat treated (4 hours at 105 °C) for the trials with sugar beet and cucumber in order to reduce the effect of soil pathogens

Test soil type: loamy sand
Grain size: ≤ 2 mm
pH-value: 6.0
Temperature: 16.5 – 32.8 °C
Photoperiod: 16 h
Light intensity: 160 – 928 $\mu\text{mol/m}^2/\text{s}$
Relative humidity: 32.3 – 80.5 %

B. Study design and method

1. In life dates: June 29 to August 22, 2020
2. Test design:
Potential adverse effects of the test item ADM.03502.F.1.A to the six terrestrial plant species ryegrass (*Lolium perenne*), onion (*Allium cepa*), sugar beet (*Beta vulgaris*), oilseed rape (*Brassica napus*), tomato (*Solanum lycopersicon*) and soybean (*Glycine max*) were examined in comparison with a water control under greenhouse conditions. In the test, 30 and 32, respectively, seeds were tested per treatment group. For ryegrass 4 seeds per pot were sown with 8 replicates per treatment. For onion, 6 seeds per pot were sown with 5 replicates per treatment. For oilseed rape, 3 seeds per pot were sown with 10 replicates per treatment and for sugar beet, tomato and soybean, 2 seeds per pot were sown with 15 replicates. In the

test ADM.03502.F.1.A was applied after sowing (BBCH 00) at application rates of 0.019, 0.051, 0.137, 0.370 and 1.000 L prod./ha in 200 L water/ha.

The test solution was sprayed once in ryegrass, onion, sugar beet, oilseed rape, tomato and soybean onto the soil surface in an automatic application cabin at a spray volume equivalent to 200 L/ha. The control and highest test item solution were sampled in duplicate directly after preparation and immediately before application and the samples were analysed via HPLC-with UV-Diode-Array detection.

During the observation period of 21 days after 50 % of the control plants had emerged (DAE), the plants were assessed weekly for seedling emergence, survival (mortality) and visual phytotoxicity (chlorosis, necrosis, deformation, stunting in %). Endpoints observed after 21 days were seedling emergence, survival (mortality) of emerged seedlings, plant length, biomass (shoot dry weight) and visible phytotoxicity.

3. Analytical verification:

The control and highest test item solution were sampled in duplicate directly after preparation and immediately before application and the samples were analysed via HPLC-with UV-Diode-Array detection.

4. Statistics:

Mean and standard deviation of assessment: Data were calculated and rounded by Excel. The measurements and observations were compared to those of untreated control plants. If negative effects had been determined on 21 DAE further statistical analyses were performed by using the software ToxRat Professional (ToxRatPro Version 3.3.0). For seedling emergence Chi²-2x2 Test with Bonferroni correction ($\alpha = 0.05$, one-sided greater) was used. For statistical evaluation of plant length and shoot dry weight Dunnett's Multiple t-test ($\alpha = 0.05$, one-sided smaller) and Williams Multiple Sequential t-test Procedure ($\alpha = 0.05$, one-sided smaller) were used.

II. Results and discussion

A. Analytical data

The nominal test concentrations of prothioconazole and fenpropidin were analytically confirmed for the highest test item solution. The recovery of prothioconazole in the specimen with test item were in the range from 99.2 % to 100.8 %. The recovery of fenpropidin in the specimen with test item were in the range from 99.7 % to 101.1 %. No active ingredient was detected in the control specimen. Thus, the concentration of the test solutions from the biological test was verified.

B. Visual phytotoxicity

The pre-emergence application at rates up to 1.000 L ADM.03502.F.1.A/ha caused no visible phytotoxic effects since no chlorosis, necrosis, deformation or stunting was detected in any tested plant species

Effects of ADM.03502.F.1.A on phytotoxicity (chlorosis, necrosis, deformation and stunting) 21 DAE in the seedling emergence and growth test [mean of all replicates in %]

Plant species	Symptom		Application rate of ADM.03502.F.1.A [L test item/ha in 200 L/ha water]					
			Control	0.019	0.051	0.137	0.370	1.000
Ryegrass	Chlorosis	[%]	0.0	0.0	0.0	0.0	0.0	0.0
		SD	0.0	0.0	0.0	0.0	0.0	0.0
	Necrosis	[%]	0.0	0.0	0.0	0.0	0.0	0.0
		SD	0.0	0.0	0.0	0.0	0.0	0.0
	Deformation	[%]	0.0	0.0	0.0	0.0	0.0	0.0
		SD	0.0	0.0	0.0	0.0	0.0	0.0
	Stunting	[%]	0.0	0.0	0.0	0.0	0.0	0.0
		SD	0.0	0.0	0.0	0.0	0.0	0.0
	Chlorosis	[%]	0.0	0.0	0.0	0.0	0.0	0.0
		SD	0.0	0.0	0.0	0.0	0.0	0.0
Onion	Necrosis	[%]	0.0	0.0	0.0	0.0	0.0	0.0
		SD	0.0	0.0	0.0	0.0	0.0	0.0
	Deformation	[%]	0.0	0.0	0.0	0.0	0.0	0.0
		SD	0.0	0.0	0.0	0.0	0.0	0.0
	Stunting	[%]	0.0	0.0	0.0	0.0	0.0	0.0
		SD	0.0	0.0	0.0	0.0	0.0	0.0
	Chlorosis	[%]	0.0	0.0	0.0	0.0	0.0	0.0
		SD	0.0	0.0	0.0	0.0	0.0	0.0
	Necrosis	[%]	0.0	0.0	0.0	0.0	0.0	0.0
		SD	0.0	0.0	0.0	0.0	0.0	0.0
Sugar beet	Deformation	[%]	0.0	0.0	0.0	0.0	0.0	0.0
		SD	0.0	0.0	0.0	0.0	0.0	0.0
	Stunting	[%]	0.0	0.0	0.0	0.0	0.0	0.0
		SD	0.0	0.0	0.0	0.0	0.0	0.0
	Chlorosis	[%]	0.0	0.0	0.0	0.0	0.0	0.0
		SD	0.0	0.0	0.0	0.0	0.0	0.0
	Necrosis	[%]	0.0	0.0	0.0	0.0	0.0	0.0
		SD	0.0	0.0	0.0	0.0	0.0	0.0
	Deformation	[%]	0.0	0.0	0.0	0.0	0.0	0.0
		SD	0.0	0.0	0.0	0.0	0.0	0.0
Oilseed rape	Stunting	[%]	0.0	0.0	0.0	0.0	0.0	0.0
		SD	0.0	0.0	0.0	0.0	0.0	0.0
	Chlorosis	[%]	0.0	0.0	0.0	0.0	0.0	0.0
		SD	0.0	0.0	0.0	0.0	0.0	0.0
	Necrosis	[%]	0.0	0.0	0.0	0.0	0.0	0.0
		SD	0.0	0.0	0.0	0.0	0.0	0.0
	Deformation	[%]	0.0	0.0	0.0	0.0	0.0	0.0
		SD	0.0	0.0	0.0	0.0	0.0	0.0
	Stunting	[%]	0.0	0.0	0.0	0.0	0.0	0.0
		SD	0.0	0.0	0.0	0.0	0.0	0.0
Tomato	Chlorosis	[%]	0.0	0.0	0.0	0.0	0.0	0.0
		SD	0.0	0.0	0.0	0.0	0.0	0.0
	Necrosis	[%]	0.0	0.0	0.0	0.0	0.0	0.0
		SD	0.0	0.0	0.0	0.0	0.0	0.0
	Deformation	[%]	0.0	0.0	0.0	0.0	0.0	0.0
		SD	0.0	0.0	0.0	0.0	0.0	0.0
	Stunting	[%]	0.0	0.0	0.0	0.0	0.0	0.0
		SD	0.0	0.0	0.0	0.0	0.0	0.0
	Chlorosis	[%]	0.0	0.0	0.0	0.0	0.0	0.0
		SD	0.0	0.0	0.0	0.0	0.0	0.0

