

REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: A23282A

Product name: **KAYAK ERA**

Chemical active substances:

Cyprodinil 225 g/L

Prothioconazole 75 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(New product authorisation)

Applicant: XXXX

Submission date: July 2022

Evaluation date: March 2023

MS Finalisation date: December 2023

Version history

When	What
March 2023	Version evaluated by PL zRMS
July 2023	<p>Document updated to answer demsnds from the NL:</p> <ul style="list-style-type: none">• Combination toxicity risk assessment has been conducted for soil organisms (page 138)• Supplementary information has been added to Appendix 2 regarding the phytotoxicity assessment conducted foer the <i>Lemna gibba</i> test with cyprodinil (page 194)• A statement has been added with regards to the soil microorganism test for A23282A (page 292) <p>All changes are highlighted in green</p>
December 2023	Final version by zRMS (additional comment/update are highlighted in yellow)

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9 Ecotoxicology (KCP 10)

9.1 Critical GAP and overall conclusions

Table 9.1-1: Table of critical GAPs

1	2	3	4	5	6	7	8	9	10	11a	11b	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 saf- ener/ synergist per ha	Conclusion						
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	L product/ha a) max. rate per appl. b) max. total rate per crop/season	g cyprodinil/ha a) max. rate per appl. b) max. total rate per crop/season	g prothioconazole/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms	Non-target plants
Zonal uses (field or outdoor uses, certain types of protected crops)																					
AT1****	Austria Belgium Czech Republic Germany Hungary Ireland Luxembourg Netherlands Poland Romania Slovakia Slovenia	spring wheat; TRZAS	F	Zymoseptoria tritici; SEPTTR	foliar spray	BBCH30- 69	a) 1 b) 1	N/A	a) 2 b) 2	a) 450 b) 450	a) 150 b) 150	100-400	N/A***								
AT5****	Austria Belgium Czech Republic Germany Hungary Ireland Luxembourg	winter wheat; TRZAW	F	Zymoseptoria tritici; SEPTTR	foliar spray	BBCH30- 69	a) 1 b) 1	N/A	a) 2 b) 2	a) 450 b) 450	a) 150 b) 150	100-400	N/A***								

1	2	3	4	5	6	7	8	9	10	11a	11b	12	13	14	15	16	17	18	19	20	21
	Netherlands Poland Romania Slovakia Slovenia																				

* 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

*** N/A stands for 'Not Applicable'; The PHI is covered by the conditions of use and/or the vegetation period remaining between the application of the plant protection product and the use of the commodity (e.g. harvest) and/or the setting of a PHI in days is not required

**** critical GAP covering all intended GAPs in Part B, Section 0, including all minor uses

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

9.1.1 Overall conclusions

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

Birds

The acute and long-term risks of A23282A to birds were assessed from toxicity exposure ratios between toxicity endpoints, estimated from studies with cyprodinil, prothioconazole, its major metabolite JAU 6476-desthio and maximum residues occurring on food items following applications according to the proposed use pattern.

For cyprodinil, prothioconazole and the prothioconazole metabolite JAU 6476-desthio, the acute and chronic screening step or Tier 1 TER values exceed the trigger values of 10 and 5, respectively, indicating that the risk to birds is acceptable following use of A23282A according to the proposed use pattern.

Additionally, the acute and chronic TER values for the mixture exceed the relevant triggers indicating an acceptable risk to birds from A23282A.

Risk of secondary poisoning has also been assessed, as cyprodinil, prothioconazole, and prothioconazole metabolites JAU 6476-desthio and JAU 6474 S-methyl all have log P_{OW} values >3.0 . The risk to birds from exposure via drinking water has also been assessed. All assessments indicate that the risk to birds is acceptable following use of A23282A according to the proposed use pattern.

Mammals

The acute and long-term risks of A23282A to mammals were assessed from toxicity exposure ratios between toxicity endpoints, estimated from studies with cyprodinil, prothioconazole, and its metabolite JAU 6476-desthio, and maximum residues occurring on food items following applications according to the proposed use pattern.

For cyprodinil, prothioconazole and the metabolite JAU 6476-desthio, acute and long-term TER values at either screening or Tier 1, all exceed the trigger values of 10 for acute risk and 5 for long-term risk.

For the mixture toxicity assessment, the acute and long-term risk to mammals from proposed uses of A23282A is considered acceptable when consideration is given to refined crop interception values.

Risk of secondary poisoning has also been assessed, as cyprodinil, prothioconazole, and prothioconazole metabolites JAU 6476-desthio and JAU 6474 S-methyl all have log P_{OW} values >3.0 . The risk to mammals from exposure via drinking water has also been assessed. All assessments indicate that the risk to mammals is acceptable following use of A23282A according to the proposed use pattern.

Other Terrestrial Vertebrate Species

There is currently no guidance addressing terrestrial life stages of amphibians and reptiles in PPP risk assessments. Therefore, the risk assessment provided above for birds and mammals is protective of terrestrial amphibian and reptile species.

9.1.1.2 Effects on aquatic organisms (KCP 10.2))

Cyprodinil

The PEC/RAC ratios, using worst-case PEC_{SW} values for fish (acute and chronic), invertebrates (acute), algae, macrophytes and sediment dwellers are less than the trigger value of 1, indicating that the risk to these groups of aquatic organisms is acceptable following use of A23282A in accordance with the proposed use pattern.

An acceptable long-term risk to invertebrates from exposure to cyprodinil is achieved if the below listed mitigation options are implemented (see Table 9.1-2 and Table 9.1-3).

Prothioconazole

The PEC/RAC ratios, using worst-case PEC_{SW} values, are less than the trigger value of 1, indicating that the risk is acceptable following use of A23282A in accordance with the proposed use pattern.

Metabolites

The PEC/RAC ratios, using worst-case PEC_{SW} values for metabolites of cyprodinil and prothioconazole, except for the prothioconazole metabolite JAU 6476-desthio, are less than the trigger value of 1, indicating that the risk to aquatic organisms for the metabolites is acceptable following use of A23282A in accordance with the proposed mitigation.

An acceptable long-term risk to fish from exposure to JAU 6476-desthio is achieved if the below listed mitigation options are implemented (see Table 9.1-2 and Table 9.1-3, mitigations options required for cyprodinil are also achieving an acceptable risk for metabolite JAU 6476-desthio).

The mitigation required for safe use has been consolidated into one table for each crop.

Table 9.1-2: Aquatic organisms: mitigation requirements / options for A23282A following use in winter cereals

FOCUS Scenario	Fish chronic	Invertebrate chronic (using Cyprodinil ETO-RAC)
D3 Ditch	-	5 m SD + 50 % DR or 10 m SD
D4 Stream	-	5 m SD + 50 % DR or 10 m SD
D5 Stream	-	5 m SD + 50 % DR or 10 m SD 10 m SD + 50 % DR or 20 m SD
R1 Stream	-	5 m SD 10 m SD + 10 m RO
R3 Stream	10 m SD + 10-12 m RO	10 m SD + 10 m RO 20 m SD + 20 m RO
R4 Stream	10 m SD + 10-12 m RO	10 m SD + 10 m RO 20 m SD + 20 m RO

--mitigation measures are not required

SD = spray drift buffer

RO = run-off mitigation

DR = drift reducing nozzles

Table 9.1-3: Aquatic organisms: mitigation requirements / options for A23282A following use in spring cereals

FOCUS Scenario	Fish chronic	Invertebrate chronic (using Cyprodinil ETO-RAC)
D3 Ditch	-	5 m SD + 50 % DR or 10 m SD
D4 Stream	-	5 m SD + 50 % DR or 10 m SD
D5 Stream	-	5 m SD + 50 % DR or 10 m SD 10 m SD + 50 % DR or 20 m SD
R4 Stream	10 m SD + 10-12 m RO	20 m SD + 20 m RO

An empty/grey field means that the scenario is not relevant to the crop group

“-“mitigation measures are not required

SD = spray drift buffer

RO = run-off mitigation

DR = drift reducing nozzles

A table indicating the percentage reduction required to achieve an acceptable risk to aquatic organisms are presented below:

Table 9.1-4: Aquatic organisms: percentage reduction of entry by cyprodinil into surface water to achieve acceptable risk when considering the ETO RAC of 0.75 µg/L

Scenario	Winter cereals		Spring cereals	
	BBCH 30	BBCH 69	BBCH 30	BBCH 69
	ETO RAC: 0.75 µg/L			
D3 Ditch	73.6	73.7	73.7	73.7
D4 Stream	64.3	69.5	67.8	69.4
D5 Stream	67.0	71.7	68.6	69.8
R1 Stream	59.9	60.1	-	-
R3 Stream	71.5	71.7	-	-
R4 Stream	60.1	60.1	60.1	60.1

- These scenarios are not relevant for spring cereals

A23282A and Mixture Toxicity

Cyprodinil was identified as the single driver of toxicity for both the invertebrate acute and algal formulation assessments, therefore a mixture toxicity risk assessment was not required for these groups, and the single substance assessments for cyprodinil should be referred to.

For sediment dwellers, there was not sufficient data to perform a mixture toxicity assessment.

The mixture Exposure/Toxicity Ratios, using worst-case PEC_{SW} values, for fish (acute and chronic) and macrophytes are less than the relevant trigger value, indicating that the risk to aquatic organisms for the mixture of cyprodinil, prothioconazole and JAU 6476-desthio is acceptable following use of A23282A in accordance with the proposed mitigation.

The RQ_{mix}, using worst-case PEC_{SW} values, for invertebrates (chronic) are less than 1, indicating that the chronic risk to invertebrates for the mixture of cyprodinil, prothioconazole and JAU 6476-desthio is acceptable following use of A23282A in accordance with the proposed mitigation.

An acceptable long-term risk to fish and invertebrates from exposure to the mixture of cyprodinil, prothioconazole and JAU 6476-desthio is achieved if the below listed mitigation options are implemented (see Table 9.1-2 and Table 9.1-3).

9.1.1.3 Effects on bees (KCP 10.3.1)

The acute risk to honeybees was assessed from hazard quotients and Exposure Toxicity Ratios (ETRs) following EFSA (2014), estimated from acute oral and contact studies with cyprodinil, prothioconazole, its metabolite JAU 6476-desthio and A23282A at the maximum single application rate. All the acute contact hazard quotients and Exposure Toxicity Ratios (ETRs) for cyprodinil, prothioconazole, its metabolite JAU 6476-desthio and A23282A are less than the relevant trigger, indicating that the acute oral and contact risk to honeybees is acceptable following use of A23282A according to the proposed use pattern.

The chronic adult and larval risk A23282A to honeybees was assessed from ETRs following EFSA (2014), estimated from chronic adult and larval studies with cyprodinil, prothioconazole, A23282A and the cyprodinil/prothioconazole mixture and potential exposure calculated from exposure via residues in pollen / nectar and the measure of consumption of foraging bees/drone larvae.

The ETR values are less than the relevant trigger values at the screening step or tier 1, indicating that the chronic risk to adult and larval honeybees is acceptable following use of A23282A according to the proposed use pattern.

9.1.1.4 Effects on arthropods other than bees (KCP 10.3.2)

The risk assessment using Tier II studies and an aged-residue extended laboratory test with *A. rhopalosiphi*, showed acceptable foliar in-field and off-field effects from foliar applications of A23282A for the worst-case use scenario (1 x 2 L product/ha in cereals). The risk to non-target arthropods is therefore acceptable following use of A23282A according to the proposed use pattern.

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

Soil meso- and macrofauna

The long-term risk of A23282A, cyprodinil, prothioconazole, and relevant metabolites to earthworms, Collembola and Hypoaspis was evaluated where relevant. The risk assessment demonstrated that the risk to non-target soil meso- and macrofauna is acceptable following use of A23282A according to the proposed use pattern.

Soil micro-organisms

The risk of A23282A, cyprodinil, prothioconazole and relevant metabolites to soil micro-organisms was evaluated by comparison of the maximum concentrations with effects $\leq 25\%$ derived from laboratory tests, with maximum PEC_s. All the effect levels exceeded the relevant PEC_s values, indicating that the risk to soil micro-organisms is acceptable following the use of A23282A according to the proposed use pattern(s).

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

Less than 50% effect on seedling emergence and vegetative vigour on all six species was observed at the maximum single use rate of 2 L A23282A/ha at screening step. This indicates that the risk to non-target

terrestrial plants in off-crop areas is acceptable following use of A23282A according to the proposed use pattern.

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

A risk assessment for other non-target species was not performed

9.1.2 Grouping of intended uses for risk assessment

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

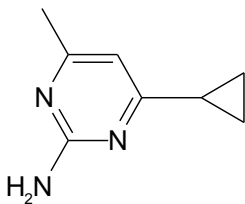
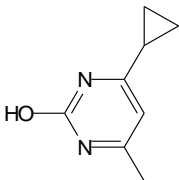
Table 9.1-5: Critical use pattern of formulation grouped according to criterion

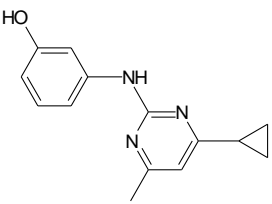
Grouping according to criterion			
Group	Intended uses	relevant use parameters for grouping	relevant parameter or value for sorting
1	Cereals (includes wheat, rye, triticale, barley and oats)	Cereals Growth stage: BBCH 30-69 Application rate: 1 x 2 L A23282A/ha	Critical use pattern for all aspects of the ecotox risk assessment.

9.1.3 Consideration of metabolites

A list of metabolites found in environmental compartments is provided below. The need for conducting a metabolite-specific risk assessment in the context of the evaluation of A23282A is indicated in the table.

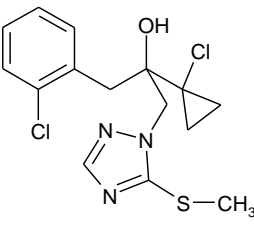
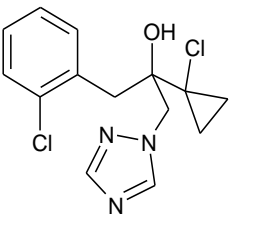

Table 9.1-6 Metabolites of Cyprodinil

Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
CGA249287		149.2	Soil: 14.3 Surface Water: 6.9 Sediment: 14.2 Whole system: 21.1	Yes, aquatic and soil organisms
CGA321915		150.2	Soil: 5.1	Yes, aquatic and soil organisms

Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
CGA275535		241.3	Soil: 21.3	Yes, aquatic and soil organisms

^a Consistent with molecular weight correct factor of 1.041 x parent molecular weight given in EFSA (2007)

Table 9.1-7 Metabolites of Prothioconazole

Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
IAU 6476-S-methyl (M01)		358.3 ^a	Major soil-metabolite (14.2%) minor aquatic metabolite (3.1%) found in animal (rat, goat, hen, fish)	Yes, soil and aquatic organisms
IAU 6476-desthio (M04)		312.2	> 10 % of the TRR on plant material major soil-metabolite (57.1%) major aquatic metabolite (whole system: 55.7%; water 32.3[AM(C1)] %) found in animal (rat, goat, hen, fish) found in plant	Yes, Birds and mammals, aquatic organisms, bees and soil organisms
1,2,4-triazole (M13)		69.1	Major aquatic metabolite (41.8%), in soil <LOQ found in animal (rat, hen)	Yes, aquatic organisms

9.2 Effects on birds (KCP 10.1.1)

zRMS Comments:	<p>The risk assessment was performed in accordance with the B & M Guidance, EFSA (2009). All relevant used endpoints were agreed at the EU level.</p> <p>Cyprodinil. In accordance with EFSA Scientific Report (2005), the lowest acute toxicity to birds is LD₅₀ > 500 mg a.s./kg bw and this endpoint was added in Table 9.2-1 and should be used acute risk assessment. The risk was corrected in relevant tables.</p> <p>The acute and long-term risk were submitted at screening and First Tier. The submitted acute and long-term risk to birds was accepted.</p>
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	<p>The TER_A and TER_{LT} values are below exceed the trigger values of 10 and 5, respectively, indicating an acceptable risk for birds.</p> <p>For consistency with considered endpoint LD₅₀ > 500 mg a.s./kg bw used in acute risk assessment for cyprodinil, the same value should be used in mixture toxicity assessment. The correction was provided in relevant tables.</p> <p>Prothioconazole. The active substance and its metabolite prothioconazole-desthio were taken into consideration. The submitted acute and long-term risk to birds was accepted. The TER_A and TER_{LT} values are below exceed the trigger values of 10 and 5, respectively, indicating an acceptable risk for birds.</p> <p>For both active substances and their relevant metabolites a risk assessment for fish-eating and earthworm-eating birds was performed and the TER_{LT} values are above the trigger value of 5.</p> <p>The risks due to bioaccumulation of prothioconazole and its metabolite via the food chain for birds is acceptable.</p> <p>For mixture toxicity evaluation please refer to p.24.</p>
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9.2.1 Toxicity data

Avian toxicity studies have been carried out with cyprodinil, prothioconazole, and the prothioconazole metabolite JAU 6476-desthio. Full details of these studies are provided in the respective EU DAR and related documents.

Effects of A23282A on birds were not evaluated as part of the EU assessment of cyprodinil or prothioconazole. However, the provision of further data on the formulation is not considered essential, because the mammalian risk assessment (Section 9.3) gives no indication of higher toxicity from the formulation, and the risk to birds from A23282A can be adequately assessed from risk assessment for the individual active substances. The risk to birds from the proposed uses of A23282A will be assessed using the endpoints for cyprodinil and prothioconazole.

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

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

Species	Substance	Exposure System	Results	Reference
Mallard Duck (<i>A. platyrhynchos</i>)	Cyprodinil	Oral 14 d Acute	LD ₅₀ = > 500 mg/kg bw ^a	EFSA Scientific Report (2005) 51, 1-78 Hakin & Rogers (1992); Report No. CBG 526/901711; CGA219417/0062; VV-369265

Species	Substance	Exposure System	Results	Reference
Mallard duck	Cyprodinil	Oral 1 d Acute	LD ₅₀ > 500 mg/kg bw Regurgitation was observed at higher dosage.	EFSA Scientific Report (2005), 51, 1-78
Bobwhite Quail (<i>C. virginianus</i>)	Cyprodinil	Oral 14 d Acute	LD ₅₀ = >2 000 mg/kg bw LD₅₀ = 3 776 mg/kg bw^b	EFSA Scientific Report (2005) 51, 1-78 Hakin & Rogers (1992); Report No. CBG 527/901712; CGA219417/0067; VV-369048
Mallard Duck (<i>A. platyrhynchos</i>)	Cyprodinil	Dietary 22 week Reproductive toxicity	NOEL = 102 mg/kg bw/d	EFSA Scientific Report (2005) 51, 1-78 Rogers (1995); Report No. CBG 671/950769; CGA219417/0477; VV-370351
Bobwhite Quail (<i>C. virginianus</i>)	Cyprodinil	Dietary 22 week Reproductive toxicity	NOEL = 64 mg/kg bw/d	EFSA Scientific Report (2005) 51, 1-78 Rogers (1995); Report No. CBG 672/950770; CGA219417/0478; VV-370354

^a Regurgitation was observed at higher dose so the endpoint derived for the bobwhite quail will be used according to EFSA 2009 guidance

^b value extrapolated by a factor of 1.888 according to EFSA/2009/1438

Values in **bold** will be used in the risk assessment

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

Species	Substance	Exposure System	Results	Reference
Bobwhite quail (<i>Colinus virginianus</i>)	Prothioconazole	Oral 1 d Acute	LD₅₀ > 2 000 mg a.s./kg bw	EFSA Conclusion 2007
Bobwhite quail (<i>Colinus virginianus</i>)	Prothioconazole	Dietary 5 d	LC ₅₀ > 1 413 mg a.s./kg bw	EFSA Conclusion 2007
Mallard duck (<i>Anas platyrhynchos</i>)	Prothioconazole	Dietary 5 d	LC ₅₀ > 2 457 mg a.s./kg bw	EFSA Conclusion 2007
Mallard duck (<i>Anas platyrhynchos</i>)	Prothioconazole	Dietary Reproductive toxicity	NOEL = 78 mg a.s./kg bw/d	EFSA Conclusion 2007
Bobwhite quail (<i>Colinus virginianus</i>)	Prothioconazole	Dietary Reproductive toxicity	NOEL ≥ 86 mg a.s./kg bw/d	EFSA Conclusion 2007
Bobwhite quail (<i>Colinus virginianus</i>)	JAU 6476-desthio	Oral 1 d Acute	LD ₅₀ > 2 000 mg a.s./kg bw	EFSA Conclusion 2007

Species	Substance	Exposure System	Results	Reference
Bobwhite quail (<i>Colinus virginianus</i>)	JAU 6476-desthio	Dietary 5 d	LC50 > 297 mg/kg bw	EFSA Conclusion 2007
Mallard duck (<i>Anas platyrhynchos</i>)	JAU 6476-desthio	Dietary Reproductive toxicity	NOEL = 63 mg/kg bw/d	EFSA Conclusion 2007
Bobwhite quail (<i>Colinus virginianus</i>)	JAU 6476-desthio	Dietary Reproductive toxicity	NOEL = 14.8 mg/kg bw/d	EFSA Conclusion 2007

Endpoints used in risk assessment are shown in **bold**
EFSA Journal, (2007) 106, 1-98.

Cyprodinil/Prothioconazole mixture

Table 9.2-3: Endpoints and effect values relevant for the risk assessment for birds – cyprodinil/prothioconazole mixture

Species	Substance	Exposure System	Results	Reference
Bobwhite Quail (<i>C. virginianus</i>)	Cyprodinil/ Prothioconazole Mixture	Oral Acute	LD₅₀ = 2 000 mg/kg bw	Refer to Section 9.2.1.1

Bold values will be considered for use in the risk assessment

Cyprodinil metabolites

Since metabolites are formed at <10% of parent level in edible crop parts and mammalian testing indicates that they are less toxic than the parent, it can be concluded that the risk to birds will be low and no further risk assessment is conducted (Cyprodinil; EFSA Scientific Report 51, 2005).

Prothioconazole metabolite

JAU 6476-desthio is a metabolite of prothioconazole that occurs in amounts of >10% of the TRR on plant material. Therefore, the metabolite is considered in the risk assessment. This is in accordance with EU conclusions (EFSA Conclusion 2007).

9.2.1.1 Justification for new endpoints

Consideration of acute endpoints for cyprodinil used in the risk assessment

Acute toxicity studies were performed with bobwhite quail and mallard duck. In all cases, no mortalities occurred, and no toxic symptoms were observed. Regurgitation occurred in the mallard duck study at the two highest dose levels of 1000 and 2000 mg a.s./kg, and the value in the LoEP was set at >500 mg a.s./kg.

EFSA/2009/1438¹ states the following:

“According to Annex II of Directive 91/414/EEC, the acute oral toxicity of an active substance to a quail species (Japanese quail, *Coturnix coturnix japonica* or bobwhite quail, *Colinus virginianus*) or to mallard duck (*Anas platyrhynchos*) must be determined. The highest dose used in tests need not normally exceed 2000 mg/kg body weight. Due to issues of regurgitation, it is recommended not to use the mallard duck (EFSA, 2007). Where regurgitation or emesis occurs at doses used for risk assessment, additional

¹European Food Safety Authority; Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA. EFSA journal 2009; 7(12):1438. [p. 14]

information is essential to complete the risk assessment. The amount of regurgitated material should be assessed for determination of the ingested dose. In the absence of this information, the lowest overall no observed effect level (NOEL) must be used for risk assessment purposes. Where more than one study has been submitted, the study/studies where no regurgitation has occurred should be used. If, however, mortalities appear in the study in which regurgitation has occurred (at dose levels at or around the LD₅₀ value for the non-regurgitation study), then it is proposed to use the NOEL (for regurgitation or mortality, whichever is lower) from the study where regurgitation has occurred.”.

Since, after regurgitation, no signs of toxicity and no mortalities were seen in the studies with the mallard, and no effects were seen in the study with the bobwhite quail, it is proposed to use an extrapolated LD₅₀ value. According to EFSA/2009/1438, for studies with a total of 10 animals exposed at the highest tested concentration (of 2000 mg a.s./kg) an extrapolation factor of 1.888 can be applied, therefore, the extrapolated LD₅₀ value is **3 776 mg a.s./kg**.

Consideration of acute mixture toxicity

According to EFSA/2009/1438¹ combined action of several toxicants must be specifically considered in the risk assessment when it is obvious that such exposure situations will occur for animals.

For the assessment of acute effects (mortality), a surrogate LD₅₀ can be calculated. The EFSA Guidance Document indicates that the following equation should be used for deriving a surrogate LD₅₀ for a mixture of active substances with known toxicity assuming dose additivity:

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

where:

X (a.s.i) = fraction of active substance (i) in the formulation mixture
LD₅₀ (a.s.i) = acute toxicity for the active substance (i)

The LD₅₀ of the mix is summarised in the table below.

Table 9.2-4: Acute LD₅₀ for the mixture of active substances

Test substance	Concentration of active substance in formulation A23282A (g/L)	Fraction of active substance in the formulation mixture ^a	Acute toxicity endpoint (mg/kg bw)	Fraction of active substance/LD ₅₀ for the active substance	LD ₅₀ mix (mg/kg bw)
Cyprodinil	225	0.75	>3 776 > 500	<0.00199 <0.0015	>3 090 > 615.4
Prothioconazole	75	0.25	>2 000	<0.000125	
Total	300	1.00	-	<0.000324 <0.001625	

^a Concentration of an active substance in the formulation, divided by, the total concentration of all active substances in the formulation.

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

A “tox per fraction” quotient can be calculated for each active substance and for the mixture according to the following equations:

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

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

Table 9.2-5: Comparison of tox per fractions for individual active substances with the tox per fraction (mix)

Active substance	LD ₅₀ (mg/kg bw)	Fraction of active substance in the formulation mixture	Tox per fraction (a.s)	% difference from tox per fraction of formulation
Cyprodinil	≥3 776 500	0.75	5 034 666.7	33
Prothioconazole	>2000	0.25	8 000	300
Tox per fraction (mix)	≥2000	1.00		

Appendix B (page 191) of the Bird and mammal guidance EFSA/2009/1438 states “If one active substance can be identified where the two quotients “tox per fraction (a.s.)” and “tox per fraction (mix)” deviate by ≤ 10%, this indicates that this active substance will contribute to ≥90% to the mixture toxicity, while the other components of the mixture will only have a marginal impact of the predicted risk. Consequently, the risk assessment can be performed for the most toxic active substance alone. No further considerations according to Steps 2-4 are necessary.”

The tox per fraction values for cyprodinil and prothioconazole deviate by >10% (33% and 300%, respectively), therefore neither of the components drive the acute risk assessment, and an acute mixture toxicity risk assessment is required according to the guidance.

9.2.2 Risk assessment for spray applications

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

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

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

Cyprodinil

Table 9.2-6: Screening assessment of the acute and long-term/reproductive risk for birds due to the use of A23282A in cereals - cyprodinil

Intended use	Cereals					
Active substance	Cyprodinil					
Application rate (g a.s./ha)	1 x 450					
Acute toxicity (mg/kg bw)	3-776 500					
TER criterion	10					
Crop scenario	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a	
Cereals	Small omnivorous bird	158.8	1.0	71.46	53 7.0	
Reprod. Toxicity (mg/kg bw/d)	64					
TER criterion	5					
Crop scenario	Indicator/generic focal species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{lt}	
Cereals	Small omnivorous bird	64.8	1 x 0.53	15.45	4.1	

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

The acute screening assessment for cyprodinil concludes a TER value greater than the trigger of 10, indicating that acute risk to birds is acceptable following use of A23282A according to the proposed use patterns.

The long-term screening assessment for cyprodinil concludes a TER value below the respective trigger, indicating that a Tier I assessment is required.

Table 9.2-7a: First-tier assessment of the acute risk for birds due to the use of A23282A in cereals - cyprodinil

Intended use	Cereals					
Active substance	Cyprodinil					
Application rate (g a.s./ha)	1 x 450					
Acute toxicity (mg/kg bw/d)	500					
TER criterion	10					
Growth stage	Indicator/generic focal species	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a	
BBCH 30-39	Small omnivorous bird "lark" Combination (invertebrates with interception) 25% crop leaves 25% weed seeds 50% ground arthropods	12.0	1	5.40	41.2	
BBCH ≥40	Small omnivorous bird "lark"	7.2	1	3.24	154	

	Combination (invertebrates with interception) 25% crop leaves 25% weed seeds 50% ground arthropods				
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Table 9.2-8: First-tier assessment of the long-term/reproductive risk for birds due to the use of A23282A in cereals - cyprodinil

Intended use	Cereals				
Active substance	Cyprodinil				
Application rate (g a.s./ha)	1 x 450				
Reprod. Toxicity (mg/kg bw/d)	64				
TER criterion	5				
Growth stage	Indicator/generic focal species	SV	MAF_m	DDD_m (mg/kg bw/d)	TER_{it}
BBCH 30-39	Small omnivorous bird “lark”	5.4	1 x 0.53	1.29	50
BBCH ≥40	Small omnivorous bird “lark”	3.3	1 x 0.53	0.79	81

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

The Tier 1 assessment for cyprodinil concludes that all TER_{it} values are greater than the trigger of 5, indicating that long term risk to birds is acceptable following use of A23282A according to the proposed use patterns.

Prothioconazole

Table 9.2-9: Screening assessment of the acute and long-term/reproductive risk for birds due to the use of A23282A in cereals - prothioconazole

Intended use	Cereals					
Active substance	Prothioconazole					
Application rate (g a.s./ha)	1 x 150					
Acute toxicity (mg/kg bw)	>2000					
TER criterion	10					
Crop scenario	Indicator/generic species	focal	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a
Cereals	Small omnivorous bird		158.8	1.0	23.82	>84
Reprod. Toxicity (mg/kg bw/d)	78					
TER criterion	5					
Crop scenario	Indicator/generic species	focal	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{lt}
Cereals	Small omnivorous bird		64.8	1 x 0.53	5.15	15

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

The acute and long-term screening assessment for prothioconazole concludes TER values greater than the triggers of 10 and 5, respectively, indicating that acute and long-term risk to birds is acceptable following use of A23282A according to the proposed use patterns.

JAU 6476-desthio

As there is not an application rate for the foliar metabolite JAU 6476-desthio, the ratio between the mass of the parent molecule and metabolite will be used to determine a worst-case surrogate application rate. The molar mass of prothioconazole is 344.26 g/mol, and the molar mass of JAU 6476-desthio is 312.2 g/mol, therefore the mass ratio between the two is 0.907. This will be used to convert the application rate of the parent active substance (150 g prothioconazole/ha) to a surrogate application rate of JAU 6476-desthio (136.05 g JAU 6476-desthio/ha).

Table 9.2-10: Screening assessment of the acute and long-term/reproductive risk for birds due to the use of A23282A in cereals – JAU 6476-desthio

Intended use	Cereals				
Metabolite	JAU 6476-desthio				
Application rate (g/ha)	1 x 136.05				
Acute toxicity (mg/kg bw)	>2000				
TER criterion	10				
Crop scenario	Indicator/generic focal species	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a
Cereals	Small omnivorous bird	158.8	1.0	21.60	>93
Reprod. Toxicity (mg/kg bw/d)	14.8				
TER criterion	5				
Crop scenario	Indicator/generic focal species	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{lt}
Cereals	Small omnivorous bird	64.8	0.53	4.67	3.2

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

The acute screening assessment for prothioconazole metabolite JAU 6476-desthio concludes a TER value greater than the trigger of 10, indicating that acute risk to birds is acceptable following use of A23282A according to the proposed use patterns.

The long-term screening assessment for prothioconazole metabolite JAU 6476-desthio concludes a TER value below the respective trigger, indicating that a Tier I assessment is required.

Table 9.2-11: First-tier assessment of the long-term/reproductive risk for birds due to the use of A23282A in cereals – JAU 6476-desthio

Intended use	Cereals				
Metabolite	JAU 6476-desthio				
Application rate (g/ha)	1 x 136.05				
Reprod. Toxicity (mg/kg bw/d)	14.8				
TER criterion	5				
Growth stage	Indicator/generic focal species	SV	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{lt}
BBCH 30-39	Small omnivorous bird “lark”	5.4	1 x 0.53	0.389	38
BBCH ≥40	Small omnivorous bird “lark”	3.3	1 x 0.53	0.238	62

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

The Tier 1 assessment for prothioconazole metabolite JAU 6476-desthio concludes that all TER values are greater than the trigger of 5, indicating that long-term risk to birds is acceptable following use of A23282A

according to the proposed use patterns.

Cyprodinil/prothioconazole mixture

zRMS
Comments:

Mixture toxicity.

For consistency with considered endpoint LD₅₀ > 500 mg a.s./kg bw used in acute risk assessment for cyprodinil, the same value should be used in mixture toxicity assessment. The corrected acute risk assessment is presented below:

Screening assessment of the acute risk for birds due to the use of A23282A in cereals - Cyprodinil/prothioconazole mixture

Intended use	Cereals					
Active substance	Cyprodinil/prothioconazole mixture					
Application rate (g a.s./ha)	1 × 600 ^a					
Acute toxicity (mg/kg bw)	>615.4					
TER criterion	10					
Crop scenario	Indicator/generic focal species	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a	
Cereals	Small omnivorous bird	158.8	1.0	95.28	> 6.5	

SV: shortcut value; MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

^a Sum of 450 g cyprodinil/ha + 150 g prothioconazole/ha

First-tier assessment of the acute risk for birds due to the use of A23282A in cereals

Intended use	Cereals					
Active substance	Cyprodinil/prothioconazole mixture					
Application rate (g a.s./ha)	1 x 600					
Acute toxicity (mg/kg bw/d)	615.4					
TER criterion	10					
Growth stage	Indicator/generic focal species	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a	
BBCH 30-39	Small omnivorous bird “lark” Combination (invertebrates with interception) 25% crop leaves 25% weed seeds 50% ground arthropods	12.0	1	7.2	85.5	
BBCH ≥40	Small omnivorous bird “lark” Combination (invertebrates with interception) 25% crop leaves 25% weed seeds 50% ground arthropods	7.2	1	4.32	142	

The proposed use of metabolite JAU 6476-desthio in chronic risk assessment as more toxic than parent was accepted.

	<p>This acute and long-term risk for birds is acceptable if formulation is used according to proposed pattern use.</p> <p>For both active substances and metabolites of prothioconazole a risk assessment for fish-eating and earthworm-eating birds was performed and the TER_{LT} values are above the trigger value of 5. The risks due to bioaccumulation of prothioconazole and its metabolite via the food chain for birds is acceptable.</p>
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Acute risk

Table 9.2-12: Screening assessment of the acute risk for birds due to the use of A23282A in cereals – Cyprodinil/prothioconazole mixture

Intended use	Cereals				
Active substance	Cyprodinil/prothioconazole mixture				
Application rate (g a.s./ha)	1 × 600 ^a				
Acute toxicity (mg/kg bw)	≥ 3 090				
TER criterion	10				
Crop scenario Growth stage	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a
Cereals	Small omnivorous bird	158.8	1.0	95.28	≥ 32

SV: shortcut value; MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

^aSum of 450 g cyprodinil/ha + 150 g prothioconazole/ha

The acute screening assessment for the cyprodinil/prothioconazole mixture concludes a TER value greater than the trigger of 10, indicating that the acute risk to birds is acceptable following use of A23282A according to the proposed use pattern.

Chronic risk

The EFSA Evaluation manual suggests that combination toxicity can be addressed based on the concentration addition model. In case of concentration addition each substance contributes to the total toxicity of a mixture in proportion to its concentration.

Since the prothioconazole metabolite JAU 6476-desthio shows greater toxicity than parent, the combination toxicity assessment is conducted with cyprodinil and JAU 6476-desthio.

A risk assessment for the potential combination toxicity was conducted using the following equation:

$$TER_{combi} = trigger / ((trigger_{cyprodinil}/TER_{cyprodinil}) + (trigger_{JAU\ 6476-desthio}/TER_{JAU\ 6476-desthio}))$$

An acceptable risk is expected when $TER_{combi} > trigger$.

In this formula, ‘triggers’ are the trigger values as mentioned in the corresponding chapter of the Evaluation Manual (these are equivalent to the EU triggers).

Table 9.2-13: Screening step assessment of the long-term/reproductive risk for birds due to the use of A23282A – Cyprodinil/Prothioconazole mixture

GAP crop	Growth stage	Indicator species	TER _{cyprodinil}	TER _{JAU 6476-desthio}	TER criterion	TER _{combi}
Cereals	BBCH 31-69	Small omnivorous bird	4.1	3.2	5	1.8

TERs shown in bold fall below the relevant trigger

The screening TER_{combi} values are below the trigger of 5, therefore, a Tier I assessment is required.

The following TER_{LT} values for cyprodinil and JAU 6476-desthio used in the Tier I mixture assessment have been previously calculated in Table 9.2-8 and Table 9.2-11, respectively.

Table 9.2-14: Tier I assessment of the long-term/reproductive risk for birds due to the use of A23282A – Cyprodinil/Prothioconazole mixture

GAP crop	Growth stage	Indicator species	TER _{cyprodinil}	TER _{JAU 6476-desthio}	TER criterion	TER _{combi}
Cereals	BBCH 30-39	Small omnivorous bird “lark”	50	38	5	22
	BBCH ≥40	Small omnivorous bird “lark”	81	62	5	35

The TER_{combi} values are greater than the trigger of 5 indicating acceptable long-term combination risks for birds for the proposed uses of A23282A.

9.2.2.2 Higher-tier risk assessment

Not required.

9.2.2.3 Drinking water exposure

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

Leaf scenario

Since A23282A 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 3 000 in the case of more sorptive substances ($K_{oc} \geq 500$ L/kg).

Cyprodinil

With a K(f)oc of 1697.7, cyprodinil belongs to the group of “more sorptive substances”. Here, the maximum use rate of 1 x 450 g a.s./ha is used to cover the risk to birds from all intended uses (see 9.1.2).

Effective application rate (g/ha) =	450		Ratio trigger
Acute toxicity (mg/kg bw) =	3 776 500	quotient = 0.12 0.9	≤ 3000
Reprod. toxicity (mg/kg bw/d) =	64	quotient = 7.03	≤ 3000

Prothioconazole

With a K(f)oc of 1765, prothioconazole belongs to the group of more sorptive substances. Here, the maximum use rate of 1 x 150 g a.s./ha is used to cover the risk to birds from all intended uses (see 9.1.2).

Effective application rate (g/ha) =	150		Ratio trigger
Acute toxicity (mg/kg bw) =	> 2 000	quotient < 0.075	≤ 3000
Reprod. Toxicity (mg/kg bw/d) =	78	quotient = 1.92	≤ 3000

JAU 6476-desthio

With a K(f)oc of 575.4, JAU 6476-desthio belongs to the group of more sorptive substances. Here, the maximum use rate of 1 x 136 g/ha is used to cover the risk to birds from all intended uses (see 9.1.2).

Effective application rate (g/ha) =	136.05 ^a		Ratio trigger
Acute toxicity (mg/kg bw) =	> 2 000	quotient < 0.068	≤ 3000
Reprod. Toxicity (mg/kg bw/d) =	14.8	quotient = 9.19	≤ 3000

^a The application rate for this metabolite was adjusted based on the ratio between the molecular mass of metabolite and the parent (i.e. metabolite 312.2 / parent 344.26 = 0.907)

The resulting ratios fall below the trigger of 3000, indicating that further assessment of the acute and long-term risk to birds from drinking water from puddles is not required for cyprodinil, prothioconazole and JAU 6476-desthio.

9.2.2.4 Effects of secondary poisoning

The log P_{ow} of cyprodinil is 4.0 and thus exceeds the trigger value of 3. The log P_{ow} of prothioconazole is 4.05 and thus also exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is required for both active substances.

The prothioconazole metabolites JAU 6476-desthio and JAU 6476-S-methyl also have log P_{ow} values >3.0 (log P_{ow} of 3.04 and 4.19, respectively), and are therefore also considered in the secondary poisoning assessment. No assessment is required for the metabolite 1,2,4-triazole, which has a log P_{ow} <3.0.

Risk assessment for earthworm-eating birds via secondary poisoning

According to EFSA/2009/1438, the risk for vermivorous birds is assessed for a bird of 100 g body weight with a daily food consumption of 104.6 g. Bioaccumulation in earthworms is estimated based on predicted concentrations in soil.

Cyprodinil

Here, the worst-case 21-day time-weighted average PEC_{soil} value following a 1 x 450 g cyprodinil/ha application to cereals was used.

Table 9.2-15: Assessment of the risk for earthworm-eating birds due to exposure to cyprodinil via bioaccumulation in earthworms (secondary poisoning) for the intended use in cereals

Parameter	Cyprodinil	Comments
PEC _{soil} (twa = 21 d) (mg/kg soil)	0.119	maximum 21 d TWA PEC _{soil} (refer Section B8)
log P _{ow} / K _{ow}	4.0 / 10000	
K _{foc}	1 697.7	Worst case geometric Mean (n = 5)
Foc	0.02	Default
BCF _{worm}	3.56	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + 0.012 \times K_{ow}) / foc \times Koc$
PEC _{worm}	0.424	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.445	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	64	
TER _{lt}	140	≥ 5; acceptable risk

Prothioconazole

Here, the worst-case 21-day time-weighted average PEC_{soil} value following a 1 x 150 g prothioconazole/ha application to cereals was used.

Table 9.2-16: Assessment of the risk for earthworm-eating birds due to exposure to prothioconazole via bioaccumulation in earthworms (secondary poisoning) for the intended uses

Parameter	Prothioconazole	Comments
PEC _{soil} (twa = 21 d) (mg/kg soil)	0.008	maximum 21 d TWA PEC _{soil} (refer Section B8)
log P _{ow} / K _{ow}	4.05 / 11 220	
Koc	1 765	Single data from aged leaching study
Foc	0.02	Default
BCF _{worm}	3.84	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + 0.012 \times K_{ow}) / foc \times Koc$
PEC _{worm}	0.031	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.033	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	78	
TER _{lt}	2 400	≥ 5; acceptable risk

JAU 6476-desthio

Here, the worst-case 21-day time-weighted average PEC_{soil} value of prothioconazole metabolite JAU 6476-desthio, following a 1 x 150 g prothioconazole/ha application to cereals was used.

Table 9.2-17: Assessment of the risk for earthworm-eating birds due to exposure to JAU 6476-desthio via bioaccumulation in earthworms (secondary poisoning) for the intended uses

Parameter	JAU 6476-desthio	Comments
PEC _{soil} (twa = 21 d) (mg/kg soil)	0.0243	maximum 21 d TWA PEC _{soil} (refer Section B8)
log P _{ow} / K _{ow}	3.04 / 1 096	
Koc	573.5	Worst case geometric mean (n = 4) based on EU agreed endpoints
Foc	0.02	Default
BCF _{worm}	1.22	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + 0.012 \times K_{ow}) / foc \times Koc$
PEC _{worm}	0.030	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.032	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	14.8	
TER _{it}	460	≥ 5; acceptable risk

JAU 6476-S-methyl

Here, the worst-case 21-day time-weighted average PEC_{soil} value of prothioconazole metabolite JAU 6476-S-methyl, following a 1 x 150 g prothioconazole/ha application to cereals was used.

Table 9.2-18: Assessment of the risk for earthworm-eating birds due to exposure to JAU 6476-S-methyl via bioaccumulation in earthworms (secondary poisoning) for the intended uses

Parameter	JAU 6476-S-methyl	Comments
PEC _{soil} (twa = 21 d) (mg/kg soil)	0.0050	maximum 21 d TWA PEC _{soil} (refer Section B8)
log P _{ow} / K _{ow}	4.19 / 15 488	
Koc	2 525.9	Worst case geometric mean (n = 4) based on EU agreed endpoints
foc	0.02	Default
BCF _{worm}	3.65	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + 0.012 \times K_{ow}) / foc \times Koc$
PEC _{worm}	0.018	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.019	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	7.8	NOEL of the parent prothioconazole divided by a factor of 10 as no data is available for this metabolite
TER _{it}	410	≥ 5; acceptable risk

Risk assessment for fish-eating birds via secondary poisoning

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

Cyprodinil

Here, the worst-case 21-day time-weighted average surface water PEC (FOCUS Step 1) following a 1 x 450 g cyprodinil/ha application to cereals was used.

Table 9.2-19: Assessment of the risk for fish-eating birds due to exposure to cyprodinil via bioaccumulation in fish (secondary poisoning) for the intended use in cereals

Parameter	Cyprodinil	Comments
PEC _{sw} (twa = 21 d) (mg/L)	0.0452	FOCUS Step 1
BCF _{fish}	393	
BMF	-	biomagnification factor (relevant for BCF ≥ 2000)
PEC _{fish}	17.76	PEC _{fish} = PEC _{water} × BCF _{fish}
Daily dietary dose (mg/kg bw/d)	2.82	DDD = PEC _{fish} × 0.159
NOEL (mg/kg bw/d)	64	
TER _{it}	23	≥ 5; acceptable risk

Prothioconazole

Here, the worst-case 21-day time-weighted average surface water PEC (FOCUS Step 1) following a 1 x 150 g prothioconazole/ha application to cereals was used.

Table 9.2-20: Assessment of the risk for fish-eating birds due to exposure to prothioconazole via bioaccumulation in fish (secondary poisoning) for the intended use in cereals

Parameter	Prothioconazole	Comments
PEC _{sw} (twa = 21 d) (mg/L)	0.0128	FOCUS Step 1
BCF _{fish}	19.7	EFSA conclusion 2007
BMF	-	biomagnification factor (relevant for BCF ≥ 2000)
PEC _{fish}	0.252	PEC _{fish} = PEC _{water} × BCF _{fish}
Daily dietary dose (mg/kg bw/d)	0.040	DDD = PEC _{fish} × 0.159
NOEL (mg/kg bw/d)	78	
TER _{it}	2 000	≥ 5; acceptable risk

JAU 6476-desthio

Here, the 21-day time weighted average surface water PEC (FOCUS Step 1) of prothioconazole metabolite JAU 6476-desthio, following a 1 x 150 g prothioconazole/ha application to cereals, was used.

Table 9.2-21: Assessment of the risk for fish-eating birds due to exposure to JAU 6476-desthio via bioaccumulation in fish (secondary poisoning) for the intended use in cereals

Parameter	JAU 6476-desthio	Comments
PEC _{sw} (twa = 21 d) (mg/L)	0.0255	FOCUS Step 1
BCF _{fish}	65	EFSA Conclusion 2007
BMF	-	biomagnification factor (relevant for BCF ≥ 2000)
PEC _{fish}	1.658	PEC _{fish} = PEC _{water} × BCF _{fish}

Parameter	JAU 6476-desthio	Comments
Daily dietary dose (mg/kg bw/d)	0.264	$DDD = PEC_{fish} \times 0.159$
NOEL (mg/kg bw/d)	14.8	
TER _{it}	56	≥ 5 ; acceptable risk

JAU 6476 S-methyl

Here, the 21-day time weighted average surface water PEC (FOCUS Step 1) of prothioconazole metabolite JAU 6476 S-methyl, following a 1 x 150 g prothioconazole/ha application to cereals, was used.

Due to the low predicted environmental concentrations of the prothioconazole metabolite JAU 6476-S-methyl in surface water, no fish-BCF-study was conducted even though this metabolite has a log P_{ow} of 4.19. The BCF for this metabolite was estimated from its log P_{ow} and the BCF and the log P_{ow} of the parent compound with a sufficient degree of precision. A worst-case BCF of 1995 for JAU 6476-S-methyl in fish (see Prothioconazole RAR B09 (2018) for further details) is used in the calculation for secondary poisoning.

Table 9.2-22: Assessment of the risk for fish-eating mammals due to exposure to JAU 6476-S-methyl via bioaccumulation in fish (secondary poisoning) for the intended uses

Parameter	JAU 6476-S-methyl	Comments
PEC _{sw} (twa = 21 d) (mg/L)	0.00273	FOCUS Step 1
BCF _{fish}	1 995	Worst-case BCF prediction as per QSAR model
BMF	-	biomagnification factor (relevant for $BCF \geq 2000$)
PEC _{fish}	5.45	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.866	$DDD = PEC_{fish} \times 0.159$
NOEL (mg/kg bw/d)	7.8	No toxicity data available, therefore metabolite assumed to be ten times more toxic than the parent substance, as a worst-case approach
TER _{it}	9.01	≥ 5 ; acceptable risk

9.2.2.5 Biomagnification in terrestrial food chains

Not relevant.

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

Not relevant.

9.2.4 Overall conclusions

The acute and long-term risks of A23282A to birds were assessed from toxicity exposure ratios between toxicity endpoints, estimated from studies with cyprodinil, prothioconazole, its major metabolite JAU 6476-desthio and maximum residues occurring on food items following applications according to the proposed use pattern.

For cyprodinil, prothioconazole and the prothioconazole metabolite JAU 6476-desthio, the acute and

chronic screening step or Tier 1 TER values exceed the trigger values of 10 and 5, respectively, indicating that the risk to birds is acceptable following use of A23282A according to the proposed use pattern.

Additionally, the acute and chronic TER values for the mixture exceed the relevant triggers indicating an acceptable risk to birds from A23282A.

Risk of secondary poisoning has also been assessed, as cyprodinil, prothioconazole, and prothioconazole metabolites JAU 6476-desthio and JAU 6474 S-methyl all have log P_{OW} values >3.0. The risk to birds from exposure via drinking water has also been assessed. All assessments indicate that the risk to birds is acceptable following use of A23282A according to the proposed use pattern.

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

<p>zRMS Comments:</p>	<p>The submitted acute and long-term risk to mammals was accepted. The risk assessment was performed in accordance with the B & M Guidance, EFSA (2009). All relevant used endpoints were agreed at the EU level. Both active substances and metabolite prothioconazole-desthio were taken into consideration.</p> <p>Cyprodinil. The acute and long-term risk were submitted at screening step. The submitted acute and long-term risk to birds was accepted. The TER_A and TER_{LT} values are below exceed the trigger values of 10 and 5, respectively, indicating an acceptable risk for mammals.</p> <p>Prothioconazole. The active substance and its metabolite prothioconazole-desthio were taken into consideration. The submitted acute and long-term risk to birds was accepted. For metabolite prothioconazole-desthio the chronic risk refinement based on deposition factor was accepted (at BBCH > 40 the interception of 90% was considered). The TER_A and TER_{LT} values are below exceed the trigger values of 10 and 5, respectively, indicating an acceptable risk for mammals.</p> <p>Mixture toxicity. The submitted risk assessment for mammals due to use of formulation A23282A was accepted. The proposed use of metabolite JAU 6476-desthio in chronic risk assessment as more toxic than parent was accepted. For active substances and its metabolite prothioconazole-desthio a risk assessment for fish-eating and earthworm-eating mammals was performed and the TER_{LT} values are above the trigger value of 5. The risk due to bioaccumulation of prothioconazole and its metabolite via the food chain for mammals is acceptable.</p>
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9.3.1 Toxicity data

Mammalian toxicity studies have been carried out with cyprodinil, prothioconazole and its relevant metabolite. Full details of these studies are provided in the respective EU DAR and related documents.

Effects of A23282A on mammals were not evaluated as part of the EU assessments of cyprodinil and prothioconazole. However, the provision of further data on the formulation is not considered essential, because the risk to mammals from A23282A can be adequately determined from the risk assessment for the individual active substances. The risk to mammals from the proposed uses of A23282A will be assessed using the endpoints for cyprodinil and prothioconazole.

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

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

Species	Substance	Exposure System	Results	Reference
Rat	Cyprodinil	Oral Acute	LD₅₀ = >2 000 mg/kg bw	EFSA Scientific Report (2005) 51, 1-78; Hartmann (1992); Report No. 901075 CGA219417/0020; VV-350984
Rat	Cyprodinil	Dietary Reproductive toxicity Two-generation study	NOAEL = 72.7 mg/kg bw/d (slight increase in F1 liver weights)^a	EFSA Scientific Report (2005) 51, 1-78; Khalil (1993); Report No. 891328; CGA219417/0162; VV-369425

^a The lowest overall mean value was calculated from all of the mean weekly consumption values for the individual sexes (72.7 mg/kg bw/day for males and 96.6 mg/kg bw/day for females)
Values in **bold** are used in the risk assessment

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

Species	Substance	Exposure System	Results	Reference
Rat	Prothioconazole	Oral 1 d Acute	LD₅₀ > 6 200 mg/kg bw	EFSA Conclusion 2007
Rat	Prothioconazole	Dietary Reproductive toxicity Two-generation study	NOAEL = 95.6 mg/kg bw/d	EFSA Conclusion 2007
Mouse	JAU 6476-desthio	Oral 1 d Acute	LD₅₀ = 2 235 mg/kg bw (male) 3459 mg/kg bw (female)	EFSA Conclusion 2007
Rat	JAU 6476-desthio	Oral 1 d Acute	LD₅₀ = 2 506 mg/kg bw (female) LD₅₀ = 2806 mg/kg bw (male)	EFSA Conclusion 2007
Rat	JAU 6476-desthio	Dietary Reproductive toxicity Two-generation study	NOAEL = 10 mg/kg bw/d	EFSA Conclusion 2007

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

Table 9.3-3: Endpoints and effect values relevant for the risk assessment for mammals – cyprodinil/prothioconazole mixture

Species	Substance	Exposure System	Results	Reference
-	Cyprodinil/Prothioconazole mixture	Oral Acute	LD₅₀ > 2408 mg/kg bw	Refer to Section 9.3.1.1

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

Cyprodinil metabolites

Since metabolites are formed at <10% of parent level in edible crop parts and mammalian testing indicates that they are less toxic than the parent, it can be concluded that the risk to birds will be low and no further risk assessment is conducted (Cyprodinil; EFSA Scientific Report 51, 2005).

Prothioconazole metabolite

JAU 6476-desthio is a metabolite of prothioconazole that occurred in amounts of > 10% of the TRR (total radioactive residues) on plant material. Wild mammals may be exposed to this metabolite mainly by consumption of contaminated feed, and therefore this metabolite is considered in the assessment. This is in accordance with EU conclusions (EFSA Conclusion 2007).

9.3.1.1 Justification for new endpoints

Consideration of acute mixture toxicity

According to EFSA/2009/1438¹ combined action of several toxicants must be specifically considered in the risk assessment when it is obvious that such exposure situations will occur for animals.

For the assessment of acute effects (mortality), a surrogate LD₅₀ can be calculated. The EFSA Guidance Document indicates that the following equation should be used for deriving a surrogate LD₅₀ for a mixture of active substances with known toxicity assuming dose additivity:

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

where:

X (a.s.i) = fraction of active substance (i) in the formulation mixture

LD₅₀ (a.s.i) = acute toxicity for the active substance (i)

The LD₅₀ of the mix is summarised in the table below.

Table 9.3-4: Acute LD₅₀ for the mixture of active substances

Test substance	Concentration of active substance in formulation A23282A (g/L)	Fraction of active substance in the formulation mixture ^A	Acute toxicity endpoint (mg/kg bw)	Fraction of active substance/LD ₅₀ for the active substance	LD ₅₀ mix (mg/kg bw)
Cyprodinil	225	0.75	>2 000	0.000375	>2 408
Prothioconazole	75	0.25	>6 200	0.0000403	
Total	300	1	-	0.000415	

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

A “tox per fraction” quotient can be calculated for each active substance and for the mixture according to the following equations:

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

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

Table 9.3-5: Comparison of tox per fractions for individual active substances with the tox per fraction (mix)

Active substance	LD ₅₀ (mg/kg bw)	Fraction of active substance in the formulation mixture	Tox per fraction (a.s)	% difference from tox per fraction of formulation
Cyprodinil	>2000	0.75	2667	11
Prothioconazole	>6200	0.25	10480	335
Tox per fraction (mix)	>2408	1.00		

Appendix B (page 191) of the Bird and mammal guidance EFSA/2009/1438 states, “If one active substance can be identified where the two quotients “tox per fraction (a.s.)” and “tox per fraction (mix)” deviate by <10%, this indicates that this active substance will contribute to >90% to the mixture toxicity, while the other components of the mixture will only have a marginal impact of the predicted risk. Consequently, the risk assessment can be performed for the most toxic active substance alone. No further considerations according to Steps 2-4 are necessary.”

The tox per fraction for cyprodinil and prothioconazole deviate by >10% (11% and 335% respectively), therefore neither of the components drive the acute risk assessment and an acute mixture toxicity risk assessment is required according to the guidance.

9.3.2 Risk assessment for spray applications

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

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

Cyprodinil

Table 9.3-6: Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of A23282A in cereals - cyprodinil

Intended use	Cereals					
Active substance	Cyprodinil					
Application rate (g a.s./ha)	1 x 450					
Acute toxicity (mg/kg bw)	>2 000					
TER criterion	10					
Crop scenario	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a	
Cereals	Small herbivorous mammal	118.4	1.0	53.28	>38	
Reprod. Toxicity (mg/kg bw/d)	72.7					
TER criterion	5					
Crop scenario	Indicator/generic focal species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{lt}	
Cereals	Small herbivorous mammal	48.3	1 x 0.53	11.52	6.3	

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

The acute and long-term screening assessment for cyprodinil concludes TER values greater than the triggers of 10 and 5, indicating that acute and long-term risks to mammals are acceptable following use of A23282A according to the proposed use patterns. However, as to conduct a Tier 1 chronic mixture toxicity risk assessment is required (see Table 9.3-13), a Tier 1 chronic risk assessment for cyprodinil has been conducted below.

Table 9.3-7: First-tier assessment of the long-term/reproductive risk for mammals due to the use of A23282A in cereals – cyprodinil (for mixture risk assessment)

Intended use	Cereals					
Active substance	Cyprodinil					
Application rate (g a.s./ha)	1 x 450					
Reprod. Toxicity (mg/kg bw/d)	72.7					
TER criterion	5					
Growth stage	Indicator/generic focal species	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{lt}	
BBCH 30-39	Small omnivorous mammal "mouse"	3.9	1 x 0.53	0.930	78	
BBCH > 20	Small insectivorous mammal "shrew"	1.9	1 x 0.53	0.453	160	
BBCH > 40	Small herbivorous mammal "vole"	21.7	1 x 0.53	5.18	14	
BBCH > 40	Small omnivorous mammal "mouse"	2.3	1 x 0.53	0.549	130	

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

The Tier 1 assessment for cyprodinil concludes that all TER values are greater than the trigger of 5, indicating that the long-term risk to mammals is acceptable following use of A23282A according to the proposed use patterns.

Prothioconazole

Table 9.3-8: Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of A23282A in cereals - prothioconazole

Intended use	Cereals					
Active substance	Prothioconazole					
Application rate (g a.s./ha)	1 x 150					
Acute toxicity (mg/kg bw)	>6 200					
TER criterion	10					
Crop scenario	Indicator/generic species	focal	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a
Cereals	Small herbivorous mammal		118.4	1.0	17.76	>350
Reprod. Toxicity (mg/kg bw/d)	95.6					
TER criterion	5					
Crop scenario	Indicator/generic species	focal	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{lt}
Cereals	Small herbivorous mammal		48.3	1 x 0.53	3.84	25

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

The acute and long-term screening assessment for prothioconazole concludes TER values greater than the triggers of 10 and 5, indicating that acute and long-term risks to mammals are acceptable following use of A23282A according to the proposed use patterns.

JAU 6476-desthio

As there is not an application rate for the foliar metabolite JAU 6476-desthio, the ratio between the mass of the parent molecule and metabolite will be used to determine a worst-case surrogate application rate. The molar mass of prothioconazole is 344.26 g/mol, and the molar mass of JAU 6476-desthio is 312.2 g/mol, therefore the mass ratio between the two is 0.907. This will be used to convert the application rate of the parent active substance (150 g prothioconazole/ha) to a surrogate application rate of JAU 6476-desthio (136.05 g JAU 6476-desthio/ha).

Table 9.3-9: Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of A23282A in cereals – JAU 6476-desthio

Intended use	Cereals					
Metabolite	JAU 6476-desthio					
Application rate (g/ha)	1 x 136.05					
Acute toxicity (mg/kg bw)	2 235					
TER criterion	10					
Crop scenario	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a	
Cereals	Small herbivorous mammal	118.4	1.0	16.1	140	
Reprod. Toxicity (mg/kg bw/d)	10					
TER criterion	5					
Crop scenario	Indicator/generic focal species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{lt}	
Cereals	Small herbivorous mammal	48.3	1 x 0.53	3.48	2.9	

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

The acute screening assessment for prothioconazole metabolite JAU 6476-desthio concludes a TER value greater than the trigger of 10, indicating that acute risk to mammals is acceptable following use of A23282A according to the proposed use patterns.

The long-term screening assessment for the prothioconazole metabolite JAU 6476-desthio concludes a TER value below the trigger of 5, indicating that a Tier I assessment is required for the long-term risk to mammals.

Table 9.3-10: First-tier assessment of the long-term/reproductive risk for mammals due to the use of A23282A in cereals – JAU 6476-desthio

Intended use	Cereals					
Metabolite	JAU 6476-desthio					
Application rate (g/ha)	1 x 136.05					
Reprod. Toxicity (mg/kg bw/d)	10					
TER criterion	5					
Growth stage	Indicator/generic focal species	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{lt}	
BBCH 30-39	Small omnivorous mammal "mouse"	3.9	1 x 0.53	0.281	36	
BBCH > 20	Small insectivorous mammal "shrew"	1.9	1 x 0.53	0.137	73	
BBCH > 40	Small herbivorous mammal "vole"	21.7	1 x 0.53	1.565	6.4	
BBCH > 40	Small omnivorous mammal "mouse"	2.3	1 x 0.53	0.166	60	

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

toxicity to exposure ratio.

The Tier 1 assessment for prothioconazole metabolite JAU 6476-desthio concludes that all TER values are greater than the trigger of 5, indicating that the long-term risk to mammals is acceptable following use of A23282A according to the proposed use patterns.

Cyprodinil/prothioconazole mixture

Acute risk

Table 9.3-11: Screening assessment of the acute risk for mammals due to the use of A23282A in cereals – cyprodinil/prothioconazole mixture

Intended use	Cereals				
Active substance	Cyprodinil/prothioconazole mixture				
Application rate (g/ha)	1 x 600 ^a				
Acute toxicity (mg/kg bw)	>2 408				
TER criterion	10				
Crop scenario	Indicator/generic focal species	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a
Cereals	Small herbivorous mammal	118.4	1.0	71.04	>34

SV: shortcut value; MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

^a Sum of 450 g cyprodinil/ha + 150 g prothioconazole/ha

The acute screening assessment for the cyprodinil/prothioconazole mixture concludes a TER value greater than the trigger of 10, indicating that the acute risk to mammals is acceptable following use of A23282A according to the proposed use patterns.

Chronic risk

The EFSA Evaluation manual suggests that combination toxicity can be addressed based on the concentration addition model. In case of concentration addition each substance contributes to the total toxicity of a mixture in proportion to its concentration.

Since the prothioconazole metabolite JAU 6476-desthio shows greater toxicity than parent, the combination toxicity assessment is conducted with cyprodinil and JAU 6476-desthio.