DAE days after 50 % emergence in the control group SD Standard deviation

C. Effects on seedling emergence

No effect on seedling emergence and plant survival were detected after pre-emergence application at rates of 0.019, 0.051, 0.137, 0.370 and 1.000 L ADM.03502.F.1.A/ha to tested plant species. The results of the individual emergence rates are listed in the table below.

Table A 59: Effects of ADM.03502.F.1.A on seedling emergence and plant survival in the seedling emergence and growth test [mean of all replicates]

Test species	Application rates in 200 L/ha	Mean number of living plants per replicate			Number of emerged plants	Emergence	Survival
	ADM.03502.F.1.A [L/ha]	7 DAE	14 DAE	21 DAE	0-21 DAE	[%] com- pared to control	21 DAE [%]
Ryegrass	control	3.6	3.6	3.8	3.8	100.0	100
	0.019	3.6	3.6	3.6	3.6	96.7	100
	0.051	3.6	3.6	3.6	3.6	96.7	100
	0.137	3.8	3.8	3.8	3.8	100.0	100
	0.370	3.8	3.8	3.8	3.8	100.0	100
	1.000	3.8	3.8	3.8	3.8	100.0	100
Onion	control	5.4	5.4	5.4	5.4	100.0	100
	0.019	5.0	5.2	5.2	5.2	96.3	100
	0.051	5.	5.4	5.4	5.4	100.0	100

	0.137	5.6	5.6	5.6	5.6	103.7	100
	0.370	5.4	5.4	5.4	5.4	100.0	100
	1.000	4.8	5.2	5.2	5.2	96.3	100
Sugar beet	control	1.7	1.8	1.8	1.8	100.0	100
	0.019	1.7	1.7	1.7	1.7	96.3	100
	0.051	1.7	1.9	1.9	1.9	103.7	100
	0.137	1.9	1.9	1.9	1.9	107.4	100
	0.370	1.6	1.8	1.8	1.8	100.0	100
	1.000	1.8	1.9	1.9	1.9	103.7	100
Oilseed rape	control	3.0	3.0	3.0	3.0	100.0	100
	0.019	3.0	3.0	3.0	3.0	100.0	100
	0.051	3.0	3.0	3.0	3.0	100.0	100
	0.137	3.0	3.0	3.0	3.0	100.0	100
	0.370	3.0	3.0	3.0	3.0	100.0	100
	1.000	3.0	3.0	3.0	3.0	100.0	100
Tomato	control	2.0	2.0	2.0	2.0	100.0	100
	0.019	2.0	2.0	2.0	2.0	100.0	100
	0.051	1.9	1.9	1.9	1.9	96.7	100
	0.137	1.9	1.9	1.9	1.9	96.7	100
	0.370	1.9	1.9	1.9	1.9	96.7	100
	1.000	1.9	1.9	1.9	1.9	96.7	100
Soybean	control	2.0	2.0	2.0	2.0	100.0	100
	0.019	1.9	1.9	1.9	1.9	96.7	100
	0.051	2.0	2.0	2.0	2.0	100.0	100
	0.137	1.9	1.9	1.9	1.9	96.7	100
	0.370	2.0	2.0	2.0	2.0	100.0	100
	1.000	1.9	1.9	1.9	1.9	96.7	100

DAE days after 50 % emergence in the control group

No statistically significant difference between control and treatment (Chi2-2x2 Test with Bonferroni correction one-sided greater, $\alpha = 0.05$)

The NOER for seedling emergence and the NOER for plant survival is ≥ 1.000 L ADM.03502.F.1.A/ha for all tested plant species. No effects for seedling emergence and plant survival could be found in any tested plant species and accordingly the ER50 is > 1.000 L ADM.03502.F.1.A/ha.

D. Plant length

No statistically significant plant length reduction was detected on tested plant species after preemergence application at application rates of 0.019, 0.051, 0.137, 0.370 and 1.000 L ADM.03502.F.1.A/ha.

Table A 60: Effects of ADM.03502.F.1.A on plant length 21 DAE in the seedling emergence and growth test [mean of all replicates in cm]

Test species	Application rate of ADM.03502.F.1.A [L prod./ha in 200 L/ha water]					
	Control	0.019	0.051	0.137	0.370	1.000
Ryegrass						
Mean [cm]	29.7	30.1	29.8	31.3	30.6	30.2
SD	1.7	2.8	1.5	2.6	2.9	2.8
CV [%]	5.6	9.3	4.9	8.3	9.5	9.2
Inhibition [%]	---	-1.4	-0.4	-5.4	-3.3	-1.9
Compared to control [%]	---	101.4	100.4	105.4	103.3	101.9
Onion						
Mean [cm]	24.6	25.1	26.4	24.9	26.0	26.9
SD	2.2	0.8	2.7	2.1	1.2	1.4
CV [%]	8.8	3.3	10.4	8.5	4.6	5.1
Inhibition [%]	---	-2.0	-7.3	-1.5	-6.0	-9.6
Compared to control [%]	---	102.0	107.3	101.5	106.0	109.6
Sugar beet						
Mean [cm]	24.1	23.8	23.4	23.1	23.3	24.2
SD	2.0	2.1	1.9	1.4	1.5	2.4

CV [%]	8.3	8.7	8.2	6.2	6.4	9.9
Inhibition [%]	---	1.5	3.0	4.3	3.6	-0.4
Compared to control [%]	---	98.5	97.0	95.7	96.4	100.4
Oilseed rape						
Mean [cm]	33.0	32.8	32.9	32.9	32.5	32.4
SD	1.3	1.7	1.4	1.3	1.4	1.7
CV [%]	4.0	5.2	4.3	4.0	4.4	5.2
Inhibition [%]	---	0.8	0.5	0.5	1.7	1.8
Compared to control [%]	---	99.2	99.5	99.5	98.3	98.2
Tomato						
Mean [cm]	23.5	23.2	23.7	22.7	22.4	24.0
SD	2.9	1.9	2.4	2.6	2.6	2.5
CV [%]	12.2	8.1	10.0	11.5	11.6	10.3
Inhibition [%]	---	1.4	-0.9	3.3	4.7	-2.1
Compared to control [%]	---	98.6	100.9	96.7	95.3	102.1
Soybean						
Mean [cm]	56.7	60.3	58.7	59.1	57.3	58.9
SD	6.3	6.1	5.3	3.3	5.7	6.2
CV [%]	11.1	10.1	9.0	5.6	10.0	10.6
Inhibition [%]	---	-6.2	-3.5	-4.2	-1.1	-3.8
Compared to control [%]	---	106.2	103.5	104.2	101.1	103.8

DAE days after 50 % emergence in the control group SD Standard deviation CV coefficient of variation

No statistically significant difference between control and treatment (for oilseed rape and tomato: Dunnett's multiple t-test, one-sided smaller, $\alpha = 0.05$)

The NOER for plant length reduction is ≥ 1.000 L ADM.03502.F.1.A/ha for all tested plant species. No dose-response for plant length reduction could be found in any tested plant species and accordingly the ER₅₀ is > 1.000 L ADM.03502.F.1.A/ha.

E. Biomass (shoot fresh weight)

No statistically significant biomass reduction was detected on tested plant species after pre-emergence application at application rates of 0.019, 0.051, 0.137, 0.370 and 1.000 L ADM.03502.F.1.A/ha.

Table A 61: Effects of ADM.03502.F.1.A on shoot dry weight 21 DAE in the seedling emergence and growth test [mean of all replicates in g]

Test species	Application rate of ADM.03502.F.1.A [L prod./ha in 200 L/ha water]					
	Control	0.019	0.051	0.137	0.370	1.000
Ryegrass						
Mean [g]	0.913	0.935	0.913	1.104	1.014	1.008
SD	0.1	0.2	0.1	0.2	0.2	0.2
CV [%]	14.4	22.8	15.1	15.2	17.6	15.2
Inhibition [%]	---	-2.4	0.1	-20.9	-11.1	-10.4
Compared to control [%]	---	102.4	99.1	120.9	111.1	110.4
Onion						
Mean [g]	0.486	0.484	0.602	0.511	0.584	0.581
SD	0.1	0.1	0.1	0.1	0.1	0.1
CV [%]	17.5	15.0	19.9	18.7	20.2	20.7
Inhibition [%]	---	0.4	-23.9	-5.2	-20.2	-19.6
Compared to control [%]	---	99.6	123.9	105.2	120.2	119.6
Sugar beet						
Mean [g]	2.627	2.562	2.511	2.370	2.426	2.556
SD	0.4	0.5	0.4	0.4	0.6	0.4
CV [%]	14.2	18.8	16.2	16.3	25.6	13.9
Inhibition [%]	---	2.5	4.4	9.8	7.7	2.7
Compared to control [%]	---	97.5	95.6	90.2	92.3	97.3

Oilseed rape						
Mean [g]	8.369	7.905	7.976	8.201	7.987	8.214
SD	0.8	0.9	0.9	0.8	1.3	0.9
CV [%]	9.3	11.3	11.8	9.3	15.9	11.1
Inhibition [%]	---	5.5	4.7	2.0	4.6	1.9
Compared to control [%]	---	94.5	95.3	98.0	95.4	98.1
Tomato						
Mean [g]	4.556	4.853	4.550	4.645	4.295	5.113
SD	0.8	0.9	1.1	0.8	1.1	0.7
CV [%]	17.4	19.2	23.4	18.0	24.7	13.7
Inhibition [%]	---	-6.5	0.1	-2.0	5.7	-12.2
Compared to control [%]	---	106.5	99.9	102.0	94.3	112.2

Soybean						
Mean [g]	8.151	8.863	8.705	8.438	8.650	8.499
SD	0.7	1.0	0.8	1.0	0.7	1.0
CV [%]	8.9	11.3	8.9	12.3	7.8	12.2
Inhibition [%]	---	-8.7	-6.8	-3.5	-6.1	-4.3
Compared to control [%]	---	108.7	106.8	103.5	106.1	104.3

DAE days after 50 % emergence in the control group SD Standard deviation CV coefficient of variation

No statistically significant difference between control and treatment (for sugar beet, oilseed rape and tomato: Dunnett's multiple t-test, one-sided smaller, $\alpha = 0.05$; for ryegrass and onion: Williams Multiple Sequential t-test, one-sided smaller $\alpha = 0.05$)

The NOER for biomass reduction is ≥ 1.000 L ADM.03502.F.1.A/ha for all tested plant species. No dose-response for biomass reduction could be found in any tested plant species and accordingly the ER₅₀ is > 1.000 L ADM.03502.F.1.A/ha.

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 ≥ 90 %.
The seedlings of the control shall not exhibit visible phytotoxic 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 survival of the plants in the control group was 100 % 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.03502.F.1.A 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 1.0 L prod./ha. The ER₂₅ and ER₅₀ of shoot height, shoot fresh weight and emergence was determined to be > 1.0 L prod./ha. The study is considered valid (see: "F. Validity of the test").