A risk assessment for the potential combination toxicity was conducted using the following equation:

$$TER_{combi} = trigger / ((trigger_{cyprodinil}/TER_{cyprodinil}) + (trigger_{JAU\ 6476-desthio}/TER_{JAU\ 6476-desthio}))$$

An acceptable risk is expected when $TER_{combi} > trigger$.

In this formula, ‘triggers’ are the trigger values as mentioned in the corresponding chapter of the Evaluation Manual (these are equivalent to the EU triggers).

Table 9.3-12: Screening step assessment of the long-term/reproductive risk for mammals due to the use of A23282A – Cyprodinil/Prothioconazole mixture

GAP crop	Growth stage	Indicator species	TER _{cyprodinil}	TER _{JAU 6476-desthio}	TER criterion	TER _{combi}
Cereals	BBCH 31-69	Small herbivorous mammal	6.3	2.9	5	2.0

TERs shown in bold fall below the relevant trigger

The screening TER_{combi} values are below the trigger of 5, therefore, a Tier I assessment is required.

The following TER_{LT} values for cyprodinil and JAU 6476-desthio used in the Tier I mixture assessment have been previously calculated in Table 9.3-7 and Table 9.3-10, respectively.

Table 9.3-13: Tier I assessment of the long-term/reproductive risk for mammals due to the use of A23282A – Cyprodinil/Prothioconazole mixture

GAP crop	Growth stage	Indicator species	TER _{cyprodinil}	TER _{JAU 6476-desthio}	TER criterion	TER _{combi}
Cereals	BBCH 30-39	Small omnivorous mammal "mouse"	78	36	5	25
	BBCH > 20	Small insectivorous mammal "shrew"	160	73	5	51
	BBCH > 40	Small herbivorous mammal "vole"	14	6.4	5	4.4
	BBCH > 40	Small omnivorous mammal "mouse"	130	60	5	41

For three out of four scenarios the TER_{combi} values are greater than the trigger of 5 indicating acceptable long-term combination risks for mammals for the proposed uses of A23282A. However, for small herbivorous mammal 'vole' further refinement is required.

9.3.2.2 Higher-tier risk assessment

For the combination of the two active substances, refined long-term/reproductive risk assessment is required for the following scenarios:

- Cereals, small herbivorous mammal "vole" (BBCH ≥ 40)

According to Appendix A of the EFSA/2009/1438, it is assumed that small herbivorous mammals (vole) are feeding on 100% grass in treated cereals. Since the food item is at ground level, it is possible to refine the default deposition factor. Within Appendix E of the EFSA/2009/1438 Risk Assessment on 'Impact of crop interception on residues on plant food items', in referring to deposition estimates for Tier I, it is stated that '*The deposition factors provided for the different crops and growth stages are likely to reflect conservative estimates. In the context of a higher-tier assessment, the more detailed values of FOCUS Groundwater guidance report (FOCUS, 2000) may therefore also be used*'. Therefore, this risk assessment will be refined using FOCUS Groundwater guidance interception values, using however a more recent version of the guidance (EFSA 2014)². At BBCH 40-69, cereals are estimated to intercept approximately 90% of residues and so the deposition factor (DF) can be adjusted from 0.3 to 0.1 in the higher tier assessment calculation for the proportion of diet represented by voles in cereals. This higher tier risk assessment is presented in the tables below for the chronic risk to mammals.

² EFSA (2014). Guidance Document for evaluating laboratory and field dissipation studies to obtain DegT50 values of active substances of plant protection products and transformation products of these active substances in soil. EFSA Journal 2014;12(5):3662, 37 pp. doi:10.2903/j.efsa.2014.3662

Table 9.3-14: Higher-tier assessment of the long-term/reproductive risk for mammals due to the use of A23282A in cereals – cyprodinil

Intended use	Cereals					
Active substance	Cyprodinil					
Application rate (g a.s./ha)	1 x 450					
Reprod. toxicity (mg/kg bw/d)	72.7					
TER criterion	5					
Generic Focal species	Food category % in diet	FIR/bw	RUD_m × DF^a (mg/kg food)	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{lt}
Small herbivorous mammal “vole”; BBCH ≥ 40	Grass, 100%	1.33	54.2 x 0.1	1 x 0.53	1.72	42

FIR/bw: Food intake rate per body weight; RUD: residue unit dose; DF: deposition factor (considering possible interception by the crop); SV: short-cut value; MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

^a Refined according to deposition factor of 0.1

Table 9.3-15: Higher-tier assessment of the long-term/reproductive risk for mammals due to the use of A23282 in cereals – JAU 6476-desthio

Intended use	Cereals					
Metabolite	JAU 6476-desthio					
Application rate (g/ha)	1 x 136.05					
Reprod. toxicity (mg/kg bw/d)	10					
TER criterion	5					
Generic Focal species	Food category % in diet	FIR/bw	RUD_m × DF^a (mg/kg food)	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{lt}
Small herbivorous mammal “vole”; BBCH ≥ 40	Grass, 100%	1.33	54.2 x 0.1	1 x 0.53	0.520	19

FIR/bw: Food intake rate per body weight; RUD: residue unit dose; DF: deposition factor (considering possible interception by the crop); SV: short-cut value; MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

^a Refined according to deposition factor of 0.1

Table 9.3-16: Refined Tier I assessment of the long-term/reproductive risk for mammals due to the use of A23282A in cereals – mixture

Crop scenario Growth stage	Generic focal species	TER_{cyprodinil}	TER_{JAU 6476- desthio}	Trigger	TER_{combi}
Cereals BBCH ≥ 40	Small herbivorous mammal “vole”	42	19	5	13

The refined TER_{combi} value for mammals is above the trigger of 5, indicating acceptable chronic risk to

mammals from the use of A23282A.

9.3.2.3 Drinking water exposure

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

Puddle scenario

Due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals, no specific calculations of exposure and TER are necessary when the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed 50 in the case of less sorptive substances ($K_{oc} < 500$ L/kg) or 3 000 in the case of more sorptive substances ($K_{oc} \geq 500$ L/kg).

Cyprodinil

With a $K(f)_{oc}$ of 1 697.7, cyprodinil belongs to the group of more sorptive substances. Here, the maximum use rate of 1 x 450 g a.s./ha is used to cover the risk to mammals from all intended uses (see 9.1.2).

Effective application rate (g/ha) =	450		Ratio trigger
Acute toxicity (mg/kg bw) =	>2 000	quotient <0.225	≤ 3000
Reprod. toxicity (mg/kg bw/d) =	72.7	quotient = 6.19	≤ 3000

Prothioconazole

With a $K(f)_{oc}$ of 1765, prothioconazole belongs to the group of more sorptive substances. Here, the maximum use rate of 1 x 150 g a.s./ha is used to cover the risk to mammals from all intended uses (see 9.1.2).

Effective application rate (g/ha) =	150		Ratio trigger
Acute toxicity (mg/kg bw) =	> 6 200	quotient < 0.024	≤ 3000
Reprod. toxicity (mg/kg bw/d) =	95.6	quotient = 1.6	≤ 3000

JAU 6476-desthio

With a $K(f)_{oc}$ of 573.5, JAU 6476-desthio belongs to the group of more sorptive substances. Here, the maximum use rate of 1 x 136 g/ha is used to cover the risk to mammals from all intended uses (see 9.1.2).

Effective application rate (g/ha) =	136.05 ^a		Ratio trigger
Acute toxicity (mg/kg bw) =	2 235	quotient = 0.061	≤ 3000
Reprod. toxicity (mg/kg bw/d) =	10	quotient = 13.6	≤ 3000

^a The application rate for this metabolite was adjusted based on the ratio between the molecular mass of the metabolite and the parent (i.e. metabolite 312.2 / parent 344.26 = 0.907)

The resulting ratios all fall below the trigger of 3000, indicating that further assessment of the acute and long-term risk to mammals from drinking water from puddles is not required for cyprodinil, prothioconazole and JAU 6476-desthio.

9.3.2.4 Effects of secondary poisoning

The log P_{ow} of cyprodinil is 4.0 and thus exceeds the trigger value of 3. The log P_{ow} of prothioconazole is 4.05 and thus also exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is required for both active substances.

The prothioconazole metabolites JAU 6476-desthio and JAU 6476-S-methyl also have log P_{ow} values >3.0 (log P_{ow} of 3.04 and 4.19, respectively), and are therefore also considered in the secondary poisoning assessment. No assessment is required for the metabolite 1,2,4-triazole, which has a log P_{ow} <3.0.

Risk assessment for earthworm-eating mammals via secondary poisoning

According to EFSA/2009/1438, the risk for vermivorous mammals is assessed for a small mammal of 10 g body weight with a daily food consumption of 12.8 g. Bioaccumulation in earthworms is estimated based on predicted concentrations in soil.

Cyprodinil

Here, the 21-day time weighted average soil PEC following a 1 x 450 g cyprodinil/ha application to cereals was used.

Table 9.3-17: Assessment of the risk for earthworm-eating mammals due to exposure to cyprodinil via bioaccumulation in earthworms (secondary poisoning) for the intended use in cereals

Parameter	Cyprodinil	Comments
PEC _{soil} (twa = 21 d) (mg/kg soil)	0.119	maximum 21 d TWA PEC _{soil} (refer Section B8)
Log P_{ow} / K_{ow}	4.0/10000	
K _{foc}	1 697.7	Worst case geometric Mean (n = 5)
F _{oc}	0.02	Default
BCF _{worm}	3.56	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + 0.012 \times K_{ow}) / f_{oc} \times K_{oc}$
PEC _{worm}	0.424	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.542	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	72.7	
TER _{lt}	130	≥ 5; acceptable risk

Prothioconazole

Here, the worst-case 21-day time-weighted average PEC_{soil} value following a 1 x 150 g prothioconazole/ha application to cereals was used.

Table 9.3-18: Assessment of the risk for earthworm-eating mammals due to exposure to prothioconazole via bioaccumulation in earthworms (secondary poisoning) for the intended uses in cereals

Parameter	Prothioconazole	Comments
PEC _{soil} (twa = 21 d) (mg/kg soil)	0.008	maximum 21 d TWA PEC _{soil} (refer Section B8)
log P_{ow} / K_{ow}	4.05 / 11220	
K _{oc}	1 765	Single data from aged leaching study

Parameter	Prothioconazole	Comments
foc	0.02	Default
BCF _{worm}	3.84	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + 0.012 \times K_{ow}) / foc \times Koc$
PEC _{worm}	0.031	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.039	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	95.6	
TER _{It}	2 400	≥ 5; acceptable risk

JAU 6476-desthio

Here, the worst-case 21-day time-weighted average PEC_{soil} value of prothioconazole metabolite JAU 6476-desthio, following a 1 x 150 g prothioconazole/ha application to cereals was used.

Table 9.3-19: Assessment of the risk for earthworm-eating mammals due to exposure to JAU 6476-desthio via bioaccumulation in earthworms (secondary poisoning) for the intended uses in cereals

Parameter	JAU 6476-desthio	Comments
PEC _{soil} (twa = 21 d) (mg/kg soil)	0.0243	maximum 21 d TWA PEC _{soil} (refer Section B8)
log P _{ow} / K _{ow}	3.04 / 1 096	
Koc	573.5	Geometric mean (n = 4) based on EU agreed endpoints
Foc	0.02	Default
BCF _{worm}	1.22	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + 0.012 \times K_{ow}) / foc \times Koc$
PEC _{worm}	0.030	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.038	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	10	
TER _{It}	260	≥ 5; acceptable risk

JAU 6476-S-methyl

Here, the worst-case 21-day time-weighted average PEC_{soil} value of prothioconazole metabolite JAU 6476-S-methyl, following a 1 x 150 g prothioconazole/ha application to cereals was used.

Table 9.3-20: Assessment of the risk for earthworm-eating mammals due to exposure to JAU 6476-S-methyl via bioaccumulation in earthworms (secondary poisoning) for the intended uses in cereals

Parameter	JAU 6476-S-methyl	Comments
PEC _{soil} (twa = 21 d) (mg/kg soil)	0.0050	maximum 21 d TWA PEC _{soil} (refer Section B8)
log P _{ow} / K _{ow}	4.19 / 15 488	
Koc	2 525.9	Geometric mean (n = 4) based on EU agreed endpoints
foc	0.02	Default

Parameter	JAU 6476-S-methyl	Comments
BCF _{worm}	3.65	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + 0.012 \times K_{ow}) / foc \times Koc$
PEC _{worm}	0.018	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.023	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	9.56	NOEL of the parent prothioconazole divided by a factor of 10 as no data is available for this metabolite
TER _{lt}	420	≥ 5 ; acceptable risk

Risk assessment for fish-eating mammals via secondary poisoning

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

Cyprodinil

Here, the worst-case 21-day time weighted average surface water PEC (FOCUS Step 1) following a 1 x 450 g cyprodinil/ha application to cereals was used.

Table 9.3-21: Assessment of the risk for fish-eating mammals due to exposure to cyprodinil via bioaccumulation in fish (secondary poisoning) for the intended use in cereals

Parameter	Cyprodinil	Comments
PEC _{sw} (twa = 21 d) (mg/L)	0.0452	FOCUS Step 1
BCF _{fish}	393	
BMF	-	biomagnification factor (relevant for $BCF \geq 2000$)
PEC _{fish}	17.76	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	2.522	$DDD = PEC_{fish} \times 0.142$
NOEL (mg/kg bw/d)	72.7	
TER _{lt}	29	≥ 5 ; acceptable risk

Prothioconazole

Here, the worst-case 21-day time weighted average surface water PEC (FOCUS Step 1) following a 1 x 150 g prothioconazole/ha application to cereals was used.

Table 9.3-22: Assessment of the risk for fish-eating mammals due to exposure to prothioconazole via bioaccumulation in fish (secondary poisoning) for the intended uses

Parameter	Prothioconazole	Comments
PEC _{sw} (twa = 21 d) (mg/L)	0.0128	FOCUS Step 1

BCF _{fish}	19.7	EFSA conclusion 2007
BMF	-	biomagnification factor (relevant for BCF ≥ 2000)
PEC _{fish}	0.252	PEC _{fish} = PEC _{water} × BCF _{fish}
Daily dietary dose (mg/kg bw/d)	0.036	DDD = PEC _{fish} × 0.142
NOEL (mg/kg bw/d)	95.6	
TER _{it}	2 700	≥ 5; acceptable risk

JAU 6476-desthio

Here, the 21-day time weighted average surface water PEC (FOCUS Step 1) of prothioconazole metabolite JAU 6476-desthio, following a 1 x 150 g prothioconazole/ha application to cereals, was used.

Table 9.3-23: Assessment of the risk for fish-eating mammals due to exposure to JAU 6476-desthio via bioaccumulation in fish (secondary poisoning) for the intended uses

Parameter	JAU 6476-desthio	Comments
PEC _{sw} (twa = 21 d) (mg/L)	0.0255	FOCUS Step 1
BCF _{fish}	65	EFSA Conclusion 2007
BMF	-	biomagnification factor (relevant for BCF ≥ 2000)
PEC _{fish}	1.658	PEC _{fish} = PEC _{water} × BCF _{fish}
Daily dietary dose (mg/kg bw/d)	0.235	DDD = PEC _{fish} × 0.142
NOEL (mg/kg bw/d)	10	
TER _{it}	43	≥ 5; acceptable risk

JAU 6476 S-methyl

Here, the 21-day time weighted average surface water PEC (FOCUS Step 1) of prothioconazole metabolite JAU 6476 S-methyl, following a 1 x 150 g prothioconazole/ha application to cereals, was used.

Due to the low predicted environmental concentrations of the prothioconazole metabolite JAU 6476-S-methyl in surface water, no fish-BCF-study was conducted even though this metabolite has a log P_{ow} of 4.19. The BCF for this metabolite was estimated from its log P_{ow} and the BCF and the log P_{ow} of the parent compound with a sufficient degree of precision. A worst-case BCF of 1995 for JAU 6476-S-methyl in fish (see Prothioconazole RAR B9 (2018) for further details) is used in the calculation for secondary poisoning.

Table 9.3-24: Assessment of the risk for fish-eating mammals due to exposure to JAU 6476-S-methyl via bioaccumulation in fish (secondary poisoning) for the intended uses

Parameter	JAU 6476-S-methyl	Comments
PEC _{sw} (twa = 21 d) (mg/L)	0.00273	FOCUS Step 1
BCF _{fish}	1995	Worst-case BCF prediction as per QSAR model
BMF	-	biomagnification factor (relevant for BCF ≥ 2000)
PEC _{fish}	5.45	PEC _{fish} = PEC _{water} × BCF _{fish}
Daily dietary dose (mg/kg bw/d)	0.774	DDD = PEC _{fish} × 0.142

NOEL (mg/kg bw/d)	9.56	No toxicity data available, therefore metabolite assumed to be ten times more toxic than the parent substance, as a worst-case approach
TER _{lt}	12	≥ 5; acceptable risk

9.3.2.5 Biomagnification in terrestrial food chains

Not relevant.

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

Not relevant.

9.3.4 Overall conclusions

The acute and long-term risks of A23282A to mammals were assessed from toxicity exposure ratios between toxicity endpoints, estimated from studies with cyprodinil, prothioconazole, and its metabolite JAU 6476-desthio, and maximum residues occurring on food items following applications according to the proposed use pattern.

For cyprodinil, prothioconazole and the metabolite JAU 6476-desthio, acute and long-term TER values at either screening or Tier 1, all exceed the trigger values of 10 for acute risk and 5 for long-term risk.

For the mixture toxicity assessment, the acute and long-term risk to mammals from proposed uses of A23282A is considered acceptable when consideration is given to refined crop interception values.

Risk of secondary poisoning has also been assessed, as cyprodinil, prothioconazole, and prothioconazole metabolites JAU 6476-desthio and JAU 6474 S-methyl all have log P_{ow} values >3.0. The risk to mammals from exposure via drinking water has also been assessed. All assessments indicate that the risk to mammals is acceptable following use of A23282A according to the proposed use pattern.

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

There is currently no guidance addressing terrestrial life stages of amphibians and reptiles in PPP risk assessments. Therefore, the risk assessment provided above for birds and mammals is considered to be protective of terrestrial amphibian and reptile species.

9.5 Effects on aquatic organisms (KCP 10.2)

zRMS Comments:	<p>The submitted information and justification were accepted.</p> <p>The following application pattern was taken into consideration:</p> <ul style="list-style-type: none"> • Winter cereals at early (BBCH 30) and late (BBCH 69) applications at 1 x 450 g cyprodinil/ha and 1 x 150 g prothioconazole/ha, • Spring cereals at early (BBCH 30) and late (BBCH 69) applications at 1 x 450 g cyprodinil/ha and 1 x 150 g prothioconazole/ha. <p>All relevant metabolites were taken into consideration.</p>
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	<p>All changes were provided in dRR in grey.</p> <p>Cyprodinil. The endpoints used for risk assessment were corrected for using in further risk assessment:</p> <ul style="list-style-type: none">fish long-term risk assessment, the NOEC = 83 µg/L;chronic risk to aquatic invertebrates, ETO-RAC = 0.50 µg/L; The assessment factor of 3 was used as it is in opinion of evaluator more relevant (please refer to guidance for risk assessment for aquatic organisms). <p>The risk assessment using ERO-RAC was not evaluated (marked on grey). The risk assessment considering the relevant endpoints was corrected in relevant tables.</p> <p>The HC₅ value was corrected: HC₅ = 15.4 µg/L and RAC value based on it is of 3.85 µg/L.</p> <p>The relevant metabolites CGA249287 and CGA275535 were taken into consideration. Metabolite CGA321915 was not taken into consideration as no endpoint was agreed at the EU level. New submitted studies should be evaluated during active substance renewal.</p> <p>The mitigation measures were proposed in risk assessment.</p> <p>Prothioconazole. The endpoints used for risk assessment were agreed at the EU level. The risk assessment for aquatic organisms indicate an acceptable risk from acute and chronic exposure to the active substance prothioconazole and its metabolites prothioconazole-S-methyl and 1,2,4-triazole. The TER-values were well above the trigger of 100 for acute exposure and the trigger of 10 for long-term exposure. The risk assessment for acute exposure to the metabolite prothioconazole-desthio also indicated an acceptable risk to aquatic organisms but a long-term risk for fish was identified and mitigation measures were proposed. The proper mitigation measures (based on prothioconazole-desthio risk assessment) should be considered at MS level in accordance with the national requirements.</p> <p>Formulation toxicity. The lower endpoint for algae was used in risk assessment as a worse case. The 72 h E_yC₅₀ = 5.24 mg/L is 3–times lower than recommended 72 h E_rC₅₀ = 16.9 mg/L.</p> <p>Mixture toxicity. The risk assessment was corrected in accordance with recalculated ETO-RAC value for chronic toxicity of 0.50 µg/L. Additionally the prothioconazole metabolite JAU 6476-desthio was also taken into consideration in mixture toxicity assessment. Based on mixture toxicity assessment the mitigation measures for winter and spring cereals were proposed:</p> <ul style="list-style-type: none">winter cereals: 20 m SD + 20 m RO (R3 and R4 scenarios);spring cereals: 20 m SD + 20 m RO (R4 scenario.) <p>The proper mitigation measures should be considered at MS level in accordance with the national requirements.</p> <p>For Poland, considering the national requirements, the following mitigation measures are proposed:</p> <table><tr><th>Crop/Application pattern</th><th>Vegetative strip (m)</th><th>No spray buffer (m)</th><th>Scenario</th><th>Notes</th></tr><tr><td>Winter cereals</td><td>10</td><td>10</td><td>R1 stream</td><td>Based on mixture toxicity assessment</td></tr></table>	Crop/Application pattern	Vegetative strip (m)	No spray buffer (m)	Scenario	Notes	Winter cereals	10	10	R1 stream	Based on mixture toxicity assessment
Crop/Application pattern	Vegetative strip (m)	No spray buffer (m)	Scenario	Notes							
Winter cereals	10	10	R1 stream	Based on mixture toxicity assessment							

		Spring cereals	10	10	R1 stream*	Based on mixture toxicity assessment
* in case of spring cereals the R1 scenario from winter one was used.						
An acceptable risk to aquatic organisms is expected if the application of the A23282A is in accordance with proposed pattern use and relevant mitigation measures are applied.						

9.5.1 Toxicity data

Studies on the toxicity to aquatic organisms have been carried out with cyprodinil, prothioconazole and their relevant metabolites. Full details of these studies are provided in the respective EU DARs and related documents.

Effects of A23282A on aquatic organisms were not evaluated as part of the EU assessment of cyprodinil and prothioconazole. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

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

Species	Substance	Exposure System	Results	Reference
Rainbow trout (<i>Oncorhynchus mykiss</i>)	Cyprodinil	96 h, s	96 h LC ₅₀ = 2.41 mg/L (mm)	EFSA Scientific Report (2005) 51, 1-78 Boeri <i>et al.</i> (1995); Report No. 543-CG; CGA219417/0486; VV-369970
Bluegill sunfish (<i>Lepomis macrochirus</i>)	Cyprodinil	96 h, s	96 h LC₅₀ = 2.17 mg/L (mm)	EFSA Scientific Report (2005) 51, 1-78 Boeri <i>et al.</i> (1995); Report No. 542-CG; CGA219417/0507; VV-370642
Bluegill sunfish (<i>Lepomis macrochirus</i>)	Cyprodinil	96 h, f	96 h LC ₅₀ = 3.2 mg/L (mm)	EFSA Scientific Report (2005) 51, 1-78 Ward <i>et al.</i> (1995); Report No. 842-CG; CGA219417/0651; VV-370643
<i>Oncorhynchus mykiss</i>	CGA249287	96 h, s	96 h LC₅₀ = 55 mg/L (nom)	EFSA Scientific Report (2005) 51, 1-78 Maetzler (1999): Report No. 983825; CGA249287/0007; VV-311865

Species	Substance	Exposure System	Results	Reference
<i>Oncorhynchus mykiss</i>	CGA275535	96 h, s	96 h LC₅₀ = 2.1 mg/L (mm)	EFSA Scientific Report (2005) 51, 1-78 Pfeifle (2001): Report No. L01-000310; CGA275535/0017; VV-311866
Rainbow trout (<i>Oncorhynchus mykiss</i>)	Cyprodinil	Chronic, f	NOEC = 0.083 mg/L (mm) ^a	EFSA Scientific Report (2005) 51, 1-78 Vial (1991); Report No. 901366; CGA219417/0009; VV-356142
Fathead minnow (<i>Pimephales promelas</i>)	Cyprodinil	Chronic, f	NOEC = 0.231 mg/L (mm)	EFSA Scientific Report (2005) 51, 1-78 Ward <i>et al.</i> (1995); Report No. 846-CG; CGA219417/0653; VV-370646
<i>Daphnia magna</i>	Cyprodinil	48 h, s	48 h EC ₅₀ = 0.033 mg/L (mm)	EFSA Scientific Report (2005) 51, 1-78 Boeri <i>et al.</i> (1995), Report No. 467-CG; CGA219417/0461; VV-370648
<i>Daphnia longispina</i>	Cyprodinil	48 h, s	48 h EC ₅₀ = 0.22 mg/L (mm)	EFSA Scientific Report (2005) 51, 1-78 Peither (2000); Report No. 777666; CGA219417/0993; VV-311859
<i>Daphniopsis sp.</i>	Cyprodinil	24 h, s	24 h EC ₅₀ = 0.21 mg/L (mm)	EFSA Scientific Report (2005) 51, 1-78 Peither (2000); Report No. 777824; CGA219417/0990; VV-311856
<i>Simocephalus vetulus</i>	Cyprodinil	48 h, s	48 h EC ₅₀ = 0.15 mg/L (mm)	EFSA Scientific Report (2005) 51, 1-78 Peither (2000); Report No. 777644; CGA219417/0994; VV-311860
<i>Gammarus sp.</i>	Cyprodinil	48 h, s	48 h EC ₅₀ = 1.8 mg/L (mm)	EFSA Scientific Report (2005) 51, 1-78 Peither (2000); Report No. 777645; CGA219417/0998; VV-311559

Species	Substance	Exposure System	Results	Reference
<i>Thamnocephalus platyurus</i>	Cyprodinil	24 h, s	24 h EC ₅₀ = 0.12 mg/L (mm)	EFSA Scientific Report (2005) 51, 1-78 Peither (2000); Report No. 777780; CGA219417/0991; VV-311857
<i>Ostracoda sp.</i>	Cyprodinil	48 h, s	48 h EC ₅₀ = 1.1 mg/L (mm)	EFSA Scientific Report (2005) 51, 1-78; Peither (2000); Report No. 777688; CGA 249417/0995; VV-311927
<i>Brachionus calyciflorus</i>	Cyprodinil	24 h, s	24 h EC ₅₀ = >9.5 mg/L (mm)	EFSA Scientific Report (2005) 51, 1-78 Peither (2000); Report No. 777767; CGA219417/0992; VV-311858
<i>Cloeon sp.</i>	Cyprodinil	48 h, s	48 h EC ₅₀ = 3.5 mg/L (mm)	EFSA Scientific Report (2005) 51, 1-78 Peither (2000); Report No. 777723; CGA219417/0996; VV-311861
<i>Chaoborus sp.</i>	Cyprodinil	48 h, s	48 h EC ₅₀ = 4.0 mg/L (mm)	EFSA Scientific Report (2005) 51, 1-78 Peither (2000); Report No. 777701; CGA219417/0999; VV-311863
Bay shrimp (<i>Mysidopsis bahia</i>)	Cyprodinil	96 h, f	96 h LC ₅₀ = 0.00805 mg/L (mm)	Ward et al. (1995); Report No. 827-CG; CGA219417/0649; VV-372679
<i>Asellus aquaticus</i> (nymphs)	Cyprodinil	96 h, s	96 h EC ₅₀ = 2.64 mg/L (nom) 48 h EC ₅₀ = 2.35 mg/L (mm)	Maynard (2011); Report No. CEMR-5069; CGA219417_11453; VV-397982
<i>Lymnea stagnalis</i>	Cyprodinil	48 h, s	48 h EC ₅₀ = 2.9 mg/L (mm)	EFSA Scientific Report (2005) 51, 1-78 Peither (2000); Report No. 777802; CGA219417/0997; VV-311862
Invertebrate Acute HC ₅	Cyprodinil	HC ₅ derived from an SSD of the 13 acute invertebrate endpoints	HC ₅ = 0.019 ° 0.0154 mg/L. AF = 4, hence RAC = 0.00475 0.00385 mg/L	Refer Section 9.5.1.1

Species	Substance	Exposure System	Results	Reference
<i>Daphnia magna</i>	CGA249287	48 h, s	48 h EC₅₀ = >100 mg/L (nom)	EFSA Scientific Report (2005) 51, 1-78 Maetzler (1999); Report No. G57114; CGA249287/0008; VV-311510
<i>Daphnia magna</i>	CGA321915 ^b	48 h, s	48 h EC₅₀ = >98 mg/L (mm)	Eckenstein (2015): Report No. D96733; CGA321915_10005; VV-411573
<i>Daphnia magna</i>	CGA275535	48 h, s	48 h EC₅₀ = 6.8 mg/L (nom)	EFSA Scientific Report (2005) 51, 1-78 Maetzler (2001); Report No. G65514; CGA275535/0016; VV-311619
<i>Mysidopsis bahia</i>	Cyprodinil	30 d, f	EC _{10 repro} = 0.00197 mg/L (mm)	Drott & Krueger (1999); Report No. 108A-205; CGA219417/0926 ; VV-311558
<i>Daphnia magna</i>	Cyprodinil	21 d, NOEC	NOEC _{repro} = 0.0082 mg/L	EFSA Scientific Report (2005) 51, 1-78, Ward et al., (1995); Report No. 468-CG; CGA219417/0543 ; VV-369966
		Statistical re-analysis	EC _{10 repro} = 0.00849 mg/L EC _{20 repro} = 0.00942 mg/L EC _{10 adult mortality} = 0.01671 mg/L EC _{20 adult mortality} = 0.01928 mg/L	Taylor & Sanchez (2015); Report No. CEA.1415 ; VV-28892
Invertebrates (<i>Daphnia magna</i> , neonates and adults)	Cyprodinil	21 d semi-static exposure + 21 d clearance for F1	NOEC adults = 0.0088 mg/L NOEC neonates = 0.0018 mg/L EC ₁₀ neonates = 0.0073 mg/L	EFSA Scientific Report (2005) 51, 1-78, Rufli (1998); Report No. 973606; CGA219417/0876; VV-372284
<i>Chironomus riparius</i>	Cyprodinil	27 d, spiked sediment	27 d NOEC = 80 mg/kg sediment (nom)	EFSA Scientific Report (2005) 51, 1-78 Grade (2000); Report No. 2003518; CGA249217/1003; VV-312265

Species	Substance	Exposure System	Results	Reference
<i>Chironomus riparius</i>	CGA249287	28 d, spiked sediment	28 d NOEC = 25.6 mg/kg sediment (nom)	EFSA Scientific Report (2005) 51, 1-78 Grade (2001); Report No. 2003768; CGA249287/0024; VV-323854
<i>Pseudokirchneriella subcapitata</i>	Cyprodinil	72 h, s	72 h E_rC_{50} = 3.28 mg/L (mm) 72 h EC_{50} = 2.6 mg/L	EFSA Scientific Report (2005) 51, 1-78 Ward <i>et al.</i> (1995); Report No. 791-CG; CGA219417/0648; VV-370655 & Taylor S., Pickering F. & Allen (2016), Report No. CEA.1424 CGA219417_11593; VV-28887
<i>Pseudokirchneriella subcapitata</i>	Cyprodinil	72 h, s	72 h E_rC_{50} = 5.2 mg/L (nom) 72 h E_bC_{50} = 2.6 mg/L	EFSA Scientific Report (2005) 51, 1-78 Maetzler (2001); Report No. 791-CG; CGA219417/1030; VV-319292 & Taylor S. & Allen M. (2016); Report No. CEA.1423 CGA219417_11598; VV-28896
<i>Anabaena flos-aquae</i>	Cyprodinil	72 h, s	96 h E_rC_{50} = 7.55 mg/L (mm) 72 h EC_{50} = 3.76 mg/L	EFSA Scientific Report (2005) 51, 1-78 Ward <i>et al.</i> (1995); Report No. 793-CG; CGA219417/0647; VV-370650
<i>Navicula pelliculosa</i>	Cyprodinil	72 h, s	72 h E_rC_{50} = 3.24 mg/L (mm) 72 h E_bC_{50} = 2.11 mg/L	EFSA Scientific Report (2005) 51, 1-78 Ward <i>et al.</i> (1995); Report No. 794-CG; CGA219417/0646; VV-370652
<i>Pseudokirchneriella subcapitata</i>	CGA249287	72 h, s	72 h E_rC_{50} = >100 mg/L (nom)	EFSA Scientific Report (2005) 51, 1-78 Maetzler (1999); Report No. 794-CG; CGA249287/0006; VV-311509 & Taylor S., Allen M. (2015); Report No. CEA.1428 CGA249287_10009; VV-28890

Species	Substance	Exposure System	Results	Reference
<i>Pseudokirchneriella subcapitata</i>	CGA275535	72 h, s	72 h ErC₅₀ = 18 mg/L (mm)	EFSA Scientific Report (2005) 51, 1-78 Maetzler (2001); Report No. G65517; CGA275535/0015; VV-311620 & Taylor S., Allen M. (2015); Report No CEA.1429 CGA275535_10006; VV-28891*
<i>Pseudokirchneriella subcapitata</i>	CGA321915 ^b	72 h, s	72 h ErC₅₀ = >99 mg/L (mm)	Eckenstein (2015); Report No. D96711; CGA321915_10004 ; VV-411271
<i>Lemna gibba</i>	Cyprodinil	72 h, ss	72 h E_yC₅₀ = 7.42 mg/L (im)	EFSA Scientific Report (2005) 51, 1-78 ; Ward <i>et al.</i> (1995); Report No. 792-CG; CGA219417/0645; VV-373819
Higher-tier studies (micro- or mesocosm studies)				
Invertebrates + fish	Cyprodinil	Microcosm	NOEC/NOEAEC = 0.013 mg/L	EFSA Scientific Report (2005) 51, 1-78 Kennedy and Reed, (1995), Report No. CMP4
Invertebrates	A14325E	Microcosm	NOAEAC = 10 µg a.s./L _{nom} , ERO = 3.33 µg a.s./L (AF = 3) NOEC = 1.5 µg a.s./L _{nom} , ETO = 0.75 µg a.s./L (AF=2) ETO = 0.50 µg a.s./L (AF = 3)	Ashwell <i>et al.</i> (2007); Report No. T008777-05-REG; CGA219417/1683; VV-339018 Statistical Re-analysis: Taylor S., Dark R. (2015), Report No CEA.1464; A14325E_10079 / VV-889899

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

* it is no longer appropriate to use the endpoint derived from a fish long term in the chronic risk assessment, therefore the early life stage value of 0.231 mg/L will be used

^b Metabolite CGA 321915 was not considered during EU review, but is proposed for the submission according to Regulation (EC) No. 283/2013 (present at >5% at two consecutive timepoints - Strassenacker soil, Schaffer, 1994)

^c HC₅ derived from an SSD of all 13 invertebrate endpoints see 9.5.1.1

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

Species	Substance	Exposure System	Results	Reference
<i>Oncorhynchus mykiss</i>	Prothioconazole	96 h, ss	LC₅₀ = 1 830 µg a.s./L_{mm}	EFSA Conclusion 2007

Species	Substance	Exposure System	Results	Reference
<i>Cyprinus carpio</i>	Prothioconazole	96 h	LC ₅₀ = 6 910 µg a.s./L	EFSA Conclusion 2007
<i>Lepomis macrochirus</i>	Prothioconazole	96 h	LC ₅₀ = 4 590 µg a.s./L	EFSA Conclusion 2007
<i>Oncorhynchus mykiss</i>	JAU 6476-desthio	96 h, s	LC ₅₀ = 6 630 µg/L nom	EFSA Conclusion 2007
<i>Leucisus idus melanotus</i>	JAU 6476-desthio	96 h	LC ₅₀ = 13 200 µg a.s./L	EFSA Conclusion 2007
<i>Oncorhynchus mykiss</i>	JAU 6476-S-methyl	96 h, ss	LC ₅₀ = 1 790 µg/L mm	EFSA Conclusion 2007
<i>Oncorhynchus mykiss</i>	1,2,4-Triazole	96 h, s	LC ₅₀ = 498 000 µg/L nom	EFSA Conclusion 2007
<i>Oncorhynchus mykiss</i>	Prothioconazole	97 d, f	NOEC = 308 µg a.s./L mm	EFSA Conclusion 2007
<i>Oncorhynchus mykiss</i>	JAU 6476-desthio	96 d, f	NOEC = 3.34 µg/L mm	EFSA Conclusion 2007
<i>Oncorhynchus mykiss</i>	1,2,4-Triazole	96 d, ss	NOEC = 3 200 µg/L nom	EFSA Conclusion 2007
<i>Daphnia magna</i>	Prothioconazole	48 h, s	EC ₅₀ = 1 300 µg a.s./L nom	EFSA Conclusion 2007
<i>Daphnia magna</i>	JAU 6476-desthio	48 h, s	EC ₅₀ > 10 000 µg/L nom	EFSA Conclusion 2007
<i>Daphnia magna</i>	JAU 6476-S-methyl	48 h, s	EC ₅₀ = 2 800 µg/L nom	EFSA Conclusion 2007
<i>Daphnia magna</i>	1,2,4-Triazole	48 h, s	EC ₅₀ = 900 mg/L	EFSA Conclusion 2007
<i>Americamysis bahia</i>	JAU 6476-desthio	96 h, f	LC ₅₀ = 60 µg/L mm	Drott et al, 2002 ^a Document No: M-083055-01-1
<i>Americamysis bahia</i>	JAU 6476-desthio	96 h, f	LC ₅₀ > 1 009 µg/L nom	Blankinship A.S., Kendall T.Z., Krueger H.O., 2003 ^a Document No: M-104620-01-1
<i>Americamysis bahia</i>	JAU 6476-desthio	96 h, f	Geomean LC ₅₀ = 246 µg/L	Refer Section 9.5.1.1
<i>Daphnia magna</i>	Prothioconazole	21 d, ss	NOEC = 560 µg a.s./L nom	EFSA Conclusion 2007
<i>Daphnia magna</i>	JAU 6476-desthio	21 d, ss	NOEC = 100 µg/L nom	EFSA Conclusion 2007
<i>Americamysis bahia</i>	JAU 6476-desthio	29 d, ss	NOEC = 64 µg/L nom	Blankinship A.S., Kendall T.Z., Krueger H.O., 2003 ^a Document No: M-104620-01-1

Species	Substance	Exposure System	Results	Reference
<i>Chironomus riparius</i> (spiked water)	Prothioconazole	28 d, s	NOEC = 9 140 µg a.s./L_{im}	EFSA Conclusion 2007
<i>Chironomus riparius</i> (spiked water)	JAU 6476-desthio	28 d, s	NOEC = 2 000 µg/L_{nom}	EFSA Conclusion 2007
<i>Chironomus riparius</i> (spiked water)	JAU 6476-S-methyl	28 d, s	NOEC = 100 µg/L_{nom}	Bruns E., 2006 ^a Document No: M-266605-01-1
<i>Pseudokirchneriella subcapitata</i>	Prothioconazole	72 h, s	E _b C ₅₀ = 1 100 µg a.s./L _{im} E _r C ₅₀ = 2 180 µg a.s./L _{im}	EFSA Conclusion 2007
<i>Skeletonema costatum</i>	Prothioconazole	96 h, s	E_rC₅₀ = 46 µg a.s./L_{nom}	Kern M.E., De Haan R.A., 2004 ^a Document No: M-000954-01-1
<i>Pseudokirchneriella subcapitata</i>	JAU 6476-desthio	72 h, s	E _b C ₅₀ = 73 µg/L _{im} E_rC₅₀ = 550 µg/L_{im}	EFSA Conclusion 2007
<i>Pseudokirchneriella subcapitata</i>	JAU 6476-S-methyl	72 h, s	E _b C ₅₀ = 3 770 µg/L _{im} E_rC₅₀ = 47 400 µg/L_{im}	EFSA Conclusion 2007
<i>Pseudokirchneriella subcapitata</i>	1,2,4-Triazole	72 h, s	E _b C ₅₀ = 8 200 µg/L _{nom} E_rC₅₀ = 22 500 µg/L_{nom}	EFSA Conclusion 2007
<i>Lemna gibba</i>	Prothioconazole	7 d, ss	E_rC₅₀ > 404 µg a.s./L_{mm}	Kern M. E., Banman C. S., Lam C. V., 2004 ^a Document No: M-000532-01-1,
<i>Lemna gibba</i>	JAU 6476-desthio	7 d, ss	E_rC₅₀ = 80.9 µg/L_{mm}	Kern M. E., Banman C. S., Lam C. V., 2003 ^a Document No: M-104599-01-1
Higher-tier studies (micro- or mesocosm studies)				
Not required				

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

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

^aEndpoints not included in EFSA Journal, (2007) 106, 1-98.

Table 9.5-3: Endpoints and effect values relevant for the risk assessment for aquatic organisms – A23282A

Species	Substance	Exposure system	Results	Reference
<i>Daphnia magna</i>	A23282A	48 h, s	EC₅₀ = 0.223 mg/L_{nom}	Schuler, L. 2021; Report No. S21-05725

Species	Substance	Exposure system	Results	Reference
				VV-931771
<i>Raphidocelis subcapitata</i> (= <i>Pseudokirchneriella subcapitata</i>)	A23282A	96 h, s	72 h E _r C ₁₀ = 5.84 mg/L _{mm} 72 h E _y C ₁₀ = n.d. mg/L _{mm} ^a 72 h E _r C ₂₀ = 8.93 mg/L _{mm} 72 h E _y C ₂₀ = 1.59 mg/L _{mm} 72 h E_rC₅₀ = 16.9 mg/L_{mm} 72 h E _y C ₅₀ = 5.24 mg/L _{mm}	Schuler, L. 2021; Report No. S21-05724 VV-931772

s: static; nom: based on nominal concentrations; mm: based on mean measured concentrations

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

^aInhibition at all test item concentrations was above 10 %, therefore the EC₁₀ could not be determined

9.5.1.1 Justification for new endpoints

New studies are available for formulation A23282A which are required to fulfil the data requirements for plant protection products in accordance with Regulation (EC) No 1107/2009. The endpoints are summarised in Table 9.5-3.

Cyprodinil

Refinement of the acute risk to aquatic invertebrates

Given that the RAC for aquatic invertebrates represents the lowest endpoint for the acute risk assessment, refinement of the risk to this group will be protective of acute toxicity to other groups.

The lowest acute invertebrate endpoint for cyprodinil is based on a 96-hour LC₅₀ of 8.05 µg a.s./L for *Mysidopsis bahia*. This value is the lowest endpoint generated from tests with 12-other species, where EC₅₀ values range between 0.033 and 9.5 mg a.s./L.

For convenience, the list of endpoints for acute invertebrates is presented in the table below.

Table 9.5-4: Acute cyprodinil toxicity endpoints for aquatic invertebrates, for probabilistic risk assessment

Test organism	Taxonomy		EC/LC ₅₀ (mg a.s./L)	Reference
	Subphylum	Order		
<i>Mysidopsis bahia</i>	Crustacean	Mysida	0.00805	<i>Ward (1995)</i>
<i>Daphnia magna</i>	Crustacean	Cladocera	0.033	<i>Boeri et al (1995)</i>
<i>Thamnocephalus platyurus</i>	Crustacean	Anostraca	0.12	<i>Peither (2000)</i>
<i>Simocephalus vetulus</i>	Crustacean	Anomopoda	0.15	<i>Peither (2000)</i>
<i>Daphniopsis sp.</i>	Crustacean	Cladocera	0.21	<i>Peither (2000)</i>
<i>Daphnia longispina</i>	Crustacean	Cladocera	0.22	<i>Peither (2000)</i>
<i>Ostracoda sp.</i>	Crustacean	Podocopa	1.1	<i>Peither (2000)</i>
<i>Gammarus sp.</i>	Crustacean	Amphipoda	1.8	<i>Peither (2000)</i>
<i>Asellus aquaticus (nymphs)</i>	Crustacean	Isopoda	2.35	<i>Maynard (2011)</i>
<i>Lymnea stagnalis</i>	Mollusca	Hygrophila	2.9	<i>Peither (2000)</i>
<i>Cloeon sp.</i>	Hexapoda	Ephemeroptera	3.5	<i>Peither (2000)</i>
<i>Chaoborus sp.</i>	Hexapoda	Diptera	4.0	<i>Peither (2000)</i>
<i>Brachionus calyciflorus</i>	Rotifera	Ploima	>9.5	<i>Peither (2000)</i>

As discussed in the **aquatic guidance document**, when considering the quality of acute toxicity data used to construct the SSD:

‘If the toxicity data comprise several different genera/families/orders of the potentially sensitive taxonomic group (see section 8.4.3 for further guidance), including Ephemeroptera/Plecoptera/Trichoptera taxa (EPT) for insecticides, a lower AF in the proposed range may be selected. However, if another valid SSD can be constructed with a more limited dataset containing the most sensitive species, and the HC₅ derived from this SSD curve is lower than that of the SSD curve using toxicity data for a wider array of taxa, a higher AF in the proposed range may be selected to be applied to the SSD from the wider set.’

Given the number of endpoints that are available, a species sensitivity distribution has been constructed using the program Mosaic, 2017³. The SSD distribution is presented in Figure 9.5-1.

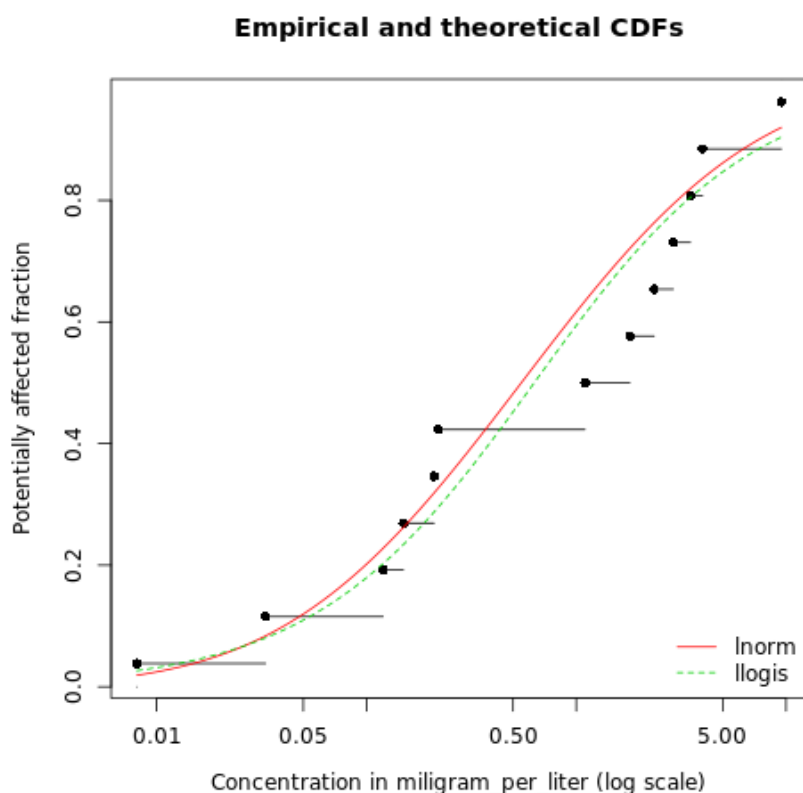


Figure 9.5-1: Species sensitivity distribution (SSD) for acute exposure of invertebrates to cyprodinil

The resulting median HC₅ value is **19 15.4** µg a.s./L (95% CI: 3.9 – 120 µg a.s./L).

According to the aquatic guidance document an assessment factor (AF) of 3 – 6 is recommended for this type of data. Several aspects need to be considered when selecting an appropriate AF from an SSD distribution. For ease of reference these are directly quoted below:

1. *The quality of the acute toxicity data used to construct the SSD.* If the toxicity data comprise several different genera/families/orders of the potentially sensitive taxonomic group (see section 8.4.3 for further guidance), including Ephemeroptera/Plecoptera/Trichoptera taxa (EPT) for insecticides, a lower AF in the proposed range may be selected. However, if another valid SSD can be constructed

³ Charles S, Veber P, Delignette-Muller ML. 2017. MOSAIC: a web-interface for statistical analyses in ecotoxicology. Environ. Sci. Pollut. Res. <https://doi.org/10.1007/s11356-017-9809-4>.

with a more limited dataset containing the most sensitive species, and the HC_5 derived from this SSD curve is lower than that of the SSD curve using toxicity data for a wider array of taxa, a higher AF in the proposed range may be selected to be applied to the SSD from the wider set.

2. *The lower limit value of the HC_5 .* If the lower limit HC_5 derived from the curve is less than 1/3 of the median HC_5 , a higher AF in the proposed range may be warranted.
3. *The lower tier RACs on the basis of standard toxicity data (tier 1), standard and additional toxicity data (Geomean approach) and tier 3 data.* The size of the AF should ideally not result in an SSD- $RAC_{sw;ac}$ higher than the tier 3 RAC derived from effect class 1 and 2 of micro- mesocosm studies, nor should it result in an SSD- $RAC_{sw;ac}$ lower than the tier 1 $RAC_{sw;ac}$ on the basis of standard test species and/or the Geomean- $RAC_{sw;ac}$ and/or method 3 to 5 (EFSA, 2006a) on the basis of the same toxicity data that were used to construct the SSD. Note that according to EFSA (2006a), the Geomean approach aims to achieve the same average level of protection as in the tier 1 effect assessment but can be predicted more accurately because of the availability of additional toxicity data for the relevant taxonomic groups.
4. *The position of the toxicity data in the lower tail of the SSD (around the HC_5).* If in the lower tail the toxicity data, overall, are positioned on the right side of the SSD curve, the derived HC_5 estimate may be considered relatively “conservative” for the most sensitive species. This may be a reason to adopt a lower AF from the proposed range. In contrast, if in the lower tail the toxicity data are, overall, positioned on the left side of the SSD curve, this may be a reason to adopt a higher AF from the proposed range.
5. *The steepness of the SSD curve.* In the case of a relatively steep SSD curve (e.g. less than a factor of 100 between lowest and highest $L(E)C_{50}$ value used to construct the SSD curve), a higher AF from the proposed range is recommended since exposure concentrations that exceed the $RAC_{sw;ac}$ may have ecotoxicological consequences for a larger number of taxa.
6. *Considering information on chronic effects.* If acute to chronic ratio (acute EC_{50} /chronic EC_{10}) is larger than 10, then an AF in the higher range may be warranted.

It is proposed that an **AF of 4** is applied to the HC_5 of **19 15. 4** $\mu\text{g a.s./L}$, giving an SSD- $RAC_{sw;ac}$ of **4.75 3.85** $\mu\text{g a.s./L}$. Justification is provided below by considering the data set presented in Table 9.5-4 against the above aspects:

1. The most sensitive taxa have been used to construct the SSD and several different orders are represented – **therefore a lower assessment factor can be justified here.**
2. The lower limit of the HC_5 is less than 1/3 of the median HC_5 (lower limit is 3.9 $\mu\text{g/L}$ and the HC_5 is 19 15. 4 $\mu\text{g/L}$, i.e., approximately a fifth of the HC_5) - **therefore a higher assessment factor should be considered here.**
3. The size of the AF should ideally not result in an SSD- $RAC_{sw;ac}$ higher than the tier 3 RAC derived from effect class 1 and 2 of micro- mesocosm studies, nor should it result in an SSD- $RAC_{sw;ac}$ lower than the tier 1 $RAC_{sw;ac}$ on the basis of standard test species – **therefore a lower assessment factor can be justified here.**
4. In the lower tail, the toxicity data, overall, are positioned on the left side of the SSD curve - **therefore a higher assessment factor should be considered here.**
5. The SSD curve is relatively shallow in that there is greater than a factor of 100 between lowest and highest $L(E)C_{50}$ - **therefore a lower assessment factor can be justified here.**
6. The acute to chronic ratio for *Mysidopsis bahia* is 4 - **therefore a lower assessment factor can be justified here.**

In addition to these points, the test for normality was acceptable for all three tests (Anderson-Darling,

Kolmogorov-Smirnov and Cramer von Mises) for all significance levels.

PEC_{SW} values for all relevant cyprodinil application scenarios have been compared with the cyprodinil SSD-RAC_{SW;ac} of **4.75 3.85 µg a.s./L** for the invertebrate acute group. These are shown in the tables in section 9.5.2.

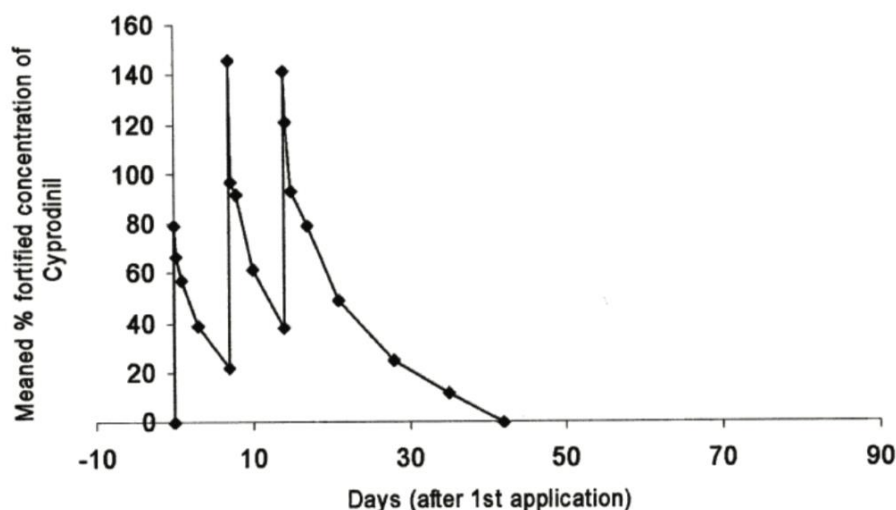
Refinement of the long-term risk to aquatic invertebrates (RAC_{SW;ch})

Following the EU review for cyprodinil, a new microcosm study was conducted with the cyprodinil 300 EC formulation A14325E (Ashwell *et al.* 2007; VV-339018). to a community typical for a lentic freshwater community, containing phyto- and zooplankton and macroinvertebrates. Previous lab studies had shown that microcrustaceans were expected to be the most sensitive group. A previous microcosm study (treatment levels: 1.3 – 7 µg a.s./L) with technical cyprodinil in 1995 reported only minor transient effects on some macroinvertebrates and zooplankton. This study differed from the Kennedy & Reed study by the inclusion of fish and is difficult to interpret and results are therefore considered inconclusive.

Intended initial concentrations in the present study (Ashwell *et al.* 2007; VV-339018) were 0 – 1.5 – 5 – 10 – 20 – 50 µg a.s./L. Immediately after each of the three applications the test compound was mixed in the water layer of the microcosms. Measurements in dosing solutions and water indicated that the test systems received the intended doses. Shortly after the applications 75-80%, 119-154% and 118-156% of the target amount was measured in the water of the test systems. In this evaluation report, the observed treatment-related responses are assigned to the nominal concentrations of the active substance cyprodinil to ensure a worst-case risk assessment. For the phytoplankton, zooplankton and macroinvertebrate community, the NOEC_{population} as well as the NOEC_{community} is 1.5 µg a.s./L, and the NOEAEC is 10 µg a.s./L.

Three applications were made to the mesocosm study conducted by Ashwell *et al.* 2007, hence this would represent an extreme worst case exposure profile in comparison to one application of 450 g cyprodinil/L for A23262A. The screenshot below represents an excerpt from the report for the nominal concentration of 10 µg cyprodinil/L.

Figure 3 Meaned measured concentrations of 10µg/L Microcosms



The figures below illustrate the exposure profiles for cyprodinil following application of A23282A in spring and winter cereals.

Figure 9.5-2: EPAT profile for 1 x 450 g/ha – Step 3, D3 Ditch, Spring Cereals

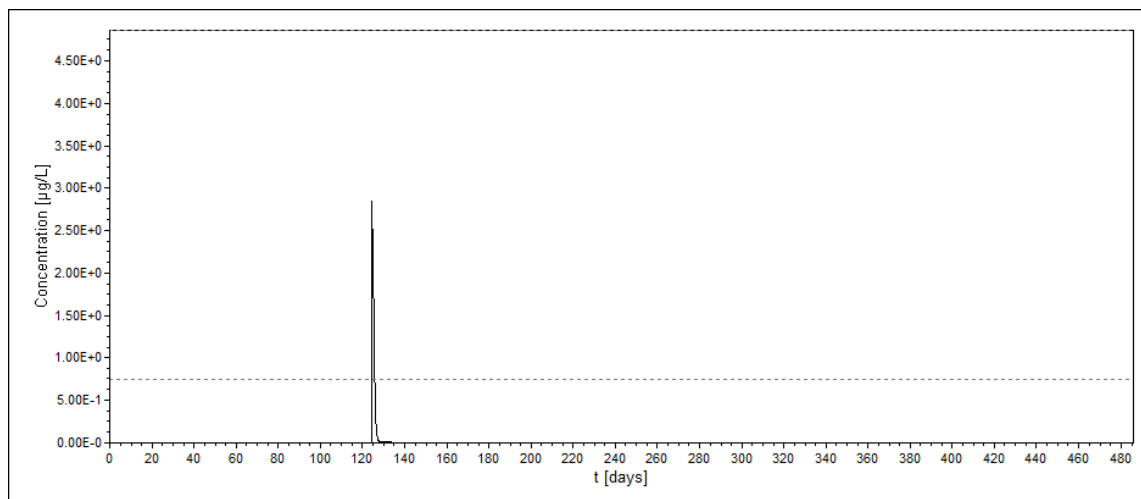


Figure 9.5-3: EPAT profile for 1 x 450 g/ha – Step 3, D4 Pond, Spring Cereals

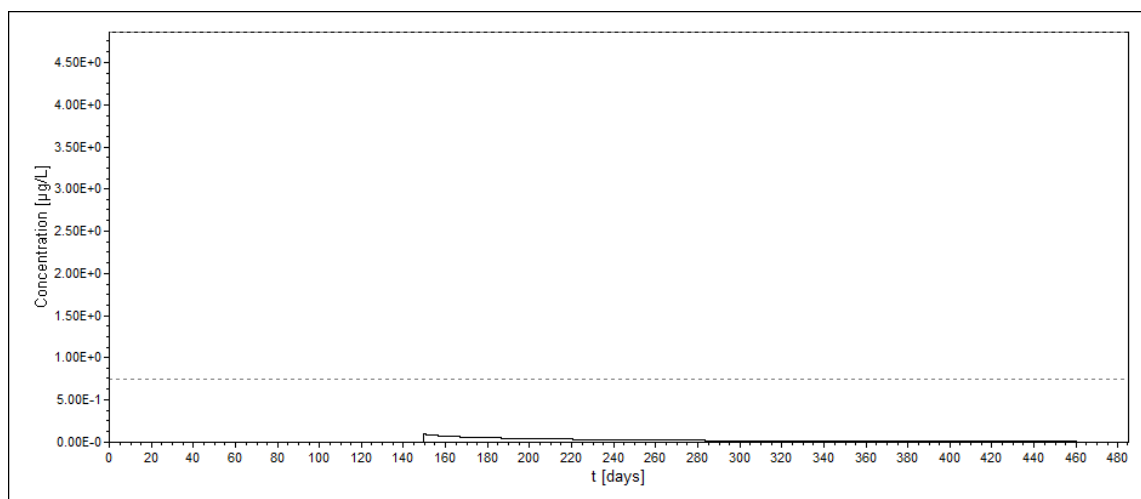


Figure 9.5-4: EPAT profile for 1 x 450 g/ha – Step 3, R4 Stream, Spring Cereals

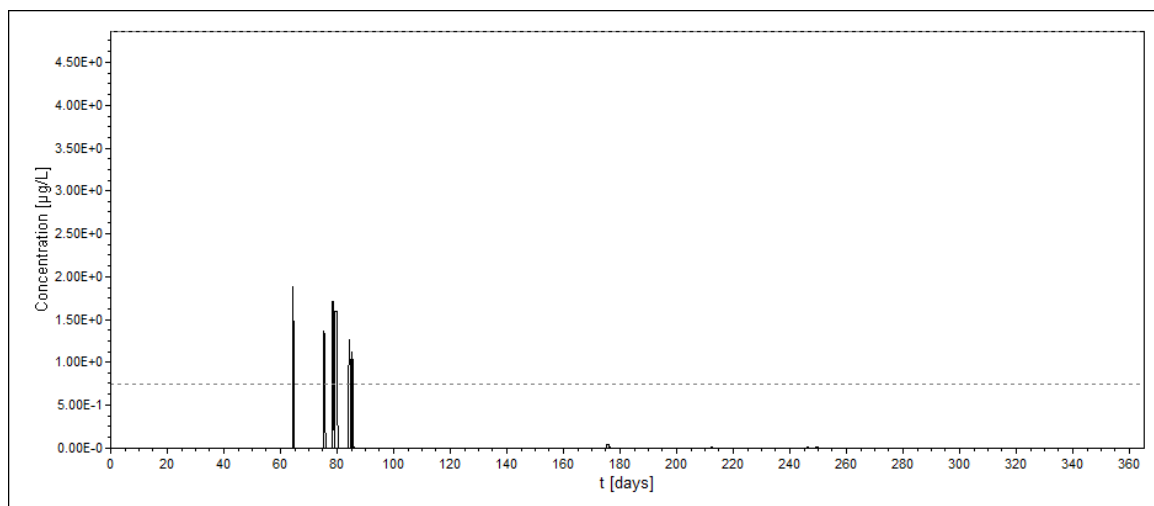


Figure 9.5-5: EPAT profile for 1 x 450 g/ha – Step 3, D2 Ditch, Winter Cereals

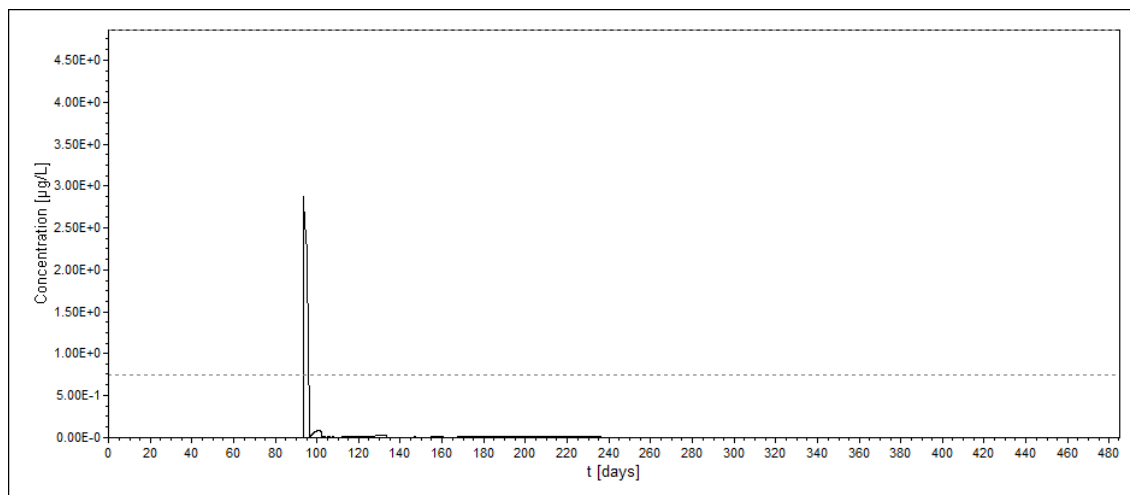


Figure 9.5-6: EPAT profile for 1 x 450 g/ha – Step 3, D2 Stream, Winter Cereals

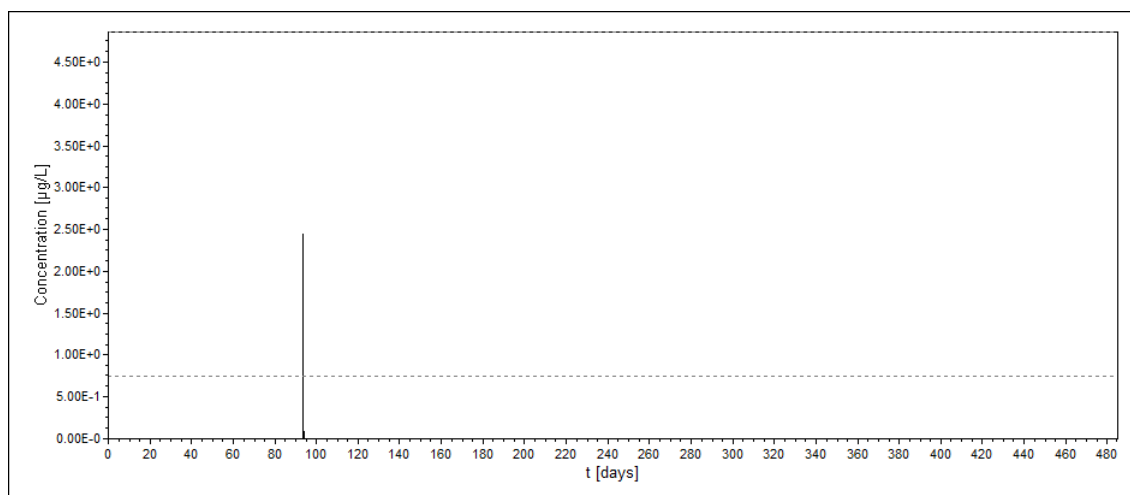


Figure 9.5-7: EPAT profile for 1 x 450 g/ha – Step 3, D5 Pond, Winter Cereals

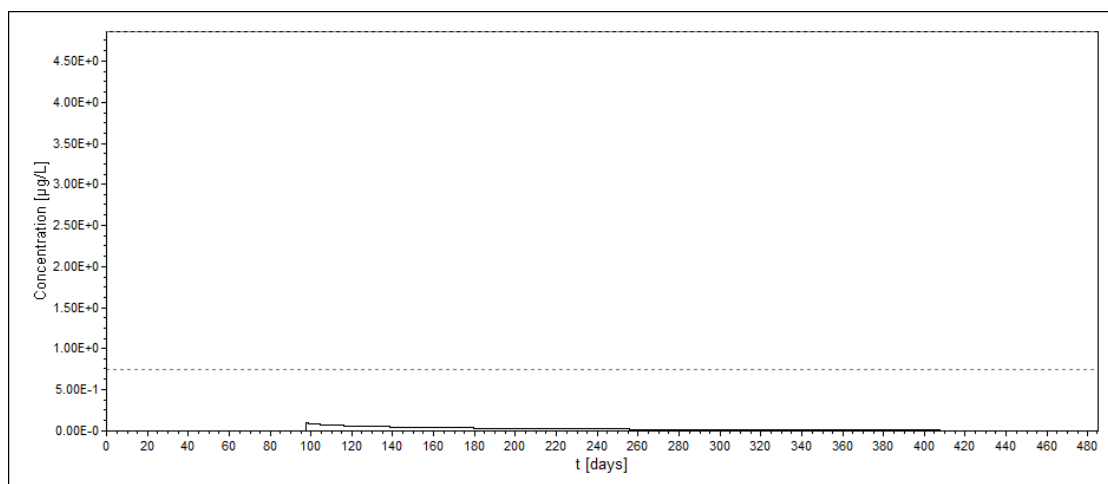


Figure 9.5-8: EPAT profile for 1 x 450 g/ha – Step 3, D5 Stream, Winter Cereals

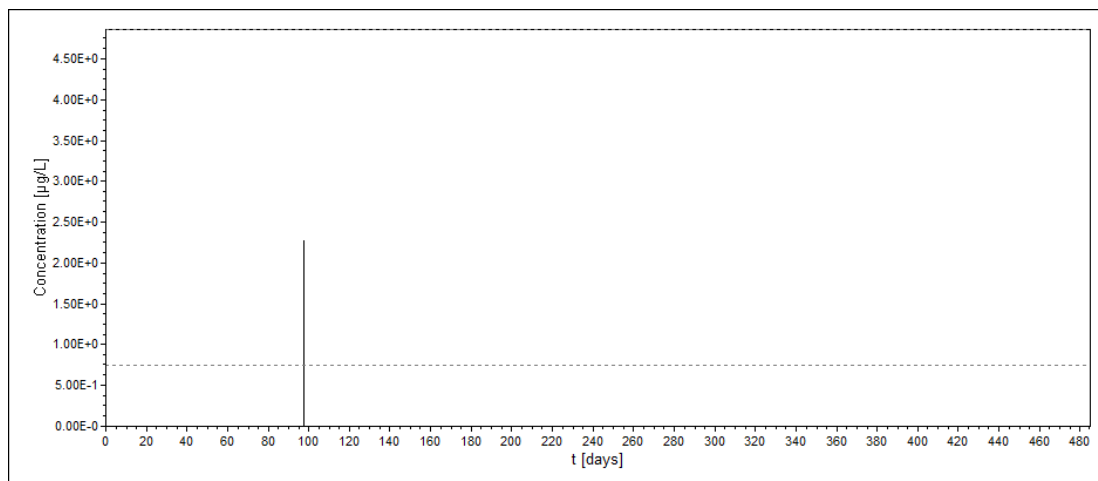


Figure 9.5-9: EPAT profile for 1 x 450 g/ha – Step 3, D6 Ditch, Winter Cereals

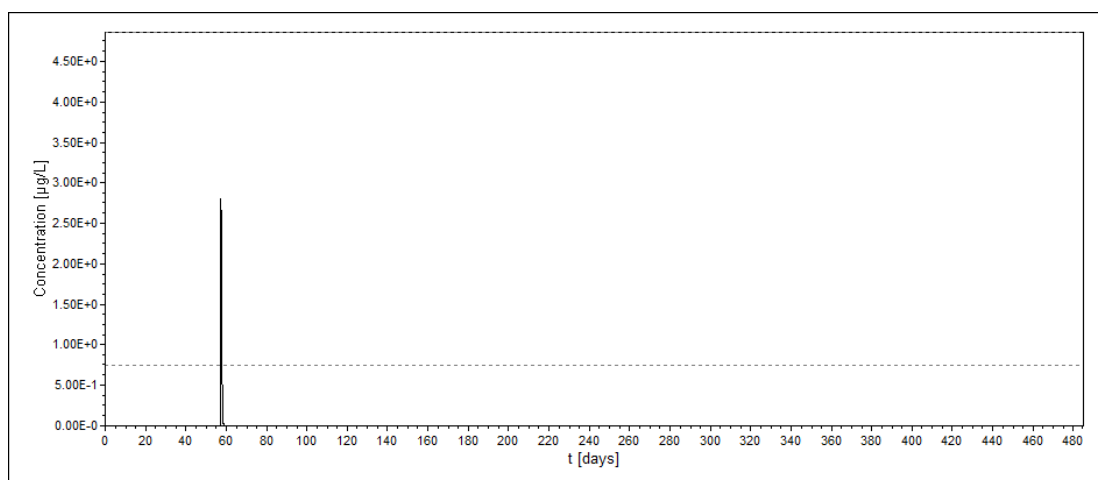


Figure 9.5-10: EPAT profile for 1 x 450 g/ha – Step 3, R1 Pond, Winter Cereals

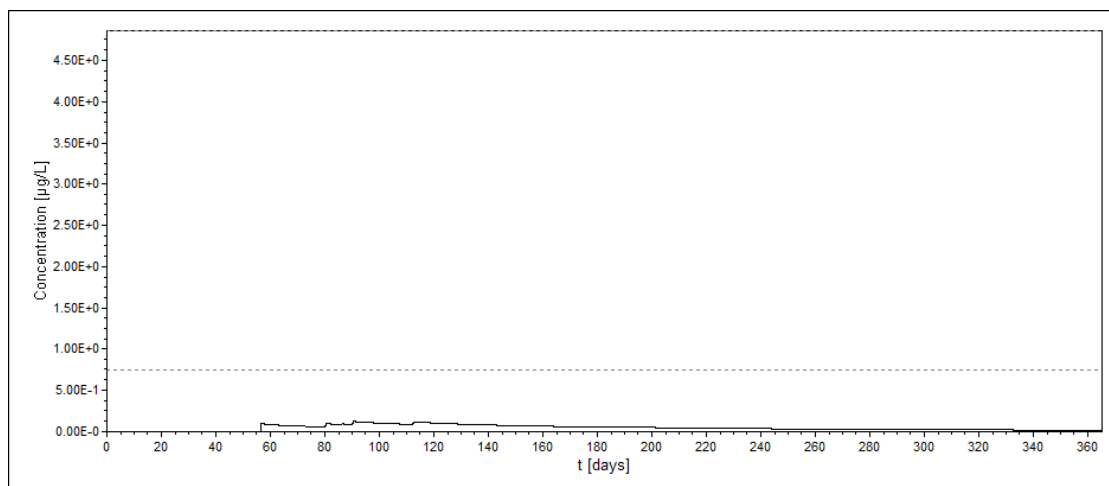


Figure 9.5-11: EPAT profile for 1 x 450 g/ha – Step 3, R1 Stream, Winter Cereals

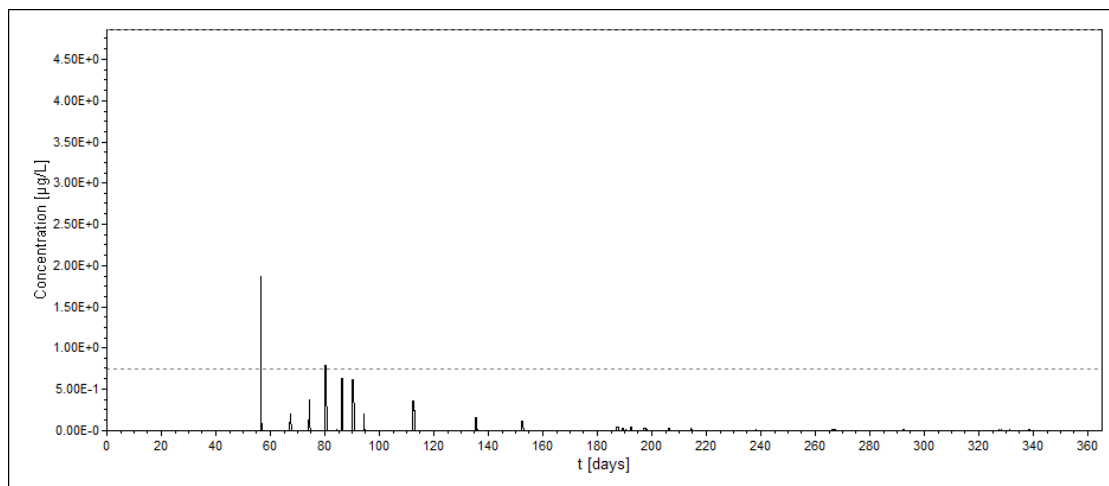


Figure 9.5-12: EPAT profile for 1 x 450 g/ha – Step 3, R3 Stream, Winter Cereals

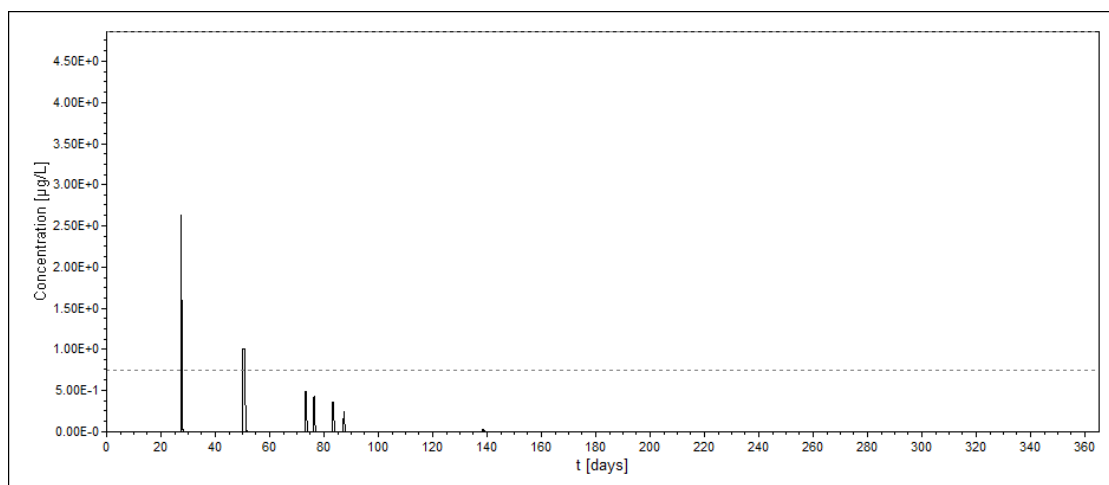
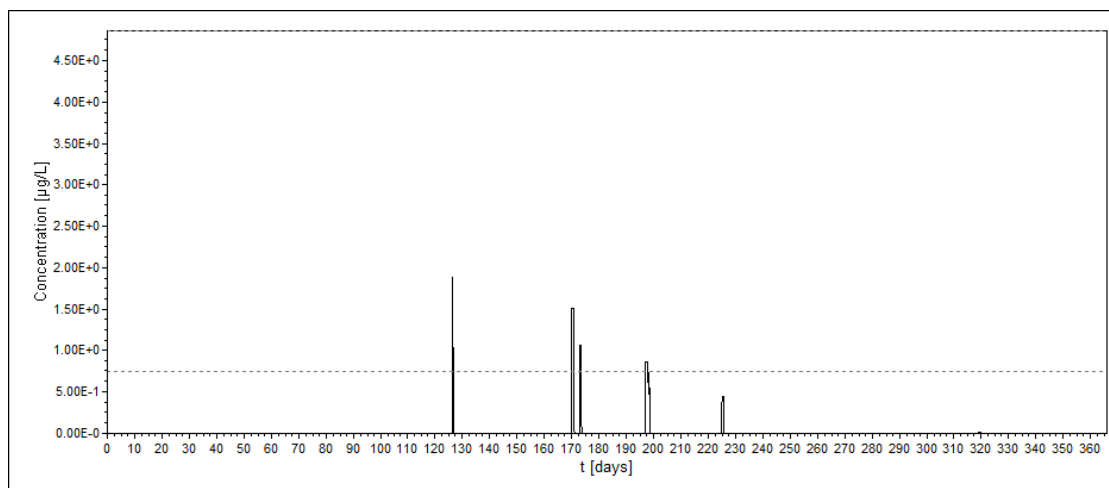


Figure 9.5-13: EPAT profile for 1 x 450 g/ha – Step 3, R4 Stream, Winter Cereals



As is clear, the concentrations illustrated in FOCUS exposure profiles obtained for both winter and spring cereals, peaks are well below the ERO-RAC of 3.33 $\mu\text{g/L}$, and for all scenarios (except Pond scenarios

where concentrations are $< 0.10 \mu\text{g/L}$), all peaks are short-lived (concentration returning to 0 within 5 days).

Based on all available data, an uncertainty factor of 3 applied to the concentration of $10 \mu\text{g/L}$ (max measured) from the mesocosm (Ashwell et al. 2007; VV-339018) would provide a reliable endpoint for refined risk assessment. This would give an **ERO-RAC** (Regulatory Acceptable Concentration) of **$3.33 \mu\text{g a.s./L}$** .

For completeness, the **ETO-RAC** of **0.75 $0.50 \mu\text{g a.s./L}$** has been included in the risk assessment, as required by the aquatic guidance document.

JAU 6476-desthio

A geometric mean value of **$\text{LC}_{50} = 246 \mu\text{g a.s./L}$** can be calculated from the two acute studies for *Americamysis bahia*; the 96 h LC_{50} value of $60 \mu\text{g a.s./L}$ from Drottar *et al.*, 2002 (Document No. M-083055-01-1) and the acute 96 h LC_{50} value of $1009 \mu\text{g a.s./L}$ from Blankinship *et al.*, 2003 (Document No. M-104620-01-1).

A23282A

According to the recommendations in the “Guidance document on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters in the context of Regulation (EC) No 1107/2009”, as provided by the Commission Services (SANTE-2015-00080, 15 January 2015), focus on growth rate endpoints for algae are recommended for European risk assessment. The advantages of using growth rate are that growth rate is less dependent on study duration and is relevant to ‘recovery potential’. Based on the recommendations from the aquatic guidance, XXXX propose that the E_rC_{50} value of 16.9 mg/L for A23282A is used in the algal risk assessment.

An acute toxicity test was not conducted with fish with A23282A since they are the least sensitive to cyprodinil out of invertebrates and algae by a factor of $>10 \times$. Aquatic invertebrates are the most sensitive group as the LC_{50} for *Mysidopsis bahia* was $8.05 \mu\text{g/L}$, in comparison with that for Bluegill sunfish (*Lepomis macrochirus*), the most sensitive fish species, for which an LC_{50} of $2\,170 \mu\text{g/L}$ was derived.

For prothioconazole, the algal species *Skeletonema costatum* was the most sensitive out of the three groups with an E_rC_{50} of $460 \mu\text{g/L}$ compared to the most sensitive fish species (*Oncorhynchus mykiss*) endpoint of $1\,830 \mu\text{g/L}$.

9.5.2 Risk assessment

The evaluation of the risk for aquatic and sediment-dwelling organisms was performed in accordance with the recommendations of the “Guidance document on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters in the context of Regulation (EC) No 1107/2009”, as provided by the Commission Services (SANTE-2015-00080, 15 January 2015).

The relevant global maximum FOCUS Step 1, 2 and 3 PEC_{SW} for risk assessments covering the proposed use pattern and the resulting PEC/RAC ratios are presented in the tables below.

Here, for A23282A, cyprodinil, prothioconazole, and relevant metabolites, the relevant endpoint values are compared to the maximum FOCUS PEC_{SW} ensuring that the aquatic risk from all intended uses is covered (see 9.1.2).

Table 9.5-5: Derivation of RAC values used in the risk assessment – cyprodinil and relevant metabolites

Species	Substance	Exposure System	Results (µg/L)	Assessment Factor	RAC (µg/L)
<i>Oncorhynchus mykiss</i>	Cyprodinil	96 h, f	LC ₅₀ = 2 170	100	21.7
<i>Oncorhynchus mykiss</i>	CGA249287	96 h, s	LC ₅₀ = 55 000	100	550
<i>Oncorhynchus mykiss</i>	CGA275535	96 h, s	LC ₅₀ = 2 100	100	21
<i>Oncorhynchus mykiss</i>	Cyprodinil	21 d, f	NOEC = 231	10	23.1
			NOEC = 83	10	8.3
Invertebrate – Species Sensitivity Distribution	Cyprodinil	Acute	HC ₅ = 19	4 ^a	4.75
			HC ₅ = 15.4		3.85
<i>Daphnia magna</i>	CGA249287	48 h, s	48 h EC ₅₀ = > 100 000	100	>1 000
<i>Daphnia magna</i>	CGA321915	48 h, s	48 h EC₅₀ = > 98 000	100	>980
<i>Daphnia magna</i>	CGA275535	48 h, s	48 h EC ₅₀ = 6 800	100	68
<i>Chironomus riparius</i>	Cyprodinil	27 d, spiked sediment	27 d NOEC = 80 000 µg/kg	10	8 000 µg/kg
<i>Chironomus riparius</i>	CGA249287	28 d, spiked sediment	28 d NOEC = 25 600 µg/kg	10	2 560 µg/kg
<i>Navicula pelliculosa</i>	Cyprodinil	72 h, s	72 h E _b C ₅₀ = 2 110	10	211
<i>Pseudokirchneriella subcapitata</i>	CGA249287	72 h, s	72 h E _r C ₅₀ = >100 000	10	>10 000
<i>Pseudokirchneriella subcapitata</i>	CGA321915	72 h, s	72 h E_rC₅₀ = >99 000	10	>9 900
<i>Pseudokirchneriella subcapitata</i>	CGA275535	72 h, s	72 h E _r C ₅₀ = 18 000	10	1 800
<i>Lemna gibba</i>	Cyprodinil	72 h, ss	72 h E _y C ₅₀ = 7 420	10	742
Higher-tier studies (micro- or mesocosm studies)					
Invertebrates	Cyprodinil	Mesocosm	NOAEAC = 10 NOEC = 1.5	3 ^a 2^a 3	ERO = 3.33 ETO = 0.75 ETO = 0.50

s = static system
ss = semi-static system
f = flow-through system
^a Refer Section 9.5.1.1

Table 9.5-6: Derivation of RAC values used in the risk assessment – prothioconazole and relevant metabolites

Species	Substance	Exposure System	Results (µg/L)	Assessment Factor	RAC (µg/L)
<i>Oncorhynchus</i>	Prothioconazole	96 h, ss	LC ₅₀ = 1 830	100	18.3

Species	Substance	Exposure System	Results (µg/L)	Assessment Factor	RAC (µg/L)
<i>mykiss</i>					
<i>Oncorhynchus mykiss</i>	JAU 6476-desthio	96 h, s	LC ₅₀ = 6 630	100	66.3
<i>Oncorhynchus mykiss</i>	JAU 6476-S-methyl	96 h, ss	LC ₅₀ = 1 790	100	17.9
<i>Oncorhynchus mykiss</i>	1,2,4-Triazole	96 h, s	LC ₅₀ = 498 000	100	4 980
<i>Oncorhynchus mykiss</i>	Prothioconazole	97 d, f	NOEC = 308	10	30.8
<i>Oncorhynchus mykiss</i>	JAU 6476-desthio	96 d, f	NOEC = 3.34	10	0.334
<i>Oncorhynchus mykiss</i>	1,2,4-Triazole	96 d, ss	NOEC = 3 200	10	320
<i>Daphnia magna</i>	Prothioconazole	48 h, s	EC ₅₀ = 1 300	100	13
<i>Americamysis bahia</i>	JAU 6476-desthio	96 h, f	Geomean LC ₅₀ = 246	100	2.46
<i>Daphnia magna</i>	JAU 6476-S-methyl	48 h, s	EC ₅₀ = 2 800	100	28
<i>Daphnia magna</i>	1,2,4-Triazole	48 h, s	EC ₅₀ > 100 000	100	> 1 000
<i>Daphnia magna</i>	Prothioconazole	21 d, ss	NOEC = 560	10	56
<i>Americamysis bahia</i>	JAU 6476-desthio	29 d, ss	NOEC = 64	10	6.4
<i>Chironomus riparius</i>	Prothioconazole	28 d, spiked water, s	NOEC = 9 140	10	914
<i>Chironomus riparius</i>	JAU 6476-desthio	28 d, spiked water, s	NOEC = 2 000	10	200
<i>Chironomus riparius</i>	JAU 6476-S-methyl	28 d, spiked water, s	NOEC = 100	10	10
<i>Skeletonema costatum</i>	Prothioconazole	96 h, s	E _r C ₅₀ = 46	10	4.6
<i>Pseudokirchneriella subcapitata</i>	JAU 6476-desthio	72 h, s	E _r C ₅₀ = 550	10	55
<i>Pseudokirchneriella subcapitata</i>	JAU 6476-S-methyl	72 h, s	E _r C ₅₀ = 47 400	10	4 740
<i>Pseudokirchneriella subcapitata</i>	1,2,4-Triazole	72 h, s	E _r C ₅₀ = 22 500	10	2 250
<i>Lemna gibba</i>	Prothioconazole	7 d, ss	E _r C ₅₀ > 404	10	> 40.4
<i>Lemna gibba</i>	JAU 6476-desthio	7 d, ss	E _r C ₅₀ = 80.9	10	8.09
Higher-tier studies (micro- or mesocosm studies)					
Not required					

Table 9.5-7: Derivation of RAC values used in the risk assessment – A23282A

Species	Substance	Exposure system	Results (µg/L)	Assessment Safety factor	RAC (µg/L)
Tier 1					
<i>Daphnia magna</i>	A23282A	48 h, s	EC ₅₀ = 223 µg/L	100	2.23
<i>Pseudokirchneriella subcapitata</i>	A23282A	96 h, s	72 h E_xC₅₀ = 16 900 72 h E _y C ₅₀ = 5240	10	1 690 524

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

9.5.2.1 Risk assessment with FOCUS STEP 1-2 and 3

Cyprodinil

Table 9.5-8: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for cyprodinil for each organism group based on maximum FOCUS Steps 1, 2 and 3 calculations for the use of A23282A in winter cereals (application at BBCH 30; 1 x 450 g a.s./ha)

Group		Fish acute	Fish prolonged	Fish prolonged	Inverteb. acute (SSD-HC₅)	Inverteb. acute (SSD-HC ₅)	Inverteb. prolonged (ETO-RAC)	Inverteb. prolonged (ETO-RAC)	Inverteb. prolonged (ERO-RAC)	Algae	Aquatic macrophyte		Sed. dwell. prolonged
RAC (µg/L)		21.7	23.1	8.3	4.75	3.85	0.75	0.50	3.33	211	742	RAC (µg/kg)	8000
FOCUS Scenario	PEC _{gl-max} (µg/L)											PEC _{SED} (µg/kg)	
Step 1													
	50.10	2.3	2.2	6.0	11	13	67	102	15	0.24	0.068	798	0.10
Step 2													
N-Europe	18.20	0.84	0.79	2.2	3.8	4.7	24	36.4	5.5	0.086	0.025	302	0.038
S-Europe	14.90	0.69	0.65	1.8	3.1	3.9	20	29.8	4.5	0.071	0.020	245	0.031
Step 3													
D3 Ditch	2.84	-	-	0.34	0.60	0.7	3.8	5.7	0.85	-	-	-	-
D4 Pond	0.098	-	-	0.01	0.021	0.0	0.13	0.2	0.029	-	-	-	-
D4 Stream	2.10	-	-	0.28	0.44	0.5	2.8	4.2	0.63	-	-	-	-
D5 Pond	0.098	-	-	0.01	0.021	0.0	0.13	0.2	0.029	-	-	-	-

Group		Fish acute	Fish prolonged	Fish prolonged	Inverteb. acute (SSD-HC ₅)	Inverteb. acute (SSD-HC ₅)	Inverteb. prolonged (ETO-RAC)	Inverteb. prolonged (ETO-RAC)	Inverteb. prolonged (ERO-RAC)	Algae	Aquatic macrophyte		Sed. dwell. prolonged
D5 Stream	2.27	-	-	0.27	0.48	0.6	3.0	4.5	0.68	-	-	-	-
R1 Pond	0.128	-	-	0.02	0.027	0.0	0.17	0.3	0.038	-	-	-	-
R1 Stream	1.87	-	-	0.23	0.39	0.5	2.5	3.7	0.56	-	-	-	-
R3 Stream	2.63	-	-	0.32	0.55	0.7	3.5	5.3	0.79	-	-	-	-
R4 Stream	1.88	-	-	0.23	0.40	0.5	2.5	3.8	0.56	-	-	-	-

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

For the intended use in winter cereals at BBCH 30, calculated PEC/RAC ratios for cyprodinil indicated an acceptable long-term risk for aquatic invertebrates at Step 3 when considering the ERO RAC of 3.33 µg/L.

When considering the ETO RAC of ~~0.75~~ 0.50 µg/L, for several FOCUS Step 3 scenarios (D3 ditch, D4 stream, D5 stream, R1 stream, R3 stream and R4 stream) the PEC/RAC ratios were below the trigger value of 1. Therefore, further PEC/RAC ratios were calculated based on maximum FOCUS Step 4 PEC_{SW} values for scenarios with unacceptable risk at Step 3, considering reduced exposure of surface water bodies.

Table 9.5-9: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for cyprodinil for each organism group based on maximum FOCUS Steps 1, 2 and 3 calculations for the use of A23282A in winter cereals (application at BBCH 69; 1 x 450 g a.s./ha)

Group		Fish acute	Fish prolonged	Fish prolonged	Inverteb. acute (SSD-HC ₅)	Inverteb. acute (SSD-HC ₅)	Inverteb. prolonged (ETO-RAC)	Inverteb. prolonged (ETO-RAC)	Inverteb. prolonged (ERO-RAC)	Algae	Aquatic macrophyte		Sed. dwell. prolonged
RAC (µg/L)		21.7	23.4	8.3	4.75	3.85	0.75	0.50	3.33	211	742	RAC (µg/kg)	8000
FOCUS Scenario	PEC _{gl-max} (µg/L)											PEC _{SED} (µg/kg)	
Step 1													

Group		Fish acute	Fish prolonged	Fish prolonged	Inverteb. acute (SSD-HC ₅)	Inverteb. acute (SSD-HC ₅)	Inverteb. prolonged (ETO-RAC)	Inverteb. prolonged (ETO-RAC)	Inverteb. prolonged (ERO-RAC)	Algae	Aquatic macrophyte		Sed. dwell. prolonged
	50.10	2.3	2.2	6.0	11	13	67	102	15	0.24	0.068	798	0.10
Step 2													
N-Europe	18.20	0.84	0.79	2.2	3.8	4.7	24	36.4	5.5	0.086	0.025	302	0.038
S-Europe	14.90	0.69	0.65	1.8	3.1	3.9	20	29.8	4.5	0.071	0.020	245	0.031
Step 3													
D3 Ditch	2.85	-	-	0.34	0.60	0.74	3.8	5.7	0.86	-	-	-	-
D4 Pond	0.098	-	-	0.01	0.021	0.03	0.13	0.20	0.029	-	-	-	-
D4 Stream	2.46	-	-	0.30	0.52	0.64	3.3	4.9	0.74	-	-	-	-
D5 Pond	0.098	-	-	0.01	0.021	0.03	0.13	0.20	0.029	-	-	-	-
D5 Stream	2.65	-	-	0.32	0.56	0.69	3.5	5.3	0.80	-	-	-	-
R1 Pond	0.148	-	-	0.02	0.031	0.04	0.20	0.30	0.044	-	-	-	-
R1 Stream	1.88	-	-	0.23	0.40	0.49	2.5	3.8	0.56	-	-	-	-
R3 Stream	2.65	-	-	0.32	0.56	0.69	3.5	5.3	0.79	-	-	-	-
R4 Stream	1.88	-	-	0.23	0.40	0.49	2.5	3.8	0.56	-	-	-	-

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

For the intended use in winter cereals at BBH 69, calculated PEC/RAC ratios for cyprodinil indicated an acceptable long-term risk for aquatic invertebrates at Step 3 when considering the ERO RAC of 3.33 µg/L.

When considering the ETO RAC of ~~0.75~~ 0.50 µg/L, for several FOCUS Step 3 scenarios (D3 ditch, D4 stream, D5 stream, R1 stream, R3 stream and R4 stream) the PEC/RAC ratios were below the trigger value of 1. Therefore, further PEC/RAC ratios were calculated based on maximum FOCUS Step 4 PEC_{SW} values for scenarios with unacceptable risk at Step 3, considering reduced exposure of surface water bodies.

Table 9.5-10: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for cyprodinil for each organism group based on maximum FOCUS Steps 1, 2 and 3 calculations for the use of A23282A in spring cereals (application at BBCH 30; 1 x 450 g a.s./ha)

Group		Fish acute	Fish prolonged	Fish prolonged	Inverteb. acute (SSD-HC ₅)	Inverteb. acute (SSD-HC ₅)	Inverteb. prolonged (ETO-RAC)	Inverteb. prolonged (ETO-RAC)	Inverteb. prolonged (ERO-RAC)	Algae	Aquatic macrophyte		Sed. dwell. prolonged
RAC (µg/L)		21.7	23.4	8.3	4.75	3.85	0.75	0.50	3.33	211	742	RAC (µg/kg)	8000
FOCUS Scenario	PEC _{gl-max} (µg/L)											PEC _{SED} (µg/kg)	
Step 1													
	50.10	2.3	2.2	6.0	11	13	67	102	15	0.24	0.068	798	0.10
Step 2													
N-Europe	18.20	0.84	0.79	2.2	3.8	4.7	24	36.4	5.5	0.086	0.025	302	0.038
S-Europe	14.90	0.69	0.65	1.8	3.1	3.9	20	29.8	4.5	0.071	0.020	245	0.031
Step 3													
D3 Ditch	2.85	-	-	0.34	0.60	0.74	3.8	5.7	0.86	-	-	-	-
D4 Pond	0.098	-	-	0.01	0.021	0.03	0.13	0.20	0.029	-	-	-	-
D4 Stream	2.33	-	-	0.28	0.49	0.61	3.1	4.7	0.70	-	-	-	-
D5 Pond	0.098	-	-	0.01	0.021	0.03	0.13	0.20	0.029	-	-	-	-
D5 Stream	2.39	-	-	0.29	0.50	0.62	3.2	4.8	0.72	-	-	-	-
R4 Stream	1.88	-	-	0.23	0.40	0.49	2.5	3.8	0.56	-	-	-	-

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

For the intended use in spring cereals at BBCH 30, calculated PEC/RAC ratios for cyprodinil indicated an acceptable long-term risk for aquatic invertebrates at Step 3 when considering the ERO RAC of 3.33 µg/L.

When considering the ETO RAC of ~~0.75~~ 0.50 µg/L, for several FOCUS Step 3 scenarios (D3 ditch, D4 stream, D5 stream, and R4 stream) the PEC/RAC ratios were

below the trigger value of 1. Therefore, further PEC/RAC ratios were calculated based on maximum FOCUS Step 4 PEC_{SW} values for scenarios with unacceptable risk at Step 3, considering reduced exposure of surface water bodies.

Table 9.5-11: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for cyprodinil for each organism group based on maximum FOCUS Steps 1, 2 and 3 calculations for the use of A23282A in spring cereals (application at BBCH 69; 1 x 450 g a.s./ha)

Group		Fish acute	Fish prolonged	Fish prolonged	Inverteb. acute (SSD-HC₅)	Inverteb. acute (SSD-HC ₅)	Inverteb. prolonged (ETO-RAC)	Inverteb. prolonged (ETO-RAC)	Inverteb. prolonged (ERO-RAC)	Algae	Aquatic macrophyte		Sed. dwell. prolonged
RAC (µg/L)		21.7	23.1	8.3	4.75	3.85	0.75	0.50	3.33	211	742	RAC (µg/kg)	8000
FOCUS Scenario	PEC _{gl-max} (µg/L)											PEC _{SED} (µg/kg)	
Step 1													
	50.10	2.3	2.2	6.0	11	13	67	102	15	0.24	0.068	798	0.10
Step 2													
N-Europe	18.20	0.84	0.79	2.2	3.8	4.7	24	36.4	5.5	0.086	0.025	302	0.038
S-Europe	14.90	0.69	0.65	1.8	3.1	3.9	20	29.8	4.5	0.071	0.020	245	0.031
Step 3													
D3 Ditch	2.85	-	-	0.34	0.60	0.74	3.8	5.7	0.85	-	-	-	-
D4 Pond	0.098	-	-	0.01	0.021	0.03	0.13	0.20	0.029	-	-	-	-
D4 Stream	2.45	-	-	0.30	0.52	0.64	3.3	4.9	0.74	-	-	-	-
D5 Pond	0.098	-	-	0.01	0.021	0.03	0.13	0.20	0.029	-	-	-	-
D5 Stream	2.48	-	-	0.30	0.52	0.64	3.3	5.0	0.75	-	-	-	-
R4 Stream	1.88	-	-	0.02	0.40	0.49	2.5	3.8	0.56	-	-	-	-

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

For the intended use in spring cereals at BBCH 69, calculated PEC/RAC ratios for cyprodinil indicated an acceptable long-term risk for aquatic invertebrates at Step 3 when considering the ERO RAC of 3.33 µg/L.

When considering the ETO RAC of ~~0.75~~ 0.50 µg/L, for several FOCUS Step 3 scenarios (D3 ditch, D4 stream, D5 stream, and R4 stream) the PEC/RAC ratios were below the trigger value of 1. Therefore, further PEC/RAC ratios were calculated based on maximum FOCUS Step 4 PEC_{SW} values for scenarios with unacceptable risk at Step 3, considering reduced exposure of surface water bodies.

Cyprodinil metabolite CGA249287

Table 9.5-12: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for cyprodinil metabolite CGA249287 for each organism group based on maximum FOCUS Step 1 and 2 calculations for the use of A23282A in cereals (1 x 450 g a.s./ha)

Group		Fish acute	Inverteb. acute	Algae		Sed. dwell. prolonged
RAC (µg/L)		550	>1000	10000	RAC (µg/kg)	2560
FOCUS Scenario	PEC _{gl-max} (µg/L)				PEC _{SED} (µg/kg)	
Step 1						
	29.20	0.053	<0.029	0.0029	50.2	0.020

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration

For the intended use of A23262A in cereals, calculated PEC/RAC ratios for cyprodinil metabolite CGA249287 indicated an acceptable risk for all groups of aquatic organisms at FOCUS Step 1.

Cyprodinil metabolite CGA321915

Table 9.5-13: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for cyprodinil metabolite CGA321915 for each organism group based on maximum FOCUS Step 1 and 2 calculations for the use of A23282A in cereals (1 x 450 g a.s./ha)

Group		Inverteb. acute	Algae
RAC (µg/L)		>980	>9900
FOCUS Scenario	PEC _{gl-max} (µg/L)		
Step 1			
-	4.33	<0.0044	<0.00044

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration

For the intended use of A23262A in cereals, calculated PEC/RAC ratios for cyprodinil metabolite CGA321915 indicated an acceptable risk for invertebrates and algae at FOCUS Step 1.

Cyprodinil metabolite CGA275535

Table 9.5-14: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for cyprodinil metabolite CGA275535 for each organism group based on maximum FOCUS Step 1 and 2 calculations for the use of A23282A in cereals (1 x 450 g a.s./ha)

Group		Fish acute	Inverteb. acute	Algae
RAC (µg/L)		21	68	1800
FOCUS Scenario	PEC _{gl-max} (µg/L)			
Step 1				
	10.00	0.48	0.15	0.0056

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration

For the intended use of A23262A in cereals, calculated PEC/RAC ratios for cyprodinil metabolite CGA249287 indicated an acceptable risk for all groups of aquatic organisms at FOCUS Step 1.

Prothioconazole

Table 9.5-15: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prothioconazole for each organism group based on maximum FOCUS Steps 1 and 2 calculations for the use of A23282A in cereals (1 x 150 g a.s./ha)

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Aquatic macrophyte	Sed. dwell. prolonged
RAC (µg/L)		18.3	30.8	13	56	4.6	>40.4	914
FOCUS Scenario	PEC _{gl-max} (µg/L)							
Step 1								
	16.30	0.89	0.53	1.3	0.29	3.5	<0.40	0.018
Step 2								
N-Europe	1.38	0.075	0.045	0.11	0.025	0.30	<0.034	0.0015
S-Europe	1.38	0.075	0.045	0.11	0.025	0.30	<0.034	0.0015

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

For the intended use of A23262A in cereals, calculated PEC/RAC ratios for prothioconazole indicated an acceptable risk for all groups of aquatic organisms at FOCUS Step 2.

Prothioconazole metabolite JAU 6476-desthio

Table 9.5-16: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prothioconazole metabolite JAU 6476-desthio for each organism group based on maximum FOCUS Steps 1, 2 and 3 calculations for the use of A23282A in winter cereals (application at BBCH 30; 1 x 150 g a.s./ha)

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Aquatic macrophyte	Sed. dwell. prolonged
RAC (µg/L)		66.3	0.334	2.46	6.4	55	8.09	200
FOCUS Scenario	PEC ^{gl-max} (µg/L)							
Step 1								
	29.70	0.45	89	12	4.6	0.54	3.7	0.15
Step 2								
N-Europe	5.90	0.089	18	2.4	0.92	0.11	0.73	0.030
S-Europe	4.81	0.073	14	2.0	0.75	0.087	0.59	0.024
Step 3								
D3 Ditch	0.003	-	0.0090	0.0012	-	-	-	-
D4 Pond	0.005	-	0.015	0.0020	-	-	-	-
D4 Stream	0.004	-	0.012	0.0016	-	-	-	-
D5 Pond	0.008	-	0.024	0.0033	-	-	-	-
D5 Stream	0.008	-	0.024	0.0033	-	-	-	-
R1 Pond	0.030	-	0.090	0.012	-	-	-	-
R1 Stream	0.265	-	0.79	0.11	-	-	-	-
R3 Stream	0.323	-	0.97	0.13	-	-	-	-
R4 Stream	0.477	-	1.40	0.19	-	-	-	-

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

For the intended use of A23262A in winter cereals at BBCH 30, calculated PEC/RAC ratios for prothioconazole metabolite JAU 6476-desthio indicated an acceptable risk for all groups of aquatic organisms at FOCUS Step 3 for all scenarios except R4 stream. Further consideration is therefore required.

Table 9.5-17: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prothioconazole metabolite JAU 6476-desthio for each organism group based on maximum FOCUS Steps 1, 2 and 3 calculations for the use of A23282A in winter cereals (application at BBCH 69; 1 x 150 g a.s./ha)

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Aquatic macrophyte	Sed. dwell. prolonged
RAC (µg/L)		66.3	0.334	2.46	6.4	55	8.09	200
FOCUS Scenario	PEC ^{gl-max} (µg/L)							
Step 1								
	29.70	0.45	89	12	4.6	0.54	3.7	0.15
Step 2								
N-Europe	5.90	0.089	18	2.4	0.92	0.11	0.73	0.030
S-Europe	4.81	0.073	14	2.0	0.75	0.087	0.59	0.024
Step 3								
D3 Ditch	0.009	-	0.027	0.0037	-	-	-	-
D4 Pond	0.008	-	0.024	0.0033	-	-	-	-
D4 Stream	0.006	-	0.018	0.0024	-	-	-	-
D5 Pond	0.008	-	0.024	0.0033	-	-	-	-
D5 Stream	0.010	-	0.030	0.0041	-	-	-	-
R1 Pond	0.024	-	0.072	0.0098	-	-	-	-
R1 Stream	0.169	-	0.51	0.069	-	-	-	-

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Aquatic macrophyte	Sed. dwell. prolonged
R3 Stream	0.314	-	0.94	0.13	-	-	-	-
R4 Stream	0.383	-	1.10	0.16	-	-	-	-

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

For the intended use of A23262A in winter cereals at BBCH 69, calculated PEC/RAC ratios for prothioconazole metabolite JAU 6476-desthio indicated an acceptable risk for all groups of aquatic organisms at FOCUS Step 3 for all scenarios except R4 stream. Further consideration is therefore required.

Table 9.5-18: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prothioconazole metabolite JAU 6476-desthio for each organism group based on maximum FOCUS Steps 1, 2 and 3 calculations for the use of A23282A in spring cereals (application at BBCH 30; 1 x 150 g a.s./ha)

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Aquatic macrophyte	Sed. dwell. prolonged
RAC (µg/L)		66.3	0.334	2.46	6.4	55	8.09	200
FOCUS Scenario	PEC_{gl-max} (µg/L)							
Step 1								
	29.70	0.45	89	12	4.6	0.54	3.7	0.15
Step 2								
N-Europe	5.90	0.089	18	2.4	0.92	0.11	0.73	0.030
S-Europe	4.81	0.073	14	2.0	0.75	0.087	0.59	0.024
Step 3								
D3 Ditch	0.006	-	0.018	0.0024	-	-	-	-
D4 Pond	0.007	-	0.021	0.0028	-	-	-	-
D4 Stream	0.005	-	0.015	0.0020	-	-	-	-

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Aquatic macrophyte	Sed. dwell. prolonged
D5 Pond	0.008	-	0.024	0.0033	-	-	-	-
D5 Stream	0.008	-	0.024	0.0033	-	-	-	-
R4 Stream	0.427	-	1.3	0.17	-	-	-	-

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

For the intended use of A23262A in spring cereals at BBCH 30, calculated PEC/RAC ratios for prothioconazole metabolite JAU 6476-desthio indicated an acceptable risk for all groups of aquatic organisms at FOCUS Step 3 for all scenarios except R4 stream. Further consideration is therefore required.

Table 9.5-19: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prothioconazole metabolite JAU 6476-desthio for each organism group based on maximum FOCUS Steps 1, 2 and 3 calculations for the use of A23282A in spring cereals (application at BBCH 69; 1 x 150 g a.s./ha)

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Aquatic macrophyte	Sed. dwell. prolonged
RAC (µg/L)		66.3	0.334	2.46	6.4	55	8.09	200
FOCUS Scenario	PEC ^{gl-max} (µg/L)							
Step 1								
	29.70	0.45	89	12	4.6	0.54	3.7	0.15
Step 2								
N-Europe	5.90	0.089	18	2.4	0.92	0.11	0.73	0.030
S-Europe	4.81	0.073	14	2.0	0.75	0.087	0.59	0.024
Step 3								
D3 Ditch	0.006	-	0.017	0.0022	-	-	-	-

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Aquatic macrophyte	Sed. dwell. prolonged
D4 Pond	0.007	-	0.022	0.0030	-	-	-	-
D4 Stream	0.006	-	0.018	0.0024	-	-	-	-
D5 Pond	0.008	-	0.023	0.0031	-	-	-	-
D5 Stream	0.009	-	0.027	0.0037	-	-	-	-
R4 Stream	0.443	-	1.3	0.18	-	-	-	-

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

For the intended use of A23262A in spring cereals at BBCH 69, calculated PEC/RAC ratios for prothioconazole metabolite JAU 6476-desthio indicated an acceptable risk for all groups of aquatic organisms at FOCUS Step 3.

Prothioconazole metabolite JAU 6476-S-methyl

Table 9.5-20: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prothioconazole metabolite JAU 6476-S-methyl for each organism group based on maximum FOCUS Steps 1 and 2 calculations for the use of A23282A in cereals (1 x 150 g a.s./ha)

Group		Fish acute	Inverteb. acute	Algae	Sed. dwell. prolonged
RAC (µg/L)		17.9	28	4 740	10
FOCUS Scenario	PEC _{gl-max} (µg/L)				
Step 1					
	3.39	0.19	0.12	0.00072	0.34

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration

For the intended use of A23262A in cereals, calculated PEC/RAC ratios for prothioconazole metabolite JAU 6476-S-methyl indicated an acceptable risk for all groups of aquatic organisms at FOCUS Step 1.

Prothioconazole metabolite 1,2,4-triazole

Table 9.5-21: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prothioconazole metabolite 1,2,4-triazole for each organism group based on maximum FOCUS Steps 1 and 2 calculations for the use of A23282A in cereals (1 x 150 g a.s./ha)

Group		Fish acute	Fish Prolonged	Inverteb. acute	Algae
RAC (µg/L)		4 980	320	1 000	2 250
FOCUS Scenario	PEC _{gl-max} (µg/L)				
Step 1					
	3.89	0.00078	0.012	0.0039	0.0017
Step 2					
N-Europe	0.180	0.000036	0.00056	0.00018	0.000080
S-Europe	0.164	0.000033	0.00051	0.00016	0.000073

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration

For the intended use of A23262A in cereals, calculated PEC/RAC ratios for prothioconazole metabolite 1,2,4-triazole indicated an acceptable risk for all groups of aquatic organisms at FOCUS Step 1.

Formulation A23282A

Table 9.5-22: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the formulation A23282A for each organism group based on the maximum instantaneous PEC_{SW} for the use of A8272C in cereals

Group			Inverteb. acute	Algae
RAC (µg/L)			2.23	1 690
Buffer Distance (m)	Drift Reducing Nozzle (%)	PEC _{gl-max} (µg/L)	PEC/RAC	
1 m	-	18.34	8.2	0.011
	50	9.17	4.1	-
	75	4.584	2.1	-
	90	1.834	0.82	-
3 m	-	6.29	2.8	-
	50	3.145	1.4	-
	75	1.572	0.70	-
4 m	-	4.700	2.1	-
	50	2.350	1.1	-
	75	1.175	0.53	-
5 m	-	3.773	1.7	-
	50	1.887	0.85	-
6 m	-	3.178	1.4	-
	50	1.589	0.71	-
7 m	-	2.714	1.2	-
	50	1.357	0.61	-

Group			Inverteb. acute	Algae
RAC (µg/L)			2.23	1 690
Buffer Distance (m)	Drift Reducing Nozzle (%)	PEC _{gl-max} (µg/L)	PEC/RAC	
8 m	-	2.383	1.1	-
	50	1.192	0.53	-
9 m	-	2.118	0.95	-

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration

9.5.2.2 Risk assessment with FOCUS STEP 4

In the following tables, the ratios between the maximum predicted environmental concentrations in surface water bodies (PEC_{SW}) at FOCUS Step 4 have been taken from Section 8 (Environmental Fate) and regulatory acceptable concentrations (RAC) for aquatic organisms are given for each FOCUS scenario and each organism group. The application conditions under which the maximum PEC_{SW} values for that FOCUS scenario occur are noted for each table.

Cyprodinil

Table 9.5-23: Aquatic organisms: higher-tier risk assessment for acceptability of risk (PEC/RAC < 1) for cyprodinil incorporating exposure mitigation options for winter cereals (1 x 450 g a.s./ha)

Group					Invertebrate Prolonged (ETO-RAC)	Invertebrate Prolonged (ETO-RAC)
RAC (µg/L)					0.75	0.50
Step 4 FOCUS Scenario	Spray drift buffer (m)	Drift- reducing nozzles (%)	Vegetative Filter Strip (m)	Max PEC _{SW} (µg/L)	PEC/RAC	PEC / RAC
D3 Ditch	5	0	0	0.811	1.1	1.62
		50		0.483	0.64	0.97
	10	0	0	0.466	0.62	0.93
D4 Stream	5	0	0	0.930	1.2	1.86
		50		0.495	0.66	0.99
	10	0	0	0.507	0.68	1.01
	10	50	0	0.254		0.51
D5 Stream	5	0	0	0.991	1.3	1.98
		50		0.527	0.70	1.05
	10	0	0	0.540	0.72	1.08
	10	50	0	0.270		0.54
R1 Stream	5	0	0	0.745	0.99	1.49
	10	0	10	0.409		0.82
R3 Stream	5	0	0	1.02	1.4	2.04
		50		0.922	1.2	1.84
	10	0	0	0.922	1.2	1.84
	10	0	10 m	0.560	0.75	1.12
	20	0	20	0.295		0.59
R4 Stream	5	0	0	1.62	2.2	3.24
		50	0	1.62	2.2	3.24

Group					Invertebrate Prolonged (ETO-RAC)	Invertebrate Prolonged (ETO-RAC)
RAC (µg/L)					0.75	0.50
Step 4 FOCUS Scenario	Spray drift buffer (m)	Drift- reducing nozzles (%)	Vegetative Filter Strip (m)	Max PEC _{sw} (µg/L)	PEC / RAC	PEC / RAC
	10	0	0	1.62	2.2	3.24
	10	0	10 m	0.736	0.98	1.47
	20	0	20	0.386		0.19

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

Table 9.5-24: Aquatic organisms: higher-tier risk assessment for acceptability of risk (PEC/RAC < 1) for cyprodinil incorporating exposure mitigation options for spring cereals (1 x 450 g a.s./ha)

Group					Invertebrate Prolonged (ETO-RAC)	Invertebrate Prolonged (ETO-RAC)
RAC (µg/L)					0.75	0.50
Step 4 FOCUS Scenario	Spray drift buffer (m)	Drift- reducing nozzles (%)	Vegetative Filter Strip (m)	Max PEC _{sw} (µg/L)	PEC / RAC	
D3 Ditch	5	0	0	0.805	1.1	1.61
		50		0.474	0.63	0.95
	10	0	0	0.457	0.61	0.91
D4 Stream	5	0	0	0.927	1.2	1.85
		50		0.495	0.66	0.99
	10	0	0	0.505	0.67	1.01
	10	50	0	0.253		0.51
D5 Stream	5	0	0	0.945	1.3	1.89
		50		0.492	0.66	0.98
	10	0	0	0.510	0.68	1.02
	10	50	0	0.255		0.51
R4 Stream	5	0	0	1.80	2.4	3.60
	10	0	0	1.80	2.4	3.60
	10	0	10 m	0.82	1.1	1.64
	20	0	20 m	0.43	0.57	0.86

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

Prothioconazole metabolite JAU 6476-desthio

Table 9.5-25: Aquatic organisms: higher-tier risk assessment for acceptability of risk (PEC/RAC < 1) for prothioconazole metabolite JAU 6476-desthio incorporating exposure mitigation options for winter cereals (1 x 150 g a.s./ha)

Group					Fish Prolonged
RAC (µg/L)					0.334
Step 4 FOCUS Scenario	Spray drift buffer (m)	Drift-reducing nozzles (%)	Vegetative Filter Strip (m)	Max PEC _{sw} (µg/L)	PEC / RAC
R4 Stream	5	0	0	0.477	1.4
		50		0.477	1.4
	10	0	10 m	0.477	1.4
	10	0	10 m	0.217	0.65

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

Table 9.5-26: Aquatic organisms: higher-tier risk assessment for acceptability of risk (PEC/RAC < 1) for prothioconazole metabolite JAU 6476-desthio incorporating exposure mitigation options for spring cereals (1 x 150 g a.s./ha)

Group					Fish Prolonged
RAC (µg/L)					0.334
Step 4 FOCUS Scenario	Spray drift buffer (m)	Drift-reducing nozzles (%)	Vegetative Filter Strip (m)	Max PEC _{sw} (µg/L)	PEC / RAC
R4 Stream	5	0	0	0.443	1.3
		50		0.443	1.3
	10	0	10 m	0.443	1.3
	10	0	10 m	0.201	0.60

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

Mitigation Requirements

Table 9.5-27: Aquatic organisms: mitigation requirements / options for cyprodinil following use in cereals

Scenario	Winter cereals	Spring cereals
	Inverteb. prolonged (ETO)	Inverteb. prolonged (ETO)
D3 Ditch	5 m SD + 50 % DR or 10 m SD	5 m SD + 50 % DR or 10 m SD
D4 Stream	5 m SD + 50 % DR or 10 m SD	5 m SD + 50 % DR or 10 m SD
D5 Stream	5 m SD + 50 % DR or 10 m SD	5 m SD + 50 % DR or 10 m SD
R1 Stream	5 m SD	-
R3 Stream	10 m SD + 10 m RO	-
R4 Stream	10 m SD + 10 m RO	20 m SD + 20 m RO

Scenario	Winter cereals	Spring cereals
	Inverteb. prolonged (ETO)	Inverteb. prolonged (ETO)
D3 Ditch	5 m SD + 50 % DR or 10 m SD	5 m SD + 50 % DR or 10 m SD
D4 Stream	5 m SD + 50 % DR or 10 m SD	5 m SD + 50 % DR or 10 m SD
D5 Stream	10 m SD + 50 % DR or 10 m SD	5 m SD + 50 % DR or 10 m SD
R1 Stream	10 m SD + 10 m RO	-
R3 Stream	20 m SD + 20 m RO	-
R4 Stream	20 m SD + 20 m RO	20 m SD + 20 m RO

An empty/grey field means that the scenario is not relevant to the crop group

“-“mitigation measures are not required

SD = spray drift buffer

RO = run-off mitigation

DR = drift reducing nozzles

A table indicating the percentage reduction required to achieve an acceptable risk to aquatic organisms are presented below:

Table 9.5-28: Aquatic organisms: percentage reduction of entry by cyprodinil into surface water to achieve acceptable risk when considering the ETO RAC of 0.75 µg/L

Scenario	Winter cereals		Spring cereals	
	BBCH 30	BBCH 69	BBCH 30	BBCH 69
	ETO RAC: 0.75 µg/L			
D3 Ditch	73.6	73.7	73.7	73.7
D4 Stream	64.3	69.5	67.8	69.4
D5 Stream	67.0	71.7	68.6	69.8
R1 Stream	59.9	60.1	-	-

Scenario	Winter cereals		Spring cereals	
	BBCH 30	BBCH 69	BBCH 30	BBCH 69
	ETO RAC: 0.75 µg/L			
R3 Stream	71.5	71.7	-	-
R4 Stream	60.1	60.1	60.1	60.1
These scenarios are not relevant for spring cereals				

Prothioconazole (JAU 6476-desthio)

For the R4 scenario, to ensure that the long-term risk to fish is low, a 10 m spray drift buffer + 10 m run-off mitigation is required (which is covered already by the proposed above cyprodinil mitigation options).

9.5.2.3 Calculation of the Mixture Toxicity of A23282A

The mixture toxicity risk assessment will be performed in a stepwise approach and will be conducted according to Section 10.3.11 of the EFSA Aquatic Guidance Document.

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

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

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

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

where:

n = number of mixture components

i = index from 1...n mixture components

p_i = the i^{th} component as a relative fraction of the mixture composition (note $\sum p_i$ must be 1)

EC_{x_i} = concentration of component i provoking x% effect (pragmatically, NOEC_i may be inserted, too)

The model deviation ratio (MDR) is then calculated using equation 15 of the EFSA Aquatic guidance (page 149).

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

According to the EFSA Guidance, MDR between 0.2 and 5 indicates that the mixture toxicity conforms to assumptions of concentration-addition; MDR >5 indicates synergy; MDR <0.2 indicates antagonism. The

same test species have been used across this comparison where possible, regardless of whether or not they provide the lowest endpoint for the individual active ingredients. Formulation studies have been performed with *Daphnia magna* and *Raphidocelis subcapitata* (formerly known as *Pseudokirchneriella subcapitata*). The expected acute toxicity of A23282A to aquatic invertebrates and algae according to the assumption of concentration addition are given in Table 9.5-29.

Table 9.5-29: Mixture toxicity of A23282A to aquatic invertebrates and algae calculated according to assumption of concentration addition and MDR analysis

Species	Test substance	Concentration of active substance in formulation A23282A (g/L)	Fraction of active substance in the formulation mixture	L/EC ₅₀ for active substance (µg/L)	Fraction of active substance / L/EC ₅₀ for the active substance	Calculated L/EC ₅₀ mix-CA (µg a.s./L) ^a	Measured L/EC ₅₀ ppp (µg form./L)	Measured L/EC ₅₀ ppp (µg a.s./L) ^b	MDR L/EC ₅₀ mix-CA / L/EC ₅₀ ppp
Aquatic invertebrates (<i>Daphnia magna</i>)	Cyprodinil	225	0.75	33	0.02273	43.63	223	65.79	0.66
	Prothioconazole	75	0.25	1300	0.00019				
Total	-	300	1.0	-	-	-	-	-	-
Algae (<i>Pseudokirchneriella subcapitata</i>)	Cyprodinil	225	0.75	3280	0.00023	2941	16900	4986	0.59
	Prothioconazole	75	0.25	2180	0.00011		5240	1545	1.90
Total	-	300	1.0	-	-	-	-	-	-

^a Predicted mixture toxicity under assumption of concentration-addition

^b Formulation endpoint calculation based on 29.5 % active ingredient in A23282A, considering a density of 0.993 g/cm³

The model deviation ratio (MDR) values in the table above indicate that acute toxicity of A23282A to aquatic invertebrates, and toxicity to algae is between **0.2 and 5** times that of the expected toxicity of the combination of cyprodinil and prothioconazole in the aquatic environment and conforms to the assumptions of concentration-addition.

The next step is to determine if the EC_Xppp and the EC_Xmix-CA are similar. If EC_Xppp divided by EC_Xmix-CA yields a value between 0.8 and 1.2, a direct comparison of PEC_{mix} with the EC_Xppp will be performed. If the mixture composition is less than 0.8 or greater than 1.2, the measured data cannot be used directly for calculating the ETR; and the mixture risk assessment will instead be based on calculated mixture toxicity.

Table 9.5-30: Determination of EC_{Xmix-CA} for PEC_{mix} at FOCUS Step 2

Species	Test substance	Concentration of active substance at FOCUS Step 2 (µg/L)	Fraction of active substance in the PEC _{mix}	L/EC ₅₀ for active substance (µg/L)	Fraction of active substance / L/EC ₅₀ for the active substance	Calculated L/EC ₅₀ mix-CA (µg a.s./L)
Aquatic invertebrates (<i>Daphnia magna</i>)	Cyprodinil	18.2	0.93	33	0.028	35.6
	Prothioconazole	1.38	0.07	1300	0.000054	
Total	-	19.58	1.0	-	0.028054	-
Algae (<i>Pseudokirchneriella subcapitata</i>)	Cyprodinil	18.2	0.93	3280	0.00028	3205
	Prothioconazole	1.38	0.07	2180	0.000032	
Total	-	19.58	1.0	-	0.000312	-

Table 9.5-31: Ratio of EC_{XPPP} and EC_{Xmix-CA} based upon FOCUS Step 2 values

Organism Group	EC _{XPPP} (µg a.s./L)	EC _{Xmix-CA} (µg a.s./L) [a.s. in PEC _{mix}]	Ratio
Acute Invertebrates	65.79	35.6	1.85
Algae	4986 1545	3205	1.56 0.48

As the EC_{XPPP} and the EC_{Xmix-CA} are not similar, for the initial step in the acute invertebrate and algal risk assessments, the toxic unit approach will be taken to evaluate if one compound is driving the risk. Equation 14 of the EFSA Aquatic Guidance Document (page 148) is as follows:

$$\sum_{i=1}^n TU_i = \sum_{i=1}^n \frac{c_i}{ECx_i}$$

Where:

c_i is the concentration of a mixture component

ECx_i is the measured mixture toxicity endpoint (EC_{XPPP}), expressed as active substance

If a single driver of toxicity is identified, then the risk assessment previously conducted for that active substance is applicable instead of a mixture risk assessment.

If no single driver of toxicity is identified, a mixture toxicity risk assessment will be conducted based upon calculated mixture toxicity.

Table 9.5-32: Toxic Units for Invertebrate Acute and Algal Risk Assessments

Species	EC _{XPPP} (µg a.s./L)	Substance	Step 2 PEC _{sw} max (µg/L)	Toxic unit (concentration/endpoint)	Sum of toxic units	% of total toxic unit
Invertebrate Acute	65.79	Cyprodinil	18.2	0.277	0.298	93
		Prothioconazole	1.38	0.021		7.0
Algal	4986	Cyprodinil	18.2	0.0037	0.00398	93

Species	EC _x PPP (µg a.s./L)	Substance	Step PEC _{sw} (µg/L)	2 max	Toxic unit (concentration/endpoint)	Sum of toxic units	% of total toxic unit
	1545				0.0128	0.01369	93.5
		Prothioconazole	1.38		0.00028 0.00089		7.0 6.5

As cyprodinil was identified as the single driver of toxicity for both the invertebrate acute and algal toxic unit assessments, a mixture toxicity risk assessment is not required for these groups, and the single substance assessments for cyprodinil should be referred to.