Comments of zRMS:	<p>The study was conducted in line with OECD 227 with minor deviations.</p> <p>The nominal test concentrations of prothioconazole and fenpropidin were analytically confirmed for the highest test item solution. The recovery of prothioconazole was 101.2% 102.2 % for fenpropidin.</p> <p>All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>ER₅₀, survival, phytotoxicity, plant length and shoot dry weight > 1 L product/ha (Beta vulgaris (sugar beet), Brassica napus (rape), Solanum lycopersicon (tomato) Glycine max (soybean), Lolium perenne (perennial ryegrass), Allium cepa (onion)).</p>
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Report:	Effects of ADM.03502.F.1.A on vegetative vigour of six non-target terrestrial plant species under greenhouse conditions, Kästner, K., 2020b, report no.: 2046PVV0009, sponsor no.: 000104853
Guideline(s):	OECD 227 (2006)
Deviations:	None
GLP:	Yes
Acceptability/Reliability:	Yes
Duplication (if vertebrate study)	Not applicable

Executive summary

Potential adverse effects of the test item ADM.03502.F.1.A to the six terrestrial plant species ryegrass (*Lolium perenne*), onion (*Allium cepa*), sugar beet (*Beta vulgaris*), oilseed rape (*Brassica napus*), tomato (*Solanum lycopersicon*), soybean (*Glycine max*) were examined in comparison with a water control under greenhouse conditions. In the test ADM.03502.F.1.A was applied at BBCH stage 12-14 (2-4 true leaf stage) at application rates of 0.019, 0.051, 0.137, 0.370 and 1.0 L/ha in 200 L water/ha. The test solution was sprayed once in ryegrass, onion, sugar beet, oilseed rape, tomato and soybean in an automatic application cabin at a spray volume equivalent to 200 L/ha. The control and highest test item solution were sampled in duplicate directly after preparation and immediately before application and the samples were analysed via HPLC with UV-Diode-Array detection. During the observation period of 21 days after treatment (DAT), the plants were assessed weekly for growth stage, plant survival and phytotoxicity (chlorosis, necrosis, deformation, stunting in %). Endpoints observed 21 DAT were growth stage, plant survival, plant length, biomass (shoot dry weight) and phytotoxicity. The effects of ADM.03502.F.1.A after application on six different plant species (ryegrass, onion, sugar beet, oilseed rape, tomato, soybean) at BBCH stage 12-14 were examined at nominal application rates of 0.019, 0.051, 0.137, 0.370 and 1.0 L prod./ha in 200 L water/ha under greenhouse conditions. The test endpoints were plant survival (mortality), plant length, shoot dry weight and visual phytotoxicity 21 days after application (DAT). An effect on plant survival could not be detected after application at BBCH stage 12-14 at rates up to 1.0 L ADM.03502.F.1.A/ha in any tested plant species. The application at BBCH stage 12-14 at rates up to 1.0 L ADM.03502.F.1.A/ha caused visible phytotoxic effects to onion, sugar beet, oilseed rape, tomato and soybean at the two highest test rates at a mean percentage of 0.3 to 8.7 %. In onion, only the highest rate induced phytotoxic effects. Chlorosis, necrosis, deformation and stunting was detected for sugar beet, oilseed rape, tomato and soybean. For onion necrosis was detected. Statistically significant plant length reduction was detected for sugar beet and oilseed rape.

I. Materials and methods

A. Materials

- Test material: ADM.03502.F.1.A
Lot/Batch no.: 1191-101219-01
Content/Purity: 250 g fenpropidin/L, 175 g prothioconazole/L (nominal)
253.7 g fenpropidin/L, 175.9 g prothioconazole/L (analysed)
Control: Tap water at 200 L/ha
Solvent/vehicle: water
Toxic reference: none
- Test organisms -
Dicotyledonous species: *Beta vulgaris* (sugar beet)
Brassica napus (rape)
Solanum lycopersicon (tomato)
Glycine max (soybean)
Monocotyledonous species: *Lolium perenne* (perennial ryegrass)
Allium cepa (onion)

Growth stage at treatment:	2 - 4 leaf stage
No. of plants:	5-15 replicates treatment and control depending on test species, 2-6 plants per pot
3. Test units and exposure –	
Test system:	vegetative vigour, dose-response test
Type and size:	The test vessels (non-porous plastic pots not used before (diameter 15 cm) with holes in the bottom to allow watering)
Test duration:	21 days
4. Test conditions –	
Test substrate:	Test soil was a natural field soil from site Gerichshain. No pesticides or fertilisers were applied on the origin plot for at least 5 years (the plot was fallow land). Before use, the soil was heat treated (4 hours at 105 °C) for the trials with sugar beet and cucumber in order to reduce the effect of soil pathogens
Test type:	loamy sand
pH-value:	6.0
Temperature:	16.9 – 35.7
Photoperiod:	16 h
Light intensity:	353 – 503 $\mu\text{mol}/\text{m}^2/\text{s}$
Relative humidity:	38.4 – 83.5 %
Watering:	bottom watering of the test containers

B. Study design and method

1. In life dates: 03 June 2020 – 02 July 2020
2. Test design:

Potential adverse effects of the test item ADM.03502.F.1.A to the six terrestrial plant species ryegrass (*Lolium perenne*), onion (*Allium cepa*), sugar beet (*Beta vulgaris*), oilseed rape (*Brassica napus*), tomato (*Solanum lycopersicon*), soybean (*Glycine max*) were examined in comparison with a water control under greenhouse conditions. During the study the greenhouse conditions were as follows: air temperature 16.9 – 35.7°C, relative humidity 38.4 – 83.5 %, light intensity 446.6 $\mu\text{mol}/\text{m}^2/\text{s}$ (daily mean of the 16 hours photoperiod). In the test 30 and 32 plants, respectively, were tested per treatment group. For ryegrass, 4 plants per pot were used with 8 replicates per treatment. For onion 6 plants, per pot were used with 5 replicates per treatment. For oilseed rape, 3 plants per pot were used with 10 replicates per treatment and for sugar beet, tomato and soybean, 2 plants per pot were used with 15 replicates. In the test ADM.03502.F.1.A was applied at BBCH stage 12-14 (2-4 true leaf stage) at application rates of 0.019, 0.051, 0.137, 0.370 and 1.0 L/ha in 200 L water/ha. The test solution was sprayed once in ryegrass, onion, sugar beet, oilseed rape, tomato and soybean in an automatic application cabin at a spray volume equivalent to 200 L/ha. The control and highest test item solution were sampled in duplicate directly after preparation and immediately before application and the samples were analysed via HPLC with UV-Diode-Array detection.

During the observation period of 21 days after treatment (DAT), the plants were assessed weekly for growth stage, plant survival and phytotoxicity (chlorosis, necrosis, deformation, stunting in %). Endpoints observed 21 DAT were growth stage, plant survival, plant length, biomass (shoot dry weight) and phytotoxicity.

3. Analytical verification:

The control and highest test item solution were sampled in duplicate directly after preparation and immediately before application and the samples were analysed via HPLC with UV-Diode-Array detection.

4. Statistics:

Mean and standard deviation of assessment data were calculated and rounded by Excel. The measurements and observations were compared to those of untreated control plants. If negative effects had been determined on 21 DAT further statistical analyses were performed by using the software ToxRat Professional (ToxRatPro Version 3.3.0). For statistical evaluation of metric data of plant length and shoot dry weight the data were tested for Normal Distribution (Shapiro-Wilk's Test, $\alpha = 0.01$), Variance Homogeneity (with Residuals) (Levene's Test, $\alpha = 0.01$) and Trend analysis by Contrasts (Monotonicity of Concentration/Response, $\alpha = 0.05$). Depending of the outcomes of the pre-testing sequences the Williams Multiple Sequential t-test (one-sided smaller, $\alpha = 0.05$), the Dunnett's multiple t-test procedure (one-sided smaller, $\alpha = 0.05$) or the multiple sequentially-rejective Welch-t-test after Bonferroni-Holm (one-sided smaller, $\alpha = 0.05$) was used. For calculation of effective rates (ER₂₅ and ER₅₀) for plant survival, plant length and shoot dry weight a Probit, Logit or Weibull analysis using linear max. likelihood regression was performed. The 95 %-confidence limits were calculated according to Fieller's theorem or by Normal Approximation.

II. Results and discussion

A. Analytical data

The nominal test concentrations of prothioconazole and fenpropidin were analytically confirmed for the highest test item solution. The recovery of prothioconazole and fenpropidin in the specimen with test item was 101.2 % and 102.2 %, respectively, of the nominal concentration. No active ingredient was detected in the control specimen. Thus, the concentration of the test solutions from the biological test was verified.

B. Visual phytotoxicity

The application at BBCH stage 12-14 at rates up to 1.000 L ADM.03502.F.1.A/ha caused visible phytotoxic effects to sugar beet, oilseed rape, tomato and soybean at the two highest test rates. In onion, only the highest rate induced phytotoxic effects. Chlorosis, necrosis, deformation and stunting was detected for these plant species at a mean percentage of 0.3 to 8.7 %.

Effects of ADM.03502.F.1.A on phytotoxicity (chlorosis, necrosis, deformation and stunting) 21 DAT in the vegetative vigour test [mean of all replicates in %].

Plant species	Symptom		Application rate of ADM.03502.F.1.A [L test item/ha in 200 L/ha water]					
			Control	0.019	0.051	0.137	0.370	1.000
Ryegrass	Chlorosis	[%]	0.0	0.0	0.0	0.0	0.0	0.0
		SD	0.0	0.0	0.0	0.0	0.0	0.0
	Necrosis	[%]	0.0	0.0	0.0	0.0	0.0	0.0
		SD	0.0	0.0	0.0	0.0	0.0	0.0
	Deformation	[%]	0.0	0.0	0.0	0.0	0.0	0.0
		SD	0.0	0.0	0.0	0.0	0.0	0.0
	Stunting	[%]	0.0	0.0	0.0	0.0	0.0	0.0
		SD	0.0	0.0	0.0	0.0	0.0	0.0
	Chlorosis	[%]	0.0	0.0	0.0	0.0	0.0	0.0
		SD	0.0	0.0	0.0	0.0	0.0	0.0
Onion	Necrosis	[%]	0.0	0.0	0.0	0.0	0.0	0.0
		SD	0.0	0.0	0.0	0.0	0.0	1.0
	Deformation	[%]	0.0	0.0	0.0	0.0	0.0	0.0
		SD	0.0	0.0	0.0	0.0	0.0	0.0
	Stunting	[%]	0.0	0.0	0.0	0.0	0.0	0.0
		SD	0.0	0.0	0.0	0.0	0.0	0.0
	Chlorosis	[%]	0.0	0.0	0.0	0.0	1.5	5.0
		SD	0.0	0.0	0.0	0.0	0.5	0.0
	Necrosis	[%]	0.0	0.0	0.0	0.0	0.4	4.6
		SD	0.0	0.0	0.0	0.0	0.5	1.1
Sugar beet	Deformation	[%]	0.0	0.0	0.0	0.0	0.3	5.7
		SD	0.0	0.0	0.0	0.0	0.7	3.7
	Stunting	[%]	0.0	0.0	0.0	0.0	5.3	8.7
		SD	0.0	0.0	0.0	0.0	6.1	8.3
	Chlorosis	[%]	0.0	0.0	0.0	0.0	0.0	5.0
		SD	0.0	0.0	0.0	0.0	0.0	0.0
	Necrosis	[%]	0.0	0.0	0.0	0.0	0.5	5.0
		SD	0.0	0.0	0.0	0.0	0.5	0.0
	Deformation	[%]	0.0	0.0	0.0	0.0	0.0	5.0
		SD	0.0	0.0	0.0	0.0	0.0	0.0
Oilseed rape	Stunting	[%]	0.0	0.0	0.0	0.0	0.0	4.0
		SD	0.0	0.0	0.0	0.0	0.0	5.2
	Chlorosis	[%]	0.0	0.0	0.0	0.0	0.0	4.0
		SD	0.0	0.0	0.0	0.0	0.0	1.5
	Necrosis	[%]	0.0	0.0	0.0	0.0	1.0	1.8
		SD	0.0	0.0	0.0	0.0	0.0	0.4
	Deformation	[%]	0.0	0.0	0.0	0.0	0.9	4.5
		SD	0.0	0.0	0.0	0.0	1.0	1.5
	Stunting	[%]	0.0	0.0	0.0	0.0	0.0	0.0
		SD	0.0	0.0	0.0	0.0	0.0	0.0
Tomato	Chlorosis	[%]	0.0	0.0	0.0	0.0	0.5	5.0
		SD	0.0	0.0	0.0	0.0	0.5	0.0
	Necrosis	[%]	0.0	0.0	0.0	0.0	1.0	4.5
		SD	0.0	0.0	0.0	0.0	0.0	1.4
	Deformation	[%]	0.0	0.0	0.0	0.0	0.0	5.1
		SD	0.0	0.0	0.0	0.0	0.0	1.7
	Stunting	[%]	0.0	0.0	0.0	0.0	0.0	0.0
		SD	0.0	0.0	0.0	0.0	0.0	0.0
	Chlorosis	[%]	0.0	0.0	0.0	0.0	0.5	5.0
		SD	0.0	0.0	0.0	0.0	0.5	0.0
Soybean	Necrosis	[%]	0.0	0.0	0.0	0.0	1.0	4.5
		SD	0.0	0.0	0.0	0.0	0.0	1.4
	Deformation	[%]	0.0	0.0	0.0	0.0	0.0	5.1
		SD	0.0	0.0	0.0	0.0	0.0	1.7
	Stunting	[%]	0.0	0.0	0.0	0.0	0.0	0.0
		SD	0.0	0.0	0.0	0.0	0.0	0.0
	Chlorosis	[%]	0.0	0.0	0.0	0.0	0.5	5.0
		SD	0.0	0.0	0.0	0.0	0.5	0.0
	Necrosis	[%]	0.0	0.0	0.0	0.0	1.0	4.5
		SD	0.0	0.0	0.0	0.0	0.0	1.4