As only active substance data is available for acute and chronic fish, chronic invertebrate and aquatic plant endpoints, a mixture toxicity risk assessment will be performed for these groups. For sediment dwellers, there is not sufficient data to perform a mixture toxicity assessment.

In order to perform a mixture toxicity assessment, a surrogate mixture toxicity value will be calculated, assuming concentration addition.

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

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

where:

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 (note Σ p_i must be 1)

ECx_i = concentration of component i provoking x% effect (pragmatically, NOEC_i may be inserted, too)

At each step of the assessment, the mixture composition is based upon the PEC_{mix}. The PEC_{mix} is then divided by the EC_{xmix-CA} to give the ETR value. If the ETR value is below the trigger, then the risk is acceptable. The trigger for the ETR calculation is 1 divided by the standard assessment factor for that organism group (e.g. 100 for acute endpoints and 10 for chronic endpoints).

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

Fish Acute Mixture Risk Assessment

Table 9.5-33: Aquatic organisms: acceptability of risk (TER < 0.01) for fish acute mixture toxicity based on maximum FOCUS Step 1 and 2 calculations for the use of A23282A (cyprodinil/prothioconazole/JAU 6476-desthio) in cereals

Focus Scenario	Test substance	PEC _{gl-max} (µg/L)	PEC _{mix} (µg/L)	Fraction of Substance in PEC _{mix}	LC ₅₀ for the substance (µg a.s./L) ^a	Fraction of substance / LC ₅₀ for the substance	Calculated LC _{50 mix-CA} (µg a.s./L)	ETR _{mix}
Focus Step 1	Cyprodinil	50.1	96.1	0.52	2170	0.0002396	2636	0.0365
	Prothioconazole	16.3		0.17	1830	0.0000929		
	JAU 6476-desthio	29.7		0.31	6630	0.0000468		
Focus Step 2 (N-Europe)	Cyprodinil	18.2	25.48	0.71	2170	0.0003272	2569	0.0099
	Prothioconazole	1.38		0.05	1830	0.0000273		
	JAU 6476-desthio	5.9		0.23	6630	0.0000347		
Focus Step 2 (S-Europe)	Cyprodinil	14.9	21.09	0.71	2170	0.0003272	2499	0.0084
	Prothioconazole	1.38		0.07	1830	0.0000383		
	JAU 6476-desthio	4.81		0.23	6630	0.0000347		

ETR: Exposure-Toxicity Ratio; ETRs above the relevant trigger of 0.01 are shown in bold
JAU 6476-desthio is an ecotoxicologically relevant metabolite of prothioconazole.

Fish Chronic Mixture Risk Assessment

Table 9.5-34: Aquatic organisms: acceptability of risk (TER < 0.1) for fish chronic mixture toxicity based on maximum FOCUS Step 1 and 2 calculations for the use of A23282A (cyprodinil/prothioconazole/JAU 6476-desthio) in cereals

Focus Scenario	Test substance	PEC _{gl-max} (µg/L)	PEC _{mix} (µg/L)	Fraction of Substance in PEC _{mix}	NOEC for the substance a.s./L) ^a	Fraction of substance / NOEC for the substance	Calculated NOEC _{mix-CA} (µg a.s./L)	ETR _{mix}
Focus Step 1	Cyprodinil	50.1	96.1	0.52	231	0.002251100	10.5	9.15
	Prothioconazole	16.3		0.17	308	0.000551900		
	JAU 6476-desthio	29.7		0.31	3.34	0.092814400		
Focus Step 2 (N-Europe)	Cyprodinil	18.2	25.48	0.71	231	0.003073600	13.9	1.83
	Prothioconazole	1.38		0.05	308	0.000162300		
	JAU 6476-desthio	5.90		0.23	3.34	0.068862300		
Focus Step 2 (S-Europe)	Cyprodinil	14.9	21.09	0.71	231	0.003073600	13.9	1.52
	Prothioconazole	1.38		0.07	308	0.000227300		
	JAU 6476-desthio	4.81		0.23	3.34	0.068862300		

ETR: Exposure-Toxicity Ratio; ETRs above the relevant trigger of 0.1 are shown in bold. JAU 6476-desthio is an ecotoxicologically relevant metabolite of prothioconazole.

Table 9.5-35: Aquatic organisms: acceptability of risk (TER < 0.1) for fish chronic mixture toxicity based on maximum FOCUS Step 3 calculations for the use of A23282A (cyprodinil/prothioconazole/JAU 6476-desthio) in winter cereals (application at BBCH 30)

Focus Scenario	Test substance	PEC _{gl-max} (µg/L)	PEC _{mix} (µg/L)	Fraction of Substance in PEC _{mix}	NOEC for the substance a.s./L) ^a	Fraction of substance / NOEC for the substance	Calculated NOEC _{mix-CA} (µg a.s./L)	ETR _{mix}
Focus Step 3 D3 Ditch	Cyprodinil	2.84	3.792	0.75	231	0.0032468	246.4	0.015
	Prothioconazole	0.949		0.25	308	0.0008117		
	JAU 6476-desthio	0.003		0	3.34	0.0000000		
Focus Step 3 D4 Pond	Cyprodinil	0.098	0.136	0.72	231	0.0031169	63	0.002
	Prothioconazole	0.033		0.24	308	0.0007792		
	JAU 6476-desthio	0.005		0.04	3.34	0.0119760		
Focus Step 3 D4 Stream	Cyprodinil	2.1	2.805	0.75	231	0.0032468	246.4	0.011
	Prothioconazole	0.701		0.25	308	0.0008117		
	JAU 6476-desthio	0.004		0	3.34	0.0000000		
Focus Step 3 D5 Pond	Cyprodinil	0.098	0.139	0.71	231	0.0030736	45.8	0.003
	Prothioconazole	0.033		0.24	308	0.0007792		
	JAU 6476-desthio	0.008		0.06	3.34	0.0179641		
Focus Step 3 D5 Stream	Cyprodinil	2.27	3.036	0.75	231	0.0032468	246.4	0.012
	Prothioconazole	0.758		0.25	308	0.0008117		
	JAU 6476-desthio	0.008		0	3.34	0.0000000		
Focus Step 3 R1 Pond	Cyprodinil	0.128	0.191	0.67	231	0.0029004	19.5	0.010
	Prothioconazole	0.033		0.17	308	0.0005519		
	JAU 6476-desthio	0.03		0.16	3.34	0.0479042		
Focus Step 3 R1 Stream	Cyprodinil	1.87	2.760	0.68	231	0.0029437	29.7	0.093
	Prothioconazole	0.625		0.23	308	0.0007468		
	JAU 6476-desthio	0.265		0.1	3.34	0.0299401		

Focus Scenario	Test substance	PEC _{gl-max} (µg/L)	PEC _{mix} (µg/L)	Fraction of Substance in PEC _{mix}	NOEC for the substance a.s./L) ^a	Fraction of substance / NOEC for the substance	Calculated NOEC _{mix-CA} (µg a.s./L)	ETR _{mix}
Focus Step 3 R3 Stream	Cyprodinil	2.63	3.831	0.69	231	0.0029870	36.1	0.106
	Prothioconazole	0.878		0.23	308	0.0007468		
	JAU 6476-desthio	0.323		0.08	3.34	0.0239521		
Focus Step 3 R4 Stream	Cyprodinil	1.88	3.985	0.63	231	0.0027273	19.1	0.153
	Prothioconazole	0.628		0.21	308	0.0006818		
	JAU 6476-desthio	0.477		0.16	3.34	0.0479042		

ETR: Exposure-Toxicity Ratio; ETRs above the relevant trigger of 0.1 are shown in bold. JAU 6476-desthio is an ecotoxicologically relevant metabolite of prothioconazole.

Table 9.5-36: Aquatic organisms: acceptability of risk (TER < 0.1) for fish chronic mixture toxicity based on maximum FOCUS Step 3 calculations for the use of A23282A (cyprodinil/prothioconazole/JAU 6476-desthio) in winter cereals (application at BBCH 69)

Focus Scenario	Test substance	PEC _{gl-max} (µg/L)	PEC _{mix} (µg/L)	Fraction of Substance in PEC _{mix}	NOEC for the substance a.s./L) ^a	Fraction of substance / NOEC for the substance	Calculated NOEC _{mix-CA} (µg a.s./L)	ETR _{mix}
Focus Step 3 D3 Ditch	Cyprodinil	2.85	3.811	0.75	231	0.0032468	246.4	0.015
	Prothioconazole	0.952		0.25	308	0.0008117		
	JAU 6476-desthio	0.009		0	3.34	0.0000000		
Focus Step 3 D4 Pond	Cyprodinil	0.098	0.139	0.71	231	0.0030736	45.8	0.003
	Prothioconazole	0.033		0.24	308	0.0007792		
	JAU 6476-desthio	0.008		0.06	3.34	0.0179641		
Focus Step 3 D4 Stream	Cyprodinil	2.46	3.287	0.75	231	0.0032468	246.4	0.013
	Prothioconazole	0.821		0.25	308	0.0008117		
	JAU 6476-desthio	0.006		0	3.34	0.0000000		

Focus Scenario	Test substance	PEC _{gl-max} (µg/L)	PEC _{mix} (µg/L)	Fraction of Substance in PEC _{mix}	NOEC for the substance a.s./L) ^a	Fraction of substance / NOEC for the substance	Calculated NOEC _{mix-CA} (µg a.s./L)	ETR _{mix}
Focus Step 3 D5 Pond	Cyprodinil	0.098	0.139	0.71	231	0.0030736	45.8	0.003
	Prothioconazole	0.033		0.24	308	0.0007792		
	JAU 6476-desthio	0.008		0.06	3.34	0.0179641		
Focus Step 3 D5 Stream	Cyprodinil	2.65	3.546	0.75	231	0.0032468	246.4	0.014
	Prothioconazole	0.886		0.25	308	0.0008117		
	JAU 6476-desthio	0.01		0	3.34	0.0000000		
Focus Step 3 R1 Pond	Cyprodinil	0.148	0.221	0.67	231	0.0029004	27.4	0.008
	Prothioconazole	0.049		0.22	308	0.0007143		
	JAU 6476-desthio	0.024		0.11	3.34	0.0329341		
Focus Step 3 R1 Stream	Cyprodinil	1.88	2.676	0.70	231	0.0030303	46	0.058
	Prothioconazole	0.627		0.23	308	0.0007468		
	JAU 6476-desthio	0.169		0.06	3.34	0.0179641		
Focus Step 3 R3 Stream	Cyprodinil	2.65	3.848	0.69	231	0.0029870	36.1	0.107
	Prothioconazole	0.884		0.23	308	0.0007468		
	JAU 6476-desthio	0.314		0.08	3.34	0.0239521		
Focus Step 3 R4 Stream	Cyprodinil	1.88	2.891	0.65	231	0.0028139	23.6	0.123
	Prothioconazole	0.628		0.22	308	0.0007143		
	JAU 6476-desthio	0.383		0.13	3.34	0.0389222		

ETR: Exposure-Toxicity Ratio; ETRs above the relevant trigger of 0.1 are shown in bold. JAU 6476-desthio is an ecotoxicologically relevant metabolite of prothioconazole.

Table 9.5-37: Aquatic organisms: acceptability of risk (TER < 0.1) for fish chronic mixture toxicity based on maximum FOCUS Step 3 calculations for the use of A23282A (cyprodinil/prothioconazole/JAU 6476-desthio) in spring cereals (application at BBCH 30)

Focus Scenario	Test substance	PEC _{gl-max} (µg/L)	PEC _{mix} (µg/L)	Fraction of Substance in PEC _{mix}	NOEC for the substance a.s./L) ^a	Fraction of substance / NOEC for the substance	Calculated NOEC _{mix-CA} (µg a.s./L)	ETR _{mix}
Focus Step 3 D3 Ditch	Cyprodinil	2.85	3.806	0.75	231	0.0032468	246.4	0.015
	Prothioconazole	0.95		0.25	308	0.0008117		
	JAU 6476-desthio	0.006		0	3.34	0.0000000		
Focus Step 3 D4 Pond	Cyprodinil	0.098	0.138	0.71	231	0.0030736	53.1	0.003
	Prothioconazole	0.033		0.24	308	0.0007792		
	JAU 6476-desthio	0.007		0.05	3.34	0.0149701		
Focus Step 3 D4 Stream	Cyprodinil	2.33	3.112	0.75	231	0.0032468	246.4	0.013
	Prothioconazole	0.777		0.25	308	0.0008117		
	JAU 6476-desthio	0.005		0	3.34	0.0000000		
Focus Step 3 D5 Pond	Cyprodinil	0.098	0.139	0.71	231	0.0030736	45.8	0.003
	Prothioconazole	0.033		0.24	308	0.0007792		
	JAU 6476-desthio	0.008		0.06	3.34	0.0179641		
Focus Step 3 D5 Stream	Cyprodinil	2.39	3.196	0.75	231	0.0032468	246.4	0.013
	Prothioconazole	0.798		0.25	308	0.0008117		
	JAU 6476-desthio	0.008		0	3.34	0.0000000		
Focus Step 3 R4 Stream	Cyprodinil	1.88	2.935	0.64	231	0.0027706	20.7	0.142
	Prothioconazole	0.628		0.21	308	0.0006818		
	JAU 6476-desthio	0.427		0.15	3.34	0.0449102		

ETR: Exposure-Toxicity Ratio; ETRs above the relevant trigger of 0.1 are shown in bold. JAU 6476-desthio is an ecotoxicologically relevant metabolite of prothioconazole.

Table 9.5-38: Aquatic organisms: acceptability of risk (TER < 0.1) for fish chronic mixture toxicity based on maximum FOCUS Step 3 calculations for the use of A23282A (cyprodinil/prothioconazole/JAU 6476-desthio) in spring cereals (application at BBCH 69)

Focus Scenario	Test substance	PEC _{gl-max} (µg/L)	PEC _{mix} (µg/L)	Fraction of substance in PEC _{mix}	NOEC for the substance a.s./L) ^a	Fraction of substance / NOEC for the substance	Calculated NOEC _{mix-CA} (µg a.s./L)	ETR _{mix}
Focus Step 3 D3 Ditch	Cyprodinil	2.85	3.807	0.75	231	0.0032468	246.4	0.015
	Prothioconazole	0.951		0.25	308	0.0008117		
	JAU 6476-desthio	0.006		0	3.34	0.0000000		
Focus Step 3 D4 Pond	Cyprodinil	0.098	0.138	0.71	231	0.0030736	53.1	0.003
	Prothioconazole	0.033		0.24	308	0.0007792		
	JAU 6476-desthio	0.007		0.05	3.34	0.0149701		
Focus Step 3 D4 Stream	Cyprodinil	2.45	3.275	0.75	231	0.0032468	246.4	0.013
	Prothioconazole	0.819		0.25	308	0.0008117		
	JAU 6476-desthio	0.006		0	3.34	0.0000000		
Focus Step 3 D5 Pond	Cyprodinil	0.098	0.139	0.71	231	0.0030736	45.8	0.003
	Prothioconazole	0.033		0.24	308	0.0007792		
	JAU 6476-desthio	0.008		0.06	3.34	0.0179641		
Focus Step 3 D5 Stream	Cyprodinil	2.48	3.318	0.75	231	0.0032468	246.4	0.013
	Prothioconazole	0.829		0.25	308	0.0008117		
	JAU 6476-desthio	0.009		0	3.34	0.0000000		
Focus Step 3 R4 Stream	Cyprodinil	1.88	2.951	0.64	231	0.0027706	20.7	0.143
	Prothioconazole	0.628		0.21	308	0.0006818		
	JAU 6476-desthio	0.443		0.15	3.34	0.0449102		

ETR: Exposure-Toxicity Ratio; ETRs above the relevant trigger of 0.1 are shown in bold. JAU 6476-desthio is an ecotoxicologically relevant metabolite of prothioconazole.

Table 9.5-39: Aquatic organisms: acceptability of risk (TER < 0.1) for fish chronic mixture toxicity based on maximum FOCUS Step 4 calculations for the use of A23282A (cyprodinil/prothioconazole/JAU 6476-desthio) in winter cereals

Focus Step 4 Scenario	Mitigation	Test substance	PEC _{gl-max} (µg/L)	PEC _{mix} (µg/L)	Fraction of Substance in PEC _{mix}	NOEC for the substance (µg a.s./L) ^a	Fraction of substance / NOEC for the substance	Calculated NOEC _{mix-CA} (µg a.s./L)	ETR _{mix}
R3 Stream	5 m Spray Drift Buffer	Cyprodinil	1.02	1.666	0.61	231	0.0026407	16.6	0.100
		Prothioconazole	0.323		0.19	308	0.0006169		
		JAU 6476-desthio	0.323		0.19	3.34	0.0568862		
R3 Stream	5 m Spray Drift Buffer + 50% Drift Reduction	Cyprodinil	0.922	1.406	0.66	231	0.0028571	13.9	0.101
		Prothioconazole	0.161		0.11	308	0.0003571		
		JAU 6476-desthio	0.323		0.23	3.34	0.0688623		
R3 Stream	10 m Spray Drift Buffer	Cyprodinil	0.922	1.416	0.65	231	0.0028139	13.9	0.102
		Prothioconazole	0.171		0.12	308	0.0003896		
		JAU 6476-desthio	0.323		0.23	3.34	0.0688623		
R3 Stream	10 m Spray Drift Buffer + 10 m VFS	Cyprodinil	0.56	0.879	0.64	231	0.0027706	18.4	0.048
		Prothioconazole	0.171		0.19	308	0.0006169		
		JAU 6476-desthio	0.148		0.17	3.34	0.0508982		
R4 Stream	5 m Spray Drift Buffer	Cyprodinil	1.62	2.512	0.64	231	0.0027706	16.6	0.151
		Prothioconazole	0.415		0.17	308	0.0005519		
		JAU 6476-desthio	0.477		0.19	3.34	0.0568862		
R4 Stream	5 m Spray Drift Buffer + 50% Drift Reduction	Cyprodinil	1.62	2.512	0.64	231	0.0027706	16.6	0.151
		Prothioconazole	0.415		0.17	308	0.0005519		
		JAU 6476-desthio	0.477		0.19	3.34	0.0568862		
R4 Stream	10 m Spray Drift Buffer	Cyprodinil	1.62	2.512	0.64	231	0.0027706	16.6	0.151
		Prothioconazole	0.415		0.17	308	0.0005519		
		JAU 6476-desthio	0.477		0.19	3.34	0.0568862		

Focus Step 4 Scenario	Mitigation	Test substance	PEC ^{gl-max} (µg/L)	PEC _{mix} (µg/L)	Fraction of Substance in PEC _{mix}	NOEC for the substance (µg a.s./L) ^a	Fraction of substance / NOEC for the substance	Calculated NOEC _{mix-CA} (µg a.s./L)	ETR _{mix}
R4 Stream	10 m Spray Drift Buffer + 10 m VFS	Cyprodinil	0.736	1.140	0.65	231	0.0028139	16.6	0.069
		Prothioconazole	0.187		0.16	308	0.0005195		
		JAU 6476-desthio	0.217		0.19	3.34	0.0568862		

ETR: Exposure-Toxicity Ratio; ETRs above the relevant trigger of 0.1 are shown in bold. JAU 6476-desthio is an ecotoxicologically relevant metabolite of prothioconazole.

Table 9.5-40: Aquatic organisms: acceptability of risk (TER < 0.1) for fish chronic mixture toxicity based on maximum FOCUS Step 4 calculations for the use of A23282A (cyprodinil/prothioconazole/JAU 6476-desthio) in spring cereals

Focus Step 4 Scenario	Mitigation	Test substance	PEC ^{gl-max} (µg/L)	PEC _{mix} (µg/L)	Fraction of Substance in PEC _{mix}	NOEC for the substance (µg a.s./L) ^a	Fraction of substance / NOEC for the substance	Calculated NOEC _{mix-CA} (µg a.s./L)	ETR _{mix}
R4 Stream	5 m Spray Drift Buffer	Cyprodinil	1.80	2.660	0.68	231	0.0029437	18.4	0.145
		Prothioconazole	0.417		0.16	308	0.0005195		
		JAU 6476-desthio	0.443		0.17	3.34	0.0508982		
R4 Stream	5 m Spray Drift Buffer + 50% Drift Reduction	Cyprodinil	1.80	2.660	0.68	231	0.0029437	18.4	0.145
		Prothioconazole	0.417		0.16	308	0.0005195		
		JAU 6476-desthio	0.443		0.17	3.34	0.0508982		
R4 Stream	10 m Spray Drift Buffer	Cyprodinil	1.80	2.660	0.68	231	0.0029437	18.4	0.145
		Prothioconazole	0.417		0.16	308	0.0005195		
		JAU 6476-desthio	0.443		0.17	3.34	0.0508982		
R4 Stream	10 m Spray Drift Buffer + 10 m VFS	Cyprodinil	0.82	1.209	0.68	231	0.0029437	18.4	0.066
		Prothioconazole	0.188		0.16	308	0.0005195		
		JAU 6476-desthio	0.201		0.17	3.34	0.0508982		

ETR: Exposure-Toxicity Ratio; ETRs above the relevant trigger of 0.1 are shown in bold. JAU 6476-desthio is an ecotoxicologically relevant metabolite of prothioconazole.

Aquatic Plant Mixture Risk Assessment

Table 9.5-41: Aquatic organisms: acceptability of risk (TER < 0.1) for aquatic plant mixture toxicity based on maximum FOCUS Step 1 and 2 calculations for the use of A23282A (cyprodinil/prothioconazole/JAU 6476-desthio) in cereals

Focus Scenario	Test substance	PEC _{gl-max} (µg/L)	PEC _{mix} (µg/L)	Fraction of Substance in PEC _{mix}	LC ₅₀ for the substance (µg a.s./L) ^a	Fraction of substance / LC ₅₀ for the substance	Calculated LC _{50 mix-CA} (µg a.s./L)	ETR _{mix}
Focus Step 1	Cyprodinil	50.1	96.1	0.52	7420	0.0000701	231.3	0.415
	Prothioconazole	16.3		0.17	404	0.0004208		
	JAU 6476-desthio	29.7		0.31	80.9	0.0038319		
Focus Step 2 (N-Europe)	Cyprodinil	18.2	25.48	0.71	7420	0.0000957	326.5	0.078
	Prothioconazole	1.38		0.05	404	0.0001238		
	JAU 6476-desthio	5.9		0.23	80.9	0.0028430		
Focus Step 2 (S-Europe)	Cyprodinil	14.9	21.09	0.71	7420	0.0000957	321.3	0.066
	Prothioconazole	1.38		0.07	404	0.0001733		
	JAU 6476-desthio	4.81		0.23	80.9	0.0028430		

ETR: Exposure-Toxicity Ratio; ETRs above the relevant trigger of 0.1 are shown in bold. JAU 6476-desthio is an ecotoxicologically relevant metabolite of prothioconazole.

Invertebrate Chronic Mixture Risk Assessment

As the invertebrate chronic endpoints used in the cyprodinil risk assessment were based upon higher tier assessments with amended assessment factors, the following equation will be used in each of the invertebrate chronic mixture risk assessments (for the ERO-RAC of 3.33 µg/L and the ETO-RAC of 0.75 0.50 µg/L):

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

if $RQ_{mix} < 1$, the risk is acceptable.

The mixture risk assessments will begin at FOCUS Step 3 due to the outcome of the individual active substance assessments.

Table 9.5-42: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for invertebrate chronic (ERO-RAC) mixture toxicity based on maximum FOCUS Step 3 calculations for the use of A23282A (cyprodinil/prothioconazole/JAU 6476-desthio) in winter cereals (application at BBCH 30)

Step 3 Scenario	Cyprodinil			Prothioconazole			JAU 6476-desthio			RQ _{mix}
	Step 3 PEC _{sw} max (µg/L)	ERO-RAC (µg/L)	PEC/RAC	Step 3 PEC _{sw} max (µg/L)	RAC (µg/L)	PEC/RAC	Step 3 PEC _{sw} max (µg/L)	RAC (µg/L)	PEC/RAC	
D3 Ditch	2.84	3.33	0.85	0.949	56	0.017	0.003	6.4	0.00041	0.87
D4 Pond	0.098	3.33	0.029	0.033	56	0.00058	0.005	6.4	0.00078	0.030
D4 Stream	2.1	3.33	0.63	0.701	56	0.013	0.004	6.4	0.00064	0.64
D5 Pond	0.098	3.33	0.029	0.033	56	0.00059	0.008	6.4	0.0012	0.031
D5 Stream	2.27	3.33	0.68	0.758	56	0.014	0.008	6.4	0.0013	0.70
R1 Pond	0.128	3.33	0.038	0.033	56	0.00059	0.030	6.4	0.0047	0.043
R1 Stream	1.87	3.33	0.56	0.625	56	0.011	0.265	6.4	0.041	0.61
R3 Stream	2.63	3.33	0.79	0.878	56	0.016	0.323	6.4	0.051	0.86
R4 Stream	1.88	3.33	0.56	0.628	56	0.011	0.477	6.4	0.075	0.65

PEC/RAC ratios and RQ_{mix} values above the relevant trigger of 1 are shown in bold. JAU 6476-desthio is an ecotoxicologically relevant metabolite of prothioconazole.

Table 9.5-43: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for invertebrate chronic (ERO) mixture toxicity based on maximum FOCUS Step 3 calculations for the use of A23282A (cyprodinil/prothioconazole/JAU 6476-desthio) in winter cereals (application at BBCH 69)

Step 3 Scenario	Cyprodinil			Prothioconazole			JAU 6476-desthio			RQ _{mix}
	Step 3 PEC _{sw} max (µg/L)	ERO-RAC (µg/L)	PEC/RAC	Step 3 PEC _{sw} max (µg/L)	RAC (µg/L)	PEC/RAC	Step 3 PEC _{sw} max (µg/L)	RAC (µg/L)	PEC/RAC	
D3 Ditch	2.85	3.33	0.86	0.952	56	0.017	0.009	6.4	0.0015	0.88
D4 Pond	0.098	3.33	0.03	0.033	56	0.00059	0.008	6.4	0.0012	0.031
D4 Stream	2.46	3.33	0.74	0.821	56	0.015	0.006	6.4	0.00091	0.76
D5 Pond	0.098	3.33	0.03	0.033	56	0.00059	0.008	6.4	0.0013	0.032
D5 Stream	2.65	3.33	0.80	0.886	56	0.016	0.010	6.4	0.0016	0.82
R1 Pond	0.148	3.33	0.04	0.047	56	0.00083	0.024	6.4	0.0037	0.049
R1 Stream	1.88	3.33	0.56	0.627	56	0.011	0.169	6.4	0.026	0.60
R3 Stream	2.65	3.33	0.79	0.884	56	0.016	0.314	6.4	0.049	0.86
R4 Stream	1.88	3.33	0.56	0.628	56	0.011	0.383	6.4	0.060	0.63

PEC/RAC ratios and RQ_{mix} values above the relevant trigger of 1 are shown in bold. JAU 6476-desthio is an ecotoxicologically relevant metabolite of prothioconazole.

Table 9.5-44: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for invertebrate chronic (ERO-RAC) mixture toxicity based on maximum FOCUS Step 3 calculations for the use of A23282A (cyprodinil/prothioconazole/JAU 6476-desthio) in spring cereals (application at BBCH 30)

Step 3 Scenario	Cyprodinil			Prothioconazole			JAU 6476-desthio			RQ _{mix}
	Step 3 PEC _{sw} max (µg/L)	ERO-RAC (µg/L)	PEC/RAC	Step 3 PEC _{sw} max (µg/L)	RAC (µg/L)	PEC/RAC	Step 3 PEC _{sw} max (µg/L)	RAC (µg/L)	PEC/RAC	
D3 Ditch	2.85	3.33	0.86	0.950	56	0.017	0.006	6.4	0.00086	0.88
D4 Pond	0.098	3.33	0.029	0.033	56	0.001	0.007	6.4	0.0012	0.03
D4 Stream	2.33	3.33	0.70	0.777	56	0.014	0.005	6.4	0.00072	0.71
D5 Pond	0.098	3.33	0.029	0.033	56	0.001	0.008	6.4	0.0012	0.03
D5 Stream	2.39	3.33	0.72	0.798	56	0.014	0.008	6.4	0.0013	0.74
R4 Stream	1.88	3.33	0.56	0.628	56	0.011	0.427	6.4	0.067	0.64

PEC/RAC ratios and RQ_{mix} values above the relevant trigger of 1 are shown in bold. JAU 6476-desthio is an ecotoxicologically relevant metabolite of prothioconazole.

Table 9.5-45: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for invertebrate chronic (ERO-RAC) mixture toxicity based on maximum FOCUS Step 3 calculations for the use of A23282A (cyprodinil/prothioconazole/JAU 6476-desthio) in spring cereals (application at BBCH 69)

Step 3 Scenario	Cyprodinil			Prothioconazole			JAU 6476-desthio			RQ _{mix}
	Step 3 PEC _{sw} max (µg/L)	ERO-RAC (µg/L)	PEC/RAC	Step 3 PEC _{sw} max (µg/L)	RAC (µg/L)	PEC/RAC	Step 3 PEC _{sw} max (µg/L)	RAC (µg/L)	PEC/RAC	
D3 Ditch	2.85	3.33	0.85	0.951	56	0.017	0.006	6.4	0.0010	0.87
D4 Pond	0.098	3.33	0.029	0.033	56	0.00059	0.007	6.4	0.0012	0.03
D4 Stream	2.45	3.33	0.74	0.819	56	0.015	0.006	6.4	0.00089	0.76
D5 Pond	0.098	3.33	0.029	0.033	56	0.001	0.008	6.4	0.0013	0.03

Step 3 Scenario	Cyprodinil			Prothioconazole			JAU 6476-desthio			RQ _{mix}
	Step 3 PEC _{sw} max (µg/L)	ERO-RAC (µg/L)	PEC/RAC	Step 3 PEC _{sw} max (µg/L)	RAC (µg/L)	PEC/RAC	Step 3 PEC _{sw} max (µg/L)	RAC (µg/L)	PEC/RAC	
D5 Stream	2.48	3.33	0.75	0.829	56	0.015	0.009	6.4	0.0014	0.77
R4 Stream	1.88	3.33	0.56	0.628	56	0.011	0.443	6.4	0.069	0.64

PEC/RAC ratios and RQ_{mix} values above the relevant trigger of 1 are shown in bold. JAU 6476-desthio is an ecotoxicologically relevant metabolite of prothioconazole.

Table 9.5-46: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for invertebrate chronic (ETO-RAC) mixture toxicity based on maximum FOCUS Step 3 calculations for the use of A23282A (cyprodinil/prothioconazole/JAU 6476-desthio) in winter cereals (application at BBCH 30)

Step 3 Scenario	Step 3 PEC _{sw} max (µg/L)	Cyprodinil				Prothioconazole			JAU 6476-desthio			RQ _{mix}	RQ _{mix}
		ETO-RAC (µg/L)	PEC/RAC	ETO-RAC (µg/L)	PEC/RAC	Step 3 PEC _{sw} max (µg/L)	RAC (µg/L)	PEC/RAC	Step 3 PEC _{sw} max (µg/L)	RAC (µg/L)	PEC/RAC		
D3 Ditch	2.84	0.75	3.8	0.50	5.68	0.949	56	0.017	0.003	6.4	0.00041	3.8	5.70
D4 Pond	0.098	0.75	0.131	0.50	0.196	0.033	56	0.00058	0.005	6.4	0.00078	0.13	0.20
D4 Stream	2.1	0.75	2.8	0.50	4.2	0.701	56	0.013	0.004	6.4	0.00064	2.8	4.21
D5 Pond	0.098	0.75	0.13	0.50	0.196	0.033	56	0.00059	0.008	6.4	0.0012	0.13	0.20
D5 Stream	2.27	0.75	3.0	0.50	4.54	0.758	56	0.014	0.008	6.4	0.0013	3.1	4.56
R1 Pond	0.128	0.75	0.171	0.50	0.256	0.033	56	0.00059	0.030	6.4	0.0047	0.18	0.26
R1 Stream	1.87	0.75	2.5	0.50	3.74	0.625	56	0.011	0.265	6.4	0.041	2.5	3.79
R3 Stream	2.63	0.75	3.5	0.50	5.26	0.878	56	0.016	0.323	6.4	0.051	3.6	5.33
R4 Stream	1.88	0.75	2.5	0.50	3.76	0.628	56	0.011	0.477	6.4	0.075	2.6	3.85

PEC/RAC ratios and RQ_{mix} values above the relevant trigger of 1 are shown in bold. JAU 6476-desthio is an ecotoxicologically relevant metabolite of prothioconazole.

Table 9.5-47: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for invertebrate chronic (ETO-RAC) mixture toxicity based on maximum FOCUS Step 3 calculations for the use of A23282A (cyprodinil/prothioconazole/JAU 6476-desthio) in winter cereals (application at BBCH 69)

Step 3 Scenario	Step 3 PEC _{sw max} (µg/L)	Cyprodinil				Prothioconazole			JAU 6476-desthio			RQ _{mix}	RQ _{mix}
		ETO-RAC (µg/L)	PEC/RAC	ETO-RAC (µg/L)	PEC/RAC	Step 3 PEC _{sw max} (µg/L)	RAC (µg/L)	PEC/RAC	Step 3 PEC _{sw max} (µg/L)	RAC (µg/L)	PEC/RAC		
D3 Ditch	2.85	0.75	3.8	0.50	5.7	0.952	56	0.017	0.009	6.4	0.0015	3.8	5.72
D4 Pond	0.098	0.75	0.13	0.50	0.196	0.033	56	0.00059	0.008	6.4	0.0012	0.13	0.20
D4 Stream	2.46	0.75	3.3	0.50	4.92	0.821	56	0.015	0.006	6.4	0.00091	3.3	4.94
D5 Pond	0.098	0.75	0.13	0.50	0.196	0.033	56	0.00059	0.008	6.4	0.0013	0.13	0.20
D5 Stream	2.65	0.75	3.5	0.50	5.3	0.886	56	0.016	0.010	6.4	0.0016	3.6	5.32
R1 Pond	0.148	0.75	0.20	0.50	0.296	0.047	56	0.00083	0.024	6.4	0.0037	0.20	0.30
R1 Stream	1.88	0.75	2.5	0.50	3.76	0.627	56	0.011	0.169	6.4	0.026	2.5	3.80
R3 Stream	2.65	0.75	3.5	0.50	5.3	0.884	56	0.016	0.314	6.4	0.049	3.6	5.37
R4 Stream	1.88	0.75	2.5	0.50	3.76	0.628	56	0.011	0.383	6.4	0.060	2.6	3.83

PEC/RAC ratios and RQ_{mix} values above the relevant trigger of 1 are shown in bold. JAU 6476-desthio is an ecotoxicologically relevant metabolite of prothioconazole.

Table 9.5-48: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for invertebrate chronic (ETO-RAC) mixture toxicity based on maximum FOCUS Step 3 calculations for the use of A23282A (cyprodinil/prothioconazole/JAU 6476-desthio) in spring cereals (application at BBCH 30)

Step 3 Scenario	Step 3 PEC _{sw max} (µg/L)	Cyprodinil				Prothioconazole			JAU 6476-desthio			RQ _{mix}	RQ _{mix}
		ETO-RAC (µg/L)	PEC/RAC	ETO-RAC (µg/L)	PEC/RAC	Step 3 PEC _{sw max} (µg/L)	RAC (µg/L)	PEC/RAC	Step 3 PEC _{sw max} (µg/L)	RAC (µg/L)	PEC/RAC		
D3 Ditch	2.85	0.75	3.8	0.50	5.7	0.950	56	0.017	0.006	6.4	0.00086	3.8	5.72
D4 Pond	0.098	0.75	0.13	0.50	0.196	0.033	56	0.00059	0.007	6.4	0.0012	0.13	0.20
D4 Stream	2.33	0.75	3.1	0.50	4.66	0.777	56	0.014	0.005	6.4	0.00072	3.1	4.67
D5 Pond	0.098	0.75	0.13	0.50	0.196	0.033	56	0.00059	0.008	6.4	0.0012	0.13	0.20
D5 Stream	2.39	0.75	3.2	0.50	4.78	0.798	56	0.014	0.008	6.4	0.0013	3.2	4.80
R4 Stream	1.88	0.75	2.5	0.50	3.76	0.628	56	0.011	0.427	6.4	0.067	2.6	3.84

PEC/RAC ratios and RQ_{mix} values above the relevant trigger of 1 are shown in bold. JAU 6476-desthio is an ecotoxicologically relevant metabolite of prothioconazole.

Table 9.5-49: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for invertebrate chronic (ETO-RAC) mixture toxicity based on maximum FOCUS Step 3 calculations for the use of A23282A (cyprodinil/prothioconazole/JAU 6476-desthio) in spring cereals (application at BBCH 69)

Step 3 Scenario	Step 3 PEC _{sw max} (µg/L)	Cyprodinil				Prothioconazole			JAU 6476-desthio			RQ _{mix}	RQ _{mix}
		ETO-RAC (µg/L)	PEC/RAC	ETO-RAC (µg/L)	PEC/RAC	Step 3 PEC _{sw max} (µg/L)	RAC (µg/L)	PEC/RAC	Step 3 PEC _{sw max} (µg/L)	RAC (µg/L)	PEC/RAC		
D3 Ditch	2.85	0.75	3.8	0.50	5.7	0.951	56	0.017	0.006	6.4	0.00096	3.8	5.72
D4 Pond	0.098	0.75	0.13	0.50	0.196	0.033	56	0.00059	0.007	6.4	0.0012	0.13	0.20
D4 Stream	2.45	0.75	3.3	0.50	4.9	0.819	56	0.015	0.006	6.4	0.00089	3.3	4.92

Step 3 Scenario	Step 3 PEC _{sw max} (µg/L)	Cyprodinil				Prothioconazole			JAU 6476-desthio			RQ _{mix}	RQ _{mix}
		ETO-RAC (µg/L)	PEC/RAC	ETO-RAC (µg/L)	PEC/RAC	Step 3 PEC _{sw max} (µg/L)	RAC (µg/L)	PEC/RAC	Step 3 PEC _{sw max} (µg/L)	RAC (µg/L)	PEC/RAC		
D5 Pond	0.098	0.75	0.13	0.50	0.196	0.033	56	0.00059	0.008	6.4	0.0013	0.13	0.20
D5 Stream	2.48	0.75	3.3	0.50	4.96	0.829	56	0.015	0.009	6.4	0.0014	3.3	4.98
R4 Stream	1.88	0.75	2.5	0.50	3.76	0.628	56	0.011	0.443	6.4	0.069	2.6	3.84

PEC/RAC ratios and RQ_{mix} values above the relevant trigger of 1 are shown in bold. JAU 6476-desthio is an ecotoxicologically relevant metabolite of prothioconazole.

Table 9.5-50: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for invertebrate chronic (ETO-RAC) mixture toxicity based on maximum FOCUS Step 4 calculations for the use of A23282A (cyprodinil/prothioconazole/JAU 6476-desthio) in winter cereals

Scenario	Spray Drift Buffer (m)	Nozzle reduction (%)	VFS (m)	Cyprodinil					Prothioconazole			JAU 6476-desthio			RQ _{mix}	RQ _{mix}
				Step 4 PEC _{sw max} (µg/L)	ETO-RAC (µg/L)	PEC/RAC	ETO-RAC (µg/L)	PEC/RAC	Step 4 PEC _{sw max} (µg/L)	RAC (µg/L)	PEC/RAC	Step 4 PEC _{sw max} (µg/L)	RAC (µg/L)	PEC/RAC		
D3 Ditch	5	0	0	0.811	0.75	1.1	0.50	1.62	0.258	56	0.0046	0.003	6.4	0.00047	1.1	1.63
	5	50	0	0.483	0.75	0.64	0.50	0.97	0.129	56	0.0023	0.001	6.4	0.00016	0.64	0.97
	10	0	0	0.466	0.75	0.62	0.50	0.93	0.137	56	0.0024	0.001	6.4	0.00016	0.62	0.93
D4 Stream	5	0	0	0.930	0.75	1.2	0.50	1.86	0.300	56	0.0054	0.004	6.4	0.00063	1.25	1.87
	5	50	0	0.495	0.75	0.66	0.50	0.99	0.150	56	0.0027	0.004	6.4	0.00063	0.66	0.99
	10	0	0	0.507	0.75	0.68	0.50	1.01	0.159	56	0.0028	0.004	6.4	0.00063	0.68	1.01
	10	50	0	0.254			0.50	0.51	0.080	56	0.0014	0.002	6.4	0.00031		0.01
D5 Stream	5	0	0	0.991	0.75	1.3	0.50	1.98	0.324	56	0.0058	0.004	6.4	0.00063	1.3	1.99
	5	50	0	0.527	0.75	0.70	0.50	1.05	0.162	56	0.0029	0.002	6.4	0.00031	0.70	1.05
	10	0	0	0.540	0.75	0.72	0.50	1.08	0.172	56	0.0031	0.002	6.4	0.00031	0.72	1.08

Scenario	Spray Drift Buffer (m)	Nozzle reduction (%)	VFS (m)	Cyprodinil					Prothioconazole			JAU 6476-desthio			RQ _{mix}	RQ _{mix}
				Step 4 PEC _{sw} max (µg/L)	ETO-RAC (µg/L)	PEC/RAC	ETO-RAC (µg/L)	PEC/RAC	Step 4 PEC _{sw} max (µg/L)	RAC (µg/L)	PEC/RAC	Step 4 PEC _{sw} max (µg/L)	RAC (µg/L)	PEC/RAC		
	10	50	0	0.270			0.50	0.54	0.086	56	0.0015	0.001	6.4	0.00016		0.54
R1 Stream	5	0	0	0.745	0.75	0.99	0.50	1.49	0.229	56	0.0041	0.265	6.4	0.041	0.99	1.54
	10	0	10	0.409			0.50	0.82	0.122	56	0.0022	0.121	6.4	0.019		0.84
R3 Stream	5	0	0	1.020	0.75	1.4	0.50	2.04	0.323	56	0.0058	0.323	6.4	0.050	1.4	2.10
	5	50	0	0.922	0.75	1.2	0.50	1.84	0.161	56	0.0029	0.323	6.4	0.050	1.2	1.89
	10	0	0	0.922	0.75	1.2	0.50	1.84	0.171	56	0.0031	0.323	6.4	0.050	1.2	1.89
	10	0	10	0.560	0.75	0.75	0.50	1.12	0.171	56	0.0031	0.148	6.4	0.023	0.75	1.15
	20	0	20	0.295			0.50	0.59	0.089	56	0.0016	0.077	6.4	0.012		0.60
R4 Stream	5	0	0	1.620	0.75	2.2	0.50	3.24	0.415	56	0.0074	0.477	6.4	0.075	2.2	3.32
	5	50	0	1.620	0.75	2.2	0.50	3.24	0.415	56	0.0074	0.477	6.4	0.075	2.2	3.32
	10	0	0	1.620	0.75	2.2	0.50	3.24	0.415	56	0.0074	0.477	6.4	0.075	2.2	3.32
	10	0	10	0.736	0.75	0.98	0.50	1.47	0.187	56	0.0033	0.217	6.4	0.034	0.98	1.51
	20	0	20	0.386			0.50	0.19	0.098	56	0.0018	0.114	6.4	0.018		0.21

PEC/RAC ratios and RQ_{mix} values above the relevant trigger of 1 are shown in bold. JAU 6476-desthio is an ecotoxicologically relevant metabolite of prothioconazole.

Table 9.5-51: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for invertebrate chronic (ETO-RAC) mixture toxicity based on maximum FOCUS Step 4 calculations for the use of A23282A (cyprodinil/prothioconazole/JAU 6476-desthio) in spring cereals

Scenario	Spray Drift Buffer (m)	Nozzle reduction (%)	VFS (m)	Cyprodinil					Prothioconazole			JAU 6476-desthio			RQ _{mix}	RQ _{mix}
				Step 4 PEC _{sw} max (µg/L)	ETO-RAC (µg/L)	PEC/RAC	ETO-RAC (µg/L)	PEC/RAC	Step 4 PEC _{sw} max (µg/L)	RAC (µg/L)	PEC/RAC	Step 4 PEC _{sw} max (µg/L)	RAC (µg/L)	PEC/RAC		
D3 Ditch	5	0	0	0.805	0.75	1.1	0.50	1.61	0.258	56	0.0046	0.002	6.4	0.00031	1.1	1.61
	5	50	0	0.474	0.75	0.63	0.50	0.948	0.129	56	0.0023	0.001	6.4	0.00016	0.63	0.95
	10	0	0	0.457	0.75	0.61	0.50	0.914	0.137	56	0.0024	0.001	6.4	0.00016	0.61	0.92
D4 Stream	5	0	0	0.927	0.75	1.2	0.50	1.854	0.299	56	0.0053	0.005	6.4	0.00078	1.3	1.86
	5	50	0	0.495	0.75	0.66	0.50	0.99	0.150	56	0.0027	0.005	6.4	0.00078	0.66	0.99
	10	0	0	0.505	0.75	0.67	0.50	1.01	0.159	56	0.0028	0.005	6.4	0.00078	0.67	1.01
	10	50	0	0.253			0.50	0.506	0.080	56	0.0014	0.003	6.4	0.00047		0.51
D5 Stream	5	0	0	0.945	0.75	1.3	0.50	1.89	0.303	56	0.0054	0.003 0.010	6.4	0.00047 0.00156	1.3	1.90
	5	50	0	0.492	0.75	0.66	0.50	0.984	0.151	56	0.0027	0.002 0.005	6.4	0.00031 0.00078	0.66	0.99
	10	0	0	0.510	0.75	0.68	0.50	1.02	0.161	56	0.0029	0.002 0.005	6.4	0.00031 0.00078	0.68	1.02
	10	50	0	0.255			0.50	0.51	0.081	56	0.0014	0.002	6.4	0.00031		0.51
R4 Stream	5	0	0	1.80	0.75	2.4	0.50	3.6	0.417	56	0.0074	0.443	6.4	0.069	2.4	3.68
	5	50	0	0.90	0.75	2.4	0.50	3.6	0.417	56	0.0074	0.443	6.4	0.069	2.4	3.68
	10	0	0	1.80	0.75	2.4	0.50	3.6	0.417	56	0.0074	0.443	6.4	0.069	2.4	3.68
	10	0	10 m	0.820	0.75	1.1	0.50	1.64	0.188	56	0.0034	0.201	6.4	0.0314	1.1	1.67
	20	0	20 m	0.430	0.75	0.57	0.50	0.86	0.098	56	0.0018	0.106	6.4	0.0166	0.57	0.88

PEC/RAC ratios and RQ_{mix} values above the relevant trigger of 1 are shown in bold. JAU 6476-desthio is an ecotoxicologically relevant metabolite of prothioconazole.

Mitigation Requirements

Table 9.5-52: Aquatic organisms: mitigation requirements / options for A23282A (based upon mixture toxicity)

Scenario	Winter Cereals		Spring Cereals	
	Fish prolonged	Inverteb. prolonged (using Cyprodinil ETO-RAC)	Fish prolonged	Inverteb. prolonged (using Cyprodinil ETO-RAC)
D3 Ditch	-	5 m SD + 50 % DR or 10 m SD	-	5 m SD + 50 % DR or 10 m SD
D4 Stream	-	5 m SD + 50 % DR or 10 m SD	-	5 m SD + 50 % DR or 10 m SD
D5 Stream	-	5 m SD + 50 % DR or 10 m SD 10 m SD + 50 % DR or 20 m SD	-	5 m SD + 50 % DR or 10 m SD 10 m SD + 50 % DR or 20 m SD
R1 Stream	-	5 m SD 10 m SD + 10 m RO	-	-
R3 Stream	10 m SD + 10 m RO	10 m SD + 10 m RO 20 m SD + 20 m RO	-	-
R4 Stream	10 m SD + 10 m RO	10 m SD + 10 m RO 20 m SD + 20 m RO	10 m SD + 10 m RO	20 m SD + 20 m RO

“-“mitigation measures are not required

SD = spray drift buffer

RO = run-off mitigation

DR = drift reducing nozzles

9.5.3 Overall conclusions

Cyprodinil

The PEC/RAC ratios, using worst-case PEC_{SW} values for fish (acute and chronic), invertebrates (acute), algae, macrophytes and sediment dwellers are less than the trigger value of 1, indicating that the risk to these groups of aquatic organisms is acceptable following use of A23282A in accordance with the proposed use pattern.

An acceptable long-term risk to invertebrates from exposure to cyprodinil is achieved if the below listed mitigation options are implemented (see Table 9.5-53 and Table 9.5-54).

Prothioconazole

The PEC/RAC ratios, using worst-case PEC_{SW} values, are less than the trigger value of 1, indicating that the risk is acceptable following use of A23282A in accordance with the proposed use pattern.

Metabolites

The PEC/RAC ratios, using worst-case PEC_{SW} values for metabolites of cyprodinil and prothioconazole, except for the prothioconazole metabolite JAU 6476-desthio, are less than the trigger value of 1, indicating that the risk to aquatic organisms for the metabolites is acceptable following use of A23282A in accordance

with the proposed mitigation.

An acceptable long-term risk to fish from exposure to JAU 6476-desthio is achieved if the below listed mitigation options are implemented (see Table 9.5-53 and Table 9.5-54, mitigations options required for cyprodinil are also achieving an acceptable risk for Metabolite JAU 6476-desthio).

The mitigation required for safe use has been consolidated into one table for each crop.

Table 9.5-53: Aquatic organisms: mitigation requirements / options for A23282A following use in winter cereals

FOCUS Scenario	Fish chronic	Invertebrate chronic (using Cyprodinil ETO-RAC)
D3 Ditch	-	5 m SD + 50 % DR or 10 m SD
D4 Stream	-	5 m SD + 50 % DR or 10 m SD
D5 Stream	-	5 m SD + 50 % DR or 10 m SD 10 m SD + 50 % DR or 20 m SD
R1 Stream	-	5 m SD 10 m SD + 10 m RO
R3 Stream	10 m SD + 10-12 m RO	10 m SD + 10 m RO 20 m SD + 20 m RO
R4 Stream	10 m SD + 10-12 m RO	10 m SD + 10 m RO 20 m SD + 20 m RO

--"mitigation measures are not required

SD = spray drift buffer

RO = run-off mitigation

DR = drift reducing nozzles

Table 9.5-54: Aquatic organisms: mitigation requirements / options for A23282A following use in spring cereals

FOCUS Scenario	Fish chronic	Invertebrate chronic (using Cyprodinil ETO-RAC)
D3 Ditch	-	5 m SD + 50 % DR or 10 m SD
D4 Stream	-	5 m SD + 50 % DR or 10 m SD
D5 Stream	-	5 m SD + 50 % DR or 10 m SD 10 m SD + 50 % DR or 20 m SD
R4 Stream	10 m SD + 10-12 m RO	20 m SD + 20 m RO

An empty/grey field means that the scenario is not relevant to the crop group

--"mitigation measures are not required

SD = spray drift buffer

A table indicating the percentage reduction required to achieve an acceptable risk to aquatic organisms are presented below.

Table 9.5-55: Aquatic organisms: percentage reduction of entry by cyprodinil into surface water to achieve acceptable risk when considering the ETO RAC of 0.75 µg/L

Scenario	Winter cereals		Spring cereals	
	BBCH 30	BBCH 69	BBCH 30	BBCH 69
	ETO RAC: 0.75 µg/L			
D3 Ditch	73.6	73.7	73.7	73.7
D4 Stream	64.3	69.5	67.8	69.4
D5 Stream	67.0	71.7	68.6	69.8
R1 Stream	59.9	60.1	-	-
R3 Stream	71.5	71.7	-	-
R4 Stream	60.1	60.1	60.1	60.1

- These scenarios are not relevant for spring cereals

A23282A and Mixture Toxicity

Cyprodinil was identified as the single driver of toxicity for both the invertebrate acute and algal formulation assessments, therefore a mixture toxicity risk assessment was not required for these groups, and the single substance assessments for cyprodinil should be referred to.

For sediment dwellers, there was not sufficient data to perform a mixture toxicity assessment.

The mixture Exposure/Toxicity Ratios, using worst-case PEC_{SW} values, for fish (acute and chronic) and macrophytes are less than the relevant trigger value, indicating that the risk to aquatic organisms for the mixture of cyprodinil, prothioconazole and JAU 6476-desthio is acceptable following use of A23282A in accordance with the proposed mitigation.

The RQ_{mix}, using worst-case PEC_{SW} values, for invertebrates (chronic) are less than 1, indicating that the chronic risk to invertebrates for the mixture of cyprodinil, prothioconazole and JAU 6476-desthio is acceptable following use of A23282A in accordance with the proposed mitigation.

An acceptable long-term risk to fish and invertebrates from exposure to the mixture of cyprodinil, prothioconazole and JAU 6476-desthio is achieved if the below listed mitigation options are implemented (see Table 9.5-53 and Table 9.5-54).

9.6 Effects on bees (KCP 10.3.1)

zRMS Comments:	<p>The submitted risk assessment is based new EU guidance (2013). As this guidance is not obligatory yet, the risk assessment following this approach will be considered at the Member State level according to national requirements. This approach was not evaluated.</p> <p>New studies for acute and chronic toxicity for formulation were submitted and accepted. The EU agreed endpoints and accepted endpoints from submitted studies were used in risk assessment performed in accordance with the SANCO guidance, 2002,</p> <p>The acute risk assessment is presented in the tables below:</p>
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Acute oral exposure of A23282A			
Test substance	Application rate (g/ha)	Oral LD ₅₀ (µg/bee)	Hazard quotient
Cyprodinil	450	> 112.5	< 4.0
Prothioconazole	150	> 71	< 2.1
A23282A	1986	445	4.5
calculated based on an application rate of 2.0 L/ha and density of 0.993 kg/m ³ of A23282A			
Acute contact exposure of A23282A			
Test substance	Application rate (g/ha)	Contact LD ₅₀ (µg/bee)	Hazard quotient
Cyprodinil	450	> 75	< 6.0
Prothioconazole	150	> 200	< 0.75
A23282A	1986	645	3.1
<p>The hazard quotients are below the trigger value of 50 considering SANCO guidance indicating that the active substances and formulation pose an acceptable acute risk to bees.</p> <p>The chronic risk assessment was not performed.</p> <p>Therefore, an acceptable risk to bees is expected from the application of A23282A.</p> <p>Taking into consideration the acute toxicity of formulation to bees and non-target arthropods other than bees, the negative effect on larvae pupation is not expected.</p>			

9.6.1 Toxicity data

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

Effects on bees of A23282A were not evaluated as part of the EU assessment of cyprodinil and prothioconazole. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

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

Species	Substance	Exposure System	Results	Reference
<i>Apis mellifera</i> (adults)	Cyprodinil (tested as UNIX 75 WG)	Oral	LD ₅₀ = >112.5 µg a.s./bee	EFSA Scientific Report (2005) 51, 1-78; Schmitzer (1995); Report No. 541036 CGA219417/0771; VV-370443

Species	Substance	Exposure System	Results	Reference
<i>Apis mellifera</i> (adults)	Cyprodinil (tested as A8637C)	Oral	LD₅₀ = >125 µg a.s./bee	EFSA Scientific Report (2005) 51, 1-78; Candolfi (1995); Report No. 95-053-1008 CGA219417/0375; VV-370665
<i>Apis mellifera</i> (adults)	Cyprodinil (tested as A8637C)	Contact	LD₅₀ = >125 µg a.s./bee	EFSA Scientific Report (2005) 51, 1-78; Candolfi (1995); Report No. 95-053-1008 CGA219417/0375; VV-370665
<i>Apis mellifera</i> (adults)	Cyprodinil	Contact	LD₅₀ = >784 µg a.s./bee	EFSA Scientific Report (2005) 51, 1-78; Boeri & Ward (1995); Report No. 664-CG CGA219417/0532; VV-370506
<i>Apis mellifera</i> (adults)	Cyprodinil (tested as UNIX 75 WG)	Contact	LD₅₀ = >75 µg a.s./bee	EFSA Scientific Report (2005) 51, 1-78; Schmitzer (1995); Report No. 541036 CGA219417/0771; VV-370443
<i>Apis mellifera</i> (adults)	Cyprodinil (tested as A14325E)	Oral Adult Chronic	10d LDD₅₀ = 69.7 µg a.s./bee/day NOED = 44.2 µg consumed a.s./bee	Ruhland (2014); Report No. 14-10-48-147-B A14325E_10065; VV-410413
<i>Apis mellifera</i> (larvae)	Cyprodinil (tested as A8637C)	Oral Larval Development	NOED = 13.3 a.s./larva/development period	Kleebaum (2014); Report No. 14-10-48-145-B A8637C_10330; VV-411059
Higher-tier studies (tunnel test, field studies): not required				

Table 9.6-2: Endpoints and effect values relevant for the risk assessment for bees - prothioconazole

Species	Substance	Exposure System	Results	Reference
<i>Apis mellifera</i>	Prothioconazole	Oral	LD₅₀ > 71 µg a.s./bee	EFSA Conclusion 2007
<i>Apis mellifera</i>	Prothioconazole	Contact	LD₅₀ > 200 µg a.s./bee	EFSA Conclusion 2007

Species	Substance	Exposure System	Results	Reference
<i>Bombus terrestris</i>	Prothioconazole	Oral	LD₅₀ > 214.32 µg a.s./bee	Kling, 2016 Document No: M-557946-01-1 Refer to the EFSA request for additional information for prothioconazole
<i>Bombus terrestris</i>	Prothioconazole	Contact	LD₅₀ > 100 µg a.s./bee	Pfeiffer, 2015 Document No: M-521802-01-1 Refer to RAR for prothioconazole
<i>Apis mellifera</i>	IAU-6476-desthio	Oral	LD₅₀ > 106.5 µg/bee	Sekine, 2015 Report No: M-528139-01-1
<i>Apis mellifera</i>	IAU-6476-desthio	Contact	LD₅₀ > 100 µg/bee	Refer to RAR for prothioconazole
<i>Apis mellifera</i>	Prothioconazole (tested as formulation SC-480 G)	Oral Adult-Chronic	10d-LDD₅₀ > 3.8 µg a.s./bee/day 10d-NOEDD = 3.8 µg a.s./bee/day	Pfeiffer, 2015 Document No: M-528888-01-1 Refer to RAR for prothioconazole
<i>Apis mellifera</i>	Prothioconazole (tested as formulation SC-480 G)	Oral Adult-Chronic	10d-LDD₅₀ = 40.5 µg a.s./bee/day 10d-NOEDD = 23.3 µg a.s./bee/day	Ruhland, 2018 Document No: M-631888-01-1 Refer to the EFSA request for additional information for prothioconazole
<i>Apis mellifera</i>	Prothioconazole	Oral Larval-Development	22d-NOED > 25 µg a.s./larva/development period	Kleebaum, 2018 Document No: M-631888-01-1 Refer to the EFSA request for additional information for prothioconazole
Higher-tier studies (tunnel test, field studies): not required				

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

Table 9.6-3: Endpoints and effect values relevant for the risk assessment for bees – A23282A

Species	Substance	Exposure system	Results	Reference
<i>Apis mellifera</i>	A23282A	Contact	LD₅₀ = 645 µg A23282A/bee	Franke M, 2021; Report No. 21 48 BAA 0103 VV-932551
<i>Apis mellifera</i>	A23282A	Oral	LD₅₀ = 445 µg A23282A/bee	
<i>Apis mellifera</i>	A23282A	Oral Adult Chronic	10d LDD₅₀ = 43.8 µg A23282A/bee/day	Ripperger D, 2021; Report No. S21-2794 VV-946992
<i>Apis mellifera</i>	A23282A	Oral Larval Development	22d NOED = 69.3 µg A23282A/larva/development period	Ripperger D, 2021; Report No. S21-2796 VV-947029

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

Table 9.6-4: Endpoints and effect values relevant for the risk assessment for bees - cyprodinil/prothioconazole mixture

Exposure system	Species	Proposed mixture endpoint	Reference
Acute contact	<i>Apis mellifera</i>	LD ₅₀ mix >1500 µg product/bee	Refer to 9.6.1.1
Acute oral		LD ₅₀ mix > 348 µg product/bee	
Adult chronic		LDD ₅₀ mix = 196 µg product/bee	
Larval chronic		NOED = 50.3 µg product/larva/development period	

9.6.1.1 Justification for new endpoints

New studies are available for formulation A23282A which are required to fulfil the data requirements for plant protection products in accordance with Regulation (EC) No 1107/2009.

Since Annex I Submission/inclusion, new acute and chronic bee studies have been conducted with cyprodinil and prothioconazole in accordance with Regulation (EC) No 1107/2009, and as a result there are new endpoints for use in the risk assessment. These studies are summarised in Table 9.6-1 and Table 9.6-2.

Acute and chronic mixture toxicity

According to the draft (EFSA Journal 2014;11(7):3295) combined action of several toxicants must be specifically considered in the risk assessment when it is obvious that such exposure situations will occur for bees.

For the assessment of effects, surrogate endpoints can be calculated. The EFSA Guidance Document indicates that the following equation should be used for deriving a surrogate endpoint for a mixture of active substances with known toxicity assuming dose additivity:

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

where:

X (a.s.i) = fraction of active substance (i) in the formulation mixture

LD₅₀ (a.s.i) = acute toxicity for the active substance (i)

The mixture endpoints are summarised in the table below.

Table 9.6-5: Endpoints for the Cyprodinil/Prothioconazole mixture

Exposure system	Test substance	Concentration of active substance in formulation A23282A (g/L)	Fraction of active substance in the formulation mixture ^a	Toxicity endpoint (µg a.s./bee)	Predicted endpoint for mixture (µg a.s./bee)	Predicted endpoint for mixture (µg product/bee) ^b
Acute contact	Cyprodinil	225	0.75	>784 >112.5	>453 >126.3	>1500
	Prothioconazole	75	0.25	>200		
	Total	300	1	-		
Acute oral	Cyprodinil	225	0.75	>125	>105	>348
	Prothioconazole	75	0.25	>71		
	Total	300	1	-		
Adult chronic	Cyprodinil	225	0.75	69.7	59.1	196
	Prothioconazole	75	0.25	40.5		
	Total	300	1	-		
Larval chronic	Cyprodinil	225	0.75	13.3	15.2	50.3
	Prothioconazole	75	0.25	>25		
	Total	300	1	-		

^a Concentration of an active substance in the formulation divided by the total concentration of all active substances in the formulation.

^b Used for comparison with measured toxicity of product; density of product is taken into account.

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

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

Where:

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

EC_x or NOEC(a.s.i) = acute toxicity value for active substance [i]

EC_x or NOEC (mix) = measured acute toxicity value for the mixture.