DAT days after treatment SD Standard deviation

C. Plant survival

No effect on plant survival was detected after application at BBCH stage 12-14 at rates up to 1.0 L ADM.03502.F.1.A/ha for all tested plant species. The results are listed in the table below.

Table A 62: Effect of ADM.03502.F.1.A on plant survival in the vegetative vigour test [mean of all replicates]

Test species	Application rates in 200 L/ha	Mean number of living plants per replicate			Survival
	ADM.03502.F.1.A [L/ha]	7 DAT	14 DAT	21 DAT	21 DAT [%]
Ryegrass	control	4.0	4.0	4.0	100.0
	0.019	4.0	4.0	4.0	100.0
	0.051	4.0	4.0	4.0	100.0
	0.137	4.0	4.0	4.0	100.0

	0.370	4.0	4.0	4.0	100.0
	1.000	4.0	4.0	4.0	100.0
Onion	control	6.0	6.0	6.0	100.0
	0.019	6.0	6.0	6.0	100.0
	0.051	6.0	6.0	6.0	100.0
	0.137	6.0	6.0	6.0	100.0
	0.370	6.0	6.0	6.0	100.0
	1.000	6.0	6.0	6.0	100.0
Sugar beet	control	2.0	2.0	2.0	100.0
	0.019	2.0	2.0	2.0	100.0
	0.051	2.0	2.0	2.0	100.0
	0.137	2.0	2.0	2.0	100.0
	0.370	2.0	2.0	2.0	100.0
	1.000	2.0	2.0	2.0	100.0
Oilseed rape	control	3.0	3.0	3.0	100.0
	0.019	3.0	3.0	3.0	100.0
	0.051	3.0	3.0	3.0	100.0
	0.137	3.0	3.0	3.0	100.0
	0.370	3.0	3.0	3.0	100.0
	1.000	3.0	3.0	3.0	100.0
Tomato	control	2.0	2.0	2.0	100.0
	0.019	2.0	2.0	2.0	100.0
	0.051	2.0	2.0	2.0	100.0
	0.137	2.0	2.0	2.0	100.0
	0.370	2.0	2.0	2.0	100.0
	1.000	2.0	2.0	2.0	100.0
Soybean	control	2.0	2.0	2.0	100.0
	0.019	2.0	2.0	2.0	100.0
	0.051	2.0	2.0	2.0	100.0
	0.137	2.0	2.0	2.0	100.0
	0.370	2.0	2.0	2.0	100.0
	1.000	2.0	2.0	2.0	100.0

DAT days after treatment

The NOER for plant survival is ≥ 1.000 L ADM.03502.F.1.A/ha for all tested plant species.

D. Plant length

Significant plant length reduction was detected for sugar beet and oilseed rape after application up to 1.000 L ADM.03502.F.1.A/ha at BBCH 12-14, but not for the other tested species.

Table A 63: Effects of ADM.03502.F.1.A on plant length 21 DAT in the vegetative vigour test [mean of all replicates in cm]

Test species	Application rate of ADM.03502.F.1.A [L prod./ha in 200 L/ha water]					
	Control	0.019	0.051	0.137	0.370	1.000
Ryegrass						
Mean [cm]	36.4	39.0	37.1	38.3	37.6	36.7
SD	1.7	2.7	0.8	2.0	2.1	3.2
CV [%]	4.8	6.8	2.2	5.1	5.5	8.8
Inhibition [%]	---	-7.1	-1.9	-5.4	-3.3	-0.9
Compared to control [%]	---	107.1	101.9	105.4	103.3	100.9
Onion						
Mean [cm]	50.1	50.5	50.6	51.4	49.9	50.8
SD	5.3	5.2	6.2	6.4	5.2	5.7
CV [%]	10.6	10.2	12.2	12.3	10.4	11.3
Inhibition [%]	---	-0.9	-1.1	-2.7	0.4	-1.5
Compared to control [%]	---	100.9	101.1	102.7	99.6	101.5
Sugar beet						
Mean [cm]	33.5	32.6	30.4*	29.8*	29.2*	28.5*

SD	2.7	3.4	4.3	3.4	3.7	4.9
CV [%]	8.2	10.5	14.3	11.3	12.6	17.1
Inhibition [%]	---	2.7	9.1	10.9	12.7	14.7
Compared to control [%]	---	97.3	90.9	89.1	87.3	85.3
Oilseed rape						
Mean [cm]	40.5	40.4	40.1	40.0	39.6	38.2*
SD	1.3	1.8	1.6	1.6	1.1	2.7
CV [%]	3.3	4.4	4.0	4.0	2.9	7.1
Inhibition [%]	---	0.1	0.9	1.1	2.2	5.7
Compared to control [%]	---	99.9	99.1	98.9	97.8	94.3
Tomato						
Mean [cm]	61.9	61.7	59.0	62.4	63.5	63.8
SD	5.0	4.6	7.0	5.2	4.8	4.8
CV [%]	8.0	7.4	11.9	8.3	7.5	7.4
Inhibition [%]	---	0.3	4.6	-0.9	-2.6	-3.2
Compared to control [%]	---	99.7	95.4	100.9	102.6	103.2
Soybean						
Mean [cm]	123.2	122.6	121.3	123.5	121.2	118.2
SD	8.4	9.2	8.0	8.6	6.8	9.4
CV [%]	6.8	7.5	6.6	6.9	5.6	8.0
Inhibition [%]	---	0.5	1.5	-0.2	1.6	4.1
Compared to control [%]	---	99.5	98.5	100.2	98.4	95.9

DAT days after treatment SD Standard deviation CV coefficient of variation

* statistically significant different to control (Williams Multiple Sequential t-test (one-sided smaller, $\alpha = 0.05$))

The NOER for plant length reduction for sugar beet is 0.019 L ADM.03502.F.1.A/ha and for oilseed rape 0.370 L ADM.03502.F.1.A/ha. The NOER for ryegrass, onion, tomato and soybean is ≥ 1.0 L ADM.03502.F.1.A/ha

E. Biomass (shoot fresh weight)

Significant biomass reduction was detected for sugar beet and oilseed rape after application up to 1.0 L ADM.03502.F.1.A/ha at BBCH 12-14, but not for the other tested species.

Table A 64: Effects of ADM.03502.F.1.A on shoot dry weight 21 DAT in the vegetative vigour test [mean of all replicates in g]

Test species	Application rate of ADM.03502.F.1.A [L prod./ha in 200 L/ha water]					
	Control	0.019	0.051	0.137	0.370	1.000
Ryegrass						
Mean [g]	3.064	3.471	3.189	3.174	3.135	3.549
SD	0.2	0.4	0.3	0.2	0.7	0.5
CV [%]	7.6	11.5	8.5	7.8	21.0	13.6
Inhibition [%]	---	-13.3	-4.1	-3.6	-2.3	-15.8
Compared to control [%]	---	113.3	104.1	103.6	102.3	115.8
Onion						
Mean [g]	11.520	11.007	10.334	10.537	10.345	10.351
SD	0.9	1.5	0.8	0.9	1.9	1.4
CV [%]	8.0	13.7	7.4	8.6	18.0	13.9
Inhibition [%]	---	4.5	10.3	8.5	10.2	10.1
Compared to control [%]	---	95.5	89.7	91.5	89.8	89.9
Sugar beet						
Mean [g]	15.162	14.944	12.758**	12.367**	12.360**	11.266**
SD	2.2	2.1	3.6	3.1	3.0	4.2
CV [%]	14.8	13.8	27.9	25.4	24.7	37.2
Inhibition [%]	---	1.4	15.9	18.4	18.5	25.7
Compared to control [%]	---	98.6	84.1	81.6	81.5	74.3

Oilseed rape						
Mean [g]	35.519	36.306	34.890	35.288	34.181	32.328*
SD	2.3	2.0	2.4	2.2	3.0	3.7
CV [%]	6.5	5.5	6.8	6.3	8.8	11.4
Inhibition [%]	---	-2.2	1.8	0.6	3.8	9.0
Compared to control [%]	---	102.2	98.2	99.4	96.2	91.0
Tomato						
Mean [g]	25.2	24.8	24.8	25.9	25.3	24.4
SD	2.7	2.7	2.3	2.6	2.6	2.3
CV [%]	10.8	10.8	9.4	9.9	10.4	9.3
Inhibition [%]	---	1.4	1.3	-2.7	-0.4	3.3
Compared to control [%]	---	98.6	98.7	102.7	100.4	96.7

Soybean						
Mean [g]	27.4	28.2	28.6	29.3	28.5	28.2
SD	4.3	2.9	2.9	3.5	2.8	2.8
CV [%]	15.5	10.1	10.0	11.9	9.8	10.0
Inhibition [%]	---	-2.9	-4.2	-7.0	-4.1	-3.0
Compared to control [%]	---	102.9	104.2	107.0	104.1	103.0

DAT days after treatment SD Standard deviation CV coefficient of variation

* statistically significant different to control (Williams Multiple Sequential t-test (one-sided smaller, $\alpha=0.05$))

** statistically significant different to control (Multiple sequentially-rejective Welch-t-test after Bonferroni-Holm (one-sided smaller, $\alpha = 0.05$))

The NOER for biomass reduction for sugar beet is 0.019 L ADM.03502.F.1.A/ha and for oilseed rape it is 0.370 L ADM.03502.F.1.A/ha. The NOER for ryegrass, onion, tomato and soybean is ≥ 1.0 L ADM.03502.F.1.A/ha. The ER₂₅ of sugar beet was calculated to be 0.464 L ADM.03502.F.1.A/ha. The ER₂₅ for the other tested species is > 1.0 L ADM.03502.F.1.A/ha

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 ≥ 92 %.
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 100 % at the end of the test.
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

The effects of ADM.03502.F.1.A after application on six different plant species (ryegrass, onion, sugar beet, oilseed rape, tomato, soybean) at BBCH stage 12-14 were examined at nominal application rates of 0.019, 0.051, 0.137, 0.370 and 1.0 L prod./ha in 200 L water/ha under greenhouse conditions. The test endpoints were plant survival (mortality), plant length, shoot dry weight and visual phytotoxicity 21 days after application (DAT). An effect on plant survival could not be detected after application at BBCH stage 12-14 at rates up to 1.0 L ADM.03502.F.1.A/ha in any tested plant species. The application at BBCH stage 12-14 at rates up to 1.0 L ADM.03502.F.1.A/ha caused visible phytotoxic effects to onion, sugar beet, oilseed rape, tomato, and soybean at the two highest test rates at a mean percentage of 0.3 to 8.7 %. In onion, only the highest rate induced phytotoxic effects. Chlorosis, necrosis, deformation, and stunting was detected for sugar beet, oilseed rape, tomato and soybean. For onion necrosis was detected. Statistically significant plant length reduction was detected for sugar beet and oilseed rape.

The NOER for plant length reduction for sugar beet is 0.019 L ADM.03502.F.1.A/ha and for oilseed rape 0.370 L ADM.03502.F.1.A/ha. The NOER for ryegrass, onion, tomato, and soybean is greater or equal the highest tested application rate of 1.0 L ADM.03502.F.1.A/ha. Statistically significant biomass reduction was detected for sugar beet and oilseed rape after application of 1.000 L ADM.03502.F.1.A/ha. The NOER for biomass reduction for sugar beet was 0.019 L ADM.03502.F.1.A/ha and for oilseed rape 0.370 L ADM.03502.F.1.A/ha. The NOER for ryegrass, onion, tomato, and soybean is greater or equal the highest tested application rate of 1.000 L ADM.03502.F.1.A/ha. The ER₂₅ of sugar beet was calculated to be 0.464 L ADM.03502.F.1.A/ha.