A greater value on the right side of the equation indicates that the formulation is more toxic than predicted from the toxicity of the individual components (active substances and co-formulants of known toxicity).

If endpoints are available from both active substance and formulation studies, then the lower endpoint should be used in the risk assessment. Dismissing the EC₅₀ of the formulation from the risk assessment would only be acceptable at a Higher Tier if any observed greater toxicity in the test could be clearly and unambiguously ascribed to a factor that would not be relevant under environmental exposure conditions.

If, in contrast, the measured toxicity of a formulation is lower than predicted, the predicted mixture toxicity should be used in the first-tier risk assessment, together with appropriate exposure estimates.

Table 9.6-6: Comparison of Experimental and Predicted Endpoints for the cyprodinil/prothioconazole mixture

Exposure system	Test substance	Fraction of active substance in formulation A23282A	Active substance toxicity endpoints (µg a.s./bee)	Sum of Active substance fraction/ active substance endpoint	1 / Formulation endpoint (µg product/bee)	Endpoint to be used in the mixture risk assessment
Acute contact	Cyprodinil	0.75	>784 >112.5	<0.00211 <0.00792	0.00155	Formulation Toxicity
	Prothioconazole	0.25	>200			
	Total	1	-			
Acute oral	Cyprodinil	0.75	>125	<0.00952	0.00225	Formulation Toxicity
	Prothioconazole	0.25	>71			
	Total	1	-			
Adult chronic	Cyprodinil	0.75	69.7	0.0169	0.0228	Formulation Toxicity
	Prothioconazole	0.25	40.5			
	Total	1	-			
Larval chronic	Cyprodinil	0.75	13.3	<0.0664	0.0144	Predicted toxicity
	Prothioconazole	0.25	>25			
	Total	1	-			

As is clear, endpoints derived for the product is lower than for the predicted toxicity for three out of four endpoints. However, as a conservative approach risk assessment will be conducted using both the predicted and measured (using product endpoints) toxicity.

9.6.2 Risk assessment

For the purposes of this risk assessment, the evaluation of the risk for honeybees was performed in accordance with the principles of the “EFSA Guidance Document on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees) “(EFSA Journal 2013;11(7):3295 – updated 2014)”.

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

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

All screening step calculations were performed using the EFSA Bee calculator Tool (Bee-Tool v.3; Date accessed: 13 Jan 2022) available at <http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2013.3295/full>.

Where the screening step indicates a potential risk for acute or chronic exposure to bees, a Tier I risk assessment will be performed.

For acute contact and oral, a Tier I risk assessment will be conducted using the EFSA bee calculator Tool (Bee-Tool v.3; Date accessed: 13 Jan 2022) available at

<http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2013.3295/full>. The treated crop scenario is considered by the applicant to represent the worst-case exposure. All other scenarios are considered to have lower exposure e.g., field margins, adjacent crop etc. Therefore, only contact and oral exposure in the treated crop is considered at Tier 1 and where this indicates ETR values below the triggers acceptable risk to bees is considered to be demonstrated.

For chronic exposure to adult bees and honeybee larvae, the Tier I risk assessment is conducted following the EFSA Bee Guidance Document (2013) modified according to the ECPA approach⁴. A detailed explanation of the methods is provided under the ‘Tier 1 - Chronic Risk Assessment’ utilizing the EFSA bee calculator tool.

Table 9.6-7: Critical use patterns relevant to the use of A23282A

Test substance	GAP crop species	Application category	Critical use pattern		
			Rate (kg a.s./ha)	No. of apps	App. Interval (days)
Cyprodinil	Cereals BBCH 30-69	Downward Spray	0.450	1	n/a
Prothioconazole			0.150		
A23282A			2 L product/ha 0.600 kg a.s./ha		

9.6.2.1 Hazard quotients for bees

Screening step – Acute and Chronic Risk Assessment - cyprodinil

Acute, chronic adult and larval honeybee studies have been conducted with cyprodinil, prothioconazole and A23282A according to the data requirements under 1107/2009. The endpoints from these studies have been assessed by using EFSA Bee Guidance (2013) and EFSA Bee Tool.

Table 9.6-8: Screening step assessment of the risk for bees due to the use of formulation A23282A in cereals - cyprodinil

Intended use	Downward				
Active substance	Cyprodinil				
Application rate (g a.s./ha)	1 × 450				
Test design	Endpoint (lab.) (µg/bee)	Single application rate	Shortcut Value (downward spray)	HQ/ETR	Trigger
Acute contact toxicity LD ₅₀	>784	450 g/ha	1	<0.6	42
Acute oral toxicity LD ₅₀	>125	0.450 kg/ha	7.6	<0.03	0.2

⁴ ECPA (2017) Proposal for a protective and workable regulatory European bee risk assessment scheme based on the EFSA bee guidance and other new data and available approaches http://www.ecpa.eu/sites/default/files/document_policy/28028_ECPA%20Proposal%20for%20a%20protective%20and%20workable%20EU%20Bee%20Risk%20Assessment%20-%20Version%2009%20June%2017.pdf

Intended use	Downward				
Active substance	Cyprodinil				
Application rate (g a.s./ha)	1 × 450				
Test design	Endpoint (lab.) (µg/bee)	Single application rate	Shortcut Value (downward spray)	HQ/ETR	Trigger
Chronic adult oral toxicity LDD ₅₀	69.7 µg a.s./bee/day	0.450 kg/ha	7.6	0.049	0.03
Larval development oral toxicity NOED	13.3 µg a.s./larva /development period	0.450 kg/ha	4.4	0.15	0.2

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

The HQ/ETR values for cyprodinil are less than the screening step trigger values for downward sprays indicating that the acute contact and oral risk to honeybees, and the chronic oral risk to larvae is acceptable following use of A23282A according to the proposed use pattern. However, there is a potential chronic oral risk to adult bees for cyprodinil, and therefore a Tier 1 assessment has been provided.

Screening step – Acute and Chronic Risk Assessment - Prothioconazole

Table 9.6-9: Screening step assessment of the risk for bees due to the use of formulation A23282A in cereals - prothioconazole

Intended use	Downward Spray				
Active substance	Prothioconazole				
Application rate (g a.s./ha)	1 × 150				
Test design	Endpoint (lab.) (µg/bee)	Single application rate	Shortcut Value (downward spray)	HQ/ETR	Trigger
Acute contact toxicity LD ₅₀	>200	150 g/ha	1	<0.8	42
Acute oral toxicity LD ₅₀	>71	0.150 kg/ha	7.6	<0.02	0.2
Chronic adult oral toxicity LDD ₅₀	40.5 µg a.s./bee/day	0.150 kg/ha	7.6	0.028	0.03
Larval development oral toxicity NOED	>25 µg a.s./larva /development period	0.150 kg/ha	4.4	<0.03	0.2

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

The HQ/ETR values for prothioconazole are less than the screening step trigger values for downward sprays, indicating that the acute and chronic risk to honeybee adults and larvae is acceptable following use of A23282A according to the proposed use pattern.

Screening step – Acute Risk Assessment – JAU 6476-desthio

As there is not an application rate for the foliar metabolite JAU 6476-desthio, the ratio between the mass of the parent molecule and metabolite will be used to determine a worst-case surrogate application rate. The molar mass of prothioconazole is 344.26 g/mol, and the molar mass of JAU 6476-desthio is 312.2 g/mol, therefore the mass ratio between the two is 0.907. This will be used to convert the application rate of the parent active substance (150 g prothioconazole/ha) to a surrogate application rate of JAU 6476-desthio (136.05 g JAU 6476-desthio/ha).

Table 9.6-10: Screening step assessment of the risk for bees due to the use of formulation A23282A in cereals – JAU 6476-desthio

Intended use	Downward				
Active substance	JAU 6476-desthio				
Application rate (g a.s./ha)	1 × 136.05				
Test design	Endpoint (lab.) (µg/bee)	Single application rate	Shortcut Value (downward spray)	HQ/ETR	Trigger
Acute contact toxicity LD ₅₀	>100	136.05 g/ha	1	<1.4	42
Acute oral toxicity LD ₅₀	>106.5	0.13605 kg/ha	7.6	<0.01	0.2

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

The HQ/ETR values for JAU 6476-desthio are less than the screening step trigger values for downward sprays indicating that the acute risk to honeybee adults is acceptable following use of A23282A according to the proposed use pattern.

Tier 1 – Chronic Risk Assessment – Cyprodinil

Table 9.6-11: First-tier assessment of the chronic risk for bees due to the use of formulation A23282A in Cereals for the treated crop - cyprodinil

Intended use		Downward spray					
Active substance		Cyprodinil					
Application rate (g/ha)		1 × 450					
Test design	Endpoint (lab.) (µg/bee)	Single application rate	Shortcut Value (downward spray)	TWA	Ef	ETR	Trigger
Chronic adult oral toxicity LDD ₅₀	69.7 µg a.s./bee/day	0.450 kg/ha	0.92	0.72	1.0	0.004	0.03

ETR (exposure toxicity ratio) for oral exposure.

The Tier 1 ETR value for cyprodinil is less than the trigger value for downward sprays, indicating that the chronic oral risk to adult honeybees is acceptable following use of A23282A according to the proposed use pattern.

The risk assessment for the treated crop does not represent the worst-case exposure scenario according to the EFSA Bee Tool. The worst-case exposure is from weeds in crop. However, exposure to treated weeds is not considered a relevant exposure scenario according to the guidance, as evidence is available to demonstrate that in arable crops flowering attractive weeds are not present at >10% of the area of use⁵.

Mixture Assessment

Screening step – Acute and Chronic Risk Assessment

Table 9.6-12: Screening step assessment of the risk for bees due to the use of formulation A23282A in cereals – A23282A

Intended use		Downward spray				
Active substance		A23282A				
Application rate (g product/ha)		1 x 2L A23282A/ha (equivalent to 1.986 kg/ha; product density is 0.993 g/cm ³)				
Test design	Endpoint (lab.) (µg/bee)	Single application rate	Calculation factor (downward spray)	HQ/ ETR	Trigger	
Acute contact toxicity, LD ₅₀	645	1986 g/ha	1	3.1	42	
Acute oral toxicity, LD ₅₀	445	1.986 kg/ha	7.6	0.03	0.2	

⁵ Maynard et al., 2015 Weeds in the treated field - a realistic scenario for pollinator risk assessment? Proceedings of 12th International Symposium of the ICP-PR Bee Protection Group, Ghent (Belgium), September 15-17, 2014. Available at: <https://ojs.openagrar.de/index.php/JKA/article/view/5318>

Intended use	Downward spray				
Active substance	A23282A				
Application rate (g product/ha)	1 x 2L A23282A/ha (equivalent to 1.986 kg/ha; product density is 0.993 g/cm ³)				
Test design	Endpoint (lab.) (µg/bee)	Single application rate	Calculation factor (downward spray)	HQ/ ETR	Trigger
Chronic adult oral toxicity, LDD ₅₀	43.8 µg A23282A/bee/day	1.986 kg/ha	7.6	0.345	0.03
Larval development oral toxicity, NOED	69.3 µg /larva/development period	1.986 kg/ha	4.4	0.13	0.2

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

The HQ/ETR values for A23282A are less than the screening step trigger values for downward sprays indicating that the acute risk to honeybees and the chronic oral risk to larvae is acceptable following use of A23282A according to the proposed use pattern. However, there is a potential chronic oral risk to adult bees and therefore a Tier 1 assessment has been provided.

Table 9.6-13: First-tier assessment of the chronic risk for bees due to the use of formulation A23282A in Cereals for the treated crop – A23282A

Intended use	Downward spray							
Product	A23282A							
Application rate (g/ha)	1 x 2L A23282A/ha (equivalent to 1.986 kg/ha; product density is 0.993 g/cm ³)							
Test design	Endpoint (lab.) (µg/bee)	Single application rate	Crop/ Growth Stage	Shortcut Value (downward spray)	TWA	Ef	ETR	Trigger
Chronic adult oral toxicity LDD ₅₀	43.8 µg A23282A/bee/day	1.986 kg/ha	BBCH 30-69	0.92	0.72	1	0.03	0.03

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

The Tier 1 ETR value for A23282A is less than the trigger value for downward sprays, indicating that the chronic oral risk to adult honeybees is acceptable following use of A23282A according to the proposed use pattern.

Table 9.6-14: Screening step assessment of the risk for bees due to the use of formulation A23282A in cereals –Cyprodinil / Prothioconazole mixture

Intended use	Downward spray				
Mixture	Cyprodinil /prothioconazole				
Application rate (g product/ha)	1 x 600 g/ha				
Test design	Endpoint (lab.) (µg/bee)	Single application rate	Calculation factor (downward spray)	HQ/ ETR	Trigger
Acute contact toxicity, LD ₅₀	>1500	600 g/ha	1	<0.4	42
Acute oral toxicity, LD ₅₀	>348	0.6 kg/ha	7.6	0.01	0.2
Chronic adult oral toxicity, LDD ₅₀	196 µg A23282A/bee/day	0.6 kg/ha	7.6	0.023	0.03
Larval development oral toxicity, NOED	50.3 µg /larva/development period	0.6 kg/ha	4.4	0.05	0.2

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

The HQ/ETR values for A23282A are less than the screening step trigger values for downward sprays indicating that the risk to honeybees is acceptable following use of A23282A according to the proposed use pattern.

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

Not relevant.

9.6.3 Effects on bumble bees

No data or information is currently available for bumble bees.

9.6.4 Effects on solitary bees

No data or information is currently available for solitary bees.

9.6.5 Overall conclusions

The acute risk to honeybees was assessed from hazard quotients and Exposure Toxicity Ratios (ETRs) following EFSA (2014), estimated from acute oral and contact studies with cyprodinil, prothioconazole, its metabolite JAU 6476-desthio and A23282A at the maximum single application rate. All the acute contact hazard quotients and Exposure Toxicity Ratios (ETRs) for cyprodinil, prothioconazole, its metabolite JAU 6476-desthio and A23282A are less than the relevant trigger, indicating that the acute oral and contact risk to honeybees is acceptable following use of A23282A according to the proposed use pattern.

The chronic adult and larval risk A23282A to honeybees was assessed from ETRs following EFSA (2014), estimated from chronic adult and larval studies with cyprodinil, prothioconazole, A23282A and the cyprodinil/prothioconazole mixture and potential exposure calculated from exposure via residues in pollen / nectar and the measure of consumption of foraging bees/drone larvae.

The ETR values are less than the relevant trigger values at the screening step or tier 1, indicating that the chronic risk to adult and larval honeybees is acceptable following use of A23282A according to the proposed use pattern.

9.7 Effects on arthropods other than bees (KCP 10.3.2)

zRMS Comments:	<p>The submitted risk assessment based on the “Guidance Document on Terrestrial Ecotoxicology”, 2002, was accepted. New studies for formulation at Tier 1 and Tier 2 were submitted and accepted.</p> <p>The risk assessment is based on accepted studies endpoints for formulation A23282A.</p> <p>The following endpoints at Tier 1 were used in risk assessment:</p> <ul style="list-style-type: none"> <i>Typhlodromus pyri</i>: LR₅₀ = 988.16 mL/ha <i>Aphidius rhopalosiphi</i>: : LR₅₀ = 106.5 mL/ha <p>As the hazard quotients are higher than trigger value (HQ ≤ 2) at tier 1, the refinement at higher tier was provided. indicating that the formulation poses an acceptable risk to arthropods other than bees.</p> <p>Therefore, an acceptable risk to arthropods other than bees is expected if the application of the A23282A is in accordance with intended uses.</p>
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9.7.1 Toxicity data

Effects on non-target arthropods of A23282A were not evaluated as part of the EU assessment of cyprodinil or prothioconazole. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

Table 9.7-1: Endpoints and effect values relevant for the risk assessment for non-target arthropods – A23282A

Species	Substance	Exposure System	Results	Reference
<i>Typhlodromus pyri</i> (protonymphs)	A23282A	Laboratory test glass plates (2D)	LR ₅₀ = 988.16 mL A23282A/ha ER ₅₀ = 617.82 mL A23282A/ha	Fallowfield, L., 2021; Report No. SYN-21- 28; VV-918193
<i>Aphidius rhopalosiphi</i> (adults)	A23282A	Laboratory test glass plates (2D)	LR ₅₀ = 106.5 mL A23282A/ha ER ₅₀ > 75 mL A23282A/ha	Stevens, J., 2021 Report No. SYN-21- 29; VV-917627
<i>Typhlodromus pyri</i> (protonymphs)	A23282A	Extended laboratory test french bean	LR ₅₀ = 2637.99 mL A23282A/ha ER ₅₀ = 2200.80 mL	Fallowfield, L., 2022 Report No. SYN-21-

Species	Substance	Exposure System	Results	Reference
		(<i>Phaseolus vulgaris</i>) leaves (2D)	A23282A/ha	30; VV-935534
<i>Aphidius rhopalosiphi</i> (adults)	A23282A	Aged residue Extended laboratory test french bean plants (<i>Phaseolus vulgaris</i>) (3D)	LR ₅₀ > 2 L A23282A/ha Mortality: 2.5% (14DAT) at 2 L A23282A/ha 2.6% (28DAT) at 2 L A23282A/ha ER ₅₀ >2 L A23282A/ha Reproduction: -6.8% (14 DAT) at 2 L A23282A/ha -0.6% (28 DAT) at 2 L A23282A/ha	Stevens, J., 2021 Report No. SYN-21- 31; VV-930411
<i>Chrysoperla carnea</i>	A23282A	Extended laboratory test french bean (<i>Phaseolus vulgaris</i>) leaves (2D)	LR ₅₀ > 2 L A23282A/ha (highest rate tested) Mortality _{corr.} : 17.6% at 2 L A23282A/ha ER ₅₀ >2 L A23282A/ha	Vaughan, R., 2021 Report No. SYN-21- 32; VV-933833
<i>Aleochara bilineata</i>	A23282A	Extended laboratory test (2D)	NOER > 2 L A23282A/ha (highest rate tested) Mortality _{corr.} : -4.1% at 2 L A23282A/ha ER ₅₀ >2 L A23282A/ha	Tew, G., 2022 Report No. SYN-21- 33; VV-936487
Field or semi-field tests				
None				

9.7.1.1 Justification for new endpoints

A23282A

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

The toxicity of A23282A to non-target arthropods has been investigated by carrying out Tier I tests on the representative non-target arthropods *Aphidius rhopalosiphi* and *Typhlodromus pyri*. Additionally, Tier II tests were carried out on *T. pyri*, *Chrysoperla carnea* and *Aleochara bilineata* in accordance with ESCORT 2. An aged residue extended laboratory test on *Aphidius rhopalosiphi* was also conducted in accordance with ESCORT 2.

9.7.2 Risk assessment

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

9.7.2.1 Risk assessment for in-field exposure

The $PER_{in-field}$ values according to ESCORT 2 were calculated as:
Application rate \times MAF.

Table 9.7-2: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of A23282A in cereals - A23282A

Intended use	Cereals		
Product	A23282A		
Application rate (mL/ha)	1 \times 2000		
MAF	1.0 (foliar/soil)		
Test species Tier I	LR₅₀ (lab.) (mL/ha)	PER_{in-field} (mL/ha)	HQ_{in-field} criterion: HQ \leq 2
<i>Typhlodromus pyri</i>	988.16	2 000 (foliar) 2 000 (soil)	2.02 (foliar) 2.02 (soil)
<i>Aphidius rhopalosiphi</i>	106.5	2 000 (foliar) 2 000 (soil)	19 (foliar) 19 (soil)
Test species Higher-tier	Rate with \leq 50 % effect¹ (mL/ha)	PER_{in-field} (mL/ha)	PER_{in-field} below rate with \leq 50 % effect?
<i>Typhlodromus pyri</i>	2 200.80	2 000 (foliar) 2 000 (soil)	Yes
<i>Aphidius rhopalosiphi</i> ²	>2 000 mL/ha at 14 DAT	2 000 (foliar) 2 000 (soil)	Yes
<i>Chrysoperla carnea</i>	>2 000 mL/ha	2 000 (foliar) 2 000 (soil)	Yes
<i>Aleochara bilineata</i>	>2 000 mL/ha	2 000 (foliar) 2 000 (soil)	Yes

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

¹ The most conservative endpoint between LR₅₀ and ER₅₀ is used

² Series of Aged-Residue Extended Laboratory Tests on 14-day and 28-day field-aged residues of A23282A

The Tier II, extended laboratory studies with *Typhlodromus pyri*, *Chrysoperla carnea* and *Aleochara bilineata* and the Tier II, Aged-residue extended laboratory study (14-day and 28-day) with *Aphidius rhopalosiphi* indicated that the risk to in-field non-target arthropods is acceptable following the use of A23282A according to the proposed use pattern.

9.7.2.2 Risk assessment for off-field exposure

The risks to non-target arthropods for all intended uses are presented.

The $PER_{\text{off-field}}$ value according to ESCORT 2 was calculated as:
Application rate \times MAF \times (drift factor/vegetation distribution factor).

The corrected $PER_{\text{off-field}}$ values according to ESCORT 2 was calculated as:
 $\text{corr. } PER_{\text{off-field}} = PER_{\text{off-field}} \times \text{correction factor}$

Table 9.7-3: First- tier assessment of the off-field risk for non-target arthropods due to the use of A23282A in cereals - A23282A

Intended use		Cereals			
Active substance/product		A23282A			
Application rate (mL/ha)		1 \times 2000			
MAF		1.0			
Drift rate (%)		2.77 ^a			
Vdf		10 for <i>T. pyri</i> and <i>A. rhopalosiphi</i> (Tier 1)			
Test species	LR₅₀ (lab.)	Drift factor	PER_{off-field}	CF	HQ_{off-field}
Tier I	(mL/ha)		(mL/ha)		criterion: HQ \leq 2
<i>Typhlodromus pyri</i>	988.16	0.0277	5.54	10	0.056
<i>Aphidius rhopalosiphi</i>	106.5				0.52

^a – based on 90th percentile drift rate for one application

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

The Tier I laboratory studies for *Aphidius rhopalosiphi* and *Typhlodromus pyri* showed acceptable off-field effects from foliar applications of A23282A.

9.7.2.3 Additional higher-tier risk assessment

Not relevant.

9.7.2.4 Risk mitigation measures

No risk mitigation needed.

9.7.3 Overall conclusions

The risk assessment using Tier II studies and an aged-residue extended laboratory test with *A. rhopalosiphi*, showed acceptable foliar in-field and off-field effects from foliar applications of A23282A for the worst-case use scenario (1 x 2 L/ha in cereals). The risk to non-target arthropods is therefore acceptable following use of A23282A according to the proposed use pattern.

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

zRMS Comments:	<p>The submitted risk assessment was accepted.</p> <p>New laboratory studies were submitted and partially accepted. The new studies concerning cyprodinil metabolite CGA321915 were not taken into consideration in risk assessment (please refer to Appendix 2). The study considering the toxicity of active substance cyprodinil or its metabolite should be evaluated at EU level during substance renewal.</p> <p>The statistical re-analysis for toxicity endpoints for active substance and its metabolites was not used in risk assessment.</p> <p>The risk assessment for formulation is based on accepted endpoints.</p> <p>The max PECs values for active substances, their metabolites and formulation (see Section 8. Fate and behavior) were used for acute and long-term risk assessment. Since risk assessment for non-target soil meso- and macrofauna (earthworm and other organisms) is acceptable at Tier 1, then no further assessment was required.</p> <p>An acceptable risk to non-target soil organisms meso- and macrofauna is expected if the A23282A formulation is used in accordance with proposed uses.</p>
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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 cyprodinil, prothioconazole and their relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents as well as in Appendix 2 of this document (new studies).

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of A23282A were not evaluated as part of the EU assessment of cyprodinil or prothioconazole. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

The selection of studies and endpoints for the risk assessment is not in line with the results of the EU review process. Justifications are provided below.

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

Species	Substance	Exposure System	Results	Reference
Chronic earthworm				
<i>Eisenia fetida</i>	Cyprodinil ^g	Mixed into substrate 56 d, chronic 10% peat content	NOEC = 3.75 kg a.s./ha (≅ 5 mg a.s./kg) EC ₁₀ /EC ₂₀ estimation Not possible due to lack of a significant concentration response	EFSA Scientific Report (2005) 51, 1-78, Nienstedt (2001); Report No. 1047.094.631 CGA219417/1029; VV-319545 EC ₁₀ /EC ₂₀ estimate Taylor & Pickering (2015); Report No. CEA.1411 A8779A_10237; VV-28980

Species	Substance	Exposure System	Results	Reference
<i>Eisenia fetida</i>	Cyprodinil ^g	Mixed into substrate 56 d, chronic 10% peat content	NOEC = 15 kg a.s./ha (≅ 20 mg a.s./kg) EC ₁₀ /EC ₂₀ estimation Not possible due to lack of a significant concentration response NOEC_{corr} = 10 mg a.s./kg soil d.w.*	EFSA Scientific Report (2005) 51, 1-78, Ehlers (2001); Report No. 1047.094.631 CGA219417/1028; VV-318911 EC ₁₀ /EC ₂₀ estimate Taylor & Joyce (2015); Report No. CEA.1410; A8779A_10236; VV-28979
<i>Eisenia fetida</i>	CGA249287	Mixed into substrate 56 d, chronic 10% peat content	NOEC = 1.13 mg/kg soil d.w. EC ₁₀ /EC ₂₀ estimation Not possible due to lack of a significant concentration response	EFSA Scientific Report (2005) 51, 1-78, Pfeifle (2001); Report No. 1047.094.631; CGA249287/0020; VV-311852 EC₁₀/EC₂₀ estimate Taylor & Pickering (2015); Report No. CEA.1427; CGA249287_10008; VV-28889
<i>Eisenia fetida</i>	CGA321915	Mixed into substrate 56 d, chronic 5% peat content	NOEC/EC₁₀/EC₂₀ (reproduction) = 1000 mg/kg soil d.w.	Lührs (2015); Report No. 96341022 CGA321915_10012; VV-411784
<i>Eisenia fetida</i>	CGA275535	Mixed into substrate 56 d, chronic 10% peat content	NOEC (reproduction) = 556 mg/kg soil d.w.; EC ₁₀ = 385 mg/kg; EC ₂₀ = 638 mg/kg EC_{10,corr} = 192.5 mg/kg soil d.w.*	Lührs (2014); Report No. 92791022; CGA275535_10002; VV-410370
Collembola				
<i>Folsomia candida</i>	Cyprodinil ^a	Mixed into substrate 28 d, chronic 5 % peat content	NOEC = 29.4 mg A14325E/kg (8.67 mg a.s./kg); EC ₁₀ = 53.2 mg A14325E/kg (15.7 mg a.s./kg); EC ₂₀ = 67.7 mg A14325E (20 mg a.s./kg) ^e NOEC_{corr} = 4.34 mg/kg soil d.w.*	Lührs (2014); Report No. 92781016; A14325E_10061; VV-410669
<i>Folsomia candida</i>	Cyprodinil ^b	Mixed into substrate 28 d, chronic 5 % peat content	NOEC/EC ₁₀ /EC ₂₀ = 105 mg A8637C/kg (52.5 mg a.s./kg) ^d ; ^e EC ₁₀ /EC ₂₀ estimation Not possible due to lack of a significant concentration	Lührs (2014); Report No. 92771016; A8637C_10314; VV-410108 EC ₁₀ /EC ₂₀ estimate Taylor & Pickering (2016); Report No. CEA.1772 A8637C_10368; VV-134367

Species	Substance	Exposure System	Results	Reference
			response	
<i>Folsomia candida</i>	CGA249287	Mixed into substrate 28 d, chronic 5 % peat content	NOEC = 31 mg/kg soil; EC₁₀ = 7.9 mg/kg ; EC ₂₀ = 22.7 mg/kg	Vinall (2012); Report No. SYN-12-40 CGA249287_10003; VV-402680
<i>Folsomia candida</i>	CGA321915	Mixed into substrate 28 d, chronic 5 % peat content	NOEC/EC₁₀/EC₂₀ = 1000 mg/kg soil d.w.^e	Lührs (2015); Report No. 96341016 CGA321915_10010; VV-412025
<i>Folsomia candida</i>	CGA275535	Mixed into substrate 28 d, chronic 5 % peat content	NOEC = 171.5 mg/kg soil ^f NOEC_{corr} = 85.75 mg/kg soil[‡]	Lührs (2014); Report No. 92791016 CGA275535_10004; VV-410751
<i>Hypoaspis aculeifer</i>				
<i>Hypoaspis aculeifer</i>	Cyprodinil ^a	Mixed into substrate 14 d, chronic 5 % peat content	NOEC/EC ₁₀ /EC ₂₀ = 1000 mg A14325E/kg soil (295 mg a.s./kg) ^{c, e}	Lührs (2014); Report No. 92781089 A14325E_10062; VV-410670
<i>Hypoaspis aculeifer</i>	Cyprodinil ^b	Mixed into substrate 14 d, chronic 5 % peat content	NOEC/EC ₁₀ /EC ₂₀ = 555.6 mg A8637C/kg (277.8 mg a.s./kg) ^{d, f} NOEC_{corr} = 138.9 mg a.s./kg soil[‡]	Lührs (2014); Report No. 92771089; A8637C_10312; VV-410371
<i>Hypoaspis aculeifer</i>	CGA249287	Mixed into substrate 14 d, chronic 5 % peat content	NOEC = 74 mg/kg soil; EC₁₀ = 70.5 mg/kg ; EC ₂₀ = 321.3 mg/kg	Schultz (2014); Report No. 14-10-48-194-S CGA249287_10005; VV-410243
<i>Hypoaspis aculeifer</i>	CGA321915	Mixed into substrate 14 d, chronic 5 % peat content	NOEC/EC₁₀/EC₂₀ = 1000 mg/kg soil	Lührs (2015); Report No. 96341089; CGA321915_10011; VV-412028
<i>Hypoaspis aculeifer</i>	CGA275535	Mixed into substrate 14 d, chronic 5 % peat content	NOEC = 171.5; EC ₁₀ = 104.6 mg/kg; EC ₂₀ = 272.5 mg/kg NOEC_{corr} = 85.75 mg/kg soil[‡]	Lührs (2014); Report No. 92791089; CGA275535_10000; VV-410172
Field studies				
Not conducted				
Litter bag test				
Not conducted				

* Corrected value derived by dividing the endpoint by a factor of 2 in accordance with EFSA, 2015 (Outcome of pesticides peer review meeting on recurring issues in ecotoxicology; Supporting publication 2015:EN-924)

^a Tested as A14325E

^b Tested as A8637C

^c Concentrations converted to active substance content based on nominal formulation composition of 295 g cyprodinil/L

^d Concentrations converted to active substance content based on nominal formulation composition of 500 g cyprodinil/kg

^e It was not possible to estimate EC₁₀ or EC₂₀ values as the NOEC was derived for the highest concentration tested

^f It was not possible to estimate EC₁₀ or EC₂₀ values as a significant concentration response could not be derived

^g Tested as A8779A

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

Species	Substance	Exposure System	Results	Reference
Chronic worm				
<i>Eisenia fetida</i>	Prothioconazole	Sprayed onto substrate 56 d, chronic 10% peat content	NOEC = 1.33 mg a.s./kg NOEC _{corr.} = 3.96 mg a.s./kg ^a NOEC_{corr} = 1.98 mg a.s./kg*	EFSA Conclusion 2007
<i>Eisenia fetida</i>	JAU 6476-desthio	Mixed into substrate 56 d, chronic 10% peat content	NOEC = 1.0 mg/kg NOEC_{corr} = 0.5 mg/kg*	EFSA Conclusion 2007
<i>Eisenia fetida</i>	JAU 6476-S-methyl	Mixed into substrate 56 d, chronic 10% peat content	NOEC = 100 mg/kg NOEC_{corr} = 50 mg/kg*	EFSA Conclusion 2007
Collembola				
<i>Folsomia candida</i>	Prothioconazole	Mixed into substrate 28 d 10% peat content	NOEC = 64 mg a.s./kg NOEC_{corr} = 32 mg/kg*	EFSA Conclusion 2007
<i>Folsomia candida</i>	JAU 6476-desthio	Mixed into substrate 28 d 10% peat content	NOEC = 62.5 mg/kg NOEC_{corr} = 31.25 mg/kg*	EFSA Conclusion 2007
<i>Folsomia candida</i>	JAU 6476-S-methyl	Mixed into substrate 28 d 10% peat content	NOEC = 31.6 mg/kg NOEC_{corr} = 15.8 mg/kg*	EFSA Conclusion 2007
Hypoaspis aculeifer				
<i>Hypoaspis aculeifer</i>	Prothioconazole	Mixed into substrate 34-day LUF 2.1 soil 0.9% peat content	NOEC = 100 mg a.s./kg NOEC_{corr} = 50 mg/kg*	EFSA Conclusion 2007
<i>Hypoaspis aculeifer</i>	JAU 6476-desthio	Mixed into substrate 34-day 5% peat content	NOEC = 100 mg/kg	Schulz, 2014 ^b Document No. M-491764-01-1 refer to RAR (2018) of prothioconazole
<i>Hypoaspis aculeifer</i>	JAU 6476-S-methyl	Mixed into substrate 34-day 5% peat content	NOEC = 100 mg/kg	Schulz, 2014 ^b Document No. M-491804-01-1 refer to RAR (2018) of prothioconazole
Field studies				
<i>Lumbricus terrestris</i> , <i>L. rubellus</i> , <i>L. castanea</i> , <i>Aporrectodea caliginosa</i> ,	Prothioconazole	3 x 200 g a.s./ha to grassland site.	46 % reduction in the number of <i>A. caliginosa</i> juveniles 7 weeks after first application. No	EFSA Conclusion 2007

Species	Substance	Exposure System	Results	Reference
<i>A terrestris longa</i> .			adverse effect 5 months after first application.	
Litter bag test				

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

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

^a The listed endpoint of 1.33 mg as/kg is based on the standard conversion. In the actual study the test material had been sprayed onto the soil, the recalculated endpoint according to the actual test conditions (area 198 cm² and 500 g dry weight soil) results in 3.96 mg as/kg.

^b Since active substance EU approval new studies on the active substance and metabolites have been performed and as a result there are new endpoints which are used in the risk assessment. RAR (Feb 2018).

Table 9.8-3: Endpoints and effect values relevant for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) – A23282A

Species	Substance	Exposure System	Results	Reference
<i>Eisenia andrei</i>	A23282A	Mixed into substrate 56 d, chronic 5% peat content	NOEC = 52.9 mg A23282A/kg dw NOEC_{corr} = 26.45 mg A23282A/kg dw* EC ₁₀ = 66.3 mg A23282A/kg dw EC ₂₀ = 94.9 mg A23282A/kg dw	Friedrich, S., 2021; Report No. 21 48 TEC 0042; VV-925821
<i>Folsomia candida</i> ^a	A23282A	Mixed into substrate 28 d, chronic 5% peat content	NOEC = 95.3 mg A23282A/kg dw NOEC_{corr} = 47.65 mg A23282A/kg dw* EC ₁₀ = 92.6 mg A23282A/kg dw EC ₂₀ = 127 mg A23282A/kg dw	Friedrich, S., 2021; Report No. 21 48 TCC 0029; VV-925508
<i>Hypoaspis aculeifer</i>	A23282A	Mixed into substrate 14 d, acute 5% peat content	NOEC = 171 mg A23282A/kg dw NOEC_{corr} = 85.5 mg A23282A/kg dw* EC ₁₀ = 257 mg A23282A/kg dw EC ₂₀ = 325 mg A23282A/kg dw	Schulz, L., 2021; Report No. 21 48 THC 0030; VV-931773

^a Studies on *Folsomia* are not strictly required according to the data requirements of Regulation (EC) No 1107/2009, since the non-target arthropod risk assessment was passed at Tier 1; however, they have been conducted for reassurance.

*Corrected value derived by dividing the endpoint by a factor of 2.

9.8.1.1 Justification for new endpoints

New studies are available for formulation A23282A which are required to fulfil the data requirements for plant protection products in accordance with Regulation (EC) No 1107/2009. The endpoints are summarised in Table 9.8-3.

9.8.2 Risk assessment

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

9.8.2.1 First-tier risk assessment

The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2.

Table 9.8-4: First-tier assessment of the acute and chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of A23282A in cereals – cyprodinil and relevant metabolites

Intended use	Cereals		
Chronic effects on earthworms			
Product/active substance	Endpoint (mg/kg dw)	PEC _{soil} ^a (mg/kg dw)	TER _{lt} (criterion TER ≥ 5)
Cyprodinil	NOEC _{corr} = 10	0.224 ^a	45
CGA249287	NOEC = 1.13	0.028 ^a	40
CGA321915	NOEC = 1000	0.014 ^b	71 000
CGA275535	EC _{10corr} = 192.5	0.129 ^b	1 500
Chronic effects on other soil macro- and mesofauna – <i>Folsomia candida</i>			
Product/active substance	Endpoint (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{lt} (criterion TER ≥ 5)
Cyprodinil	NOEC _{corr} = 4.34	0.224 ^a	19
CGA249287	EC ₁₀ = 7.9	0.028 ^a	280
CGA321915	NOEC/EC ₁₀ = 1000	0.014 ^b	71 000
CGA275535	NOEC _{corr} = 85.75	0.129 ^b	660
Chronic effects on other soil macro- and mesofauna – <i>Hypoaspis aculeifer</i>			
Product/active substance	Endpoint (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{lt} (criterion TER ≥ 5)
Cyprodinil	NOEC/EC _{10,corr} = 138.9	0.224 ^a	620
CGA249287	EC ₁₀ = 70.5	0.028 ^a	2 500
CGA321915	NOEC/EC ₁₀ = 1000	0.014 ^b	71 000
CGA275535	NOEC _{corr} = 85.75	0.129 ^b	660

^a PEC_{acc}

^a PEC_{ini}

Table 9.8-5: First-tier assessment of the acute and chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of A23282A in cereals – prothioconazole and relevant metabolites

Intended use	Cereals		
Chronic effects on earthworms			
Product/active substance	NOEC/NOEC _{corr} (mg/kg dw)	PEC _{soil} ^a (mg/kg dw)	TER _{lt} (criterion TER ≥ 5)
Prothioconazole	1.98	0.040	50
JAU 6476-desthio	0.5	0.027	19
JAU 6476-S-methyl	50	0.006	8 300
Chronic effects on other soil macro- and mesofauna – <i>Folsomia candida</i>			
Product/active substance	NOEC/NOEC _{corr} (mg/kg dw)	PEC _{soil} ^a (mg/kg dw)	TER _{lt} (criterion TER ≥ 5)
Prothioconazole	32	0.040	800
JAU 6476-desthio	31.25	0.027	1 200
JAU 6476-S-methyl	15.8	0.006	2 600
Chronic effects on other soil macro- and mesofauna – <i>Hypoaspis aculeifer</i>			
Product/active substance	NOEC/NOEC _{corr} (mg/kg dw)	PEC _{soil} ^a (mg/kg dw)	TER _{lt} (criterion TER ≥ 5)
Prothioconazole	50	0.040	1 300
JAU 6476-desthio	100	0.027	3 700
JAU 6476-S-methyl	100	0.006	17 000

^a PEC_{ini}

Table 9.8-6: First-tier assessment of the acute and chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of A23282A - A23282A

Intended use	Cereals		
Chronic effects on earthworms			
Product	NOEC _{corr} (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{lt} (criterion TER ≥ 5)
A23282A	26.45	0.53	50
Chronic effects on other soil macro- and mesofauna – <i>Folsomia candida</i>			
Product	NOEC _{corr} (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{lt} (criterion TER ≥ 5)
A23282A	47.65	0.53	90
Chronic effects on other soil macro- and mesofauna – <i>Hypoaspis aculeifer</i>			
Product	NOEC _{corr} (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{lt} (criterion TER ≥ 5)
A23282A	85.50	0.53	160

At the request of the NL a combination toxicity risk assessment is required for soil organisms:

TER-values based on combination toxicity should be calculated for soil organisms when data are available for the a.s., even if an endpoint for the formulation is available (since the lowest TER- value is taken for the conclusion).

The risk assessment for the potential combination toxicity will be conducted using the following equation for earthworms given that the TER is lower for the desthio metabolite:

$$TER_{combi} = trigger / (((trigger_{cyprodinil} / TER_{cyprodinil}) + (trigger_{JAU\ 6476-desthio} / TER_{JAU\ 6476-desthio})))$$

An acceptable risk is expected when $TER_{combi} > trigger$.

For *Folsomia candida* and *Hypoaspis aculeifer* the combination of cyprodinil and prothioconazole will be calculated

In this formula, ‘triggers’ are the trigger values as mentioned in the corresponding chapter of the Evaluation Manual (these are equivalent to the EU triggers).

Table 9.8-7: First-tier assessment of the acute and chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of A23282A - A23282A

Organism	TER _{cyprodinil}	TER _{JAU 6476-desthio / prothioconazole}	TER criterion	TER _{combi}
Earthworm	45	19	5	13
<i>Folsomia candida</i>	19	800	5	19
<i>Hypoaspis aculeifer</i>	620	1 300	5	420

9.8.2.2 Higher-tier risk assessment

Not relevant.

9.8.3 Overall conclusions

The long-term risk of A23282A to earthworms was assessed from acute and long-term toxicity exposure ratios (TERs) between the selected toxicity endpoints for A23282A, cyprodinil, prothioconazole, and relevant metabolites, and the maximum PEC_{soil}. The acute and chronic TER values for A23282A, cyprodinil, prothioconazole, and metabolites are greater than the Regulation (EU) 546/2011 trigger of 5, respectively, indicating that the risk to earthworms is acceptable following use of A23282A according to the proposed use pattern.

The risk of A23282A to other non-target soil macro-organisms, as represented by Collembola and *Hypoaspis*, was assessed from long-term toxicity exposure ratios (TERs) between the selected no-effect concentrations, derived from laboratory tests on A23282A, cyprodinil, prothioconazole and metabolites, and the maximum PEC_s. The TER_{LT} values for A23282A, cyprodinil, prothioconazole, and relevant metabolites are all greater than the recommended trigger value of 5, indicating that the risk to soil macro-organisms, as represented by Collembola and *Hypoaspis*, is acceptable following use of A23282A according to the proposed use pattern.

9.9 Effects on soil microbial activity (KCP 10.5)

zRMS Comments:	<p>The submitted information and data were accepted.</p> <p>New study conducted on formulation was submitted and accepted.</p> <p>The formulation A23282A pose no adverse effect on nitrate formation in soil.</p> <p>An acceptable risk to soil microorganisms is expected if the application of the A23282A is in accordance with proposed pattern use.</p>
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9.9.1 Toxicity data

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

Effects on soil microorganisms of A23282A were not evaluated as part of the EU assessment of cyprodinil or prothioconazole.

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

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

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	Cyprodinil	28 d, aerobic soil type	NOEC = 26.7 mg a.s./kg	EFSA Scientific Report (2005) 51, 1-78, Wütrich (1993):

Endpoint	Substance	Exposure System	Results	Reference
				Report No. 329038 CGA219417/0209; VV-369337
N-mineralisation	CGA249287	28 d, aerobic soil type	NOEC = 3.33 mg a.s./kg	EFSA Scientific Report (2005) 51, 1- 78, Grade (2000); Report No. 329038 CGA249287/0010; VV-311511
N-mineralisation	CGA321915	28 d, aerobic soil type	NOEC = 5.10 mg a.s./kg	Hammesfahr (2015); Report No. 96341080; CGA321915_10008; VV-412058
N-mineralisation	CGA275535	28 d, aerobic soil type	NOEC = 1.15 mg a.s./kg	EFSA Scientific Report (2005) 51, 1- 78, Seyfried (2001); Report No. 789761 CGA275535/0020; VV- 319206

Table 9.9-2: Endpoints and effect values relevant for the risk assessment for soil microorganisms – prothioconazole and metabolites

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	Prothioconazole	28 d	< 25 % effect up to 2 kg a.s./ha soil dw < 25 % effect up to 2.71 mg a.s./kg	EFSA Conclusion 2007
C-mineralisation				
N-mineralisation	JAU 6476-desthio	28 d	< 25 % effect up to 1 kg/ha soil dw < 25 % effect up to 1.37 mg/kg	EFSA Conclusion 2007
C-mineralisation				
N-mineralisation	JAU 6476-S-methyl	28 d	< 25 % effect up to 2 kg/ha soil dw < 25 % effect up to 2.7 mg/kg	EFSA Conclusion 2007
C-mineralisation				

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

Table 9.9-3: Endpoints and effect values relevant for the risk assessment for soil microorganisms – A23282A

Endpoint	Substance	Exposure System	Results	Reference
N and C-mineralisation	A23282A	28 days, aerobic soil type	<25% effects up to 13.24 mg A23282A/kg soil	Schulz, L., 2021; Report No. 21 48 SMO 0018 VV-933827

9.9.1.1 Justification for new endpoints

New studies are available for formulation A23282A which are required to fulfil the data requirements for plant protection products in accordance with Regulation (EC) No 1107/2009. The endpoints are summarised in Table 9.9-3 and Appendix 2.

9.9.2 Risk assessment

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

The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2, Table 8.7-3 and were already used in the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) (see 9.8).

Table 9.9-4: Assessment of the risk for effects on soil micro-organisms due to the use of A23282A in cereals - cyprodinil and relevant metabolites

Intended use	Cereals		
N-mineralisation			
Product/active substance	Max. conc. with effects ≤ 25 % (mg/kg dw)	PEC _{soil} (mg/kg dw)	Risk acceptable?
Cyprodinil	26.7	0.224 ^a	yes
CGA249287	3.33	0.028 ^a	yes
CGA321915	5.10	0.014 ^b	yes
CGA275535	1.15	0.129 ^b	yes

^a PEC_{acc}

^b PEC_{ini}

Table 9.9-5: Assessment of the risk for effects on soil micro-organisms due to the use of A23282A in cereals – prothioconazole and relevant metabolites

Intended use	Cereals		
N and C-mineralisation			
Product/active substance	Max. conc. with effects ≤ 25 % (mg/kg dw)	PEC _{soil} ^a (mg/kg dw)	Risk acceptable?
Prothioconazole	2.71	0.040	yes
JAU 6476-desthio	1.37	0.027	yes
JAU 6476-S-methyl	2.7	0.006	yes

^a PEC_{ini}

Table 9.9-6: Assessment of the risk for effects on soil micro-organisms due to the use of A23282A in cereals – A23282A

Intended use	Cereals		
N and C-mineralisation			
Product/active substance	Max. conc. with effects ≤ 25 % (mg/kg dw)	PEC _{soil} (mg/kg dw)	Risk acceptable?
A23282A	13.24	0.53	yes

9.9.3 Overall conclusions

The risk of A23282A, cyprodinil, prothioconazole and relevant metabolites to soil micro-organisms was evaluated by comparison of the maximum concentrations with effects $\leq 25\%$ derived from laboratory tests, with maximum PEC_s.

All the effect levels exceeded the relevant PEC_s values, indicating that the risk to soil micro-organisms is acceptable following the use of A23282A according to the proposed use pattern(s).

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

9.10.1 Toxicity data

Effects on non-target terrestrial plants of formulation were not evaluated as part of the EU assessment of cyprodinil and prothioconazole. New data submitted with this application are listed in Appendix 1 summarised in Appendix 2.

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

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

Species	Substance	Exposure System	Results	Reference
<i>Amaranthus retroflexus</i>	Prothioconazole	Seedling emergence	ER ₅₀ > 200 g a.s./ha	EFSA Conclusion 2007
<i>Amaranthus retroflexus</i> , <i>Beta vulgaris</i> ^a	Prothioconazole	Vegetative vigour	ER ₅₀ > 250 g a.s./ha	EFSA Conclusion 2007

^a Most sensitive species

Table 9.10-2: Endpoints and effect values relevant for the risk assessment for non-target terrestrial plants – A23282A

Species	Substance	Exposure System	Results	Reference
Onion (<i>Allium cepa</i>) _m Wheat (<i>Triticum aestivum</i>) _m Sugar beet (<i>Beta vulgaris</i>) _d Oilseed rape (<i>Brassica napus</i>) _d	A23282A	28 d phytotoxicity screen, seedling emergence	No phytotoxic effects at rates up to and including 2000 mL/ha , the highest rate tested	Jones K, 2021; Report No. ACE-21-258 VV-921642

Species	Substance	Exposure System	Results	Reference
Cucumber (<i>Cucumis sativus</i>) _d Soybean (<i>Glycine max</i>) _d				
Onion (<i>Allium cepa</i>) _m Wheat (<i>Triticum aestivum</i>) _m Sugar beet (<i>Beta vulgaris</i>) _d Oilseed rape (<i>Brassica napus</i>) _d Cucumber (<i>Cucumis sativus</i>) _d Soybean (<i>Glycine max</i>) _d	A23282A	21 d phytotoxicity screen, vegetative vigour	No phytotoxic effects above 32 (in a scale of 0 to 100%) at 2000 mL/ha , the highest rate tested	

m: monocotyledonous; d: dicotyledonous

9.10.1.1 Justification for new endpoints

A screening study with formulated product A23282A has been conducted. The data are summarised in Table 9.10-2.

9.10.2 Risk assessment

9.10.2.1 Tier-1 risk assessment (based screening data)

Effects at test rates up to and including 2000 mL A23282A/ha were below the critical threshold as defined by the “Guidance Document on Terrestrial Ecotoxicology”, (SANCO/10329/2002 rev.2 final, 2002). The test rates equal the highest single field application rate of 2000 mL A23282A/ha for all uses and are thus considered an indicator for an acceptable risk.

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

Not relevant.

9.10.2.3 Higher-tier risk assessment

Not relevant.

9.10.2.4 Risk mitigation measures

No risk mitigation needed.

9.10.3 Overall conclusions

Less than 50% effect on seedling emergence and vegetative vigour on all six species was observed at the maximum single use rate of 2000 mL A23282A/ha at screening step. This indicates that the risk to non-target terrestrial plants in off-crop areas is acceptable following use of A23282A according to the proposed use pattern.

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

Tests on other non-target species are not required.

9.12 Monitoring data (KCP 10.8)

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

9.13 Classification and Labelling

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

Classification according to regulation 1272/2008:	
Acute 1	H400: Very toxic to aquatic life
Chronic 1	H410: Very toxic to aquatic life with long lasting effects

Labelling according to regulation 1272/2008:	
Hazard symbol:	GHS09
Signal word:	Warning
Hazard statement:	H410: Very toxic to aquatic life with long lasting effects

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on (A23282A)

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
XXX	XXXX	XXX	XXXX	XX	XX

List of data submitted by the applicant and relied on - prothioconazole

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
XXX	XXXX	XXX	XXXX	XX	XX

The following tables are to be completed by MS

List of data submitted by the applicant and not relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
XXX	XXXX	XXX	XXXX	XX	XX

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
KCP XX	Author	YYYY	Title Company Report N Source GLP/non GLP/GEP/non GEP Published/Unpublished	Y/N	Owner

Appendix 2 Detailed evaluation of the new studies

A 2.1 KCP 10.1 Effects on birds and other terrestrial vertebrates

A 2.1.1 KCP 10.1.1 Effects on birds

A 2.1.1.1 KCP 10.1.1.1 Acute oral toxicity

A 2.1.1.2 KCP 10.1.1.2 Higher tier data on birds

A 2.1.2 KCP 10.1.2 Effects on terrestrial vertebrates other than birds

A 2.1.2.1 KCP 10.1.2.1 Acute oral toxicity to mammals

A 2.1.2.2 KCP 10.1.2.2 Higher tier data on mammals

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

A 2.2 KCP 10.2 Effects on aquatic organisms

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

Comments of zRMS:	<p>The study is acceptable for risk assessment. The study was conducted according to OECD guideline 202. The validity criteria were met:</p> <ul style="list-style-type: none"> control immobilisation of 0 % and no daphnid showed signs of disease or stress; the dissolved oxygen concentration in the test media was ≥ 7.37 mg O₂/L at the end of the test. <p>No deviation was reported.</p> <p>The formulation A23282A was the test item. The following endpoints were derived: 48h EC₅₀ = 0.223 mg formulation/L NOEC = 0.171 mg formulation/L</p>
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Reference: KCP 10.2.1

Report Schuler L, 2021, Cyprodinil/prothioconazole EC (A23282A) - Toxicity to the

Water Flea *Daphnia magna* Straus under Laboratory Conditions (Acute Immobilisation Test – Static), Eurofins Agrosience Services Ecotox GmbH Eutinger Str. 24, 75223 Niefern-Öschelbronn, Germany, Report Number S21-05725, (XXXX File No. VV-931771).

Guideline(s):	OECD Guidelines for Testing of Chemicals, Method 202 (2004)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Executive Summary

The acute toxicity of Cyprodinil/prothioconazole EC (A23282A) to *Daphnia magna* was determined under static conditions. Daphnids were exposed to nominal concentrations of 1.00, 0.556, 0.309, 0.171, 0.0953 mg/L alongside a dilution water control. Based on nominal concentration the 48-hour EC₅₀ was 0.223 mg test item/L.

Materials

Test Material

Name/code:	Cyprodinil/prothioconazole EC (A23282A)
Lot/Batch #:	LCR001-021-001
Actual content of active ingredient:	219 g/L, 22.1 % w/w cyprodinil 73.5 g/L, 7.40 % w/w prothioconazole
Description:	Liquid / yellow
Stability of test compound:	Sufficient for the test purpose (at least 1 h)
Reanalysis/Expiry date:	30 Sep 2023
Density:	0.993 g/cm ³

Treatments

Test concentrations:	1.00, 0.556, 0.309, 0.171, 0.0953 mg A23282A/L and control Equivalent to 0.0211, 0.0378, 0.0683, 0.123 and 0.221 mg Cyprodinil/L
Solvent:	None
Positive control:	Two concentrations of the reference item potassium dichromate were tested around the same time period as the study.
Analysis of test concentrations:	Analysis of cyprodinil in all test concentrations and the control at 0 h fresh and 48 hours (aged) from samples by HPLC-MS/MS detection.

Test organisms

Species:	<i>Daphnia magna</i> STRAUS, Clone V
Source:	Continuously bred in the laboratory, originally obtained from Federal Environment Agency in Berlin/Germany
Feeding:	The animals were fed on single cell green algae (<i>Desmodesmus subspicatus</i> , formerly <i>Scenedesmus subspicatus</i>) at least three times a week. No feeding during testing

Test design

Test vessels:	100 mL glass beaker, filled with ≥ 50 mL test solution
Test medium:	Elendt M4
Replication:	4 test vessels each with 5 test organisms at all test concentrations and the control (individual organisms were randomly assigned to the treatments and

	test vessels)
Exposure regime:	Static
Duration:	48 hours
Environmental conditions	
Test temperature:	20.0 – 20.7 °C
pH range:	7.76 – 8.20
Dissolved oxygen:	7.37 – 8.91 mg/L
Total hardness of dilution water:	232 mg/L as CaCO ₃
Lighting:	16 hours photoperiod / 8 hours darkness daily; mean light intensity: 1209 lux

Study Design and Methods

Test facility: Eurofins Agrosience Services, Ecotox GmbH Eutingen Str. 24, 75223 Niefern-Öschelbronn, Germany

Experimental dates: 10th to 17th Aug 2021

The test medium was prepared by dissolving 20.0 mg of the test item completely in 1000 mL of test medium. The stock solution was homogenised by shaking and treated with 5 minutes of ultrasonication. Afterwards the stock solution was turbid. Using this stock solution, the remaining nominal test concentrations as stated above were prepared by serial dilution. The control consisted of dilution water only. Test solutions were added to the test vessels and the *Daphnia* added without conscious bias.

The immobility of the daphnids was determined by visual observations after 24 and 48 hours of exposure. Organisms unable to swim within 15 seconds after gentle agitation of the test beaker were considered to be immobile.

The pH, temperature and dissolved oxygen were measured at the start, after 24 hours and end of the test in each test concentration and the control. Light intensity was measured at test start at five different points.

The test concentrations were verified by chemical analysis of cyprodinil at 0 and 48 hours using high performance liquid chromatography with MS/MS detection.

The median effect concentration (EC₅₀) is defined as the concentration resulting in 50 % immobilisation of the *Daphnia* in the time period specified. The 24-hour EC₅₀ together with the 95 % confidence intervals were determined with Probit analysis. The 48-hour EC₅₀ together with the 95 % confidence intervals were determined by trimmed Spearman-Kärber procedure. The NOEC (No Observed Effect Concentration) is defined as the highest tested concentration which did not produce an adverse effect when compared to the control and was determined directly from the raw data.

Results

At the start of the test, the analytically determined concentrations of cyprodinil were in the range 94 to 101 % of the nominal values and at the end of the test were in the range 94 to 100 %. The limit of quantification for cyprodinil in this study was 0.00211 mg a.s./L (see table below).

Table A 1: Analytical results of cyprodinil

Nominal concentrations of A23282A (mg /L)	Nominal concentrations of Cyprodinil (mg /L)	Determined concentration at 0 hours (% of nominal)	Determined concentration at 48 hours (% of nominal)
Control	Control	<LOD	<LOD

0.0953	0.0211	0.0201 (95)	0.0204 (97)
0.171	0.0378	0.0382 (101)	0.0371 (98)
0.309	0.0683	0.0642 (94)	0.0674 (99)
0.556	0.123	0.121 (98)	0.116 (94)
1.00	0.221	0.219 (99)	0.211 (100)

n.a.: not applicable

LOD = limit of detection 0.0004 mg a.s./L

There was no immobility observed in the dilution water control. Immobility data and estimated EC₅₀ values are shown in the table below:

Table A 2: Effects of A23282A on *Daphnia magna* following exposure for 48-hours in a static test

Nominal concentration of A23282A (mg/L)	Immobilised daphnids after 24 hours		Immobilised daphnids after 48 hours	
	Number	%	Number	%
Control	0	0	0	0
0.0953	0	0	0	0
0.171	0	0	1	5
0.309	3	15	20	100
0.556	7	35	20	100
1.00	9	45	20	100
EC ₅₀ (mg /L)	0.954 ¹⁾		0.223 ²⁾	
95% Confidence limits	0.687-1.89 ¹⁾		0.211-0.236 ²⁾	
NOEC (mg /L)	0.171		0.171	

¹⁾ Calculated with probit analysis

²⁾ Calculated by trimmed Spearman-Kärber

Validity criteria

The test was considered valid since:

- There was no immobilization or other signs of disease or stress in the control (must be ≤ 10%)
- Oxygen concentration at the end of the test were ≥ 7.37 mg/L in the control and test vessels (must be ≥ 3 mg/L)

Conclusion

The acute toxicity of Cyprodinil/prothioconazole EC (A23282A) to *Daphnia magna* was determined under static conditions. Daphnids were exposed to nominal concentrations of 1.00, 0.556, 0.309, 0.171, 0.0953 mg/L alongside a dilution water control. Based on nominal concentration the 48-hour EC₅₀ was 0.223 mg/L.

(Schuler, L., 2021

Comments of zRMS:	<p>The study is acceptable for risk assessment.</p> <p>The study was conducted according to OECD guideline 201. The formulation A23282A was the test item.</p> <p>The validity criteria were met</p> <ul style="list-style-type: none"> • cell density increase in control cultures: 158.2- and 429-fold increase within 72 h and 96 h, respectively;
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	<ul style="list-style-type: none"> coefficient of variation of sectional (daily) growth rates in control cultures: 10% and 24 % after 72 h and 96 h, respectively; coefficient of variation of average growth between control replicates: 2.8% and 1.4% within 72 h and 96 h, respectively; <p>and no significant deviation was reported.</p> <p>The initial measured concentrations were between 99 % and 105 % of nominal. The measured concentrations after 96 hours were between 60 % and 103 % of nominal. Therefore, toxicological endpoints were evaluated using the nominal and mean measured concentrations of the test item A23282A. The following endpoints were derived:</p>																																																															
	<table border="1"> <thead> <tr> <th></th><th colspan="2">72 h</th><th colspan="2">96 h</th></tr> <tr> <th></th><th>Growth rate</th><th>Yield</th><th>Growth rate</th><th>Yield</th></tr> <tr> <th></th><th colspan="4">nominal concentration mg formulation/L</th></tr> </thead> <tbody> <tr> <td>EC₁₀</td><td>6.72</td><td>not derived</td><td>10.4</td><td>2.79</td></tr> <tr> <td>EC₅₀</td><td>17.9</td><td>6.13</td><td>21.0</td><td>9.46</td></tr> <tr> <td>NOEC</td><td>not derived</td><td>not derived</td><td>2.56</td><td>not derived</td></tr> <tr> <td>LOEC</td><td>2.56</td><td>2.56</td><td>6.40</td><td>2.56</td></tr> <tr> <th></th><th colspan="4">measured concentration mg formulation/L</th></tr> <tr> <td>EC₁₀</td><td>5.84</td><td>not derived</td><td>9.39</td><td>2.21</td></tr> <tr> <td>EC₅₀</td><td>16.9</td><td>5.24</td><td>20.2</td><td>8.42</td></tr> <tr> <td>NOEC</td><td>not derived</td><td>not derived</td><td>2.02</td><td>not derived</td></tr> <tr> <td>LOEC</td><td>2.02</td><td>2.02</td><td>5.48</td><td>2.02</td></tr> </tbody> </table>					72 h		96 h			Growth rate	Yield	Growth rate	Yield		nominal concentration mg formulation/L				EC ₁₀	6.72	not derived	10.4	2.79	EC ₅₀	17.9	6.13	21.0	9.46	NOEC	not derived	not derived	2.56	not derived	LOEC	2.56	2.56	6.40	2.56		measured concentration mg formulation/L				EC ₁₀	5.84	not derived	9.39	2.21	EC ₅₀	16.9	5.24	20.2	8.42	NOEC	not derived	not derived	2.02	not derived	LOEC	2.02	2.02	5.48	2.02
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Reference: KCP 10.2.1

Report Schuler L. (2021), Cyprodinil/prothioconazole EC (A23282A) -Toxicity to the Single Cell Green Alga *Raphidocelis subcapitata* Korshikov under Laboratory Conditions, Report Number S21-05724, Eurofins Agroscience Services Ecotox GmbH, Eutinger Str. 24, 75223 Niefern-Öschelbronn, Germany
(XXXX File No. VV-931772)

Guideline(s): OECD Guidelines for Testing of Chemicals, Method 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test (2011)

Deviations: No

GLP: Yes

Acceptability: Yes

Executive Summary

The toxicity of Cyprodinil/prothioconazole EC (A23282A) to the green alga *Raphidocelis subcapitata* was investigated in a 96-hour static test. Algae were exposed to nominal concentrations of 2.56, 6.40, 16.0, 40.0 and 100 mg A23282A/L equivalent to measured concentrations of 2.02, 5.48, 15.0, 40.4, and 102, alongside a culture medium control.

Based on nominal concentrations, the 72-hour E_rC₅₀ was 17.9 mg A23282A/L, the E_yC₅₀ was 6.13 mg A23282A/L and the E_bC₅₀ was 6.06 mg A23282A/L. The 96-hour E_rC₅₀ was 21.0 mg A23282A/L, the E_yC₅₀ was 9.46 mg A23282A/L and the E_bC₅₀ was 7.84 mg A23282A/L. NOEC could not be determined for growth rate, yield and biomass integral after 72 hours.