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.03502.F.1.A 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.03502.F.1.A. 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 substances or the product on organisms in the environment generated from monitoring schemes.

Appendix 3 ~~EC_x (based on prod.) / EC_x (based on PEC)~~

~~Check whether the mixture composition in the formulation study giving the measured mixture toxicity (EC_x PPP) in terms of the relative proportions of the individual a.s. is similar to the mixture composition at the PEC_{mix}. As a direct comparison on the basis of the relative proportions of the a.s. at the EC_x PPP with the relative proportion at the PEC_{mix} is not informative as such, the comparison is done based on calculated mixture toxicity (assuming CA) for both mixture compositions. Therefore, calculate EC_{x-mix} CA for the mixture composition of the a.s. at the PEC_{mix} and compare with the estimate calculated for the formulation.~~

Spring cereals, BBCH 30-65

Table A 65: Fish, acute: Comparison of the mixture composition in the formulation and the mixture composition at PEC_{mix} (highlighted in green = between ratio 0.8-1.2, indicating that compositions are similar)

Test item	LC ₅₀ /EC ₅₀ measured for a.s. [µg/L]	PEC _{sw} [µg a.s./L]	% of a.s. in mixture	LC ₅₀ /EC _{50mix} calculated at PEC _{mix} [µg/L]	LC ₅₀ /EC _{50mix} calculated for a.s. [µg/L]	Ratio
Step-3, D3, ditch						
Prothioconazole	1830	1.107	0.42	1870	1871	1.0
Fenpropidin	1900	1.555	0.58			
Step-3, D4, stream						
Prothioconazole	1830	0.905	0.42	1870	1871	1.0
Fenpropidin	1900	1.27	0.58			
Step-3, D5, stream						
Prothioconazole	1830	0.929	0.42	1870	1871	1.0
Fenpropidin	1900	1.305	0.58			
Step-3, R4, stream						
Prothioconazole	1830	0.732	0.42	1870	1871	1.0
Fenpropidin	1900	1.026	0.58			

Table A 66: Aquatic invertebrate, acute: Comparison of the mixture composition in the formulation and the mixture composition at PEC_{mix} (highlighted in green = between ratio 0.8-1.2, indicating that compositions are similar)

Test item	LC ₅₀ /EC ₅₀ measured for a.s. [µg/L]	PEC _{sw} [µg a.s./L]	% of a.s. in mixture	LC ₅₀ /EC _{50mix} calculated at PEC _{mix} [µg/L]	LC ₅₀ /EC _{50mix} calculated for a.s. [µg/L]	Ratio
Step-3, D3, ditch						
Prothioconazole	1300.0	1.107	0.42	713.4	711.2	1.0
Fenpropidin	540.0	1.555	0.58			
Step-3, D4, stream						
Prothioconazole	1300.0	0.905	0.42	713.6	711.2	1.0
Fenpropidin	540.0	1.27	0.58			
Step-3, D5, stream						
Prothioconazole	1300.0	0.929	0.42	713.4	711.2	1.0
Fenpropidin	540.0	1.305	0.58			
Step-3, R4, stream						
Prothioconazole	1300.0	0.732	0.42	713.7	711.2	1.0
Fenpropidin	540.0	1.026	0.58			

Winter cereals, BBCH 30-65

Table A 67: **Fish, acute: Comparison of the mixture composition in the formulation and the mixture composition at PEC_{mix} (highlighted in green = between ratio 0.8-1.2, indicating that compositions are similar)**

Test item	LC ₅₀ /EC ₅₀ measured for a.s. [µg/L]	PEC _{sw} [µg a.s./L]	% of a.s. in mixture	LC ₅₀ /EC _{50mix} calculated at PEC _{mix} [µg/L]	LC ₅₀ /EC _{50mix} calculated for a.s. [µg/L]	Ratio
Step-3, D3, ditch						
Prothioconazole	1830	1.106	0.42	1870	1871	1.0
fenpropidin	1900	1.554	0.58			
Step-3, D4, stream						
Prothioconazole	1830	0.817	0.42	1870	1871	1.0
fenpropidin	1900	1.147	0.58			
Step-3, D5, stream						
Prothioconazole	1830	0.883	0.42	1870	1871	1.0
fenpropidin	1900	1.239	0.58			
Step-3, R1, stream						
Prothioconazole	1830	0.726	0.42	1870	1871	1.0
fenpropidin	1900	1.017	0.58			
Step-3, R3, stream						
Prothioconazole	1830	1.023	0.42	1870	1871	1.0
fenpropidin	1900	1.437	0.58			
Step-3, R4, stream						
Prothioconazole	1830	0.732	0.42	1870	1871	1.0
fenpropidin	1900	1.026	0.58			

Table A 68: **Aquatic invertebrate, acute: Comparison of the mixture composition in the formulation and the mixture composition at PEC_{mix} (highlighted in green = between ratio 0.8-1.2, indicating that compositions are similar)**

Test item	LC ₅₀ /EC ₅₀ measured for a.s. [µg/L]	PEC _{sw} [µg a.s./L]	% of a.s. in mixture	LC ₅₀ /EC _{50mix} calculated at PEC _{mix} [µg/L]	LC ₅₀ /EC _{50mix} calculated for a.s. [µg/L]	Ratio
Step-3, D3, ditch						
Prothioconazole	1300	1.106	0.42	713.4	711.2	1.0
fenpropidin	540	1.554	0.58			
Step-3, D4, stream						
Prothioconazole	1300	0.817	0.42	713.5	711.2	1.0
fenpropidin	540	1.147	0.58			
Step-3, D5, stream						
Prothioconazole	1300	0.883	0.42	713.6	711.2	1.0
fenpropidin	540	1.239	0.58			
Step-3, D6, ditch						
Prothioconazole	1300	1.093	0.42	713.4	711.2	1.0
fenpropidin	540	1.536	0.58			
Step-3, R1, stream						
Prothioconazole	1300	0.726	0.42	713.8	711.2	1.0
fenpropidin	540	1.017	0.58			
Step-3, R3, stream						
Prothioconazole	1300	1.023	0.42	713.5	711.2	1.0
fenpropidin	540	1.437	0.58			
Step-3, R4, stream						
Prothioconazole	1300	0.732	0.42	713.7	711.2	1.0
fenpropidin	540	1.026	0.58			