Based on mean measured concentrations, the 72-hour E_rC_{50} was 16.9 mg A23282A/L, the E_yC_{50} was 5.25 g A23282A/L and the E_bC_{50} was 5.18 mg A23282A/L. The 96-hour E_rC_{50} was 20.2 mg A23282A/L, the E_yC_{50} was 8.42 mg A23282A/L and the E_bC_{50} was 6.86 mg A23282A/L. NOEC could not be determined for growth rate, yield and biomass integral after 72 hours.

Materials

Test Material

Name/Code:	Cyprodinil/prothioconazole EC (A23282A)
Lot/Batch #:	LCR001-021-001
Actual content of active ingredients:	219 g/L 22.1 % w/w cyprodinil 73.5 g/L, 7.40 % w/w prothioconazole
Description:	Liquid / yellow
Stability of test compound:	Stable under test conditions
Reanalysis/expiry date:	30 Sep 2023
Density:	0.993 g/cm ³

Treatments

Test concentrations:	Nominal: 100, 40.0, 16.0, 6.40, 2.56 mg A23282A/L and control (Equivalent to measured concentrations of 102, 40.4, 15.0 5.48 and 2.02 mg A23282A/L and control)
Solvent:	None
Positive control:	Reference test was carried out as a separate GLP study with Potassium dichromate
Analysis of test concentrations:	Analysis of cyprodinil in all test concentrations and the control at 0 h (fresh) and 96 hours (aged) samples by HPLC-MS/MS detection.

Test organism

Species:	<i>Raphidocelis subcapitata</i> Korshikov, Strain SAG 61.81
Source:	MBM Sciencebridge GmbH, Hans-Adolf-Krebs-Weg 1, D-37077 Göttingen, Germany

Test design

Test vessels:	100 mL Erlenmeyer flasks with aluminium caps
Test medium:	AAP medium
Replication:	The control was prepared with six replicates and the concentrations were prepared with three replicates at each concentration. Three additional replicates without algae were employed for test item concentration 16.0 mg/L.
Starting cell density:	5000 cells per mL (nominal)
Exposure regime:	Static
Aeration:	no
Duration:	96 h

Environmental conditions

Test temperature:	22.6 – 23.3 °C
pH of control:	7.69 – 8.34 within 72 h, up to 9.90 after 96 h
Lighting:	Continuous; Light intensity 86.4 – 97.7 $\mu E\ m^{-2}\ s^{-1}$

Study Design and Methods

Test facility: Eurofins Agrosience Services Ecotox GmbH, Eutinger Str. 24, 75223 Niefern-Öschelbronn, Germany

Experimental dates: 9th August to 9th September 2021

One stock solution (S1) containing 100 mg was prepared by direct weighing into 1000 mL test medium. The solution was homogenised by shaking and treated with 5 minutes of ultrasonication. The stock solution appeared to be turbid. The further concentrations V1 – V4 were made by diluting the appropriate solutions in test medium to give the required test concentrations. Dilution V1 and V2 appeared to be turbid, V3 – V4 were clear and transparent. Algae were added to each solution individually, targeting nominal cell densities of 0.5×10^4 cells per mL in each solution. The control consisted of culture medium only.

An aliquot of approximately ~ 50 mL test solution was placed into each test vessel and the test was started by inoculation of 5,000 algal cells per mL of test medium. The test flasks were continuously agitated in a temperature controlled light incubator on a shaker (105 rpm).

Small volumes of all test concentrations and controls were taken from all test flasks after 24, 48, 72 and 96 hours of exposure. The algal cell densities in these samples were determined by fluorescence measurements. In addition, after 96 hours exposure, a sample was taken from the control and all test item concentrations and the shape of the algal cells was examined microscopically in these samples.

The pH at test start was recorded in bulk test solutions, after 72 and 96 hours in one replicate. The temperature was measured continuously in the climate cabinet and recorded daily. The appearance of the test media was also recorded daily.

The test concentrations were verified by chemical analysis of cyprodinil at 0 (fresh) and 96 hours (aged), using high performance liquid chromatography with HPLC-MS/MS.

The algal cell densities were measured at 24, 48, 72 and 96 hours and the mean biomass, growth rate and yield calculated. The EC_{10, 20, 50}-values for growth rate after 72 h and 96 h were determined by Weibull analysis using maximum likelihood regression. Since the inhibitions of yield was above 20 % after 72 h and above 10 % after 96 h in all test item concentrations no EC₁₀-value after 72 h could be determined. The EC₁₀-value after 96 h and the EC₂₀-value after 72 h could be extrapolated. The EC₅₀-value for yield after 72 h and the EC_{20, 50}-values after 96 h were determined by Weibull analysis using maximum likelihood regression. Since the inhibitions of biomass integral was above 20 % after 72 h and above 10 % after 96 h in all test item concentrations no EC_{10, 20}-values could be determined after 72 h and no EC₁₀-value could be determined after 96 h. The EC₅₀-values for biomass integral after 72 h and 96 h were determined by Weibull analysis using maximum likelihood regression.

The test concentrations were verified by chemical analysis of Cyprodinil by HPLC-MS/MS. Analytical samples were taken in duplicate at 0 hours (initial value) from fresh bulk solutions at test start and after 96 hours aliquots of each replicate per treatment group were pooled and centrifuged before the analytical samples were taken. The samples were stored deep frozen. For the control and all test item concentrations, one of the duplicate samples from each sample set was analysed and reported. The second duplicate of each set of samples was kept for further analysis if required.

Additionally, samples were taken and analysed from test item concentration 16.0 mg/L without algae from fresh test solutions and after 72 and 96 hours from aged test solutions.

For determination of the LOEC (Lowest Observed Effect Concentration) and NOEC (No Observed Effect Concentration) values multiple sequentially-rejective Welsh-t-test after Bonferroni-Holm for yield (72 h)

and biomass integral (72 h) and Williams multiple sequential t-test for growth rate (72 and 96 h), yield (96 h) and biomass integral (96 h) (all left sided) were used to identify significant differences in the calculated growth rate, yield and biomass integral of test item treatments compared to the control.

Results

The analytical verification of test item concentrations in test medium was done by analysing the content of cyprodinil at 0 and 96 hours. The initial measured concentrations were between 99 % and 105 % of nominal. The measured concentrations after 96 hours were between 60 % and 103 % of nominal. Therefore, toxicological endpoints were evaluated using the nominal and mean measured concentrations of the test item A23282A (see table below). The limit of quantification for cyprodinil in this study was 0.0566 mg/L.

Table A 3: Analytical results based on the analysis of cyprodinil in A23282A

Nominal concentrations of A23282A (mg/L)	Nominal concentrations of Cyprodinil (mg/L)	Determined concentration of cyprodinil at 0 hours (mg a.s./L) (and % of nominal)	Determined concentration of cyprodinil at 96 hours (mg a.s./L) (and % of nominal)	Mean measured concentrations of A23282 (mg/L)
Control (0)	Control (0)	<LOD	<LOD	-
2.56	0.566	0.588 (104)	0.338 (60)	2.02
6.40	1.41	1.39 (99)	1.05 (74)	5.48
16.0	3.54	3.63 (103)	3.04 (86)	15.0
16.0 ¹⁾	3.54	3.52 (99)	3.66 (103)	16.2
40.0	8.84	9.24 (105)	8.64 (98)	40.4
100	22.1	23.2 (105)	21.9 (99)	102

n.a.: not applicable

LOQ: Limit of quantification: 0.0566 mg a.s./L corresponds to 0.256 mg A23282A/L

LOD: Limit of detection: 0.0100 mg a.s./L

¹⁾ Measurements from replicates without algae

The morphology of the algae cells was observed microscopically after 96 hours. The cells were considered normal for the control and up to a test item concentration of 16.0 mg/L. No cells were observed at 40.0 and 100 mg/L test item concentration.

Since some aged concentrations fall below 80 % of nominal, the results were calculated using the nominal and mean measured concentrations.

Algal Biomass

The algal biomass at 24, 48, 72 and 96 hours were calculated for each replicate culture and the means are shown below.

Table A 4: Mean values for the control and test item treatment of Cyprodinil/prothioconazole EC (A23282A) for the density of algal cultures at 24, 48, 72 and 96 hours for *Raphidocelis subcapitata*

Nominal concentrations of A23282A (mg /L)	Density of algal cells ^a			
	24 h	48 h	72 h	96 h
Control	2.75	17.51	82.26	223.06
2.56	1.81	12.58	59.89	196.15
6.40	1.74	8.81	43.02	157.52
16.0	1.26	3.65	11.24	45.49
40.0	0.07	0.00	0.00	0.16
100	0.00	0.00	0.00	0.00

^a The biomass was determined by fluorescence measurement and is given as number of cells (x 10⁴) per milliliter. At the start of the test, the initial cell density was 5200 algal cells/mL.

Growth rate, yield and biomass (area under the growth curve (AUC))

Table A 5: Mean values for the control and test item treatment of A23282A for the percent inhibition of growth rate, yield and AUC at 72 hours for *Raphidocelis subcapitata*

Nominal concentrations of A23282A (mg /L)	0 to 72 h					
	AUC (10 ⁴ *day)	Percentage inhibition of AUC	Growth rate (1/day)	Percentage inhibition of growth rate	Yield (x 10 ⁴)	Percentage inhibition of yield
Control	60.11	0.0	1.69009	0.0	81.75	0.0
2.56	43.04	28.4*	1.58098	6.5*	59.37	27.4*
6.40	30.76	48.8*	1.47022	13.0*	42.50	48.0*
16.0	9.23	84.6*	1.01569	39.9*	10.72	86.9*
40.0	-1.24	≥100* ¹⁾	0.00000	≥100* ¹⁾	-0.52	≥100* ¹⁾
100	-1.30	≥100* ¹⁾	0.00000	≥100* ¹⁾	-0.52	≥100* ¹⁾

* Statistically significant different to the control following Williams Multiple Sequential t-test (left-sided, p≤0.05) for growth rate and Multiple Sequentially-rejective Welsh-t-test after Bonferroni-Holm (left sided, p≤0.05) for yield and biomass integral

¹⁾ Values higher than 100 were replaced by 100. Although mathematically possible to calculate, growth rate, yield and biomass cannot be inhibited more than 100 %

Table A 6: Mean values for the control and test item treatment of A23282 for the percent inhibition of growth rate, yield and AUC at 96 hours for *Raphidocelis subcapitata*

Nominal concentrations of A23282A (mg /L)	0 to 96 h					
	AUC (10 ⁴ *day)	Percentage inhibition of AUC	Growth rate (1/day)	Percentage inhibition of growth rate	Yield (x 10 ⁴)	Percentage inhibition of yield
Control	212.25	0.0	1.51792	0.0	222.55	0.0
2.56	170.54	19.7*	1.48297	2.3	195.63	12.1*
6.40	130.51	38.5*	1.42557	6.1*	157.00	29.5*
16.0	37.07	82.5*	1.11041	26.8*	44.97	79.8*
40.0	-1.68	≥100* ¹⁾	-0.13868	≥100* ¹⁾	-0.36	≥100* ¹⁾
100	-1.82	≥100* ¹⁾	0.00000	≥100* ¹⁾	-0.52	≥100* ¹⁾

* Statistically significant different to the control following Williams multiple sequential t-test (left-sided, p<0.05) for growth rate / yield / biomass integral

¹⁾ Values higher than 100 were replaced by 100. Although mathematically possible to calculate, growth rate, yield and biomass cannot be inhibited more than 100 % .

Table A 7: Summary of biological results for toxicity of A23282A to *Raphidocelis subcapitata* after 72 and 96 hours based on nominal concentrations

Parameter	after 72 h (mg A23282A/L)			after 96 h (mg A23282A/L)		
	Growth rate	Yield	AUC	Growth rate	Yield	AUC
EC ₁₀	6.72	n.d.	n.d.	10.4	2.79*	n.d.
95% C.I.	5.47 – 8.27	n.d.	n.d.	9.50 – 11.4	1.87 – 3.63	n.d.
EC ₂₀	9.93	2.07*	n.d.	13.8	4.54	3.11
95% C.I.	8.66 – 11.4	1.60 – 2.67	n.d.	12.9 – 14.7	3.45 – 5.47	2.46 – 3.93
EC ₅₀	17.9	6.13	6.06	21.0	9.46	7.84
95% C.I.	16.5 – 19.5	5.43 – 6.91	5.32 – 6.91	20.0 – 22.1	8.33 – 10.6	6.96 – 8.84
NOEC	-	-	-	2.56	-	-
LOEC	2.56	2.56	2.56	6.40	2.56	2.56

- NOEC could not be determined

n.d. not determined due to mathematical reasons or inappropriate data

*Based on extrapolation

C.I.: confidence limits

Table A 8: Summary of biological results for toxicity of A23282A to *Raphidocelis subcapitata* after 72 and 96 hours based on mean measured concentrations

Parameter	after 72 h (mg A23282A/L)			after 96 h (mg A23282A/L)		
	Growth rate	Yield	AUC	Growth rate	Yield	AUC
EC ₁₀	5.84	n.d.	n.d.	9.39	2.21*	n.d.
95% C.I.	1.73 – 8.73	n.d.	n.d.	7.14 – 11.2	1.47 – 2.90	n.d.
EC ₂₀	8.93	1.59*	n.d.	12.7	3.76	2.49
95% C.I.	4.18 – 11.9	0.90 – 2.26	n.d.	10.6 – 14.6	2.85 – 4.57	1.58 – 3.31
EC ₅₀	16.9	5.24	5.18	20.2	8.42	6.86
95% C.I.	13.1 – 22.9	4.16 – 6.37	4.24 – 6.15	17.9 – 23.0	7.39 – 9.49	5.65 – 8.16
NOEC	-	-	-	2.02	-	-
LOEC	2.02	2.02	2.02	5.48	2.02	2.02

- NOEC could not be determined

n.d. not determined due to mathematical reasons or inappropriate data

*Based on extrapolation

C.I.: confidence limits

In a separate reference study (experimental start date 11th January 2021), algae were exposed to nominal concentrations of 0.0512, 0.128, 0.320, 0.800 and 2.00 mg potassium dichromate/L for 72-hours. The 72-hour E_rC₅₀ was 1.55 mg potassium dichromate/L, the E_yC₅₀ was 0.847 mg Potassium dichromate/L and the E_bC₅₀ was 0.869 mg Potassium dichromate/L. The NOEC was determined to be 0.320 mg potassium dichromate/L. The results of the reference test demonstrate the sensitivity of the test system.

Validity criteria

The test was considered valid since;

- The algal biomass in the control increased by a factor of 158.19 over 72 hours and 428.96 over 96 hours (must be at least a factor of 16 after 72 hours and 100 after 96 hours)
- The mean coefficient of variation of the daily growth rates in the control was 10 and 24 % over 72 and 96 hours, respectively (must be ≤ 35%).

- The coefficient of variation of average specific growth rates in replicate control cultures was 2.8 and 1.4 % after 72 and 96 hours, respectively (must be <7%).

Conclusion

The toxicity of Cyprodinil/prothioconazole EC (A23282A) to the green alga *Raphidocelis subcapitata* was investigated in a 96-hour static test. Algae were exposed to nominal concentrations of 2.56, 6.40, 16.0, 40.0 and 100 mg A23282A/L equivalent to measured concentrations of 2.02, 5.48, 15.0, 40.4, and 102, alongside a culture medium control.

Based on nominal concentrations, the 72-hour E_rC_{50} was 17.9 mg A23282A/L, the E_yC_{50} was 6.13 mg A23282A/L and the E_bC_{50} was 6.06 mg A23282A/L. The 96-hour E_rC_{50} was 21.0 mg A23282A/L, the E_yC_{50} was 9.46 mg A23282A/L and the E_bC_{50} was 7.84 mg A23282A/L.

Based on mean measured concentrations, the 72-hour E_rC_{50} was 16.9 mg A23282A/L, the E_yC_{50} was 5.24 mg A23282A/L and the E_bC_{50} was 5.18 mg A23282A/L. The 96-hour E_rC_{50} was 20.2 mg A23282A/L, the E_yC_{50} was 8.42 mg A23282A/L and the E_bC_{50} was 6.86 mg A23282A/L.

(Schuler, 2021)

Comments of zRMS:	The study was evaluated at the EU level.
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Reference:	KCP 10.2.1
Report	Ward T.J., Magazu J.P. & Borei R.L. (1995a) Acute flow-through toxicity of CGA219417 to the mysid (<i>Mysidopsis bahia</i>). Report Number 827-CG. T. R. Wilbury Laboratories, Inc. 40 Doaks Lane, Marblehead, Massachusetts. (XXXX File No. CGA219417/0649 / VV-372679)
Guideline:	US EPA FIFRA Guideline No. 72-3(c)
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

The acute toxicity of CGA219417 to the saltwater mysid (*Mysidopsis bahia*) was determined under flow-through conditions. This study was run with mean measured concentrations of 4.90, 6.25, 8.94, 14.5, and 23.5 µg a.s./L together with negative and solvent controls.

The LC_{50} was 8.05 µg a.s./L based on mean measured concentrations.

Materials

Test material	CGA219417
Description:	Off-white granules
Lot/Batch #:	P012012
Purity:	99.2%
Recertification date:	April 1995

Treatments

Test concentrations:	3.9, 6.6, 11, 18 and 30 µg/L nominal (4.90, 6.25, 8.94, 14.5, and 23.5 µg a.s./L mean measured)
Dilution water:	Carbon filtered, natural seawater
Vehicle and/or positive control:	None
Analysis of test concentrations:	Yes at 0 and 96 hours using HPLC analysis

Test organisms

Species:	Saltwater mysid (<i>Mysidopsis bahia</i>)
Source:	Test facility (originally: Aquatic Biosystems, Inc. Fort Collins, Colorado.)
Acclimatisation period:	Adults acclimated 10 days before collection of juveniles
Treatment for disease:	None
Life stage of test organism:	Juvenile (<24 hours)
Feeding:	Live brine shrimp (<i>Artemia salina</i> nauplii) daily during test

Test design

Test vessels:	20L glass aquaria containing 15 L test solution.
Replication:	2 replicates, 10 mysids per replicate
Exposure regime:	Flow-through
Duration:	96 hours

Environmental conditions

Test temperature:	21.4 to 22.5 at start and 21.4 to 22.6 at test end
pH range:	8.2 to 8.5 measured daily
Dissolved oxygen:	5.5 to 8.3 mg/L measured daily
Salinity of dilution water:	15‰ at test start
Lighting:	16 hours fluorescent light and 8 hours dark daily, with 30 minute dawn and dusk transition periods. Light intensity ~ 215 lux at water surface.

Study Design and Methods

Experimental dates: 14th to 18th December 1995

Test chambers were suspended in the aquaria which were filled with 15 L test water. A continuous flow diluter was used to deliver each concentration of the test substance, solvent control and negative control. A syringe pump was used to deliver the five test substance stocks and the solvent control stock into the mixing chambers assigned to each treatment group.

The test chambers were impartially positioned within a water bath to maintain temperature. Two replicate tanks were prepared for the controls and each test solution. Ten mysids were randomly allocated to each prepared test vessel.

A primary stock of 300 mg a.s./mL test material was prepared by combining 0.151 g of test substance with dimethylformamide in a 500 ml volumetric flask. Appropriate amounts of the stock solution were added

directly to the dilution water to formulate test media. Nominal concentrations were 3.9, 6.6, 11, 18 and 30 µg/L.

The concentrations of test material in the test solutions were measured at the beginning, and at 96 hours using HLPC analysis.

Observations were made for mortality and clinical symptoms of toxicity at approximately 6, 24, 48, 72 and 96 hours.

Results and Discussion

The measured concentrations are shown in the table below in terms of nominal concentrations. The test concentrations were maintained throughout the study. Mean measured concentrations were used for the calculation and reporting of results.

Table A 9: Analytical results

Nominal concentration (µg a.s./L)	% of nominal 0 hours	% of nominal 96 hours	Mean measured concentration (µg a.s./L)
3.9	142	108	4.90
6.6	96	95	6.25
11	80	81	8.94
18	81	81	14.5
30	82	78	23.5

Mysids in the controls the three lowest treatment groups appeared normal throughout the test. Lethargy was observed in the 14.5 µg/L treatment after 48 hours.

Table A 10: Effects of test material on the survival of saltwater mysids (*Mysidopsis bahia*) following exposure for 96 hours in a flow-through test

Mean measured concentration (mg a.s./L)	Mean number alive (n = 10)			
	24 hour	48 hour	72 hour	96 hour
Dilution water control	10	10	10	10
Solvent control	9.5	9.5	9.5	9.5
4.90	10	10	9.5	9
6.25	10	10	9	8.5
8.94	10	10	5.5	3.5
14.5	10	4.5	0	0
23.5	10	0	0	0
LC ₅₀ (µg a.s./L)	>23.4	14.0	8.85	8.05
95% confidence limits	n.d.	8.94 – 23.5	7.96 - 9.99	7.22 – 9.05
Method	-	Binomial	Probit	Probit

a Ten mysids were exposed in each test vessel, two replicates per treatment.

n.d. –not determined

Conclusions

The 96 hour LC₅₀ for test material to the saltwater mysid (*Mysidopsis bahia*) was calculated to be 8.05 µg a.s./L, based on mean measured concentrations.

(Ward *et al.*, 1995)

Comments of zRMS:	<p>The study was not evaluated.</p> <p>The study for metabolite was not used in risk assessment as no data gap was agreed at the EU level.</p> <p>The study considering the toxicity of active substance should be evaluated at EU level during substance renewal.</p>
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Reference:	KCP 10.2.1
Report	Eckenstein H. (2015). CGA321915 – Acute Toxicity to <i>Daphnia magna</i> in a 48-Hour Immobilization Test. Report number D96733. CEMAS, Harlan Laboratories Ltd., Zelgliweg 1, 4452 Itingen, Switzerland, (XXXX file no. VV-411573; CGA321915_10005)
Guideline(s):	OECD Guidelines for Testing of Chemicals, Section 2 - Effects on Biotic Systems, Method 202: <i>Daphnia</i> sp., Acute Immobilisation Test (2004)
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

The acute toxicity of CGA321915 to *Daphnia magna* was determined under static conditions. Daphnids were exposed to a range of nominal concentrations of 6.25, 12.5, 25, 50 and 100 mg CGA321915/L (mean measured: 6.1, 12, 23, 49 and 98 mg CGA321915/L) alongside a dilution water control. Based on mean measured concentrations, the 48-hour EC₅₀ was determined to be > 98 mg CGA321915/L, the highest concentration tested.

Materials

Test Material	CGA321915
	CSAA400257
Parent:	CGA219417 (Cyprodinil)
Lot/Batch #:	MES 356/1
Purity:	98 % w/w (estimated error: ± 2 %)
Description:	White solid
Stability of test compound:	Stable under test conditions
Reanalysis/Expiry date:	30 September 2016
Density:	Not applicable
Treatments	
Test concentrations:	Dilution water control and nominal concentrations of 6.25, 12.5, 25, 50 and 100 mg CGA321915/L (mean measured: 6.1, 12, 23, 49 and 98 mg CGA321915/L)
Solvent:	None
Positive control:	Potassium dichromate used twice a year
Analysis of concentrations:	test Yes 0 and 48 hours using HPLC analysis with UV detection
Test organisms	
Species:	<i>Daphnia magna</i> Straus, Clone 5

Age:	6 - 24 hours
Source:	Continuous laboratory cultures, originally sourced from University of Sheffield UK in 1992
Feeding:	None during test
Culture medium:	Reconstituted water according to ISO 6341
Test design	
Test vessels:	100 mL glass beakers containing 50 mL test medium, covered with glass plates
Test medium:	Reconstituted test water according to ISO 6341. The test water was aerated prior to the start of the study until oxygen saturation was reached.
Replication:	4 replicates of 5 daphnids
Exposure regime:	Static
Duration:	48 hours
Environmental conditions	
Test temperature:	21 – 22 °C
pH range:	7.9 to 8.1
Dissolved oxygen:	8.2 to 8.4 mg/L
Total hardness of dilution water:	250 mg/L as CaCO ₃ .
Lighting:	16 hours light (800 - 1060 Lux) and 8 hours dark with a 30 minute transition period

Study Design and Methods

Test facility: CEMAS, Harlan Laboratories Ltd., Zelgliweg 1, 4452 Itingen, Switzerland

Experimental dates: 2nd December 2014 to 6th January 2015

The test medium with the highest nominal concentration of 100 mg CGA321915/L was prepared by dissolving 100.41 mg of the test item in 1000 mL of test water under intense stirring for 15 minutes at room temperature. Appropriate volumes of this test solution were diluted with test water to prepare the test media of the lower test item concentrations. The control consisted of dilution water only. Test solutions were added to the test vessels and the *Daphnia* added without conscious bias.

The immobility of the daphnids was determined by visual observations after 24 and 48 hours of exposure. Organisms unable to swim within 15 seconds after gentle agitation of the test beaker were considered to be immobile.

The pH, temperature and dissolved oxygen were measured at the start, after 24 hours and at the end of the test in each test concentration and the control.

The test concentrations were verified by chemical analysis of CGA321915 at 0 and 48 hours using HPLC with UV detection. The 48-hour samples were taken from pooled replicates.

Results and Discussion

At the start of the test, the analytically determined concentrations of CGA321915 were in the range 83 to 100 % of the nominal values and at the end of the test were in the range 98 to 102 %, (see table below). The limit of quantification in this study was 1.03 mg CGA321915/L. Arithmetic mean measured concentrations were used for the calculation and reporting of results.

Table A 11: Analytical results

Nominal concentrations (mg CGA321915/L)	% of nominal measured at 0 hours	% of nominal measured at 48 hours	Mean measured concentrations (mg CGA321915/L)
Control	n.a.	n.a.	n.a.
6.25	94	102	6.1
12.5	98	100	12
25	83	98	23
50	100	98	49
100	97	99	98

n.a. = not applicable

The tabulated values of the samples represent rounded results obtained by calculation using the exact raw data

The median effect concentration (EC₅₀) is defined as the concentration resulting in 50 % immobilisation of the *Daphnia* in the time period specified, and could not be calculated due to the absence of toxicity of the test item. The NOEC (No Observed Effect Concentration) is defined as the highest tested concentration which did not produce an adverse effect when compared to the control and was determined directly from the raw data. There was no immobility observed in the dilution water control. Immobility data and estimated EC₅₀ values are shown in the table below:

Table A 12: Effects of CGA321915 on *Daphnia magna* following exposure for 48-hours in a static test

Mean measured concentration (mg CGA321915/L)	Immobilised daphnids after 24 hours		Immobilised daphnids after 48 hours	
	Number	%	Number	%
Control	0	0	0	0
6.1	0	0	0	0
12	0	0	0	0
23	0 (10 A)	0	0 (8A)	0
49	0 (12 A)	0	0 (12A)	0
98	0 (13A)	0	0 (18A)	0
EC ₅₀ (mg CGA321915/L)	> 98			
95% Confidence limits	n.d.			
NOEC (mg CGA321915/L)	98			

Values in parenthesis: number of test animals with adverse effects;

A: daphnids trapped at the water surface

n.d. = could not be determined

Validity Criteria

The validity criteria for the test were met:

- Immobilisation or signs of disease or stress in the control ≤10 % (observed 0 %)
- Dissolved oxygen concentration at the end of the test ≥ 3 mg/L in the control and test vessels (measured: 8.2 to 8.4 mg/L)

Conclusions

Based on mean measured concentrations, the 48-hour EC₅₀ was determined to be > 98 mg CGA321915/L, the highest concentration tested. The 48-hour NOEC was determined to be 98 mg CGA321915/L.

(Eckenstein H, 2015)

Comments of zRMS:	The study was evaluated at zonal level and the proposed endpoints were used in risk assessment.
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Reference:	KCP 10.2.1
Report	Maynard S.K. (2011). CGA219417 – 96 hour acute toxicity to juvenile <i>Asellus aquaticus</i> . Report number CEMR-5069. CEMAS, North Ascot, Berkshire, UK, (XXXX file no. VV-397982; CGA219417_11453)
Guideline:	OECD Guidelines for Testing of Chemicals, Section 2 - Effects on Biotic Systems, Method 202: <i>Daphnia</i> sp., Acute Immobilisation Test (2004)
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

The acute toxicity of CGA219417 to juvenile *Asellus aquaticus* was determined under static conditions over 96 hours. Juvenile *Asellus* were exposed to a range of nominal concentrations of 0.116, 0.256, 0.563, 1.24, 2.73 and 6.00 mg CGA219417/L, alongside a dilution water control. Based on nominal concentrations, the 96-hour EC₅₀ was 2.64 mg CGA219417/L. Control mortality at this time point had reached 35%. At 48-hours the EC₅₀ was 2.35 mg CGA219417/L, with 5% control mortality.

Materials

Test Material	CGA219417
Lot/Batch #:	P.012011
Actual content of active ingredients:	99.2%
Description:	Light beige crumbs
Stability of test compound:	Stable under standard conditions.
Reanalysis/Expiry date:	30 April, 2014
Density:	n.a.
Treatments	
Test concentrations:	Dilution water control and nominal formulation concentrations of 0.116, 0.256, 0.563, 1.24, 2.73 and 6.00 mg CGA219417/L
Solvent:	None
Positive control:	n.a.
Analysis of concentrations:	Yes 0 and 96 hours (based on measurement of CGA219417) using LC analysis
Test organisms	
Species:	<i>Asellus aquaticus</i>
Age:	Juveniles, <5mm
Source:	Commercial supplier, Mesocosm GmbH, Homberg, Germany
Feeding:	None during test
Culture medium:	Elendt M4
Test design	

Test vessels:	300 mL crystallising dishes with ~ 5 g fine quartz sand substrate
Test medium:	Elendt M4
Replication:	4 replicates, each containing 5 <i>Asellus</i> (one replicate at 2.73 mg/L contained 6 animals)
Exposure regime:	Static
Duration:	96 hours
Environmental conditions	
Test temperature:	15.1 to 16.0°C
pH range:	7.00 to 7.20
Dissolved oxygen:	84 to 98 % air saturation value (ASV), no aeration
Total hardness of dilution water:	246 mg/L as CaCO ₃ .
Lighting:	Photoperiod - 16 hours light and 8 hours dark

Study Design and Methods

Test facility: CEMAS, North Ascot, Berkshire, UK.

Experimental dates: 21st to 25th June 2011

The day before the test a stock solution with a nominal concentration of 6 mg/L was prepared by dissolving CGA219417 in dilution water using ultrasonication for 30 minutes, then one hour of stirring followed by one further minute of ultrasonication. Using this stock solution the test concentrations were prepared on the day of the test by dilution. The control consisted of dilution water. Test solutions were added to the test vessels, together with ~ 5 g fine quartz sand substrate, then five juvenile *Asellus* were added to each of the four replicates at each treatment. An additional animal was exposed at 2.73 mg/L. Preliminary work had identified the need for an inert substrate to provide the test animals some purchase on the base of the vessels.

The immobility of the *Asellus* was determined by visual observations after 24, 48, 72 and 96 hours of exposure. Organisms unable to move within 15 seconds after gentle agitation of the test beaker were considered to be immobile. Abnormal behaviour, such as lethargy, was also noted.

The pH, temperature and dissolved oxygen were measured at the start and end of the test in each test concentration and the control.

The test concentrations were verified by chemical analysis of CGA219417 at 0 and 96 hours using liquid chromatography with ultra violet-visible detection. In addition, the 6.00 mg/L treatment was sampled for analysis at 48 hours, as all the test organisms were immobile at this time.

The median effect concentrations (EC₅₀) were calculated using maximum likelihood-probit analysis. Where control mortality was >5%, Abbott's correction was applied before conducting Maximum likelihood-Probit analysis. Abbott's correction was not applied to 96-hour data before the linear interpolation analysis. The 24 and 48-h NOECs were determined using Steel's Many-One Rank Test. At 72 & 96-h Dunnett's test was used.

Results and Discussion

At the start of the test, the concentrations of CGA219417 were in the range 82 to 92% of the nominal values, and at the end of the test were in the range 88 to 100% (see table below). The limit of quantification in this study was 0.01 mg CGA219417/L. Nominal concentrations were used for the calculation and reporting of results.

Table A 13: Analytical results

Nominal concentrations of CGA219417 (mg/L)	% of nominal measured at 0 hours	% of nominal measured at 96 hours
0 (control)	n.a.	n.a.
0.116	91	88
0.256	89	91
0.563	89	90
1.24	88	90
2.73	82	90
6.00	92	100 (96)*

n.a. = not applicable, *= 48-h sample

Immobility data and estimated EC₅₀ values are shown in the table below:

Table A 14: Effects of CGA219417 on juvenile *Asellus aquaticus* following exposure for 96-hours in a static test

Nominal concentration of CGA219417 (mg/L)	Cumulative immobilisation 24 hours		Cumulative immobilisation 48 hours		Cumulative immobilisation 72 hours		Cumulative immobilisation 96 hours	
	Number	%	Number	%	Number	%	Number	%
0 (control)	0	0	1	5	3	15	7	35
0.116	0	0	1	5	3	15	7	35
0.256	0	0	0	0	3	15	4	20
0.563	0	0	0	0	5	25	6	30
1.24	0	0	3	15	7	35	8	40
2.73*	2	9	12	56	13	62	14	67
6.00	16	80	20	100	20	100	20	100
EC ₅₀ (mg CGA219417/L)	4.41		2.35		2.27**		2.64**	
95% Confidence limits	3.65 – 5.39		1.81 – 2.92		1.38 – 3.02		0.289 – 4.42	
NOEC	2.73		1.24		1.24		2.73	

*= 11 animals tested at this treatment, **= Control immobility at 72 and 96 hours was outside the validity criteria for the study

Conclusions

Based on nominal concentrations, the 96-hour EC₅₀ was 2.64 mg CGA219417/L. Control mortality at this time point had reached 35%. At 48-hours the EC₅₀ was 2.35 mg CGA219417/L, with 5% control mortality.

(Maynard S, 2011)

Comments of zRMS:	The study was evaluated at zonal level and the proposed endpoints were used in risk assessment.
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Reference: KCP 10.2.1

Report Allen M. and Taylor S., (2015) CGA249287 – Toxicity test of CGA249287 to green algae (growth inhibition test). Statistical Re-analysis. Report Number: CEA.1428. Cambridge Environmental Assessments, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK. (XXXX File No: VV-

	28890; CGA249287_10009)
Guideline(s):	OECD Guidelines No. 201 (2011)
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Summary

Report number G 571 17 (Maetzler, 1999) did not provide EC₂₀ estimates relating to yield or average specific growth rate for *Pseudokirchneriella subcapitata*. Consequently, the data generated in this study have been re-analysed in order to provide these values.

The EC₂₀ could not be reliably determined as the EC₁₀ was > 100 mg/L, the highest concentration tested.

Statistical Analysis

The test design consisted of five nominal test concentrations: 4.3, 9.4, 21, 45 and 100 mg/L and a control. Nominal concentrations were confirmed by chemical analysis.

Results

The EC₂₀ could not be reliably calculated as the EC₁₀ and NOEC values were originally reported as > 100 mg/L and 100 mg/L respectively, the highest concentration tested.

Conclusion

The EC₂₀ could not be reliably determined as the EC₁₀ was > 100 mg/L, the highest concentration tested.

(Taylor S and Allen M, 2015)

Comments of zRMS:	The study was evaluated at zonal level and the proposed endpoints were used in risk assessment.
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Reference:	KCP 10.2.1
Report	Taylor S. and Allen M. (2015) CGA275535 – Toxicity of CGA275535 to green algae (growth inhibition test). Statistical Re-analysis. Report Number: CEA.1429. Cambridge Environmental Assessments, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK. (XXXX File No: VV-28891 CGA275535_10006)
Guideline(s):	OECD Guidelines No. 201 (2011)
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Summary

Report number G 655 17 (Maetzler, 2001) did not provide EC₂₀ estimates relating to yield or average specific growth rate for *Pseudokirchneriella subcapitata*. Consequently, the data generated in this study

have been re-analysed in order to provide these values.

A dose response relationship was observed between treatment and algal growth rate and yield at 24, 48 and 72 h. EC₂₀ values and the 95% and 99% confidence limits could therefore be reliably determined.

Statistical Analysis

The algal yield represents the cell number/biomass generated during the test or evaluation period minus the cell number/biomass at the start of the exposure or evaluation period. The average specific growth rate represents the slope of the growth curve. All computations were carried out in ToxRat Professional version 2.10 (ToxRat Solutions GmbH, 2001-2010). Probit analysis with linear maximum likelihood regression was used to determine the concentration response function. Chi² was used as a goodness of fit measure and the proportion of variance explained by the dose/response function as determined.

Results

Average Specific Growth Rate

Treatment related effects were observed and the relationship between dose and response was significant at 24 h (p(F) = 0.000), 48 h (p(F) = 0.000) and 72 h (p(F) = 0.000). The ErCx values and the 95% and 99% confidence limits were reliably determined for these time periods.

Yield

Treatment related effects were observed and the relationship between dose and response was significant at 24 h (p(F) = 0.000), 48 h (p(F) = 0.000) and 72 h (p(F) = 0.000). The EyCx values and the 95% and 99% confidence limits were reliably determined for all time periods.

EC₁₀ and EC₂₀ Estimates

Estimates of the EC₁₀ and EC₂₀ can be summarized as follows:

Table A 15: Summary of growth rate and yield results for toxicity of CGA275535 to *Pseudokirchneriella subcapitata* after 24, 48 and 72 hours

Parameter	After 24 h		After 48 h		After 72 h	
	Growth rate	Yield	Growth rate	Yield	Growth rate	Yield
EC ₂₀ (mg/L)	21.694	4.117	10.537	5.523	10.342	6.342
95% CL	(13.048-31.085)	(1.837-6.516)	(5.323-15.343)	(4.504-6.392)	(5.457-14.773)	(5.595-6.936)

CL: Confidence Limits

n.d.: Not Determined

Conclusion

A dose response relationship was observed between treatment and algal growth rate and yield at 24, 48 and 72 h. EC₂₀ values and the 95% and 99% confidence limits could therefore be reliably determined.

(Taylor S & Allen M, 2015)

Comments of zRMS:	The study was evaluated at zonal level and the proposed endpoints were used in risk assessment.
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Reference: KCP 10.2.1

Report Taylor S., Pickering F. & Allen M. (2016), Cyprodinil (CGA219417) – Growth and reproduction toxicity test with CGA219417 and the freshwater alga, *Selenastrum capricornutum*. Statistical Re-analysis. Report Number:

	CEA.1424. Cambridge Environmental Assessments, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK. Amended (XXXX File No: VV-28887; CGA219417_11593)
VVGuideline(s):	OECD Guidelines No. 201 (2011)
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Report number 791-CG (Ward et al., 1995) did not provide EC₁₀ or EC₂₀ estimates relating to yield or average specific growth rate for *Pseudokirchneriella subcapitata*. Consequently, the data generated in this study have been re-analysed in order to provide these values.

A significant dose response relationship was observed between treatment and algal growth rate at 24, 72 and 96, and for yield at 24, 48, 72, 96 and 120 h, therefore EC₁₀, EC₂₀ and EC₅₀ values and the 95% and 99% confidence limits could be reliably determined. There was no significant relationship between dose and response in the remaining parameters and therefore no EC_x could be reliably calculated.

Statistical Analysis

The algal yield represents the cell number/biomass generated during the test or evaluation period minus the cell number/biomass at the start of the exposure or evaluation period. The average specific growth rate represents the slope of the growth curve. All computations were carried out in ToxRat Professional version 2.10 (ToxRat Solutions GmbH, 2001-2010). Probit analysis with linear maximum likelihood regression was used to determine the concentration response function. Chi² was used as a goodness of fit measure and the proportion of variance explained by the dose/response relationship was determined.

Data were re-evaluated using mean measured concentrations.

Results

Average Specific Growth Rate

Treatment related effects were observed and the relationship between dose and response was significant at 24 h (p(F) = 0.01), 72 h (p(F) <0.001) and 96 h (p(F) <0.001). ErC_x values and the 95% and 99% confidence limits were reliably determined for these time periods. There was no significant relationship at 48 h (p(F) = 0.590) and therefore no reliable EC_x could be calculated for this time point.

Yield

Treatment related effects were observed and the relationship between dose and response was significant at 24 h (p(F) = 0.00), 48 h (p(F) <0.001), 72 h (p(F) <0.001), 96 h (p(F) <0.001) and 120 h (p(F) <0.001). EyC_x values and the 95% and 99% confidence limits were reliably determined for these time periods.

EC₁₀, EC₂₀ and EC₅₀ estimates

Estimates of the EC₁₀, EC₂₀ and EC₅₀ can be summarized as follows:

Table A 16: Summary of growth rate results for toxicity of cyprodinil to *Selanastrum capricornutum* after 24, 48 and 72 hours

Parameter	24 hours			48 hours			72 hours		
	ErC ₁₀	ErC ₂₀	ErC ₅₀	ErC ₁₀	ErC ₂₀	ErC ₅₀	ErC ₁₀	ErC ₂₀	ErC ₅₀
Value [mg/L]	0.814	1.900	9.620	1.682	2.115	3.276	2.336	2.824	4.058
lower 95%-cl	0.105	0.613	5.931	1.646	2.079	3.240	2.245	2.742	4.008
upper 95%-cl	1.608	3.055	31.353	1.718	2.150	3.311	2.422	2.900	4.107

Table A 17: Summary of yield results for toxicity of cyprodinil to *Selanastrum capricornutum* after 24, 48 and 72 hours

Parameter	24 hours			48 hours			72 hours		
	E _y C ₁₀	E _y C ₂₀	E _y C ₅₀	E _y C ₁₀	E _y C ₂₀	E _y C ₅₀	E _y C ₁₀	E _y C ₂₀	E _y C ₅₀
Value [mg/L]	0.688	1.478	6.377	1.405	1.931	3.548	0.432	0.673	1.568
lower 95%-cl	0.060	0.335	3.950	1.254	1.772	3.374	0.410	0.646	1.529
upper 95%-cl	1.443	2.528	16.915	1.547	2.080	3.728	0.455	0.699	1.608

Table A 18: Summary of yield results for toxicity of cyprodinil to *Selanastrum capricornutum* after 96 and 120 hours

Parameter	96 hours			120 hours		
	E _y C ₁₀	E _y C ₂₀	E _y C ₅₀	E _y C ₁₀	E _y C ₂₀	E _y C ₅₀
Value [mg/L]	1.345	1.606	2.256	0.859	1.164	2.083
lower 95%-cl	1.274	1.547	2.182	0.747	1.050	1.957
upper 95%-cl	1.407	1.660	2.342	0.960	1.268	2.220

Conclusion

A significant dose response relationship was observed between treatment and algal growth rate at 24, 72 and 96 h, and for yield at 24, 48, 72, 96 and 120 h, therefore EC₁₀, EC₂₀ and EC₅₀ values and the 95% and 99% confidence limits could be reliably determined. There was no significant relationship between dose and response in the remaining parameters and therefore no EC_x could be reliably calculated.

(Taylor S. et al, 2016)

Comments of zRMS:	The study was evaluated at zonal level and the proposed endpoints were used in risk assessment.
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Reference:	KCP 10.2.1
Report	Taylor S. &. Allen M. (2016), Cyprodinil (CGA219417) – Toxicity of CGA219417 tech to green algae (growth inhibition test). Statistical Re-analysis. Report Number: CEA.1423. Cambridge Environmental Assessments, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK. Amended (XXXX File No: VV-28896; CGA219417_11598)
Guideline(s):	OECD Guidelines No. 201 (2011)
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication	No

(if vertebrate study)

Summary

Report number L01-002763 (Maetzler, 2001) did not provide EC₂₀ estimates relating to yield or average specific growth rate for *Pseudokirchneriella subcapitata*. Consequently, the data generated in this study have been re-analysed in order to provide these values.

A dose response relationship was observed between treatment and algal growth rate and yield at 24, 48, and 72 h, therefore EC₂₀ values and the 95% and 99% confidence limits could be reliably determined.

Statistical Analysis

The algal yield represents the cell number/biomass generated during the test or evaluation period minus the cell number/biomass at the start of the exposure or evaluation period. The average specific growth rate represents the slope of the growth curve. All computations were carried out in ToxRat Professional version 2.10 (ToxRat Solutions GmbH, 2001-2010). Probit analysis with linear maximum likelihood regression was used to determine the concentration response function. Chi² was used as a goodness of fit measure and the proportion of variance explained by the dose/response relationship was determined.

Results

Average Specific Growth Rate

Treatment related effects were observed and the relationship between dose and response was significant at 24 h (p(F) = 0.000), 48 h (p(F) = 0.000) and 72 h (p(F) = 0.000). E_rC₂₀ values and the 95% and 99% confidence limits were reliably determined for these time periods.

Yield

Treatment related effects were observed and the relationship between dose and response was significant at 24 h (p(F) = 0.000), 48 h (p(F) = 0.000) and 72 h (p(F) = 0.000). E_yC₂₀ values and the 95% and 99% confidence limits were reliably determined for these time periods.

EC₁₀ and EC₂₀ Estimates

Estimates of the EC₁₀ and EC₂₀ can be summarized as follows:

Table A 19: Summary of growth rate and yield results for toxicity of cyprodinil to *Pseudokirchneriella subcapitata* after 24, 48 and 72 hours

Parameter	After 24 h		After 48 h		After 72 h	
	Growth rate	Yield	Growth rate	Yield	Growth rate	Yield
EC ₂₀ (mg/L)	2.790	2.062	3.954	1.282	4.013	1.738
95% CL	(2.294-3.227)	(1.699-2.390)	(3.747-4.145)	(1.046-1.503)	(3.904-4.117)	(1.587-1.877)

CL: Confidence Limits

Conclusion

A dose response relationship was observed between treatment and algal growth rate and yield at 24, 48, and 72 h, therefore EC₂₀ values and the 95% and 99% confidence limits could be reliably determined.

(Taylor S & Allen M, 2016)

Comments of zRMS:	The study was not evaluated. The study for metabolite was not used in risk assessment as no data gap was agreed at the EU level.
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Reference:	KCP 10.2.1
Report	Eckenstein H. (2015). CGA321915 – Toxicity to <i>Pseudokirchneriella subcapitata</i> in a 96-Hour Algal Growth Inhibition Test. Report number D96711. CEMAS, Harlan Laboratories Ltd., Zelgliweg 1, 4452 Itingen, Switzerland, (XXXX file no. VV-411271; CGA321915_10004)
Guideline(s):	OECD Guidelines for Testing of Chemicals, Section 2 - Effects on Biotic Systems, Method 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test (2011)
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

The toxicity of CGA321915 to the green alga *Pseudokirchneriella subcapitata* was determined. Algae were exposed to nominal concentrations of 2.2, 4.6, 10, 22, 46 and 100 mg CGA321915/L (mean measured: 2.1, 4.6, 9.8, 21, 45 and 99 mg CGA321915/L) alongside a culture medium control. Based on the mean measured concentrations the 72- and 96-hour E_rC_{50} , E_yC_{50} and E_bC_{50} were > 99 mg CGA321915/L, the highest concentration tested.

Materials

Test Material	CGA321915 CSAA400257
Parent:	CGA219417 (Cyprodinil)
Lot/Batch #:	MES 356/1
Purity:	98 % w/w (estimated error: ± 2 %)
Description:	White solid
Stability of test compound:	Stable under test conditions
Reanalysis/Expiry date:	30 September 2016
Density:	Not applicable
Test concentrations:	Culture medium control and nominal concentrations of 2.2, 4.6, 10, 22, 46 and 100 mg CGA321915/L (mean measured concentrations: 2.1, 4.6, 9.8, 21, 45 and 99 mg CGA321915/L)
Solvent:	None
Positive control:	Potassium dichromate is used twice a year (last study – D94966, August 2014)
Analysis of test concentrations:	Yes, 0 and 96 hours using HPLC with UV detection
Test organism	
Species:	<i>Pseudokirchneriella subcapitata</i> , Strain No. 61.81 SAG
Source:	Laboratory cultures, originally obtained from Collection of Algal Cultures (SAG, Institute for Plant Physiology, University of Göttingen, 37073 Göttingen, Germany)
Test design	
Test vessels:	50-mL plastic Erlenmeyer flasks containing 15 mL of media covered with glass dishes
Test medium:	Reconstituted test water (AAP medium) prepared according to OECD

	guideline 201
Replication:	Six vessels for the control and three vessels for each test concentration
Starting cell density:	0.5×10^4 cells/mL
Exposure regime:	Static
Aeration:	None reported
Duration:	96 hours
Environmental conditions	
Test temperature:	22 °C
pH:	test start: 7.2 test end: 8.6 to 8.7
Lighting:	Continuous illumination at an approximately 4900 Lux (range: 4360 to 5290 Lux)

Study Design and Methods

Test facility: CEMAS, Harlan Laboratories Ltd., Zelgliweg 1, 4452 Itingen, Switzerland
Experimental dates: 19th November 2014 to 6th January 2015

A stock solution of the highest nominal concentration of 100 mg CGA321915/L was prepared by mixing 50.18 mg of the test item completely in 500 mL of test water using intense stirring for 15 minutes at room temperature. This stock solution was used in a series of dilutions with test water to prepare the test media of the lower test concentrations. The control consisted of culture medium only.

An aliquot of test solution was placed into each test vessel and the test was started by inoculation of 5000 algal cells per mL of test medium. Test solutions were continuously stirred with magnetic stirrers and were held in a temperature controlled water bath under continuous illumination.

Small volumes of all test concentrations and controls were taken from all test flasks after 24, 48, 72 and 96 hours of exposure. The algal cell densities in these samples were determined by fluorescence measurement. In addition, after 96 hours exposure, a sample was taken from the control and from the nominal 100 mg CGA321915/L test concentration. The shape and size of the algal cells was examined microscopically in these samples.

The pH was measured at the start and at the end of the test. The water temperature was measured daily in a flask incubated under the same conditions as the test flasks. The appearance of the test media was also recorded daily.

The test concentrations were verified by chemical analysis of CGA321915 at 0 (without algae) and 96 hours (containing algae), using HPLC with UV detection. For sampling at the end of the test, the test medium of the treatment replicates were pooled.

Results and Discussion

At the start of the test, the analytically determined concentrations of CGA321915 were in the range 98 to 100 % of the nominal values and at the end of the test were in the range 91 to 101 % (see table below). The limit of quantification in this study was 1.03 mg CGA321915/L. Arithmetic mean measured concentrations were used for the calculation and reporting of results.

Table A 20: Analytical results

Nominal concentrations (mg CGA321915/L)	% of nominal measured at 0 hours	% of nominal measured at 96 hours*	Mean measured concentrations (mg CGA321915/L)
Control	n.a.	n.a.	n.a.
2.2	99	95	2.1
4.6	98	101	4.6
10	100	95	9.8
22	98	91	21
46	99	97	45
100	98	99	99

* Pooled treatment replicates samples were centrifuged

n.a. = not applicable

The tabulated values of the samples represent rounded results obtained by calculation using the exact raw data

The algal cell densities were measured at 24, 48, 72 and 96 hours and the mean biomass, growth rate and yield calculated. The 72-hour E_bC_{10} was calculated by Probit Analysis using linear maximum likelihood regression. No further EC_x values could be determined because none of the responses exceeded 50 %. Dunnett's t-test (one-sided smaller, $\alpha = 0.05$) was used to identify significant differences in the calculated biomass, growth rate and yield of test item treatments compared to the control.

There were no abnormalities, observed microscopically, in the control or the nominal 100 mg CGA321915/L test culture at 96 hours.

Growth rates

The growth rate 0 to 72 hours and 0 to 96 hours were calculated for each replicate culture and the means are shown below.

Table A 21: Mean values at each concentration of CGA321915 for the growth rate at 72 and 96 hours for *Pseudokirchneriella subcapitata*

Mean measured concentrations (mg CGA321915/L)	Mean growth rate (1/day) 0 – 72 hrs	Percentage inhibition	Mean growth rate (1/day) 0 – 96 hrs	Percentage inhibition
Control	1.515	0.0	1.427	0.0
2.1	1.521	-0.5	1.418	0.6
4.6	1.521	-0.4	1.423	0.3
9.8	1.492	1.5	1.399	1.9
21	1.495	1.3	1.411	1.1
45	1.503	0.8	1.411	1.1
99	1.469	3.0	1.402	1.7

No statistically significant effects compared to control (according to Dunnett t-test, one-sided smaller, $\alpha = 0.05$)

Yield

The yield 0 to 72 hours and 0 to 96 hours were calculated for each replicate culture and the means are shown below.

Table A 22: Mean values at each concentration of CGA321915 for the yield at 72 and 96 hours for *Pseudokirchneriella subcapitata*

Mean measured concentrations (mg CGA321915/L)	Mean yield (x 10 ³ cells/mL) 0 – 72 hrs	Percentage inhibition	Mean yield (x 10 ³ cells/mL) 0 – 96 hrs	Percentage inhibition
Control	54.5	0.0	175.6	0.0
2.1	55.7	-2.2	169.2	3.6
4.6	55.7	-2.3	172.7	1.6
9.8	51.0	6.5	157.9	10.1
21	51.4	5.6	165.0	6.0
45	52.7	3.4	165.4	5.8
99	47.8	12.3	159.2	9.3

No statistically significant effects compared to control (according to Dunnett t-test, one-sided smaller, $\alpha = 0.05$)

Biomass (area under the growth curve)

The areas under the growth curve for 0 to 72 hours and 0 to 96 hours were calculated for each replicate culture and the means are shown below.

Table A 23: Mean values at each concentration of CGA321915 for the biomass integral (area under the growth curve) at 72 and 96 hours for *Pseudokirchneriella subcapitata*

Mean measured concentrations (mg CGA321915/L)	Mean biomass integral (x 10 ³ *day) 0 – 72 hrs	Percentage inhibition	Mean biomass integral (x 10 ³ *day) 0 – 96 hrs	Percentage inhibition
Control	44.2	0.0	159.2	0.0
2.1	44.1	0.2	156.6	1.7
4.6	43.8	1.0	158.0	0.8
9.8	40.7	7.9	145.1	8.9
21	41.7	5.8	149.9	5.9
45	41.8	5.4	150.9	5.3
99	38.4*	13.1	141.9*	10.9

*: mean value statistically significantly lower than in the control (according to Dunnett t-test, one-sided smaller, $\alpha = 0.05$)

Validity criteria

The algal biomass in the control increased by a factor of 94 over 72 hours (must be least 16). The mean coefficient of variation of the daily growth rates in the control cultures was 13 % and 17 % over 72 and 96 hours, respectively (must be ≤ 35 %). The coefficient of variation of average specific growth rates in the control cultures was 0.8 % and 0.5 % over 72 and 96 hours, respectively (must be ≤ 7 %). Therefore, all validity criteria were met.

Conclusions

Based on the mean measured concentrations the 72- and 96-hour E_rC_{50} , E_yC_{50} and E_bC_{50} were > 99 mg CGA321915/L, the highest concentration tested.

The LOEC at 72 and 96 hours, based on biomass, was 99 mg CGA321915/L and the corresponding NOEC was 45 mg CGA321915/L. The LOEC at 72 and 96 hours, based on growth rate and yield, could not be determined. The NOEC at 72 and 96 hours, based on growth rate and yield, was 99 mg CGA321915/L.

(Eckenstein H, 2015)

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

Comments of zRMS:	The study was not evaluated. The study for metabolite active substance was not used in risk assessment as no data gap was agreed at the EU level.
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Reference:	KCP 10.2.2
Report	Taylor S and Sanchez A., (2015) Cyprodinil (CGA219417) – Chronic toxicity of CGA219417 to the daphnid <i>Daphnia magna</i> . Statistical Re-analysis. Report Number CEA.1415. Cambridge Environmental Assessments, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK. (XXXX File No: CGA219417_11594 / VV-28892)
Guideline(s):	OECD Guidelines No. 211 (2012)
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Summary

Report number 468-CG (Ward et al., 1995) did not provide estimates of the EC₁₀ and EC₂₀ for the total number of surviving adults after 21 days and the total number of offspring produced per surviving parent animal. Consequently, the data generated in this study have been re-analysed in order to provide these values.

Treatment related effects were observed and the relationship between dose and response was significant for number of surviving adults and mean number of offspring per surviving adult. EC_x values and the 95% and 99% confidence limits could be reliably determined.

Statistical Analysis

Probit analysis with linear maximum likelihood regression was used to determine the concentration response function. Chi² was used as a goodness of fit measure. All computations were carried out in the Statistical program: ToxRat Professional 2.10.05 (ToxRat Solutions GmbH, 2001-2010).

Results

Number of surviving adults

There was a significant relationship between dose and mortality ($p(F) = 0.017$). EC₁₀, 20, 50 values and the 95% and 99% confidence limits were reliably determined.

Mean number of offspring per surviving adult

There was a significant relationship between dose and reproduction ($p(F) = 0.000$). EC₁₀, 20, 50 values and the 95% and 99% confidence limits were reliably determined.

EC₁₀, EC₂₀ and EC₅₀ Estimates

Estimates of the EC₁₀ and EC₂₀ can be summarized as follows:

Parameter	Reproduction	Adult Mortality
EC ₅₀ (mg/L) 95% CL	11.47 (11.46-11.49)	25.33 (21.42-1647.29)
EC ₂₀ (mg/L) 95% CL	9.42 (9.41-9.42)	19.28 (11.49-27.14)
EC ₁₀ (mg/L) 95% CL	8.49 (8.49-8.50)	16.71 (1.36-19.28)

CL: Confidence Limits
n.d.: Not Determined

Conclusion

Treatment related effects were observed and the relationship between dose and response was significant for reproduction and adult mortality. ECx values and the 95% and 99% confidence limits could be reliably determined.

(Taylor S & Sanchez A, 2015)

Comments of zRMS:	The study was not evaluated. The study for metabolite active substance was not used in risk assessment as no data gap was agreed at the EU level.
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Reference:	KCP 10.2.2
Report	Drott K.R. & Krueger H.O. (1999) CGA219417: A flow-through life cycle toxicity test with the saltwater mysid (<i>Mysidopsis bahia</i>). Report Number 108A-205. Wildlife International Ltd, Easton, MD, USA. (XXXX File No. CGA219417/0926 /VV-311558)
Guideline:	EPA Guideline No. 72-4
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

The chronic toxicity of CGA219417 to the saltwater mysid (*Mysidopsis bahia*) was determined under flow-through conditions in a life-cycle test. This study was run with nominal concentrations of 0.5, 1.0, 2.0, 4.0, and 8.0 µg a.s./L together with negative and solvent controls.

There were no statistically significant effects on survival, reproduction and growth of mysid shrimp exposed to CGA219417 at concentrations of 1.9 µg/L and above after 30 days. The No observed effect concentration (NOEC) was 1.9 µg /L, the Lowest observed effect concentration (LOEC) based on survival was 3.7 µg /L and the Maximum acceptable toxicant concentration (MATC) was 2.7 µg /L.

Materials

Test material	CGA219417
Description:	White powder
Lot/Batch #:	P012012

Purity: 99.2%
Stability of test compound: Not given

Treatments

Test concentrations: Nominal concentrations of 0.5, 1.0, 2.0, 4.0, and 8.0 µg a.s./L
Dilution water: Saltwater (0.25 µm filtered seawater)
Vehicle and/or positive control: Dimethylformamide (DMF)
Analysis of test concentrations: Yes at test start and end using HPLC analysis

Test organisms

Species: Saltwater mysid (*Mysidopsis bahia*)
Source: Test facility
Acclimatisation period: Adults acclimated 14 days before collection of juveniles
Treatment for disease: None
Life stage at test start: Juvenile < 24 hours
Feeding: Live brine shrimp (*Artemia* sp.) 3 – 4 times a day

Test design

Test vessels: Before maturity: 2 L glass beakers covered with a nylon mesh
After maturity: 5 cm diameter glass petri dishes with sides of nylon mesh attached with silicon adhesive, placed in 9L glass aquaria test chambers containing 5L of test solution.
Replication: Before maturity: 4 replicates of 15 mysids
After maturity: 4 replicates containing differing amounts of mysids (5-14) dependant on juvenile survival.
Exposure regime: Flow-through
Duration: 96 hours

Environmental conditions

Test temperature: 25 ± 2 °C
pH range: 8.0 to 8.2 measured daily
Dissolved oxygen: > 5.9 mg/L
Salinity of dilution water: 20 % throughout test

Study Design and Methods

Experimental dates: 24th February to 27th March 1999

Mysids less than 24 hours old were exposed to a series of five test concentrations, a solvent control and a negative control for 30 days. Four replicate test chambers, each containing 15 mysids, were maintained for each treatment and control group.

A continuous flow through diluter was used to deliver each concentration of the test substance. The diluter

was adjusted so that each test chamber received approximately 14 volume additions of test water every 24 hours. Prior to sexual maturity, mysids were held in 2 L glass beakers covered with a nylon mesh for 15 days. After sexual maturity, reproductive pairs were placed in reproductive compartments (one pair per compartment). The reproductive compartments were 5 cm diameter glass petri dishes with sides of nylon mesh attached with silicon adhesive. The compartments were placed in 9 L glass aquaria test chambers containing 5 L of test solution.

The test was undertaken in a temperature controlled environmental chamber.

A primary stock of 8.0 µg a.s./mL test material was prepared by mixing 0.1614 g of the test substance with DMF, bringing the final volume to 1000 mL. This stock solution was then used to make up the test concentrations by serial dilution.

The concentrations of test material in the test solutions were measured at the beginning, and at the end of the test using HPLC analysis.

Observations were made for mortality and clinical symptoms of toxicity at test start and day 7, 14, 21, 28 and 30.

Negative and solvent control data was compared to the mortality, reproductive output per day, dry weight and survival of first brood data using 2 x 2 contingency tables and the chi squared or student's t-test. Survival was evaluated before pairing (day 0 – 15) and after pairing (day 15 - 30). Reproduction and growth data were analysed by evaluating homogeneity and assessing normality using Sharpio-Wilk's test. Treatments that were statistically significant from controls were identified using Bonferroni t-test. The results of these tests were used to determine the LOEC and NOEC. The MATC was calculated as the geometric mean of the NOEC and LOEC. All statistical tests were performed using computer software SPSS/PC Version 2.0 or TOXSTAT Release 3.5.

Results and Discussion

The measured concentrations are shown in the table below in terms of nominal concentrations. The test concentrations were maintained throughout the study. The limit of quantification in this study was 0.20 µg a.s./L. Mean measured concentrations were used for the calculation and reporting of results.

Table A 24: Analytical results

Nominal concentration (µg a.s./L)	% of nominal 0 days	% of nominal 30 days	Mean measured concentration (µg a.s./L)
0.50	109	103	0.51
1.0	106	96	0.99
2.0	95	99	1.9
4.0	95	94	3.7
8.0	93	98	7.5

Table A 25: Effects of test material on the survival of saltwater mysids (*Mysidopsis bahia*) following exposure for 96 hours in a flow-through test

Mean measured concentration (µg a.s./L)	Mean juvenile survival (%) ^a	Mean adult survival (%) ^b	Mean no. young produced per reproductive day	Mean dry weight of adult mysids at end of test (mg)	Mean total length of adult mysids at test end (mm)
Negative control	90	92	0.308	0.595	6.35
Solvent control	97	98	0.275	0.589	6.32
Pooled control	93	96	0.291	0.592	6.33
0.51	92	93	0.321	0.585	6.35
0.99	95	100	0.280	0.603	6.31
1.9	97	98	0.287	0.573	6.28
3.7	78*	84	0.0391 ¹	0.397 ¹	6.00 ¹
7.5	38*	57*	n/a ²	0.303 ¹	4.93 ¹

^a (*) indicates a significant difference from the pooled control using 2 x 2 contingency tables (p ≤ 0.05)

^b (*) indicates a significant difference from the pooled control using 2 x 2 contingency tables (p ≤ 0.05)

¹ this treatment group was not included in the statistical analysis of reproduction due to a statistically significant difference in survival from test initiation to pairing

² the treatment was not mature enough to sex on day 15 and was not paired

Conclusions

There were no statically significant effects on survival, reproduction or growth of mysid shrimp (*Mysidopsis bahia*) exposed to CGA219417 at concentrations of ≤ 1.9 µg a.s./L for 30 days. The NOEC was 1.99 µg a.s./L, the LOEC for survival was 3.79 µg a.s./L and the MATC was 2.79 µg a.s./L

(Drottar K & Krueger H, 1999)

Comments of zRMS:

Report:	KIIA 8.2.8. Ward T. J., Magazu J.P. & Boeri R. L. (1995). Growth and Reproduction Toxicity Test with CGA-219417 and the duckweed, <i>Lemna gibba</i> G3. Report Number 792-CG. T. R. Wilbury Laboratories, Inc. Massachusetts 01945. (XXXX File No. CGA219417/0645; VV-373819).
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XXXX: NL requested for supplemental information to be added to thje B9 as follows:

Nowadays also visual phytotoxicity data are taken into account when evaluating tests with macrophytes. This is an agreement from the last Central Zone Harmonisation Workshop. Please check if the endpoints based on regular parameters cover the visual phytotox. If not, a lower endpoint should be chosen for risk assessment in the opinion of NL.

Phytoxicity data (in terms of number of chlorotic fronds) are presented in the table below:

Initial measured concentration (mg/L)	Rep	Days of exposure							
		0	1	4	6	8	11	13	14
0 (control)	Mean total no. of fronds	14	16	38	73	103	153	206	234
	No. of chlorotic	1	0	0	0	1	1	2	3
		2	0	0	1	1	2	2	6

	fronds	3	0	0	0	0	0	2	4	8
	Mean no. of chlorotic fronds		0.0	0.0	0.3	0.3	0.7	1.7	2.7	5.7
0.542	Mean total no. of fronds		13	14	34	60	88	152	199	218
	No. of chlorotic fronds	1	0	0	0	1	1	1	2	5
		2	0	0	0	0	0	0	4	7
		3	0	0	0	0	1	1	3	6
	Mean no. of chlorotic fronds		0.0	0.0	0.0	0.3	0.7	0.7	3.0	6.0
	% of total		0.0	0.0	0.0	0.6	0.8	0.4	1.5	2.8
1.17	Mean total no. of fronds		14	16	36	69	109	168	207	246
	No. of chlorotic fronds	1	0	0	0	0	1	2	4	6
		2	0	0	0	0	0	1	3	8
		3	0	0	0	0	0	1	3	6
	Mean no. of chlorotic fronds		0.0	0.0	0.0	0.0	0.3	1.3	3.3	6.7
	% of total		0.0	0.0	0.0	0.0	0.3	0.8	1.6	2.7
2.21	Mean total no. of fronds		14	15	39	79	103	162	220	235
	No. of chlorotic fronds	1	0	0	0	1	2	2	5	9
		2	0	0	0	0	1	1	2	5
		3	0	0	0	0	0	2	3	3
	Mean no. of chlorotic fronds		0.0	0.0	0.0	0.3	1.0	1.7	3.3	5.7
	% of total		0.0	0.0	0.0	0.4	1.0	1.0	1.5	2.4
4.42	Mean total no. of fronds		14	15	34	64	85	141	186	221
	No. of chlorotic fronds	1	0	0	0	0	0	0	1	1
		2	0	0	0	0	0	0	1	3
		3	0	0	0	0	0	0	0	1
	Mean no. of chlorotic fronds		0.0	0.0	0.0	0.0	0.0	0.0	0.67	1.7
	% of total		0.0	0.0	0.0	0.0	0.0	0.0	0.36	0.75
9.38	Mean total no. of fronds		14	15	27	43	45	51	60	66
	No. of chlorotic fronds	1	0	0	0	0	0	1	1	2
		2	0	0	0	0	0	1	2	3
		3	0	0	0	0	1	1	1	1
	Mean no. of chlorotic fronds		0.0	0.0	0.0	0.0	0.33	1.0	1.3	2.0
	% of total		0.0	0.0	0.0	0.0	0.74	2.0	2.2	3.0

A 2.2.3 KCP 10.2.3 KCP 10.2.3 Further testing on aquatic organisms

Comments of zRMS:	<p>The submitted study was accepted.</p> <p>The microcosm study was conducted to investigate the effects of cyprodinil (CGA219417) applied as the 300 g/L EC formulation A14325E, on communities of freshwater aquatic invertebrates and algae under field conditions. Treatment concentrations, ranging from 1.5 to 50 µg cyprodinil/L, were selected to cover known toxicity levels determined from laboratory studies, predicted environmental concentrations (PEC), and the 50 µg ai/L (highest) rate was included as it was considered likely to give clear effects on a variety of taxa and thereby confirm the validity of the test system.</p> <p>A wide spectrum of aquatic organisms was tested.</p> <p>The meteorological measurements were collected: air temperature and rainfall.</p> <p>The physico-chemical measurements of the water quality were conducted: temperature, dissolved oxygen, pH, turbidity, conductivity, alkalinity, hardness, chlorophyll a content.</p> <p>The analysis of cyprodinil residues was provided.</p> <p>The aquatic organisms were represented by all trophic levels: algae (10 classes), phyto- and zooplankton, macroinvertebrates (> 40 species), macrophytes (13 species'). Over 20 plant taxons were identified in microcosm study.</p> <p>The univariate and multivariate analysis were provided.</p> <p>It could be concluded that three applications of 10 µg ai/L would not result in unacceptable ecological effects.</p> <p>Three cyprodinil applications at the 20 µg ai/L treatment rate resulted in some clear treatment effects, principally long term effects on the crustacean <i>Asellus aquaticus</i>.</p> <p>At the highest treatment rate of 50 µg ai/L three applications resulted marked and long term treatment effects chiefly on <i>Asellus</i> and zooplankton populations.</p> <p>Based on statistical analysis the NOEC of 10 µg a.s./L was derived. The Applicant has proposed the lower value of 1.5 µg a.s./L. The ETO-RAC = 0.75 µg a.s./L (with AF=2) was accepted for risk refinement.</p>
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Reference: KCP 10.2.3

Report Ashwell J., Benyon K., Powley W., Richardson M. (2007) Cyprodinil (CGA219417) 300 g/L EC formulation A14325E: Effects on aquatic organisms in an outdoor microcosm. Report Number T008777-05-REG. XXX, Jealott's Hill International Research Centre, UK. Experimental dates March 2006 – March 2007. (XXXX File No. VV-339018; CGA219417/1683)

Guideline(s): Campbell P.J., Arnold D.J.S., Brock T.C.M., Grandy N.J., Heger W., Heimbach F., Maund S.J., Streloke M. (1999) PP178: Guidance document on higher-tier aquatic risk assessment for pesticides (HARAP). Lacanau Océan, France: Setac-Europe Publication.

Giddings J.E., Brock T.C.M., Heger W., Heimbach F., Maund S.J., Norman S.M., Ratte H.T., Schäfers C., Streloke M. (2002) PP60: CLASSIC - Community - level aquatic system studies - interpretation criteria. Pensacola, FL, USA: Society of Environmental Toxicology and Chemistry (Setac).

de Jong F.M.W., Brock T.C.M., Foekema E.M., Leeuwangh P. (2007): Guidance for summarizing and evaluating aquatic micro- and mesocosm

studies - Draft version February 2007. A guidance document of the Dutch Platform for the Assessment of Higher Tier Studies, Rivm Report 601506009.

Deviations: No
GLP: Yes
Acceptability: Yes
Duplication (if vertebrate study) No

Executive Summary

This microcosm study was conducted to investigate the effects of cyprodinil (formulated as A14325E) on freshwater invertebrates and algae under field conditions, in accordance with the guidance documents on Higher Tier Aquatic Risk Assessment for Pesticides (HARAP, 1999), Community-Level Aquatic System Studies-Interpretation Criteria (CLASSIC, 2002) and the draft document “Guidance for summarizing and evaluating aquatic micro- and mesocosm studies”, (de Jong et al., 2007): A guidance document of the Dutch Platform for the Assessment of Higher Tier Studies, Rivm Report 601506009).

Materials

Test Material: CGA219417 EC (300) A14325E
Lot/Batch #: SMU5BP002
Purity: 303 g/L, corresponding to 29.9% w/w
Stability of test compound: May 2007

Treatments:

Test concentrations: 1.5, 5, 10, 20 and 50 µg a.s./L and a deionised water control
Vehicle and/or positive control: Deionised water / None
Analysis of test concentrations: Yes

Test design

Test systems: The test systems were rectangular varied depth microcosms, each containing approximately 1,300 litres of water over 10 cm of sediment, with established communities of plants and invertebrates.

Exposure regime: The replicated study design consisted of five treatments of cyprodinil (1.5, 5, 10, 20 and 50 µg a.s./L) and an untreated control. The treatment levels were chosen to incorporate known toxicity levels from laboratory studies and the maximum predicted environmental concentrations from proposed use patterns. Three, weekly, applications of each treatment were made during June 2006. Application was by direct addition to the microcosms followed by thorough mixing to rapidly distribute the formulation through the water column (taking the toxicological approach favoured by HARAP and CLASSIC).

Replication: The treatments were unequally replicated: Control (4 replicates), 1.5 µg a.s./L (3 replicates), 5 µg a.s./L (4 replicates), 10 µg a.s./L (4 replicates), 20 µg a.s./L (3 replicates), 50 µg a.s./L (2 replicates). The disproportionate allocation of replicates to treatments was to enable increased precision for statistical analysis compared to the control for the critical treatment levels (i.e. around the expected no effect levels).

Study Design and Methods

Test facility: XXXX, Jealott’s Hill International Research Centre, UK.

Experimental dates: 3rd March 2006 to 1st March 2007

Sampling procedures

Physico-chemical properties of the water, phytoplankton, zooplankton and macroinvertebrate communities were studied for approximately one month prior to application and for approximately four months after application. The following sampling methods were employed:

Water column sampling: For zooplankton, phytoplankton, chlorophyll a, alkalinity and hardness measurements

“Hydrolab” water quality multi-probe: For in situ measurements of water physico-chemical properties

ESAS (Enhanced Surface Area Substrate) samplers: Artificial colonising substrates to sample benthic and epiphytic dwellers

Sweep-netting (NETS): To sample swimming and epiphytic organisms

Emergence traps (TRAPS): To sample emergent adult insects

Litterbags (LLB): To sample organisms processing leaf litter and to determine differences in the rate of decomposition of leaf material in the microcosms

Water residue analysis: Microcosm water was sampled for measurement of cyprodinil residues at the following intervals:

-1, +1, +6 and +24 hours and 3 days after the first application.

-1 hour (7 days after first application), +1, +6 and +24 hours and 3 days after the second application.

-1 hour (7 days after second application), +1, +6 and +24 hours, 3, 7, 14 and 21 days after the third application.

From 28 days after the third application, microcosms were not sampled once residues had declined to below the LOQ. In microcosms where residues remained above the LOQ, further samples were taken 28, 35, 42, 49, 56 and 63 days after the third application.

All four control microcosms were only sampled prior to the first application (-1 hour) and then at +1 hour after each of the three applications. On all other sampling occasions just one control microcosm (M02) was sampled.

Preparation of test solutions

Treatment concentrations, nominally 1.5, 5, 10, 20, and 50 μ g a.s./L were selected to cover known toxicity levels determined from laboratory studies, maximum modelled, worst-case, predicted environmental concentrations (PEC), and the 50 μ g a.s./L (highest) rate was included as it was considered likely to give clear effects on a variety of taxa.

A stock solution of cyprodinil was prepared by taking 2 g (within \pm 1%) of formulation A14325E (300 g a.s./L) and making it up to 2000 mL with deionised water. Known volumes of this solution were then diluted with deionised water to give concentrations of cyprodinil appropriate for treating the microcosms at each of the treatment rates. For each application, the test solutions were prepared the day before application. Prior to addition to the microcosms, sub-samples of the application solutions were taken to verify concentrations.

Analytical method

Application solutions and microcosm water samples for residue analysis were analysed for cyprodinil by

liquid chromatography with mass spectroscopic detection (LC/MS/MS).

Statistical analyses

Multivariate (Principal Response Curves method) was employed to identify and treatment-related changes in community structure. Analysis of variance was also used to look at population level differences for selected taxa; those not analysed were considered to be too variable in occurrence or not abundant enough to permit meaningful conclusions to be drawn.

Results and Discussion

Analytical data

Residues of cyprodinil in the microcosm water were measured following application of the test item. One hour after the first application, cyprodinil residues were measured at between 75 and 80% of the nominal applied. Residues declined to below 50% of nominal 3 days after application and at 7 days (1 hour prior to the second application) were between 22 and 27% of nominal. Following the second application, residues of between 119 and 154% of nominal were observed. These had declined to between 38 and 80% of nominal by 7 days after the second application (1 hour prior to the third application). Residues between 118 and 156% of the nominal rates were measured 1 hour after the third application. The water column DT50 for cyprodinil was approximately 2-3 days.

Table A 26: Cyprodinil concentrations measured in microcosm water in a microcosm study with three applications of cyprodinil (CGA219417) 300 g/L EC formulation A14325E

Application number	Timepoint	Nominal treatment concentration (µg a.s./L)	Mean % of nominal
Application 1	- 1 hour	0 (Control)	ND
		1.5	ND
		5	ND
		10	ND
		20	ND
		50	ND
	+ 1 hour	0 (Control)	ND
		1.5	76
		5	75
		10	79
		20	79
		50	80
	+6 hours	0 (Control)	ND
		1.5	58
		5	66
		10	67
		20	71
		50	71
	+24 hours	0 (Control)	ND
		1.5	54
		5	57
		10	57
		20	65

Application number	Timepoint	Nominal treatment concentration (µg a.s./L)	Mean % of nominal
	+3 days	50	64
		0 (Control)	ND
		1.5	<LOQ
		5	35
		10	39
		20	43
		50	39
Application 2	-1 hour	0 (Control)	ND
		1.5	<LOQ
		5	22
		10	22
		20	24
		50	27
	+1 hour	0 (Control)	ND
		1.5	119
		5	139
		10	146
		20	154
		50	135
Application 2 (continued)	+6 hours	0 (Control)	ND
		1.5	94
		5	97
		10	97
		20	107
		50	111
	+24 hours	0 (Control)	ND
		1.5	77
		5	88
		10	92
		20	63
		50	43
	+3 days	0 (Control)	ND
		1.5	55
		5	56
		10	62
		20	67
		50	72
Application 3	-1 hour	0 (Control)	ND
		1.5	80
		5	63
		10	38
		20	66
		50	47
	+1 hour	0 (Control)	ND
		1.5	118

Application number	Timepoint	Nominal treatment concentration (µg a.s./L)	Mean % of nominal
		5	156
		10	142
		20	142
		50	120
	+6 hours	0 (Control)	ND
		1.5	112
		5	113
		10	121
		20	127
		50	115
	+24 hours	0 (Control)	ND
		1.5	83
		5	89
		10	93
		20	100
		50	104
	+3 days	0 (Control)	ND
		1.5	69
		5	77
Application 3 (continued)	+3 days (continued)	10	79
		20	90
		50	86
	+7 days	0 (Control)	ND
		1.5	<LOQ
		5	48
		10	49
		20	57
		50	62
	+14 days	0 (Control)	ND
		1.5	<LOQ
		5	22
		10	25
		20	27
		50	34
	+21 days	0 (Control)	ND
		1.5	<LOQ
		5	15
		10	12
		20	15
		50	19
	+28 days	0 (Control)	ND
		5	<LOQ
		10	<LOQ
		20	8
		50	10

Application number	Timepoint	Nominal treatment concentration (µg a.s./L)	Mean % of nominal
	+35 days	0 (Control)	ND
		20	4
		50	7
	+42 days	0 (Control)	ND
		20	<LOQ
		50	4
	+49 days	0 (Control)	ND
		50	3
	+56 days	0 (Control)	ND
		50	2
	+63 days	0 (Control)	ND
		50	<LOQ

ND = None detected (limit of detection: 0.3 µg a.s./L)

<LOQ = Less than limit of quantification (0.75 µg a.s./L)

The application solutions, prepared the day before each application, were also analysed for cyprodinil. These were measured at between 59-75%, 73-83%, and 64-82% of nominal for each of the 3 applications, respectively. Owing to the high concentrations of the application solutions, considerable dilution of these was required prior to analysis, while the water residue samples taken from the microcosms were analysed by direct injection with no dilution step. These are therefore considered a reliable measure of the concentrations achieved in the microcosms and appropriate dosing is considered to have been achieved. Effect concentrations are based on the nominal treatment rates.

Biological data

Three applications of cyprodinil at 1.5, 5 and 10 µg a.s./L (maximum measured concentrations of 1.8, 7.8 and 14.6 µg a.s./L, respectively) had no clear treatment-related effects on physico-chemical parameters, leaf litter decomposition rates, phytoplankton communities or zooplankton communities and populations. Transient effects on phytoplankton populations were not believed to be treatment effects. Similarly, for the macroinvertebrates, effects at 5 µg a.s./L on the isopod crustacean *Asellus aquaticus* were not supported by a dose response and not believed to be treatment-related.

Three applications of cyprodinil at 20 µg a.s./L (maximum measured concentration 30.8 µg a.s./L) had no clear treatment-related effects on physico-chemical parameters or on leaf litter decomposition rates. Only transient effects occurred on phytoplankton and zooplankton communities and populations. Long term effects were observed on macroinvertebrate communities, driven by reductions in *Asellus aquaticus*.

Three applications of cyprodinil at 50 µg a.s./L (maximum measured concentration 67.5 µg a.s./L) had no clear treatment-related effects on physico-chemical parameters and while a marked reduction in leaf litter decomposition was observed, this was not statistically significant. There were only slight effects on phytoplankton communities and populations. Long term and more marked effects occurred on zooplankton and macroinvertebrate communities and populations, principally on Copepoda (zooplankton) and *Asellus aquaticus* (macroinvertebrates).

The lack of recovery demonstrated in different sample types by *Asellus* at 20 and 50 µg a.s./L is likely a result of the fact that the entire population was exposed to the test item in the microcosms, due to the conservative toxicological exposure scenario. The dosing and mixing of the test item throughout the water column resulted in a lack of refugia and since *Asellus* has a completely aquatic life cycle, short term recovery by immigration was extremely unlikely.

The responses observed in this study are also summarised below according to the effect classes suggested

in the draft document “Guidance for summarizing and evaluating aquatic micro- and mesocosm studies”, (de Jong et al (2007): A guidance document of the Dutch Platform for the Assessment of Higher Tier Studies, Rivm Report 601506009).

Table A 27: Overall summary of effect classes observed for several categories of endpoints in the outdoor mesocosm study receiving three applications of cyprodinil (CGA219417) 300 g/L EC formulation A14325E

	Treatment levels (µg a.s./L nominal)				
	1.5	5	10	20	50
Population responses					
Macroinvertebrate;					
<i>Asellus aquaticus</i> (ESAS)	1	2	2	5B	5B
<i>Crangonyx pseudogracilis</i> (ESAS)	1	1	1	1	3B
<i>Asellus aquaticus</i> (NETS)	1	3A ^a	2	5B	5B
Total Planorbidae (NETS)	1	1	1	1	3A
<i>Asellus aquaticus</i> (LLB)	1	1	1	5B	5B
<i>Crangonyx pseudogracilis</i> (LLB)	1	1	1	1	3A
Total Leaf Litter bags (LLB)	1	1	1	3A	3A
Zooplankton;					
<i>Daphnia</i> sp.	1	1	1	2	2
<i>Nauplia</i> sp.	1	1	1	1	5A
<i>Cylopidae</i> (Copepodit)	1	1	1	1	3A ^b
Total Zooplankton	1	1	1	1	5A
Phytoplankton;					
<i>Carteria</i> sp. (rough wall)	2	2	2	2	2
<i>Scenedesmus aculeolatus</i>	1	1	1	2	2
<i>Rhodomonas</i> sp.	1	1	1	2	2
Community responses					
Macroinvertebrates;					
ESAS (PRC)	1	2	1	5A	5B
Sweep Nets (PRC)	1	3A ^c	2	5B	5B
Leaf Litterbag (PRC)	1	1	2	5B	5B
Zooplankton (PRC)	1	1	1	1	5A
Phytoplankton (PRC)	1	1	1	2	2
Leaf Litterbag (PRC)	1	1	2	5B	5B
Physico-chemical measurements	1	1	1	1	1

Effect classes presented are based upon reductions in response for the most sensitive endpoints.^{a,c} Statistically significant reductions seen later in the study are not supported by a dose response and are not believed to be treatment effects^b Effect class based upon a trend; no statistically significant effects observed, but a clear reduction seen at this concentration.

Conclusion

The following NOECs/NOEAECs were derived following the guidance contained in the draft document “Guidance for summarizing and evaluating aquatic micro- and mesocosm studies”, (de Jong et al., (2007): A guidance document of the Dutch Platform for the Assessment of Higher Tier Studies, Rivm Report 601506009).

Table A 28: Derived NOECs and NOEAECs from the outdoor mesocosm study receiving three applications of cyprodinil (CGA219417) 300 g/L EC formulation A14325E

Group	NOEC (µg a.s./L)	NOEAEC (µg a.s./L)
Macroinvertebrate community	10	10
Macroinvertebrate populations	10	10
Zooplankton community	20	20
Zooplankton populations	20	20
Phytoplankton community	20	50
Phytoplankton populations	20	50
Physico-chemical measurements	50	50

Considering the conservative nature of the study design (i.e. treatment of the entire water body with limited potential for internal recovery) and the type of effects observed, it was concluded that three nominal applications of 10 µg a.s./L would not result in unacceptable ecological effects.

(Ashwell, *et al.*, 2007)

Comments of zRMS:	The submitted study was submitted and not evaluated at zonal level. The relevant endpoint was calculated but will not be used in risk refinement.
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Reference:	KCP 10.2.3
Report	Taylor S & Dark R (2015). Cyprodinil (A14325E) – Statistical (MDD) Re-analysis of Existing Data from a Microcosm Study with Cyprodinil. Report Number CEA.1464. Cambridge Environmental Assessments, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK (XXXX File No. VV-889899; A14325E_10079)
Guideline(s):	None
Deviations:	No
GLP:	No
Acceptability:	Yes
Duplication (if vertebrate study)	No

For zooplankton, MDD values were calculated for Cyclopidae (Copepodit), Cyclopidae sp., Daphnia sp., Keratella quadrata, Nauplia sp. and total zooplankton.

Of these data, three taxa and the total zooplankton were considered suitable for ETO-RAC derivation, namely Daphnia sp. Keratella quadrata, Nauplia sp. and total zooplankton. Overall these data encompassed one copepod, one cyplopoid, one rotifer and total zooplankton, thus covering the three main zooplankton groups.

MDD analysis of the available data for zooplankton demonstrated that typically small to large effects could be determined throughout the study for five parameters. As these evaluations included sensitive taxa (Daphnia sp.) and organisms from the three main zooplankton groups (cladocera, copepoda and rotifera), the data generated are considered robust and reliable for ETO-RAC derivation and a NOEC (class 1) of 14.6 µg a.s./L (based on measured concentrations) is recommended for zooplankton. If an NOEAEC (class 3A) is required for ERO-RAC it can be considered to be 67.5 µg a.s./L.

MDD analysis of the available data for macroinvertebrates demonstrated that typically small to large effects

could be determined throughout the study for nine parameters (15 endpoints) and for four parameters (eight endpoints), following at least one application of the test item. As these evaluations included sensitive taxa (*Asellus aquaticus* and *Crangonyx pseudogracilis*) and organisms from one isopod, one amphipod, two insects, one gastropod, total bugs, total pond snails, total ramshorn snails, total flatworms, and total organisms from four different sampling techniques, the data generated are considered robust and reliable for ETO-RAC derivation. The overall NOEC (class 2) of 14.6 µg a.s./L is recommended for macroinvertebrates and if a NOEAEC (class 5B) is required for ERO-RAC derivation it is also considered to be 14.6 µg a.s./L.

The overall NOEC (class 2) from this study has been robustly determined as 14.6 µg a.s./L (based on measured concentrations).

(Taylor and Dark, 2015)

A 2.3 KCP 10.3 Effects on arthropods

A 2.3.1 KCP 10.3.1 Effects on bees

A 2.3.1.1 KCP 10.3.1.1 Acute toxicity to bees

A 2.3.1.1.1 KCP 10.3.1.1.1 Acute oral toxicity to bees

Comments of zRMS:	<p>The study was accepted. The study was conducted in accordance with OECD guidances 213 (acute oral) and 214 (acute contact).</p> <p>The validity criteria were met: oral test: the mean mortality of the control groups was 0% (recommended ≤ 10 %); contact test: the mean mortality of the control groups was 0% and 3.3% in water and tween solution, respectively, (recommended ≤ 10 %); The following endpoints were derived Oral: LD₅₀ 48 h = 445 µg formulation/bee NOED 48 h = 209 µg formulation/bee Contact: LD₅₀ 96 h = 645 µg formulation/bee NOED 48 h = 250 µg formulation/bee</p>
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Reference: KCP 10.3.1.1

Report Franke M., (2021), Cyprodinil/Prothioconazole EC (A23282A) - Acute toxicity to the honeybee *Apis mellifera* L. under laboratory conditions, Report No. 21 48 BAA 0103. BioChem agrar, Labor für biologische und chemische, Analytik GmbH, Kupferstr. 6, 04827 Machern OT Gerichshain, Germany.
(XXXX file No. VV-932551)

Guideline(s): OECD Guidelines No. 213. Honeybees, acute oral toxicity test (1998)

OECD Guidelines No. 214. Honeybees, acute contact toxicity test (1998)

Deviations: No
GLP: Yes
Acceptability: Yes

Executive Summary

The acute toxicity of A23282A to honeybees was assessed over 48 hours in a contact and oral test. In the contact test, bees were exposed to nominal doses of 1000, 500, 250, 125, 62.5 µg product/bee, alongside water/tween solution controls. In the oral test, bees were exposed to nominal doses of 1000, 500, 250, 125, 62.5 µg product/bee alongside sucrose solution controls. The actual consumed doses for the oral test were 865, 408, 209, 116, 61.4 µg product/bee.

In the contact toxicity test, the 48 h LD₅₀ was 645 µg A23282A/bee (with 95 % CI of 563 - 741 µg A23282A/bee), the 48-h NOED was 250 µg A23282A/bee. In the oral toxicity test, the 48 h LD₅₀ was 445 µg A23282A/bee (with 95 % CI of 364 – 567 µg A23282A/bee), the 48-h NOED was 209 µg A23282A/bee.

Materials

Test Material	Cyprodinil/Prothioconazole EC (A23282A)		
Lot/Batch #:	LCR001-021-001		
Other Batch ID:	1160912		
Actual content of active ingredients:	Cyprodinil	22.1 % w/w;	219 g/L
	Prothioconazole	7.40 % w/w;	73.5 g/L
Description:	EC (emulsifiable concentrate); yellow liquid		
Stability of test compound:	Stable under test conditions		
Reanalysis/expiry date:	End of September 2023		

Treatments

Test doses:	Contact test:	1000, 500, 250, 125, 62.5 µg product/bee
	Oral test (offered):	1000, 500, 250, 125, 62.5 µg product/bee
	Oral test (consumed):	865, 408, 209, 116, 61.4 µg product/bee
Control:	Contact test:	deionised water and wetting agent control (1% v/v Tween solution)
	Oral test:	50 % w/v sucrose solution
Toxic standard:	Dimethoate EC 400; 411.2 g/L; nominal dose levels of 0.250, 0.188, 0.141 and 0.105 µg a.s./bee in contact test and 0.250, 0.175, 0.123 and 0.086 µg a.s./bee in oral test	
Administration:	Contact:	2 µL droplet per bee on the dorsal thorax of the worker bee
	Oral:	200 µL per cage with 10 bees offered by group feeding (due to trophallaxis of honeybees this corresponds to 20 µL/bee)

Test organisms

Species:	Honey bee, <i>Apis mellifera</i> L. Buckfast
Source:	BioChem agrar GmbH, Germany
Food:	50 % w/v sucrose solution

Test design

Test cage description:	Disposable test cages; 95 mm x 50 mm x 65 mm (length x width x height) constructed of cardboard, with holes in the bottom for ventilation and a glass
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	plate in front
Replication:	Three replicates per test item treatment and control
No. of bees/rep:	10
Duration of test:	48 hours (contact test); 48 hours (oral test)
Environmental conditions	test
Temperature:	22.7 – 25.3 °C* (contact and oral)
Humidity:	48 – 78 % (contact and oral)
	*Short-term deviations (≤ 2 hours) from the recommended ranges are partly unavoidable (e.g. due to handling of the set-ups) and will normally not result in major disturbances of the test performances and as control performance met the guideline validity criteria, these short-term deviations (for maximum of approximately 1 hour, each) are considered to have no impact on the validity of the study
Photoperiod:	Darkness (except during observations where there was diffuse artificial light)

Study Design and Methods

Test facility: BioChem agrar GmbH, Kupferstr. 6, 04827 Machern OT Gerichshain, Germany

Experimental dates: 5th to 7th August 2021

Honeybees (*Apis mellifera*) were exposed to A23282A via two routes of administration: contact and oral ingestion in aqueous solutions.

A stock solution was prepared by diluting 5 g A23282A in 10 mL tween solution. This was then serially diluted to prepare the test concentrations in the contact test. For preparation of the oral test concentrations, 5 g A23282A was added to 10 mL 50 % (w/v) sucrose solution which was serially diluted.

Contact test procedures:

Bees were treated with 2 μ L of droplets of the test solution, control or toxic standard applied to the dorsal surface of the thorax using topical application with a micro applicator. The bees were returned to the test unit, allowed to recover and fed with a continuous supply of 50 % w/v aqueous sucrose solution.

The mortality [%] per treatment was calculated from the number of dead bees and the total number of introduced bees per treatment group. Mortality in the test and reference item treatments was corrected with the corresponding control mortality according to the formula of SCHNEIDER-ORELLI (1947). The LD₅₀ values with 95 % confidence limits of the reference item treatment were calculated by means of a Probit analysis, whereas for the oral test item by means of Weibull analysis.

Oral test procedures:

Bees were not fed from the time they were collected from the hives for 2 hours until treatment. Each group of bees was offered 200 μ L (equivalent to 20 μ L/bee) of the test material or toxic standard dispersed in aqueous sucrose solution. Treatments were calculated so that the target dose was contained in 200 μ L. The doses were measured into the feeding tubes and the feeding tubes were weighed before the doses were made available to the bees. Once all test solutions were consumed, or after six hours (whichever was achieved first), the feeding tubes were replaced with similar tubes containing aqueous sucrose solution. All feeding tubes with test solutions were weighed to calculate actual mean consumption per bee for each treatment.

In both the contact and oral tests there were 3 replicates per treatment. The mortality and behaviour were assessed after 4, 24, 48 hours in the contact and oral toxicity tests (following application procedure).

The LD₅₀ values with 95 % confidence limits of the test item and the reference item treatment was

calculated by Fisher's Exact Binomial Test with Bonferroni-Holm Correction ($\alpha = 0.05$; one-sided greater).

Results

Mortality data for the test material and toxic standard are summarised in the table below.

Table A 29: Summary of acute contact toxicity of A23282A to the honeybee

Treatment (target dose) [µg product/bee]	Mortality [%]		Corrected mortality [%]	
	24 h	48 h	24 h	48 h
Water control	0.0	0.0	-	-
1 % v/v Tween®80 solution control	0.0	0.0	-	-
1000	90.0*	90.0*	-	-
500	13.3	23.3*	-	-
250	0.0	0.0	-	-
125	0.0	0.0	-	-
62.5	0.0	0.0	-	-
48h LD₅₀ (µg product/bee)	645			
lower – upper 95% C.I.	563 – 741			
NOED (µg product/bee)	250			

Mortality results are averages based on 3 replicates consisting of 10 bees each; corrected mortality: according to SCHNEIDER-ORELLI 1947; Calculations are performed with non-rounded values

Sublethal effects were observed at the early 4-h assessment and at dose rates of ≥ 500 µg A23282A/ bee. In the further course up to 48 hours no behavioural impairments were found in comparison with the control.

Table A 30: Summary of acute oral toxicity of A23282A to the honeybee

Treatment (target dose) [µg product/bee]	Treatment (based in consumption [µg product/bee]	Mortality [%]		Corrected mortality [%]	
		24 h	48 h	24 h	48 h
50 % w/v sucrose solution control	--	0.0	0.0	-	-
1000	865	73.3*	80.0*	-	-
500	408	36.7*	46.7*	-	-
250	209	13.3	16.7	-	-
125	116	3.3	3.3	-	-
62.5	61.4	0.0	0.0	-	-
48h LD₅₀ [µg product/bee]	445				
lower – upper 95% C.I.	364 – 567				
NOED [µg product/bee]	209				

Mortality results are averages based on 3 replicates consisting of 10 bees each; corrected mortality: according to SCHNEIDER-ORELLI 1947;

* Significant difference in pairwise comparison between treatment and sucrose solution control (Fisher's Exact Binominal Test

with Bonferroni Correction; $\alpha=0.05$; one sided greater);
Calculations are performed with non-rounded values

Sublethal effects were observed at the early 4-h assessment and predominantly at dose rates of $\geq 209 \mu\text{g}$ A23282A/bee. In the further course up to 48 hours no behavioural impairments were found in comparison with the control.

Validity criteria

The validity criteria are listed below:

- The mortality in both the contact and oral control was 0.0% (must be $\leq 10\%$)
- The oral 24-hour LD_{50} for the reference item was $0.13 \mu\text{g a.s./bee}$ (recommended $0.10 - 0.35 \mu\text{g a.s./bee}$)
- The contact-24 hour LD_{50} for the reference item was $0.15 \mu\text{g a.s./bee}$ (recommended $0.10 - 0.30 \mu\text{g a.s./bee}$)

Conclusion

In a 48-hr-acute contact toxicity test, honey bees (*Apis mellifera*) were exposed to A23282A administered topically to adult bees. The 48-hr LD_{50} was $645 \mu\text{g}$ A23282A/bee (with 95 % CI of $563 - 741 \mu\text{g}$ A23282A/bee), the 48-h NOED was $250 \mu\text{g}$ A23282A/bee.

In a 48-hr-acute oral toxicity test, honey bees (*Apis mellifera*) were exposed to A23282A via a feeding solution. The 48-hr LD_{50} was $445 \mu\text{g}$ A23282A/bee (with 95 % CI of $364 - 567 \mu\text{g}$ A23282A/bee), the 48-h NOED was $209 \mu\text{g}$ A23282A/bee.

(Franke M., 2021)

A 2.3.1.1.2 KCP 10.3.1.1.2 Acute contact toxicity to bees

Refer to KCP 10.3.1.1.1.

A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

Comments of zRMS:	<p>The study was accepted.</p> <p>The validity criteria were met mortality in control treatment group was being 5.0 % at the end of the test (10 d) and therefore below the threshold of 15 % after 10 days of exposure.</p> <p>The short-term (less than 2 h) deviations in temperature and humidity ranges from the recommended test conditions did not affect final study results.</p> <p>The following endpoints were calculated: $\text{LC}_{50} = 3.37 \text{ g formulation/kg food}$ $\text{LDD}_{50} = 43.8 \mu\text{g formulation/bee/d}$</p> <p>$\text{NOEC} = 1.70 \text{ g formulation/kg food}$ $\text{NOEDD} = 31.2 \mu\text{g formulation/bee/day}$</p>
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Reference: KCP 10.3.1.2

Report	Ripperger D., (2022), Cyprodinil/Prothioconazole EC (A23282A) - Honey Bee <i>Apis mellifera</i> L. (Hymenoptera, Apidae) Chronic Oral Toxicity Test 10 Day Feeding in the Laboratory, Report No. S21-2794. Eurofins Agrosience Services, Ecotoxicology GmbH, Eutingen Str. 24, 75223 Niefern-Öschelbronn, Germany (XXXX file No. VV-946992)
Guideline(s):	OECD Guideline 245. Honey bee (<i>Apis mellifera</i> L.), Chronic Oral Toxicity Test (10-day feeding), 2017
Deviations:	In the reference item group, behavioural abnormalities assessments were not conducted as it can be assumed that moribund and affected bees of the reference item group would have died by the end of the test.
GLP:	Yes
Acceptability:	Yes

The toxicity of A23282A to the honeybee *Apis mellifera* was determined in a 10-day continuous oral exposure study.

The 10-day NOEC was determined to be 1700 mg product/kg feeding solution. The 10-day LC₁₀, LC₂₀ and LC₅₀ were determined to be 1846 (95 % CL: 1433 / 2191), 2270 (95 % CL: 1858 / 2627) and 3370 (95 % CL: 2941 / 3861) mg product/kg feeding solution.

The 10-day NOEDD was determined to be 31.2 µg product/bee/day. The 10-day LDD₁₀, LDD₂₀ and LDD₅₀ were determined to be 33.2 (95 % CL: 29.2 / 36.1), 36.5 (95 % CL: 32.9 / 39.2) and 43.8 (95 % CL: 41.0 / 46.5) µg product/bee/day.

Materials

Test Material	A23282A (cyprodinil/prothioconazole EC (225/075))
Lot/Batch #:	LCR001-021-001
Content of a.s. (analysed):	Cyprodinil: 219 g/L, 22.1 % w/w Prothioconazole: 73.5 g/L, 7.40 % w/w
Description:	Liquid / yellow
Stability of test compound:	Sufficient for the test purpose (at least 1 h)
Reanalysis/Expiry date:	30 Sep 2023
Treatments	2 control groups 5 test item groups 1 reference item group
Test rates:	850, 1700, 3400, 6800 and 13600 mg product/kg feeding solution 19.3, 31.2, 45.3, 60.0 and 69.3 µg product/bee/day (based on actual food consumption)
Controls:	50 % (w/v) aqueous sucrose solution (C) 50 % (w/v) aqueous sucrose solution containing 0.1 % xanthan (CC)
Reference item:	0.9 mg dimethoate/kg feeding solution
Application method:	Continuous <i>ad libitum</i> feeding of test solutions
Analysis of test concentrations:	Recovery means of cyprodinil in all feeding solutions, determined 0DBA1 – 0DBA10, between 79 and 85 % of the nominal concentrations.

Test organisms	Young adult worker bees
Species:	<i>Apis mellifera</i> L. (Hymenoptera, Apidae)
Age:	1 to 2 days old
Source:	Stock beehives maintained by the test facility
Food:	50 % (w/v) aqueous sucrose solution
Test Design	Dose-response test
Test cage description:	Stainless steel cages (base: approx. 8 cm x 4 cm, height: approx. 6 cm). The front side of the cages was equipped with a transparent pane to enable observation. The bottom of the cages consisted of perforated steel, which guaranteed sufficient air supply. The cages were lined with filter paper. At the top of the cage there was a hole to place the feeder (syringe) with the feeding solutions.
Replication:	4 replicates per treatment group
No. of bees/replicate:	10 bees/replicate
Environmental conditions	test Climatic chamber
Temperature:	Target: 33 ± 2 °C Actual: 31.9* – 34.5 °C * Short-term deviations (< 2 hours) without being of any consequence on the study outcome
Humidity:	Target: 50 – 70 % Actual: 47.7* - 65.4 % * Short-term deviations (< 2 hours) without being of any consequence on the study outcome
Photoperiod:	Constant darkness except during the exchange of feeding syringes and assessments.
Duration of test:	10 days

Study Design and Methods

Test facility: Eurofins Agrosience Services Ecotox GmbH, Eutinger Str. 24, 75223 Niefern-Öschelbronn, Germany

Experimental dates: 7th June to 2nd July 2021

The test item was dissolved in 50 % (w/v) aqueous sucrose solution containing 0.1 % xanthan. The feeding solutions were prepared by dilution from a test item stock solution. The test item stock solution and feeding solutions were prepared on each application day freshly. Bees were fed *ad libitum* with treated sugar solutions provided in syringe feeders which were renewed every day. Feeders were weighed before and after feeding and the weights were used to calculate the food consumption. The daily consumption was corrected each day by the number of surviving bees as well as by the determined evaporation of the feeding solution.

Effects (mortality and behavioural abnormalities) were assessed daily during the 10-day exposure period by visual inspection.

For statistical evaluation the statistics program ToxRat professional, Version 3.3.0 was used.

Qualitative trend analysis by contrasts (Monotonicity of Concentration/Response, $\alpha = 0.05$) revealed a linear trend in mortality. Tarone's test revealed no signs of extra-binomial variance ($\alpha = 0.01$). Hence, Cochran Armitage test (one-sided greater, $\alpha = 0.05$) was used to evaluate whether there were statistically

significant differences between the mortality data of the carrier control and each test item group and to determine the NOEDD and NOEC based on mortality of day 10 data, respectively. Comparison of control and carrier control mortality revealed no statistically significant difference (Fisher's Exact Test, two-sided, $\alpha = 0.05$).

The LC_{10, 20, 50} and LDD_{10, 20, 50} values were calculated by Probit analysis using linear maximum likelihood regression. 95 %-confidence limits were estimated using Fieller's theorem.

Results

Analytical recoveries in the diets are presented below.

Table A 31: Analytical results

Nominal Concentration (mg cyprodinil/ kg feeding solution)	Analytical Recovery in Feeding Solution ^a (mg cyprodinil/kg feeding solution in % of the expected nominal concentration)									
	0DBA1	0DBA2	0DBA3	0DBA4	0DBA5	0DBA6	0DBA7	0DBA8	0DBA9	0DBA10
0 (CC)	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
188	80	63	80	67	75	86	64	81	93	92
376	81	69	85	72	77	86	67	85	94	99
751	80	73	86	73	75	90	64	84	95	96
1500	85	71	89	73	79	90	67	83	93	96
3010	86	72	88	86	81	89	72	86	94	98

LOD: 3.15 mg/kg cyprodinil; DBA: days before application; CC: carrier control

Mortality data for the test material and reference item are summarised in the table below.

Table A 32: Summary of mortality of bees in the chronic toxicity test after 10 days

Nominal Concentration [mg product/kg feeding solution]	Daily Consumed Dose based on actual consumption [µg product/bee/day]	After 10 days	
		Mean mortality	
		absolute [%]	corrected ^a [%]
0 (control)		0.0	---
0 (carrier control)		5.0	---
850	19.3	0.0	-5.3
1700	31.2	10.0	5.3
3400	45.3	45.0 ^b	42.1
6800	60.0	95.0 ^b	94.7
13600	69.3	100 ^b	100
Reference item		100	100

^a test item mortality corrected for carrier control mortality; reference item mortality corrected for control mortality

^b statistically significantly increased compared to the carrier control (Cochran-Armitage test, one-sided greater, $\alpha = 0.05$)

During the 10-day test period no behavioural abnormalities were observed at the lowest dose of 19.3 µg product/bee/day (850 mg product/kg feeding solution). Two affected and one apathetic bee was observed at the second lowest dose of 31.2 µg product/bee/day throughout the study. At the three highest doses of 45.3,

60.0 and 69.3 several affected, apathetic (only at 45.3 µg product/bee/day), cramping and moribund bees were observed at different assessment dates from which it can be assumed in most cases that they have been dead on the following day. No behavioural abnormalities were observed during the 10-day test period for the control and carrier control group.

Study endpoints are summarised in the table below.

Table A 33: Study endpoints at 10 days

Treatment	Endpoints	After 10 d
Test item doses	LDD ₁₀ [µg product/bee/day]	33.2 [29.2 to 36.1]
	LDD ₂₀ [µg product/bee/day]	36.5 [32.9 to 39.2]
	LDD ₅₀ [µg product/bee/day] [95% CL]	43.8 [41.0 to 46.5]
	NOEDD [µg product/bee/day]	31.2
Test item concentrations	LC ₁₀ [mg product/kg feeding solution]	1846 [1433 to 2191]
	LC ₂₀ [mg product/kg feeding solution]	2270 [1858 to 2627]
	LC ₅₀ [mg product/kg feeding solution] [95% CL]	3370 [2941 to 3861]
	NOEC [mg product/kg feeding solution]	1700

Validity criteria

The study is considered valid since all validity criteria were met.

Validity criteria	Required	Actual
Control mean mortality at the end of the test [%]	≤ 15	0.0
Carrier control mean mortality at the end of the test [%]	≤ 15	5.0
Reference item mean mortality at the end of the test [%]	≥ 50	100

Conclusion

The chronic oral toxicity of A23282A to young, adult worker honey bees (*Apis mellifera* L.) was investigated under laboratory conditions over a period of 10 days in a dose-response feeding study.

The mean recoveries of cyprodinil of all test item groups were within the range of 80 to 120 % of the nominal values, except at the lowest concentration which was slightly below 80 %; therefore, endpoints are reported based on the nominal values. No residues of the active ingredient cyprodinil were found in the carrier control sample.

The 10-day NOEDD was determined to be 31.2 µg product/bee/day.

The 10-day NOEC was determined to be 1700 mg product/kg feeding solution.

The 10-day LDD_{10, 20, 50} were determined to be 33.2, 36.5 and 43.8 µg product/bee/day, respectively.

The 10-day LC_{10, 20, 50} were determined to be 1846, 2270 and 3370 mg product/kg feeding solution, respectively.

The study was deemed valid since all validity criteria were met.

(Ripperger, D., 2021)

Comments of zRMS:	The test was not evaluated as it considers the chronic toxicity of cyprodinil. This study is not relevant for risk assessment of formulation A23282A. The study considering the toxicity of active substance should be evaluated at EU level during substance renewal.
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Reference:	KCP 10.3.1.2
Report	Ruhland S. (2014) Cyprodinil EC (A14325E) - Chronic toxicity to the honeybee <i>Apis mellifera</i> L. in a 10 day continuous laboratory feeding study. Report Number 14 10 48 147 B. BioChem agrar Labor für biologische und chemische, Analytik GmbH, Kupferstraße 6 04827 Gerichshain, Germany. (XXXX file No. VV-410413; A14325E_10065).
Guideline(s):	Decourtye <i>et al.</i> (2005), Suchail <i>et al.</i> (2001), AFPP method CEB No. 230 (2012), EFSA Guidance Document (2013), Ring test protocol of the AG-Bienenschutz (2014)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

The toxicity of A14325E to honey bees was determined in a 10 day continuous feeding test in the laboratory at dose levels of 399.9, 200.0, 100.0, 50.0 and 25.0 µg a.s./bee.

The 10 day LD₅₀ was determined to be 69.7 µg consumed a.s./bee/day. The 10 day NOED was determined to be 44.2 µg consumed a.s./bee/day. The 10 day LC₅₀ was determined to be 2.062 g a.s./kg food. The 10 day NOEC was determined to be 1.284 g a.s./kg food.

Materials

Test Material	A14325E Cyprodinil EC
Lot/Batch #:	SMO3A100
Actual content of active ingredients:	29.1 % w/w corresponding to 295 g/L
Description:	Light yellow liquid
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	End of January 2017
Density:	1014 kg/m ³
Treatments	
Test rates:	399.9, 200.0, 100.0, 50.0 and 25.0 µg a.s./bee.
Control:	50 % (w/v) sucrose solution.
Toxic standard:	Dimethoate EC 400
Administration:	Ingestion in aqueous sucrose solution
Test organisms	
Species:	<i>Apis mellifera</i> L. (subspecies iberica P.)
Age and sex:	Young female workers 1-4 days old
Source:	Beekeeper Joaquin Cordero, Paseo de Moro No. 19, 41370 Cazalla (Seville), Spain

Food:	50% w/v aqueous sucrose solution
Test design	
Test cage description:	Aluminium cages with the dimensions: 20 cm (width) x 15 cm (height) x 10 cm (depth); with holes in the lateral walls for sufficient air supply and ventilation and two glass plates (one in front and one in the back) for observations of the bees
Replication:	3
No. of bees/arena:	20
Duration of test:	10 days
Environmental conditions	test
Temperature:	33.5 – 34.5 °C
Humidity:	47.2 – 54.3%
Photoperiod:	Constant darkness

Study Design and Methods

Experimental dates: 1st to 10th June 2014

Honeybees (*Apis mellifera*) were exposed to A14325E via oral ingestion for a period of 10 days. A stock solution of the highest test concentration was directly prepared in acetone. All lower test solutions were freshly prepared daily, by appropriate dilution of stock solution with 50% (w/v) aqueous sucrose solution.

The treated/untreated food was provided *ad libitum* in a plastic syringe, which had been weighed before application. The actual consumption was determined by reweighing the syringe containing the remaining test solution each day after removal from the test units. Any unconsumed food was rejected and replaced with fresh treated/untreated food. Observations for mortality, sublethal effects and behavioural abnormalities were recorded daily.

Due to their social feeding behaviour, the honeybees of a distinct group are assumed to share the application solution (trophallaxis) and thus receive similar doses of the applied respective item.

The mortality [%] per treatment was calculated from the number of dead bees and the total number of introduced bees per treatment group. Mortality in the test item treatment was corrected for corresponding control mortality, where necessary, according to the formula of Abbott (1925), modified by Schneider-Orelli (1947).

The statistical calculations were performed with a computer program (for example ToxRat Professional 2.10.06).

For statistical calculation of the mortality results the Fisher's Exact Binomial test (with Bonferroni Correction) was used. The accepted significance level was $p < 0.05$ (one-sided greater). For calculation of the LD_x/LC_x values of test and reference item a Probit/Weibull model was used (linear maximum likelihood regression).

Results and Discussion

Mortality data for the test material and control are summarised in the table below.

Table A 34: Summary of chronic toxicity of A14325E to the honeybee (*Apis mellifera* L)

Treatment		Mortality after 10 days (%)	Corrected 10-day mortality (%)
Consumed (µg / bee / day)	Dose (g /kg food)		
Control		3.3	-
219.210	10.270	100.0*	99.9
189.734	5.135	98.3*	98.3
84.533	2.567	76.7*	75.9
44.182	1.284	11.7	8.6
28.039	0.642	0.0	0.0
10 d LC ₅₀ (95% confidence limits)		2.062 g a.s. /kg food (1.623 – 2.621)	
10 d LD ₅₀ (95% confidence limits)		69.7 µg consumed a.s./bee/day (63.6 – 76.5)	
NOEC ^a		1.284 g a.s./kg food	
NOED ^a		44.2 µg consumed a.s. /bee /day	

Corrected mortality (according to SCHNEIDER-ORELLI 1947), negative values are treated as “0”

* Statistically significant difference in pairwise comparison between treatment and untreated control

Median lethal dose/conc. (and 95 % CL / lower-upper) was calculated by using Probit analysis (linear max. likelihood regression)

^a Fisher's Exact Binominal Test with Bonferroni Correction; $\alpha=0.05$; one sided greater

Validity of the test

Control mortality in the test was 3.3 % (must be <15 % to be considered valid). Therefore the test was considered valid.

Conclusions

The 10 day LD₅₀ was determined to be 69.7 µg consumed a.s./bee/day. The 10 day NOED was determined to be 44.2 µg consumed a.s./bee/day. The 10 day LC₅₀ was determined to be 2.062 g a.s./kg food. The 10 day NOEC was determined to be 1.284 g a.s./kg food.

(Ruhland, 2014)

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 accepted. The validity criteria were met:</p> <ul style="list-style-type: none"> larvae mortality in the control was below 15%; observed 14.6%; larvae mortality in the reference item mortality was ≥ 50 %; observed 93.8%; <p>The following deviations were noted:</p> <ul style="list-style-type: none"> for the toxic reference item mortality but no other observations were assessed; no emergence boxes were used from Day 15 to enable the assignment of each emerged bee to the respective replicate; short-term deviations (< 2 hours) from the recommended temperature (34 to 35 °C). <p>The following endpoints were calculated: 8-d LD₅₀ = 214 µg formulation/larva</p>
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	22-d NOED = 69.3 µg formulation/larva NOEC = 0.450 g formulation/kg diet
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Reference:	KCP 10.3.1.3
Report	Ripperger D., (2021), Cyprodinil/Prothioconazole (A23282A) Honey Bee <i>Apis mellifera</i> L. (Hymenoptera, Apidae) 22 Day Larval Toxicity Test (Repeated Exposure) Report No. S21-2796. Eurofins Agrosience Services, Ecotoxicology GmbH, Euting Str. 24, 75223 Niefern-Öschelbronn, Germany (XXXX file No. VV-947029)
Guideline(s):	OECD Guidance Document 239. Honey Bee (<i>Apis mellifera</i>) Larval Toxicity Test, Repeated Exposure, 2021
Deviations:	No emergence boxes were used from Day 15 to enable the assignment of each emerged bee to the respective replicate. This has no impact on the validity of the study.
GLP:	Yes
Acceptability:	Yes

Executive Summary

The toxicity of A23282A to the larvae of the honeybee *Apis mellifera* was determined in a chronic exposure study over a 22-day period.

The 8-day LD₅₀ for larval mortality was calculated to be 214 µg product/larva per developmental period.

The 22-day NOED for adult emergence was determined to be 69.3 µg product/larva per developmental period, equivalent to a NOEC of 450 mg product/kg diet.

The 22-day ED₁₀, ED₂₀ and ED₅₀ for adult emergence on day 22 were calculated to be 112, 130 and 162 µg product/larva per developmental period, equivalent to an EC₁₀, EC₂₀ and EC₅₀ of 729, 844 and 1052 mg product/kg diet, respectively.

Materials

Test Material	A23282A
Lot/Batch #:	LCR001-021-001
Content of a.s. (analysed):	Cyprodinil: 219 g/L, 22.1 % w/w Prothioconazole: 73.5 g/L, 7.40 % w/w
Description:	Liquid / yellow
Stability of test compound:	Stable under test conditions
Reanalysis/Expiry date:	30 th Sept 2023
Treatments	1 control group 5 test item groups 1 reference item group
Test concentrations/doses:	113, 225, 450, 900 and 1800 mg product/kg diet (equivalent to cumulative doses of 7.4, 34.7, 69.3, 139 and 277 µg product/larva per developmental period)

Control:	90 % of larval diet containing 10 % autoclaved, deionised water
Toxic standard:	48.0 mg dimethoate/kg diet (equivalent to 7.39 µg dimethoate/larva)
Analysis of concentrations:	test Cyprodinil was analysed in the larval diet of the control group and each test item group from day 3 until day 6. Mean measured recoveries of cyprodinil in the samples of the larval diet were 87, 82, 80, 81 and 81 % for the test item groups of 113, 225, 450, 900 and 1800 mg product/kg diet.
Test organisms	Honey bee larvae
Species:	<i>Apis mellifera</i> L. (Hymenoptera, Apidae)
Age:	First instar larvae (L1)
Source:	Stock beehives maintained by Eurofins Agrosience Services
Food:	Diet A: 50 % weight of fresh royal jelly + 50 % weight of an aqueous solution containing 2 % weight of yeast extract, 12 % weight of glucose and 12 % weight of fructose Diet B: 50 % weight of fresh royal jelly + 50 % weight of an aqueous solution containing 3 % weight of yeast extract, 15 % weight of glucose and 15 % weight of fructose Diet C: 50 % weight of fresh royal jelly + 50 % weight of an aqueous solution containing 4 % weight of yeast extract, 18 % weight of glucose and 18 % weight of fructose
Test Design	Dose-response test
Test cage description:	Crystal polystyrene grafting cells (NICOTPLAST) having a diameter of 9 mm and a depth of 8 mm; Each cell placed into a well of a sterile 48 well cellular culture plate (Greiner Bio One)
Replication:	3 (48 larvae from three different colonies, each hive equates to one replicate)
No. of larvae/replicate:	48, divided in 3 replicates, each containing 16 larvae
Environmental conditions	test Incubator with forced air circulation
Temperature:	Target: 34 – 35 °C (but not below 23 °C or above 40 °C) Actual: 33.7 – 35.2 °C
Humidity:	Day 1 to day 8: Target: 95 ± 5 % %, Actual range: 58.7 – 100.0 % (means 98.6 – 99.0 %) Day 8 to day 15: Target: 80 ± 5 %, Actual: 55.3 to 82.6 % (mean 80.8 %) Day 15 to day 22: Target: 50 - 80 %, Actual: 36.0 – 62.9 % (mean 61.4 %)
Photoperiod:	Constant darkness except during grafting, feeding and assessments
Duration of test:	22 days (day of grafting (day 1) to adult emergence which is assessed on day 22)

Study Design and Methods

Test facility: Eurofins Agrosience Services, Ecotoxicology GmbH, Eutinger Str. 24, 75223 Niefern-Öschelbronn, Germany

Experimental dates: 7th June to 6th August 2021

To obtain larvae of approximately the same age, the queen was confined for a maximum of 30 hours on a brood comb containing empty cells for egg laying. After removing the queen from the excluder cage, the comb in the colony for approximately 3 days for incubation. Afterwards it was brought into the laboratory for grafting larvae into the individual rearing cells (test units). The diet was deposited at the bottom of each cell, then the young larvae were grafted into the cells using a grafting tool.

During development, larvae were fed with three diet compositions containing the test item on days 3, 4, 5 and 6 of the larval rearing period. The composition of the aqueous part (D-glucose, D-fructose, yeast extract) and the amount of diet per larva varied according to the larval age. The required amount of diet was prepared and stored at $\leq -18^{\circ}\text{C}$ until use. On each feeding day the required amount of diet was pre-warmed in the incubator before feeding.

Mortality was observed and recorded throughout the study period to day 22 by visual assessment. At the end of the test period, bees were counted as successfully emerged if they showed signs of adult eclosion. This included the presence of differentiated wings and hair or the absence of the pupal skin. On Day 8, the larvae were inspected to monitor diet consumption and uneaten diet was recorded.

For statistical evaluation the statistics program ToxRat professional, Version 3.3.0 was used.

The NOEC and EC_{x} were determined for day 22 for adult emergence. But for statistical reasons, statistical evaluation (determination of NOEC and EC_{x}) was done using non-emergence data for day 22.

For determination of the NOEC of day-22 adult emergence, Qualitative Trend Analysis by Contrasts revealed a linear trend in the data ($\alpha = 0.05$). Tarone's test ($\alpha = 0.01$) found no signs of extra-binomial variance. Hence, Cochran-Armitage test (one-sided greater, $\alpha = 0.05$) was used to evaluate whether there was a significant difference between the test item groups and the control group for adult non-emergence on day 22. The corresponding NOED (No Observed Effect Dose) was calculated from the NOEC considering a density of 1.1 g/cm^3 of the diet and the cumulative feeding volume of $140 \mu\text{L}$.

The EC_{10} , EC_{20} and EC_{50} for adult emergence on day 22 were calculated using Weibull analysis. The treatment response was corrected by the control response using ABBOTT's formula (ABBOTT 1925). 95 %-confidence limits were approximated by $\pm 2 \cdot \text{SE}(\text{Ln}(\text{LC}_{10/20/50}))$. The corresponding ED_{10} , ED_{20} and ED_{50} were calculated from the $\text{EC}_{10,20,50}$ considering a density of 1.1 g/cm^3 of the diet and the cumulative feeding volume of $140 \mu\text{L}$.

Diet consumption were not evaluated for statistical significance due to the non-quantitative nature of the observations.

Results and Discussion

The analytical dose verification of cyprodinil in the test item treated larval diet for each test item group from day 3 until day 6 resulted in recoveries between 80 and 91 % of the nominal concentrations. The measured recoveries were thus within the range of 80-120 % of the nominal values and, therefore, the endpoints are reported based on nominal concentrations. Analytical recoveries in the diets are presented below.

Table A 35: Analytical results

Concentrations, nominal (mg cyprodinil/kg diet)	Analytical Recovery in Diet	
	Mean measured concentrations ^a (mg cyprodinil/kg diet)	% of nominal ^a
0 (C)	0 (< LOD)	-
25.0	21.7	87
49.7	40.6	82
99.5	79.7	80
199	160	81
398	320	81

LOD: 0.600 mg/kg of cyprodinil; C: control

^a calculated as mean of each application day

Mortality and emergence data for the test material and reference item are summarised in the table below.

Table A 36: Summary of mortality and emergence over 22 days

Concentration [mg product/ kg diet]	Cumulative dose [µg product/ larva/development period] ^a	Cumulative larval mortality day 8 [%]	Larval/ pupal mortality Day 8 – 15 [%]	Adult emergence day 22 [%] ^b	
		Actual	Actual	Actual	Inhibition Compared to Control ^c
Control		14.6	2.1	81.3	
113	17.4	0.0	6.3	91.7	-12.8
225	34.7	2.1	2.1	95.8	-17.8
450	69.3	4.2	10.4	83.3	-2.5
900	139	12.5	27.1	58.3 ^d	28.3
1800	277	89.6	10.4	0.0 ^d	100.0
Reference item [mg dimethoate/ kg diet] ^b	Reference item [µg dimethoate/ larva per developmental period] ^{b, c}	93.8	-	-	-
48	7.39				

^a Based on the cumulative feeding volume from day 3 until day 6 of 140 µL diet/larva and a density of the diet of 1.1 g/cm³

^b Statistical evaluation for non-emergence

^c Negative values indicate higher emergence compared to the control

^d Statistically significantly different compared to the control group (Cochran-Armitage test, one sided greater, $\alpha = 0.05$) based on non-emergence data

On day 8, uneaten food was observed at the test item doses of 277 (4 out of 5 living larvae) and 34.7 µg product/larva (1 out of 47 living larvae) (1800 and 225 mg product/kg diet).

When compared to the control group, all larvae of the highest test item dose of 277 µg product/larva (1800 mg product/kg diet) showed a reduced size on day 5 and day 6. On the further assessment days this was no longer observed.

Table A 37: Study endpoints

Treatment	Endpoints	After 8 d
Test item doses [µg product/larva per developmental period]	8-day LD ₅₀ [95% CL]	214 [200 / 228]

Treatment	Endpoints	After 22 d
Test item doses [µg product/larva per developmental period]	22-day ED ₁₀ [95% CL]	112 [92.6 / 126]
	22-day ED ₂₀ [95% CL]	130 [113 / 144]
	22-day ED ₅₀ [95% CL]	162 [147 / 185]
	22-day NOED	69.3
Test item concentrations [mg product/kg diet]	22-day EC ₁₀ [95% CL]	729 [601 / 816]
	22-day EC ₂₀ [95% CL]	844 [737 / 932]
	22-day EC ₅₀ [95% CL]	1052 [952 / 1203]
	22-day NOEC	450

The test was considered valid since:

- Day 8 larval mortality was 14.6 % (should be ≤ 15 %) across the control replicates
- Day 22 adult bee emergence was 81.3 % (should be $\geq 70\%$) across the control replicates
- Day 8 mortality in the dimethoate reference item group was 93 % (should be $>50\%$)

Conclusions

The day 8 LD₅₀ for larval mortality was determined to be 214 µg product/larva.

On day 22, the NOED for adult emergence was determined to be 69.3 µg product/larva per developmental period, equivalent to a NOEC of 450 mg product/kg diet.

The ED₁₀ for adult emergence on day 22 was determined to be 112 µg product/larva per developmental period, equivalent to a EC₁₀ of 729 mg product/kg diet.

The ED₂₀ for adult emergence on day 22 was determined to be 130 µg product/larva per developmental period, equivalent to a EC₂₀ of 844 mg product/kg diet.

The ED₅₀ for adult emergence on day 22 was determined to be 162 µg product/larva per developmental period, equivalent to a EC₅₀ of 1052 mg product/kg diet.

(Ripperger, D. 2021)

Comments of zRMS:	The test was not evaluated as it considers the chronic toxicity of cyprodinil. This study is not relevant for risk assessment of formulation A23282A. The study considering the toxicity of active substance should be evaluated at EU level during substance renewal.
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Reference:	KCP 10.3.1.3
Report	Kleebaum, K., (2014), Cyprodinil WG (A8637C) – Chronic toxicity to honeybee larvae <i>Apis mellifera</i> L. under laboratory conditions (<i>in vitro</i>). Report Number 14 10 48 145 B. BioChem agrar. Labor führ biologische und chemische Analytik GmbH, Kupeferstraße 6, 04827 Gerichshain, Germany. (XXXX File No. VV-411059; A8637C_10330).
Guideline(s):	OECD DRAFT Guidance Document for testing chemicals: Honey bee (<i>Apis mellifera</i>) larval toxicity test, repeated exposure (February 2014) OECD 237 Guidelines for testing chemicals: Honey bee (<i>Apis mellifera</i>) larval toxicity test, single exposure (2013)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

The purpose of this study was to determine the semi-chronic toxicity of A8637C (active ingredient:

cyprodinil) to honeybee larvae *Apis mellifera* L. in an *in vitro* test after repeated oral application. The 7 and 8 day NOED was determined to be 13.3 and 33.3 µg cyprodinil/larva, respectively. The 7 and 8 day LD₅₀ was determined to be 43.9 and 47.0 µg cyprodinil/larva, respectively.

Materials

Test Material	A8637C
	Cyprodinil WG (50)
Lot/Batch #:	SMO2C304
Actual content of active ingredients:	Cyprodinil: 50.2% w/w
Description:	Brownish granules
Stability of test compound:	Stable under the given conditions
Reanalysis/Expiry date:	31 December 2016
Density:	Not applicable

Treatments

Test rates:	Total µg A8637C/larva: 10.7, 26.6, 66.6, 166.5 and 416.3 Total µg cyprodinil/larva: 5.3, 13.3, 33.3, 83.3 and 208.2 g cyprodinil/kg diet: 0.034, 0.084, 0.211, 0.526 and 1.316
Control:	Untreated artificial diet
Toxic standard:	Dimethoate tech. (BAS 152 I), purity 99.8%
Administration:	Oral application using a sterile pipette

Test organisms

Species:	Worker honey bee larvae <i>Apis mellifera</i> L. subspecies <i>carica</i> P. (Insecta, Hymenoptera, Apoidea)
Age:	First instar (L1) during grafting
Source:	Colonies purchased from Bienenfarm Kern GmbH, Rehbacher Anger 10, 04249 Leipzig, Germany
Food:	Aqueous sugar solution (comprising yeast, glucose, fructose and water) mixed with royal jelly at a 1:1 ratio w/w

Test design

Test cage description:	Crystal polystyrene grafting cells (e.g. CNE Nicoplast, internal diameter 9 mm) were placed in 48 well plates. The well plates were filled up to 1/3 with dental roll and the grafting cells were placed on wetted and disinfected dental rolls.
Replication:	3
No. of bees/arena :	12

Environmental test conditions

Temperature:	34.2 – 35.0 °C
Humidity:	92 - 97% (RH)
Photoperiod:	Constant darkness
Duration of test:	Pre-grafting (<i>in vivo</i>): days -3 to 0 Grafting: day 1 Pre-exposure (<i>in vitro</i>): days 1 to 3 Application: days 3 to 6 Post exposure (<i>in vitro</i>): days 7 and 8

Study Design and Methods

Experimental dates: 20th June to 28th August 2014

The test/reference item was mixed into sterile filtered aqueous sugar solution (stock A). Several dilutions were prepared by adding further sugar solution (stocks B and C) and royal jelly was added to each stock solution at a ratio of 1 : 1, based on (w/w), to reach the final test concentrations. Dosages were adjusted to reflect the target amounts of A8637C.

Honeybee larvae *Apis mellifera* L. were exposed to repeated oral application of 5.3, 13.3, 33.3, 83.3 and 208.2 µg cyprodinil/larva (equivalent to 0.034, 0.084, 0.211, 0.526 and 1.316 g cyprodinil/kg diet) in an *in vitro* test. One control group was included in the test. The larvae of the control treatment were fed with untreated artificial diet, which served as a vehicle for the test item and reference item.

On Day 1 the combs containing the larvae were transported from the hive to an acclimated laboratory room. Larvae were transferred from the combs to the crystal polystyrene grafting cells using a suitable grafting tool (e.g. grafting needle Swiss type). During grafting the C-shaped larvae were placed on the surface of the artificial diet within the grafting cells. Cells were placed in 48 well plates filled up to 1/3 with a piece of dental roll. Each replicate unit consisted of 12 larvae, and there were 3 replicates per treatment and control. Each larva was fed the treated diet daily between Day 3 and Day 6 using a sterile pipette.

The number of dead larvae was recorded daily on Day 4 to Day 8. Any large amounts of unconsumed food were recorded on Day 7 and Day 8. After the last assessment (Day 8) the culture plates with all organisms were placed in a freezer.

All observations were made in comparison to the control larvae. For each concentration, the corrected mortality was calculated according to Abbott (1925) modified by Schneider-Orelli (1947).

The LD₅₀ values were calculated by Moving Average Computation. The statistical significance of the mortality values and the NOEC was calculated using Fisher's Exact Binomial Test with Bonferroni Correction ($P \leq 0.05$).

Results and Discussion

Mortality data and other observations for the test material and reference item are summarised in the table below.

Table A 38: Summary of semi-chronic toxicity of A8637C to honeybee larvae

Item applied	Dosage ¹ [µg cyprodinil/larva]	Concentration [g cyprodinil/kg diet]	Day 7			Day 8		
			Mortality mean %		OO ³	Mortality mean %		OO ³
			Absolute	Correct. ²	Mean %	Absolute	Correct. ²	Mean %
Control	-	-	2.8	-	2.8	11.1	-	2.8
Test item	5.3	0.034	5.6	2.9	0.0	5.6	0.0	0.0
	13.3	0.084	0.0	0.0	5.6	11.1	0.0	0.0
	33.3	0.211	22.2*	20.0	14.5	22.2	12.5	0.0
	83.3	0.526	100.0*	100.0	-	100.0*	100.0	-
	208.2	1.316	100.0*	100.0	-	100.0*	100.0	-
Reference item	6.2	0.039	86.1	85.7	0.0	88.9	87.5	0.0
Treatment	Endpoints		Day 7			Day 8		
Test item doses	NOED [µg cyprodinil/larva]		13.3			33.3		
	LD ₅₀ [µg cyprodinil/larva] (95 %-CL/lower-upper)		43.9 (36.9 – 52.1)			47.0 (40.7 – 54.2)		
Test item concentrations	NOEC [g cyprodinil/kg diet]		0.084			0.211		

Results were average based on 3 replicates, containing 12 larvae each

Calculations were performed with non-rounded values

* Statistically significant difference in pairwise comparison between treatment and untreated control (Fisher's Exact Binomial Test with Bonferroni Correction; one sided greater; $\alpha = 0.05$)

¹ All test item doses were based on a sum of applications on days 3 to 6

² Corrected mortality (according to Schneider-Orelli 1947), negative values are set to "0"

³ OO: Other observations (large quantities of remaining food, smaller body size of larva)

- Not applicable

Validity of the test

All of the validity criteria were met:

- Control mortality should be $\leq 15\%$ for larvae across all control replicates at day 7 and day 8 (actual values were 2.8 and 11.1)
- Reference item mortality should be $\geq 50\%$ for larvae across all reference replicates at day 7 and day 8 (actual values were 86.1 and 88.9%)
- Concentration of the active substance in analysed sample of test item stock solution A should be $\pm 20\%$ of the nominal concentration of A (actual value ranged between 108 and 110 %)

Conclusions

The 7- and 8-day NOED was determined to be 13.3 and 33.3 μg cyprodinil/larva, respectively. The 7- and 8-day LD_{50} was determined to be 43.9 and 47.0 μg cyprodinil/larva, respectively.

(Kleebaum K, 2014)

A 2.3.1.4 KCP 10.3.1.4 Sub-lethal effects

A 2.3.1.5 KCP 10.3.1.5 Cage and tunnel tests

A 2.3.1.6 KCP 10.3.1.6 Field tests with honeybees

A 2.3.2 KCP 10.3.2 Effects on non-target arthropods other than bees

A 2.3.2.1 KCP 10.3.2.1 Standard laboratory testing for non-target arthropods

Comments of zRMS:	<p>The study was accepted. The validity criteria were met.</p> <p>The following endpoints for <i>Typhlodromus pyri</i> were derived: 7-day LR_{50} = 988.16 mL formulation/ha; ER50 = 617.82 mL formulation/ha NOER = 190.73 mL formulation/L.</p> <p>The endpoints were used for risk assessment.</p>
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Reference:	KCP 10.3.2.1
Report	Fallowfield L., 2021, Cyprodinil/prothioconazole EC (A23282A) – A Rate-Response Laboratory Study to Determine the Effects of Fresh Residues on the Predatory Mite <i>Typhlodromus pyri</i> (Acari: Phytoseiidae)., Report Number SYN-21-28, Mambo-Tox, A Division of Cawood Scientific Ltd. University Science Park, Southampton SO16 7NP, United Kingdom (XXXX File No. VV-918193)
Guideline(s):	Blümel <i>et al.</i> Laboratory residual contact test with the predatory mite <i>Typhlodromus pyri</i> (Acari: Phytoseiidae) for regulatory testing of plant protection products. (2000)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Executive Summary

The effects of A23282A on the predatory mite *Typhlodromus pyri* were assessed in a laboratory test. Mites were exposed to rates equivalent to 2000, 1250, 781.25, 488.28, 305.18 and 190.73 mL A23282A/ha. The 7-day LR₅₀ value was 988.16 mL A23282A/ha, with 95% confidence intervals of 729.52 and 1477.43 mL A23282A/ha. The ER₅₀ was 617.82 mL A23282A/ha, with 95% confidence interval values of 376.84 and 1012.91 mL A23282A/ha. The overall NOER value was 190.73 mL A23282A/ha.

Materials

Test Material	Cyprodinil/prothioconazole EC (A23282A)
Lot/Batch #:	LCR001-021-001
Other batch ID:	1160912
Actual content of active ingredients:	Cyprodinil: 22.1% w/w (corresponding to 219 g/L) Prothioconazole: 7.40% w/w (corresponding to 73.5 g/L)
Description:	Yellow liquid
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	End September 2023
Density:	993 kg/m ³
Treatments	
Test rates:	2000, 1250, 781.25, 488.28, 305.18 and 190.73 mL test item/ha
Control:	Purified water
Toxic standard:	Dimethoate (an EC formulation nominally containing 400 g a.s./L), applied at a rate of 15 mL product/ha
Spray volume rate:	200 L/ha
Application method:	Calibrated track sprayer
Test organisms	
Species:	<i>Typhlodromus pyri</i> (Acari: Phytoseiidae)
Age:	< 24-hr-old protonymphs
Source:	Culture maintained at Test Facility (originally: P.K. Nützlingszuchten, Welzheim, Germany)

Feeding:	1:1 v/v almond (<i>Prunus</i> sp. var. a mix of Aldrich, Nonpareil and Wood Colony) and apple (<i>Malus</i> sp. var. Red Delicious) pollen.
Test design	
Arenas:	Glass plates formed from two microscope slide cover slips (each 2.2 cm x 4.0 cm in area) joined together with two additional cover slips glued to the top and bottom ends of the main cover slips. 12 cm ² arena created on slides by non-drying sticky insect gel barrier.
Replication:	3 per treatment
No. of mites/arena:	20
Duration of test:	14 days
Environmental conditions	test
Temperature:	24.5 to 25.1°C
Humidity:	64-74% RH
Photoperiod:	16 hours light: 8 hours dark at 450-1300 lux

Study Design and Methods

Test facility: Mambo-Tox, A Division of Cawood Scientific Ltd. University Science Park, Southampton SO16 7NP, United Kingdom

Experimental dates: 22nd June to 26th July 2021

Dilutions were prepared in purified water, shortly before applications were made and the solutions were thoroughly agitated to ensure their homogeneity. Treatments were applied to glass plates which were left to dry and then used to construct the test arenas. Mites were then introduced to the arenas and their survival assessed over a 7-day period, by which time the mites in the control were adult. The sex of the adult mites was determined. The mites in the control and from all treatment rates of the test item where corrected mortality was $\leq 60\%$, as decided by the Study Director, were then left *in situ* so that their reproduction could be assessed over a further 7 days. The mean number of eggs produced per female between 7 and 14 days after treatment (DAT) was calculated.

The mean percentage mortality after 7 days was calculated for the individual treatments and then corrected for any losses in the control treatment using Abbott's formula. To determine the no-observed-effect rate (NOER) in terms of mite survival, the percentage mortality in each test item treatment was compared to that in the control firstly by qualitative trend analysis by contrasts (monotonicity of rate/response), Tarone's test procedure and then by using step-down Cochran-Armitage test procedure ($\alpha = 0.05$, one-sided, $>$ control). The median lethal rate (LR₅₀) with respect to mortality was determined using Probit analysis using linear maximum likelihood regression.

In order to determine the NOER for reproduction, the results for the mean number of eggs per female in each replicate were compared statistically. The data were checked for normality (Shapiro-Wilk test), for homogeneity of variance (Levene's test) and by qualitative trend analysis by contrasts (monotonicity of rate/response) prior to analysis by Williams' multiple sequential t-test procedure ($\alpha = 0.05$, one-sided, $<$ control). The median effect rate (ER₅₀) with respect to reproduction was determined using non-linear regression analysis.

Results

Mortality and fecundity are summarised in the table below.

Table A 39: Effects of A23282A on mortality and fecundity of *Typhlodromus pyri*, when exposed in a laboratory test

Treatment	Mean % mortality at 7 DAT ¹	Mean corrected % mortality at 7 DAT ²	Mean eggs/female from 7 to 14 DAT ³	% reduction in reproduction compared to control ⁴
Control	6.7	-	6.63	-
2000 mL A23282A/ha	91.7 *	91.1	~	-
1250 mL A23282A/ha	56.7 *	53.6	0.15 *	97.7
781.25 mL A23282A/ha	41.7 *	37.5	3.39 *	48.9
488.28 mL A23282A/ha	15.0 *	8.9	4.24 *	36.0
305.18 mL A23282A/ha	16.7 *	10.7	4.07 *	38.6
190.73 mL A23282A/ha	10.0	3.6	5.63	15.0
Toxic reference Dimethoate EC 400	81.7 *	80.4	~	-

* Treatments that differed significantly from the control

¹ Individual test item treatments were compared to the control using step-down Cochran-Armitage test procedure and for the toxic reference treatment Fisher's exact binomial test was used ($\alpha = 0.05$, one-sided, > control).

² Calculated using Abbott's formula. A positive value indicates an increase in mortality, relative to the control.

³ Individual treatments were compared to the control by Williams' multiple sequential t-test procedure ($\alpha = 0.05$, one-sided, < control).

⁴ A positive value indicates a decrease in egg production.

~Treatment not assessed.

Validity criteria

The test was considered valid since:

- Mortality in the control treatment was 6.7% (should not exceed 20 % over the initial 7 days.)
- Corrected mortality in the toxic reference treatment was 80.4% (must be 50-100%)
- The mean cumulative number of eggs produced from 7 to 14 days was 6.63 per female in the control (should be ≥ 4.0 per female).

Conclusion

The effects of A23282A on the predatory mite *Typhlodromus pyri* were assessed in a laboratory test. Mites were exposed to rates equivalent to 2000, 1250, 781.25, 488.28, 305.18 and 190.73 mL A23282A/ha. The 7-day LR₅₀ value was 988.16 mL A23282A/ha, with 95% confidence intervals of 729.52 and 1477.43 mL A23282A/ha. In terms of mite reproduction, the ER₅₀ value was 617.82 mL A23282A/ha, with 95% confidence interval values of 376.84 and 1012.91 mL A23282A. The overall NOER value was 190.73 mL A23282A/ha.

(Fallowfield L., 2021)

Comments of zRMS:	<p>The study was accepted. The validity criteria were met.</p> <p>The following endpoints for <i>Aphidius rhopalosiphi</i> were derived: 48-h LR₅₀ = 106.5 mL formulation/ha; ER₅₀ > 75 mL formulation/ha NOER = 75 mL formulation/L.</p> <p>The endpoints were used for risk assessment.</p>
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Reference:	KCP 10.3.2.1
Report	Stevens J., 2021, Cyprodinil/Prothioconazole EC (A23282A) – A Rate-Response Laboratory Study to Determine the Effects of Fresh Residues on the Parasitic Wasp <i>Aphidius rhopalosiphi</i> (Hymenoptera, Braconidae), Mambo-Tox, A Division of Cawood Scientific Ltd, University Science Park, Southampton SO16 7NP, United Kingdom, SYN-21-29 (XXXX File No. VV-917627)
Guideline(s):	Mead-Briggs <i>et al.</i> (2000).
Deviations:	None.
GLP:	Yes
Acceptability:	Yes

Executive Summary

The effects of A23282A on the parasitic wasp *Aphidius rhopalosiphi* were assessed in a laboratory test. Wasps were exposed to rates equivalent to 300, 150, 75, 37.5 and 18.75 mL test item/ha. The 48-h LR₅₀ value was 106.5 mL test item/ha, with 95% confidence limits of 64.8 and 177.4 mL test item/ha. Based on statistical comparison with the control, the NOER value for wasp survival was 18.75 mL test item/ha. In terms of effect on the reproductive performance of surviving wasps, the ER₅₀ value for A23282A was > 75 mL test item/ha and the NOER value for reproduction was 75 mL test item/ha.

Materials

Test Material	Cyprodinil/prothioconazole EC (A23282A)
Lot/Batch #:	LCR001-021-001/1160912
Other product code	CGA219417/ASF990 EC (225/075)
Actual content of active ingredients:	Cyprodinil: 22.1% w/w, corresponding to 219 g/L Prothioconazole: 7.40% w/w, corresponding to 73.5 g/L
Description:	Yellow liquid
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	End of September 2023
Density:	993 kg/m ³
Treatments	
Test rates:	300, 150, 75, 37.5 and 18.75 mL test item/ha
Control:	Purified water
Toxic standard:	Dimethoate (an EC formulation containing nominally 400 g a.s./L), applied at a rate of 0.05 mL product/ha
Spray volume rate:	200 L spray solution/ha
Application method:	Calibrated laboratory track-sprayer
Test organisms	
Species:	<i>Aphidius rhopalosiphi</i> (Hymenoptera, Braconidae)
Age:	< 48 hours
Source:	Culture maintained at Test Facility (originally: Katz Biotech AG, Baruth, Germany)
Feeding:	1:3 v/v solution of honey in water on cotton wool

Test design - Mortality phase

Arenas:	Treated surfaces of glass plates (10 cm x 10 cm) used to form floors and ceilings of shallow test arenas, the walls of which comprised a square frame made from metal casing, with mesh-covered ventilation holes present in the side walls of the casing.
Replication:	4 arenas per treatment
No. of wasps/arena:	10 (> 5 ♀)

Test design - Fecundity phase

Arenas:	Pots containing approximately 15 barley seedlings (<i>Hordeum vulgare</i> var. Laureate) and previously infested per pot with approximately > 100 adults and nymphs of a mixed cereal aphid culture (<i>Metopolophium dirhodum</i> and <i>Rhopalosiphum padi</i>), were enclosed within clear acrylic cylinders (9 cm in diameter, 20 cm high), the tops of which were covered with nylon mesh netting.
Replication:	15 pots per treatment being assessed
No. of wasps/arena:	1 ♀
Duration of test:	13 days
Environmental conditions	test
Temperature:	Mortality phase: 18.7-20.8°C. Fecundity phase: 20.5-20.8°C
Humidity:	Mortality phase: 71-78%.
Photoperiod:	Mortality phase: 16 h (941-1002 lux). Fecundity phase: 16 h (2020-2096 lux for aphid parasitisation phase; 4177-4298 lux for pupal wasp development).

Study Design and Methods

Test facility: Mambo-Tox, A Division of Cawood Scientific Ltd, University Science Park, Southampton SO16 7NP, United Kingdom

Experimental dates: 16th June to 12th July 2021

Dilutions were prepared in purified water, shortly before applications were made and the solutions were thoroughly agitated to ensure their homogeneity. Treatments were applied to glass plates which were left to dry and then used to construct the test arenas. The wasps were introduced into these arenas and their mortality was assessed 2, 24 and 48 h later.

To assess any sub-lethal effects, reproduction assessments were then carried out for the control and for the 3 highest treatment rates that had resulted in $\leq 60\%$ corrected mortality. Female wasps were confined individually over untreated aphid-infested barley plants for 24 h, before being removed. The plants were left for a further 10 days before the number of aphid mummies that had developed on plants where wasps had been found alive after the 24-h oviposition period was recorded.

The percentage mortality of the wasps in the bioassay over 48 h was calculated. Mortality was defined as the numbers of *moribund* and *dead* wasps combined. The corrected percentage mortality (taking into account any control treatment losses) was derived using Abbott's formula. The *median lethal rate* (LR_{50}) value for the test item was estimated by Probit regression analysis. Treatment mortality at 48 h was compared to mortality in the control using step-down Cochran-Armitage test procedure (one-sided, > control, $\alpha = 0.05$).

For the reproduction assessments, the data sets from each treatment were checked for normality (Shapiro-Wilk test, $p > 0.01$) and homogeneity of variance (Levene's test, $p > 0.01$) and for trend analysis by contrasts (monotonicity of concentration/response), before being compared by Dunnett's multiple t-test

procedure (one-sided, $\alpha = 0.05$).

Results

Mortality and reproduction are summarised in the table below.

Table A 40: Effects of fresh residues of A23282A on mortality and reproduction of *Aphidius rhopalosiphi*, when exposed under laboratory test conditions

Treatment mL A23282A/ha	Mean % mortality at 48 h ¹	Mean % corrected mortality at 48 h (M-value) ²	Number of females successfully assessed for reproduction	Mean number of mummies per surviving female ³ (standard deviation)	% Reduction in reproduction compared to control ⁴
Control	0.0	-	14	49.9 (14.4)	-
300	97.5 *	97.5	~	~	~
150	80.0 *	80.0	~	~	~
75	12.5 *	12.5	15	53.5 (12.2)	-7.2
37.5	7.5 *	7.5	11	49.7 (12.9)	0.4
18.75	0.0	0.0	13	52.7 (10.5)	-5.5
Toxic reference ⁵	100	100	~	~	~

*Significant differences compared to control.

¹ The individual test item treatments were compared to the control using step-down Cochran-Armitage test procedure (one-sided, $\alpha = 0.05$);

² Derived using Abbott's formula.

³ The results were compared to the control using Dunnett's-multiple t-test procedure (one-sided, $\alpha = 0.05$). There were no significant differences.

⁴ Percentage reduction in reproduction, relative to the control. A positive value indicates a decrease, a negative value an increase in reproduction.

⁵ BAS 152 65 I, containing 400 g dimethoate/L. Application rate 0.050 mL product per 200 L water/ha.

~ Treatment not assessed.

Validity criteria

The test was considered valid since:

- Mortality within the control treatment at 48 hours was 0.0% (should not exceed 13% (i.e. 5 wasps from 40).
- Corrected mortality within the toxic-reference treatment at 48 hours was 100% (should exceed 50%).
- The mean number of mummies in the control treatment was 49.9 (must be > 5.0 per female).
- There were no zero values for reproduction in the control treatment (should be no more than 2).

Conclusion

The effects of A23282A on the parasitic wasp *Aphidius rhopalosiphi* were assessed in a laboratory test. Wasps were exposed to rates equivalent to 300, 150, 75, 37.5, and 18.75 mL test item/ha. The 48-h LR₅₀ value was 106.5 mL test item/ha, with 95% confidence limits of 64.8 and 177.4 mL test item/ha. Based on statistical comparison with the control, the NOER value for wasp survival was 18.75 mL test item/ha. In terms of effect on the reproductive performance of surviving wasps, the ER₅₀ value for A23282A was > 75 mL test item/ha and the NOER value for reproduction was 75 mL test item/ha.

(Stevens J. 2021)

Comments of zRMS:	<p>The study was accepted. The validity criteria were met.</p> <p>The following endpoints for <i>Typhlodromus pyri</i> were derived: 7-day LR₅₀ = 2637.99 mL formulation/ha; ER₅₀ = 2200.8 mL formulation/ha NOER = 1234.57 mL formulation/L.</p> <p>The endpoints were used for risk assessment.</p>
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A 2.3.2.2 KCP 10.3.2.2 Extended laboratory testing, aged residue studies with non-target arthropods

Reference:	KCP 10.3.2.2
Report	Fallowfield L., 2022, Cyprodinil/prothioconazole EC (A23282A) – A rate-response extended laboratory study to determine the effects of fresh residues on the predatory mite <i>Typhlodromus pyri</i> (Acari: Phytoseiidae), Report Number SYN-21-30. Mambo-Tox, A Division of Cawood Scientific Ltd., 2 Venture Rd., University Science Park, Southampton, SO16 7NP, UK. (XXXX File No. VV-935534)
Guideline(s):	Yes; IOBC, Blümel <i>et al.</i> (2000).
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Executive Summary

The effects of A23282A on the predatory mite *Typhlodromus pyri* were assessed in an extended laboratory test. Mites were exposed to rates equivalent to 4000, 2222.22, 1234.57, 685.87, 318.04 and 211.69 mL A23282A/ha. The 7-day LR₅₀ value was 2637.99 mL A23282A/ha, with 95% confidence intervals of 2385.45 and 2941.21 mL A23282A/ha. In terms of mite reproduction, the ER₅₀ value was 2200.80 mL A23282A/ha, with 95% confidence interval values of 1591.3 and 3043.7 mL A23282A/ha. The overall NOER value was 1234.57 mL A23282A/ha.

Materials

Test Material	Cyprodinil/prothioconazole (A23282A)
Lot/Batch #:	LCR001-021-001 (1160912)
Actual content of active ingredients:	Cyprodinil: 22.1% w/w (corresponding to 219 g/L) Prothioconazole: 7.40% w/w (corresponding to 73.5 g/L)
Description:	Yellow liquid
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	End September 2023
Density:	993 kg/m ³
Treatments	
Test rates:	4000, 2222.22, 1234.57, 685.87, 318.04 and 211.69 mL test item/ha

Control:	Purified water
Toxic standard:	Dimethoate (an EC formulation nominally containing 400 g a.s./L), applied at a rate of 37.5 mL product/ha
Spray volume rate:	200 L/ha
Application method:	Calibrated track sprayer

Test organisms

Species:	<i>Typhlodromus pyri</i> (Acari: Phytoseiidae)
Age:	< 24-hr-old protonymphs
Source:	In-house culture, originally obtained in April 1995 from P.K. Nützlingszuchten, Welzheim, Germany and supplemented with further mites from the same source in 1996 and 1997.
Feeding:	1:1 v/v almond (<i>Prunus</i> sp. var. Butte) and apple (<i>Malus</i> sp. var. Red Delicious) pollen.

Test design

Arenas:	5 cm leaf discs cut from the first true leaves taken from dwarf French bean plants. 12.5 cm ² arena created on leaf discs by non-drying sticky insect gel barrier.
Replication:	3 per treatment
No. of mites/arena :	20
Duration of test:	14 days

Environmental test conditions

Temperature:	24.4-26.6°C
Humidity:	60-76%
Photoperiod:	16 h (500-1200 lux)

Study Design and Methods

Test facility: Mambo-Tox, A Division of Cawood Scientific Ltd. University Science Park, Southampton SO16 7NP, UK.

Experimental dates: 5th to 19th October 2021

Dilutions were prepared in purified water, shortly before applications were made and the solutions were thoroughly agitated to ensure their homogeneity. Treatments were applied to leaf discs which were left to dry and then used to construct the test arenas. Mites were then introduced to the arenas and their survival assessed over a 7-day period, by which time the mites were adult. The sex of the adult mites was determined. The mites in the control and from the highest three treatment rates of the test item where corrected mortality was $\leq 60\%$, were then left *in situ* so that their reproduction could be assessed over a further 7 days. The mean number of eggs produced per female between 7 and 14 days after treatment (DAT) was calculated.

The mean percentage mortality after 7 days was calculated for the individual treatments and then corrected for any losses in the control treatment using Abbott's formula. To determine the *no-observed-effect rate* (NOER) in terms of mite survival, the percentage mortality in each test item treatment was compared to that in the control firstly by qualitative trend analysis by contrasts (monotonicity of concentration/response), Tarone's test procedure and then by using step-down Rao-Scott-Cochran-Armitage test procedure ($\alpha = 0.05$, one-sided, > control). The *median lethal rate* (LR₅₀) with respect to mortality was determined using Probit analysis using linear maximum likelihood regression.

To determine the NOER for reproduction, the results for the mean number of eggs per female in each replicate were compared statistically. The data were first checked for normality (Shapiro-Wilk test, $p > 0.001$), for homogeneity of variance (Levene's test, $P > 0.01$) and by qualitative trend analysis by contrasts (monotonicity of concentration/response) prior to analysis by Williams' multiple sequential t-test procedure ($\alpha = 0.05$, one-sided, $< \text{control}$). The *median effect rate* (ER₅₀) with respect to reproduction was determined using non-linear regression analysis.

Results

Mortality and fecundity are summarised in the table below.

Table A 41: Effects of A23282A on mortality and fecundity of *Typhlodromus pyri*, when exposed in an extended laboratory test

Treatment mL A23282A/ha	Mean % mortality at 7 DAT ^{a)}	Mean corrected % mortality at 7 DAT ^{b)}	Mean eggs/female from 7 to 14 DAT ^{c)}	% reduction in reproduction compared to control ^{d)}
Control	1.7	-	10.5	-
4000	78.3 *	78.0	~	-
2222.22	45.0 *	44.1	5.2 *	50.7
1234.57	3.3	1.7	8.9	14.9
685.87	1.7	0.0	10.3	1.7
318.04	1.7	0.0	~	-
211.69	5.0	3.4	~	-
Toxic reference	100 *	100	~	-

^{a)} Individual test item treatments were compared to the control using step-down Rao-Scott-Cochran-Armitage test procedure and for the toxic reference treatment Fisher's exact binomial test was used ($\alpha = 0.05$, one-sided, $> \text{control}$). Treatments that differed significantly from the control are indicated with an asterisk (*).

^{b)} Calculated using Abbott's formula. A positive value indicates an increase in mortality, relative to the control.

^{c)} Individual treatments were compared to the control by Williams' multiple sequential t-test procedure ($\alpha = 0.05$, one-sided, $< \text{control}$). Treatment rates that differed significantly from the control are indicated with an asterisk (*).

^{d)} A positive value indicates a decrease in egg production.

^{e)} ~ Treatment not assessed.

Validity Criteria

The test was considered valid since:

- Mortality in the control treatment was 1.7% (should not exceed 20 %.)
- Corrected mortality in the toxic reference treatment was 100% (must be 50-100%)
- The mean cumulative number of eggs produced from 7 to 14 days was 10.5 per female in the control (should be equal to or greater than 4.0 per female).

Conclusion

The effects of A23282A on the predatory mite *Typhlodromus pyri* were assessed in an extended laboratory test. Mites were exposed to rates equivalent to 4000, 2222.22, 1234.57, 685.87, 318.04 and 211.69 mL A23282A/ha. The 7-day LR₅₀ value was 2637.99 mL A23282A/ha, with 95% confidence intervals of 2385.45 and 2941.21 mL A23282A/ha. In terms of mite reproduction, the ER₅₀ value was 2200.80 mL A23282A/ha, with 95% confidence interval values of 1591.3 and 3043.7 mL A23282A. The overall NOER value was 1234.57 mL A23282A/ha.

(Fallowfield L, 2021)

Comments of zRMS:	The study was accepted. The validity criteria were met.
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	<p>The following endpoints for <i>Aleochara bilineata</i> were derived: ER > 2000 mL formulation/ha NOER = 2000 mL formulation/L.</p> <p>The endpoints were used for risk assessment.</p>
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Reference: KCP 10.3.2.2

Report Tew, G. (2022). Cyprodinil/Prothioconazole EC (A23282A) – A rate-response extended laboratory study of the effects of freshly treated substrate on the rove beetle, *Aleochara bilineata* (Coleoptera, Staphylinidae), Report Number SYN-21-33. Mambo-Tox, A Division of Cawood Scientific Ltd., 2 Venture Road, University Science Park, Southampton, SO16 7NP, UK. (XXXX File No. VV-936487).

Guidelines: Grimm *et al.* (2000)

Deviations: No

GLP: Yes

Acceptability: Yes

Executive Summary

In an extended laboratory test where adults of the rove beetle *Aleochara bilineata* were exposed to fresh residues of A23282A on a natural soil substrate, the NOER with respect to the reproductive success of the beetles was 2000 mL A23282A/ha, the maximum rate tested, and the ER₅₀ was >2000 mL A23282A/ha.

Materials

Test Material Cyprodinil/Prothioconazole EC (225/075) (A23282A)

Lot/Batch #: LCR001-021-001 (1160912)

Actual content of active ingredients: cyprodinil: 22.1 % w/w (219.0 g/L)
prothioconazole: 7.4 % w/w (73.5 g/L)

Description: Yellow liquid

Stability of test compound: Stable under standard conditions

Reanalysis/Expiry date: End September 2023

Density: 993 kg/m³

Treatments

Test rates: 2000, 1000, 500, 250 and 125 mL A23282A/ha

Control: Purified water

Toxic standard: Dimethoate, EC formulation of nominally 400 g a.s./L, applied at 3.8 L product/ha

Application method: Calibrated track-sprayer

Test organisms

Species: *Aleochara bilineata* (Coleoptera: Staphylinidae)

Age: Adult beetles ≈ 4 days old at the start of the test

Source: Commercial supplier (De Groene Vlieg, Nieuwe Tonge, the Netherlands)

Food: Raw minced beef

Host pupae for larvae to parasitize: *Delia antiqua* Meigen. (Diptera, Anthomyiidae),

Test design - Mortality phase

Arenas: Clear polystyrene boxes filled to a depth of 4 cm with loamy sand (LUFA 2.1). Beetles placed in the boxes once treatments have been applied. Onion fly pupae (500 per replicate) placed in the soil at 7, 14 and 21 days after treatment (DAT).

Substrate: Lufa 2.1 soil.

Replication: 4 arenas per treatment,

No. of beetles/arena: 10 male and 10 female beetles

Test design – Fecundity phase

Arenas: Plastic pots, 9 cm in diameter and 5 cm deep. The lid and base of each pot had a central area removed and covered with nylon netting (ca. 0.5 mm by 0.5 mm aperture size for lid, ca. 2 mm by 3 mm aperture size for base). The netting at the base acted as a sieve, so that when the adult beetles emerged, they fell through into collecting chambers below (i.e. pots 9 cm diameter by 9 cm deep).

Duration of test: 75 days.

Environmental test conditions

Temperature: 18.0-21.4°C

Humidity: 63-89% RH.

Photoperiod: 16 hours (400-800 lux).

Study Design and Methods

Test facility: Mambo-Tox, A Division of Cawood Scientific Ltd.

Experimental dates: 9th September to 8th December 2021

Treatments were applied to the test arenas and the adult beetles were introduced. At days 7, 14 and 21 during exposure, 500 *D. antiqua* pupae were carefully buried in the soil. At 28 DAT days all surviving adult beetles were removed from the substrate and the number was recorded. The substrate and the parasitized onion fly pupae were returned to the climatic room in the original test units with gentle aeration. 35 days after application the pupae were sieved out of the soil and the pupae of each replicate were transferred to a separate emergence container. Emerging beetles were counted and removed from the emergence containers at least 3 times per week; emergence of the F1-generation was monitored until the control treatment fell below a rate of two beetles in each replicate per day (at 75 DAT).

The aim of the study was to determine whether the individual test-item treatments led to a reduction in the parasitic success of the treated beetles, relative to the water control. The mean number of offspring produced per replicate and a measure of the standard deviation were calculated for each treatment. The percentage of the fly pupae provided (nominally 6000 per treatment) that gave rise to F1 beetles was determined for each treatment.

Data on the number of emerged beetles in individual treatment replicates was analysed for normality (Shapiro-Wilk test, $\alpha = 0.01$) and equality of variance (Levene's test, $\alpha = 0.01$). Trend analysis by contrasts was performed ($\alpha = 0.05$) prior to comparison of the individual treatment groups to the control using Dunnett's multiple t-test procedure, (one sided, < control, $\alpha = 0.05$). The toxic reference was compared to the control by Student's t-test for homogeneous variances (one-sided, < control, $\alpha = 0.05$).

Results

Mortality and reproduction are summarised in the table below.

Table A 42: Effects of A23282A on reproduction of *Aleochara bilineata*

Rate (mL product/ha)	% mortality	corrected % mortality ^{a)}	Mean number of F ₁ progeny ^{b)}	% reduction in reproduction ^{c)}
Control	8.8	-	904.0	-
2000	5.0	-4.1	887.0	1.9
1000	6.3	-2.7	865.0	4.3
500	15.0	6.8	859.8	4.9
250	25.0	17.8	981.5	-8.6
125	16.3	8.2	945.3	-4.6
Toxic ref	100.0	100.0	89.8*	90.1

^{a)} Values corrected using Abbott's formula. Negative values indicate a decrease and positive values an increase in mortality with respect to the control.

^{b)} Individual test-item treatments were compared to the control by Dunnett's multiple t-test procedure (one sided, < control, $\alpha = 0.05$). The toxic reference was compared to the control by Student's t-test for homogeneous variances (one-sided, < control, $\alpha = 0.05$). An asterisk (*) indicates where there was a statistically significant reduction in numbers of progeny.

^{c)} The percentage change in numbers of F₁ progeny, relative to the control. Positive values indicate a decrease and negative values an increase, relative to the control.

Validity criteria

The validity criteria were met since:

- The average number of hatched beetles per replicate of the F₁-generation in the control was 904.0 (must be > 400)
- The reduction of the reproductive capacity in the reference item treatment relative to control was 90.1% (must be ≥ 50 %.)

All of the validity criteria were met.

Conclusion

In an extended laboratory test where adults of the rove beetle *Aleochara bilineata* were exposed to fresh residues of A23282A on a natural soil substrate, the NOER with respect to the reproductive success of the beetles was 2000 mL A23282A/ha, the maximum rate tested, and the ER₅₀ value was > 2000 mL A23282A/ha.

(Tew G., 2022)

Comments of zRMS:	<p>The study was accepted. The validity criteria were met.</p> <p>The following endpoints for <i>Chrysoperla carnea</i> were derived: ER₅₀ = 2000 mL formulation/ha NOER > 2000 mL formulation/L.</p> <p>The endpoints were used for risk assessment.</p>
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Reference: KCP 10.3.2.2

Report	Vaughan R., (2021) Cyprodinil/prothioconazole EC (A23282A) – A rate-response extended laboratory study to evaluate the effects of fresh residues on the green lacewing, <i>Chrysoperla carnea</i> (Neuroptera, Chrysopidae), Report Number SYN-21-32. Mambo-Tox, A Division of Cawood Scientific Ltd., 2 Venture Road, University Science Park, Southampton SO16 7NP, UK. (XXXX File No. VV-933833).
Guideline(s):	Yes, Vogt <i>et al.</i> (2000)
Deviations:	Yes - The reproduction phase of the bioassay had to be restarted, due to the lack of adult food in the 500 mL/ha treatment rate. This compromised the egg laying in the first assessment period. This deviation does not affect integrity and validity of this study.
GLP:	Yes
Acceptability:	Yes

Executive Summary

The effects of A23282A on the green lacewing *Chrysoperla carnea* were assessed in an extended laboratory test. The LR₅₀ value was > 2000 mL A23282A/ha. The NOER value with respect to lacewing survival was 2000 mL A23282A/ha. With respect to lacewing reproduction, the ER₅₀ value was > 2000 mL A23282A/ha and the NOER value was 2000 mL A23282A/ha.

Materials

Test Material	cyprodinil/prothioconazole (A23282A)
Lot/Batch #:	LCR001-021-001 (1160912)
Actual content of active ingredients:	Cyprodinil: 22.1% w/w (corresponding to 219 g/L) Prothioconazole: 7.40% w/w (corresponding to 73.5 g/L)
Description:	yellow liquid
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	End of September 2023
Density:	993 kg/m ³
Treatments	
Test rates:	2000, 1000, 500, 250 and 125mL A23282A/ha
Control:	Purified water
Toxic standard:	Dimethoate (an EC formulation containing nominally 400 g a.s./L), applied at a rate of 40 mL product/ha
Spray volume rate:	200 L/ha
Application method:	Calibrated laboratory track-sprayer
Test organisms	
Species:	<i>Chrysoperla carnea</i> (Neuroptera, Chrysopidae)
Age:	2-3 days at start of test
Source:	Lacewing eggs obtained from culture maintained at Test Facility
Feeding:	Larvae: eggs of <i>Sitotroga cerealella</i> . Adults: artificial diet, honey water and purified water.
Test design - Exposure phase	
Arenas:	Excised dwarf French bean leaf sandwiched between 7.5 cm x 7.5 cm glass plate and Perspex sheet, with 5-cm-diameter plastic collar treated with Fluon to confine larva. Ventilated lid placed on top.

Replication:	40 per treatment rate
No. of larvae/arena:	1
Test design – Reproductive phase	
Arenas:	Clear polystyrene box (15 cm x 27 cm x 10 cm) with close fitting lid. Fibrous tissue placed under each lid as oviposition site.
Replication:	1 or 2 boxes per treatment analysed (not considered as replication for statistical purposes).
Duration of test:	43 days
Environmental conditions	test
Temperature:	23.8 - 25.7°C
Humidity:	70 - 84% RH (Relative Humidity)
Photoperiod:	16 h light (2200-3500 lux)

Study Design and Methods

Test facility: Mambo-Tox, A Division of Cawood Scientific Ltd, University Science Park, Southampton SO16 7NP, UK.

Experimental dates: 16th September to 29th October 2021

Dilutions of the test item were prepared shortly before treatments were applied, and the solutions were thoroughly agitated to ensure their homogeneity. Treatments were sprayed onto the target leaves (first true leaves of *Phaseolus vulgaris*) using a laboratory track-sprayer. Once dry, the leaves were used to line the floor of test arenas. A single larva was confined in each arena, with 40 replicates prepared for each treatment. Assessments of treatment effects were made every 1-3 days until the larvae pupated. The number of successfully emerging adult lacewings was then recorded. To assess sub-lethal effects on reproduction, assessments were then carried out for the control and for the test item treatment rates of 2000, 1000, 500, 250 and 125 mL/ha. The sex of the adult lacewings was determined, and they were transferred to oviposition boxes. Eggs were sampled and counted over two 24-h periods. The eggs were then monitored in order to assess the number that successfully hatched.

The percentage pre-imaginal mortality of the insects in the bioassay was calculated. Pre-imaginal mortality was defined as the numbers of insects that did not successfully reach adulthood. The corrected percentage mortality was derived using Abbott's formula. The LR₅₀ value was determined by extrapolation from the data, since corrected mortality did not exceed 50% in any of the test item treatments. Where there was treatment mortality, this was also compared to mortality in the control using the chi² 2x2 table test with Bonferroni correction (one-sided, > control, $\alpha = 0.05$). For the reproduction assessments, effects on lacewing reproduction in the individual test item treatments are normally assessed on the basis of 'triggers', as specified in the guideline of Vogt *et al.* (2000). Namely, if treatment effects are to be deemed harmless, there should be a mean of ≥ 15 eggs produced per female per day ($n = 2$) and the mean egg-hatching rate should be $\geq 70\%$.

Results

Mortality and fecundity are summarised in the tables below.

Table A 43: Effect of A23282A on mortality, hatching and pupation of *Chrysoperla carnea*

Treatment (mL product/ha)	% mortality ^a	% corrected mortality (M-value) ^b	Mean number eggs/female/day ^c	Mean % egg viability ^d	Mean viable eggs/female/ day
Control	15.0	-	45.0	88.3	39.8

2000	30.0	17.6	30.7	89.4	27.4
1000	20.0	5.9	37.8	90.7	34.3
500	12.5	-2.9	27.1	89.6	24.3
250	20.0	5.9	37.4	91.5	34.2
125	12.5	-2.9	-	-	-
Toxic reference	97.5 *	97.1	-	-	-

- a) The results for individual treatments were compared to the control using the chi2 2x2 table test with Bonferroni correction (one-sided, > control, $\alpha = 0.05$). The result for the toxic reference treatment was compared to the control using Fisher's exact binomial test (one-sided, > control, $\alpha = 0.05$). Significant differences are indicated by an asterisk (*).
- b) Derived using Abbott's formula.
- c) Based on two 24-h long assessments made for each oviposition box in each treatment.
- d) Based on all eggs laid on the fibrous tissue sheet lining the lid of each oviposition box.
- Treatment not assessed.

Validity Criteria

The test was considered valid since:

- Pre-imaginal mortality within the control treatment was 15.0% (should not exceed 20%, i.e. 8 lacewings from 40).
- Corrected pre-imaginal mortality within the toxic-reference treatment was 97.1% (should exceed 50%).
- Mean egg production per female per day in the control treatment was 45.0 (must be ≥ 15.0 per female per day).
- Mean egg viability in the control treatment was 88.3% (must be $\geq 70\%$).

Conclusions

The effects of A23282A on the green lacewing *Chrysoperla carnea* were assessed in an extended laboratory test. The LR₅₀ value was > 2000 mL A23282A/ha. The NOER value with respect to lacewing survival was 2000 mL A23282A/ha. With respect to lacewing reproduction, the ER₅₀ value was > 2000 mL A23282A/ha and the NOER value was 2000 mL A23282A/ha.

(Vaughan, 2021)

Comments of zRMS:	<p>The study was accepted. The validity criteria were met.</p> <p>For <i>Aphidius rhopalosiphi</i> at both 14-day and 28-day field-aged residues studies no unacceptable effects on either the survival or the subsequent reproductive capacity of the wasps, (i.e. < 50% corrected mortality and < 50% reduction in reproduction, relative to the control) were observed when the formulation was applied at 2 l formulation/ha.</p>
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Reference:

KCP 10.3.2.2

Report

Stevens J., 2021, Cyprodinil/Prothioconazole EC (A23282A) – A Series of Aged-Residue Extended Laboratory Tests to Determine Effects on the Parasitic Wasp *Aphidius rhopalosiphi* (Hymenoptera, Braconidae), SYN-21-31, XXXX File No. VV-930411

Guideline(s):

Based on Mead-Briggs *et al.* (2000). A laboratory test for evaluating the

effects of plant protection products on the parasitic wasp, *Aphidius rhopalosiphi*.

Deviations: None
GLP: Yes
Acceptability: Yes

Executive Summary

The effects of fresh dry and aged-residues of A23282A on the parasitic wasp *Aphidius rhopalosiphi* were assessed in a series of extended laboratory tests. When applied once to dwarf French bean plants at a rate equivalent to 2 L A23282A/ha, 14 and 28 day field-aged residues resulted in no unacceptable effects on either the survival or the subsequent reproductive capacity of the wasps, (i.e. < 50% corrected mortality and < 50% reduction in reproduction, relative to the control).

Materials

Test Material Cyprodinil/prothioconazole EC (225/075)
Product Code A23282A
Batch ID: LCR001-021-001
Other Batch ID: 1160912
Actual content of active ingredients: Cyprodinil: 22.1% w/w, corresponding to 219 g/L
Prothioconazole: 7.40% w/w, corresponding to 73.5 g/L
Description: Yellow liquid
Stability of test compound: Stable under standard conditions
Reanalysis/Expiry date: End September 2023
Density: 993 kg/m³

Treatments

Test rates: 2 L test item/ha
Control: Purified water
Toxic standard: Dimethoate (an EC formulation containing nominally 400 g a.s./L), applied at a rate of 60 mL product/ha
Spray volume rate: 400 L/ha
Application method: Calibrated laboratory track-sprayer (moving spray boom, fitted with a single 80 flat-fan nozzle, with a spray pressure of 3 bar)

Test organisms

Species: *Aphidius rhopalosiphi* (Hymenoptera, Braconidae)
Age: < 48 h
Source: In-house culture, originally established using wasps from a commercial supplier (Katz Biotech AG, Baruth, Germany)
Feeding: 10% w/v solution of fructose solution on central vein of treated leaves

Test design - Mortality phase

Arenas: Excised treated French bean leaves (*Phaseolus vulgaris* L. (var. The Prince)) lining two 7.5 cm x 7.5 cm glass plates with clear acrylic collar (5.1 cm diameter; 1.5 cm deep) between leaves. 4 holes in acrylic collar (3 for ventilation, 1 for

	wasp entry port). Arenas secured with elastic bands and wet cotton wool wrapped around leaf petioles.
Replication:	4 arenas per treatment
No. of wasps/arena:	10 (> 5 ♀)
Test design - Fecundity phase	
Arenas:	Pots containing approximately 15 barley seedlings (<i>Hordeum vulgare</i> var. Laureate) were previously infested with approximately > 100 adults and nymphs of a mixed cereal aphid culture (<i>Metopolophium dirhodum</i> and <i>Rhopalosiphum padi</i>). The wasps were enclosed within clear acrylic cylinders (9 cm in diameter, 20 cm high), the tops of which were covered with nylon mesh netting.
Replication:	15 pots per treatment being assessed
No. of wasps/arena:	1 ♀
Duration of test:	Three bioassays set up at 0, 14 and 28 days after treatment (lasting between 2 and 13 days each)
Environmental conditions	test
Temperature:	0 DAT: Mortality phase: 20.5-20.8°C. 14 DAT: Mortality phase: 19.9-21.8°C. Fecundity phase: 20.5-21.1°C 28 DAT: Mortality phase: 20.5-20.8°C. Fecundity phase: 19.9-20.9°C
Humidity:	0 DAT: Mortality phase: 76-77%. 14 DAT: Mortality phase: 72-81%. 28 DAT: Mortality phase: 73-77%.
Photoperiod:	0 DAT: Mortality phase: 16 h (1016-1080 lux). 14 DAT: Mortality phase: 16 h (1004-1161 lux). Fecundity phase: 16 h 2616-4539 lux. 28 DAT: Mortality phase: 16 h (994-1019 lux). Fecundity phase: 16 h 2020-4818 lux.

Study Design and Methods

Test facility: Mambo-Tox, A Division of Cawood Scientific Ltd.

Experimental dates: 24th August to 4th October 2021

Dilutions of the test item were prepared in purified water shortly before use and the solutions were thoroughly agitated to ensure their homogeneity. Treatments were applied to dwarf French bean plants. Leaves were excised as needed to construct the test arenas. The wasps were introduced into these arenas and their mortality was assessed after 2, 24 and 48 h. Bioassays were initiated at 0 days after treatment (DAT), and then at 14 and 28 DAT.

To assess any sub-lethal effects, reproduction assessments were then carried out for the control and treatment rate where the latter had resulted in ≤ 50% corrected mortality. Female wasps were confined individually over untreated aphid-infested barley plants for 24 h, before being removed. The plants were left for a further 10 days before the number of aphid mummies that had developed on plants where wasps had been found alive after the 24-h oviposition period was recorded.

The testing programme was designed to terminate when the test-item treatment had resulted in < 50% corrected mortality and < 50% reduction in reproduction, relative to the control, for two consecutive bioassays.

The percentage mortality of the wasps in the bioassay over 48 h was calculated. Mortality was defined as

the numbers of *moribund* and *dead* wasps combined. Where there was treatment mortality at 48 h, this was compared to mortality in the control using Fisher's exact binomial test (one-sided, > control, $\alpha = 0.05$).

Where reproduction assessments were carried out, the data sets from the treatment and control were checked for normality (Shapiro-Wilk test, $p > 0.01$) and homogeneity of variance (Levene's test, $p > 0.01$) before being compared by Student's t-test for homogeneous variances (one-sided, < control, $\alpha = 0.05$).

Results

Mortality and reproduction are summarised in the table below.

Table A 44: Effects of fresh and aged residues of A23282A on mortality and reproduction of *Aphidius rhopalosiphi*, when exposed under 2-D extended laboratory test conditions.

Bioassay Initiated	Treatment	Mean % mortality at 48 h ¹	Mean % corrected mortality at 48 h (M-value) ²	Number females successfully assessed for reproduction	Mean number mummies per surviving female ³ (Standard deviation)	% reduction in reproduction compared to control (R-value) ⁴
0 DAT	Control	0.0	-	~	~	~
	2 L A23282A/ha	82.5 *	82.5	~	~	~
	Toxic reference	95.0 *	95.0	~	~	~
14 DAT	Control	0.0	-	15	33.3 (8.7)	-
	2 L A23282A/ha	2.5	2.5	13	35.5 (9.5)	-6.8
28 DAT	Control	2.5	-	13	37.8 (10.9)	-
	2 L A23282A/ha	5.0	2.6	15	38.1 (10.6)	-0.6

*Significant differences compared to control.

¹ The individual treatments were compared to the control using Fisher's exact binomial test (one-sided, > control, $\alpha = 0.05$)

² Derived using Abbott's formula. A positive value indicates an increase in mortality relative to the control.

³ The results were compared to the control using Student's t-test for homogeneous variances (one-sided, < control, $\alpha = 0.05$). There were no significant differences.

⁴ Percentage change in reproduction, relative to the control. A negative value indicates an increase.

~ Treatment not assessed.

Validity criteria

The test was considered valid since:

- Mortality in the control treatment was 0.0%, 0.0% and 2.5% (0, 14 & 28 DAT) at 48 h (should not exceed 13%).
- Corrected mortality in the toxic reference treatment was 0.0% (0 DAT) at 2 h (should be < 25%) and 95.0% at 48 h (should be 50-100%).
- The mean number of mummies in the control treatment was 33.3 and 37.8 (14 & 28 DAT) (must be > 5.0 per female).
- There should be no more than 2 zero values in the control treatment (there were none).

Conclusion

The effects of freshly dried and field-aged foliar residues of A23282A on the parasitic wasp, *Aphidius rhopalosiphi* were evaluated under extended laboratory test conditions. When applied to dwarf French bean plants at a rate equivalent to 2 L test item/ha, both 14-day and 28-day field-aged residues resulted in no unacceptable effects on either the survival or the subsequent reproductive capacity of the wasps, (i.e. < 50% corrected mortality and < 50% reduction in reproduction, relative to the control).

(Stevens J. 2021)

A 2.3.2.3 KCP 10.3.2.3 Semi-field studies with non-target arthropods

A 2.3.2.4 KCP 10.3.2.4 Field studies with non-target arthropods

A 2.3.2.5 KCP 10.3.2.5 Other routes of exposure for non-target arthropods

A 2.4 KCP 10.4 Effects on non-target soil meso- and macrofauna

A 2.4.1 KCP 10.4.1 Earthworms

A 2.4.1.1 KCP 10.4.1.1 Earthworms - sub-lethal effects

Comments of zRMS:	<p>The study was accepted. The validity criteria were met: adult mortality 4 weeks: less than 10 % (being 0 % after 4 weeks); number of juveniles per replicate: more than 30; (being 166 to 284) coefficient of variation of reproduction: less than 30 % (being 18.6 %)</p> <p>The following endpoints for mortality were derived: NOEC = 556 mg formulation/kg d.w.; LC₅₀ > 1000 mg formulation/kg d.w. and for reproduction the following endpoints were derived: NOEC = 52.9 mg formulation/kg d.w.; EC₅₀ = 188 mg formulation/kg d.w.</p>
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Reference: KCP 10.4.1.1

Report Friedrich S, (2021), Cyprodinil/Prothioconazole EC (A23282A) – Sublethal Effects on the Reproduction of the Earthworm *Eisenia andrei* in Artificial Soil with 5 % Peat, Report Number 21 48 TEC 0042, Biochem agrar, Labor für biologische and chemische, Analytik GmbH, Kupferstraße 6, 04827 Machern OT Gerichshain, Germany. XXXX File No. VV-925821

Guidelines: OECD 222 (2016)

Deviations: No

GLP: Yes

Acceptability: Yes

Executive Summary

The chronic toxicity of A23282A on *Eisenia andrei* was determined in a 56 day test. Earthworms were exposed to concentrations of 16.3, 29.4, 52.9, 95.3, 171, 309, 556, 1000 mg A23282A/kg soil d.w alongside a control. The NOEC for mortality was determined to be 556 mg test item/kg soil d.w. The LC₅₀ value for mortality was determined to be > 1000 mg test item/kg soil dry weight. The NOEC for biomass change and reproduction was determined to be 556 and 52.9 mg test item/kg soil d.w., respectively. The EC₁₀,

EC₂₀ and EC₅₀ values for reproduction were calculated to be 66.3, 94.9 and 188 mg test item/kg soil d.w., respectively.

Materials

Test Material	Cyprodinil/Prothioconazole EC (A23282A)
Lot/Batch #:	LCR001-021-001
Actual content of active ingredients:	cyprodinil 22.1 % w/w corresponding to 219 g/L prothioconazole 7.40 % w/w corresponding to 73.5 g/L
Description:	yellow liquid
Stability of test compound:	stable under recommended handling and storage conditions (< 30 °C)
Recertification date:	end of September 2023
Density:	993 kg/m ³

Treatments

Test rates:	16.3, 29.4, 52.9, 95.3, 171, 309, 556, 1000 mg test item/kg soil dry weight (spacing factor: 1.8)
Control:	untreated (deionised water only)
Toxic standard:	Maypon Flow (carbendazim, SC 500) was tested at concentrations of 2.2 and 4.3 mg a.s./kg soil dry weight (separate GLP study BioChem project No.: 21 48 TEC 0011).

Test organisms

Species:	<i>Eisenia andrei</i> (BOUCHÉ, 1972)
Age and weight range at test start:	adult worms, 5 months old with clitellum 294 – 460 mg/worm
Source:	reared under ambient laboratory conditions in the test facility (originally purchased from W. Neudorff GmbH KG, An der Mühle 3, 31860 Emmerthal, Germany)
Feeding:	air-dried and finely ground horse manure

Test design

Vessels:	plastic vessel (16.5 cm x 12 cm x 6 cm) with a lid pervious to air and light.
Substrate:	artificial soil comprising 5 % sphagnum peat, 20 % kaolin clay (kaolinite content > 30 %), 74.7 % industrial quartz sand (> 50 % of the particles between 50 and 200 µm) and 0.3 % calcium carbonate. 750 g soil wet weight, corresponding to 600 g dry weight of artificial soil was added to each test vessel.
Replication:	8 replicates for the control group and 4 replicates for the treated groups
No. of worms/vessel :	10
Duration of test:	8 weeks (4 weeks adult mortality and biomass change; 4 weeks juvenile development)

Environmental conditions test

Temperature:	19.2 - 20.2 °C
pH of soil:	test start: 6.00 – 6.08 test end: 5.57 – 5.81
Water content of soil:	test start: 56.8 – 57.1% of max. WHC test end: 55.3 – 56.2 % of max. WHC

Photoperiod: 16 hours light: 8 hours dark (approximately 580 lux)

Study Design and Methods

Test facility: BioChem agrar GmbH, Kupferstraße 6, 04827 Machern OT Gerichshain, Germany.

Experimental dates: 3rd August to 28th September 2021

One day before test start (day of application), the artificial soil was prepared, and deionised water was added to the dry components to adjust the water content to approximately 40-60 % of its maximum water holding capacity (WHC). The worms were acclimatised in a separate batch of the untreated artificial substrate for approximately 27 hours before test start. On the day of the test start, the test concentrations were prepared by dispersing an exactly weighed amount of the test item in deionised water to make a stock solution. This stock solution was diluted with deionised water for each test concentration and was thoroughly mixed with the artificial soil using a laboratory mixer, achieving a final nominal water content of 40-60 % of WHC. The control was treated with deionised water only. After a randomising procedure according to the worm fresh weight, selected groups of 10 worms were then randomly assigned to each treatment group. Four replicates were used per test item concentration and eight replicates were used for the control. One day after application, 5 g air-dried and finely ground horse manure was scattered on the soil surface of each test vessel. The feeding interval was weekly during the first four weeks of the test.

After four weeks, the adult worms were removed from the test vessels, and mortality and the body weight of the surviving worms were determined. After all the adult worms had been removed, the soil in each vessel was mixed with 5 g horse manure. Four weeks later, the number of surviving juveniles and any morphological alterations were recorded. Observations of behavioural and pathological symptoms were performed weekly.

The endpoints were mortality, change of biomass (difference in fresh weight of surviving worms between test start and four weeks after treatment) and reproduction (the number of juveniles present). For identifying the NOEC values the Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm (for mortality) and the Williams-t-test (for biomass and reproduction) were used to compare the control with the independent test item groups. The EC_x values (reproduction) were calculated using the Probit analysis using linear max. likelihood regression. Confidence limits (95 %) of the EC_x values were computed by normal approximation.

Results

Mortality and reproduction are summarised in the table below.

Table A 45: Effect of Cyprodinil/Prothioconazole EC (A23282A) on mortality, growth and reproduction of *Eisenia andrei*

Endpoint	Treatment group (mg test item/kg soil dry weight)								
	Control	16.3	29.4	52.9	95.3	171	309	556	1000
Mortality of adult worms after 4 weeks (%)	0.0	0.0	0.0	0.0	0.0	2.5	0.0	0.0	20.0*
Mean biomass increase after 4 weeks (%)	27.3	26.1	27.4	29.1	28.2	27.2	25.6	23.4	6.8*
Mean number of juveniles after 8 weeks	228.5	233.3	226.3	229.8	168.8*	134.5*	54.3*	29.0*	1.0*
Change of reproduction compared to control (%)	-	-2.1	1.0	-0.5	26.1	41.1	76.3	87.3	99.6
	Endpoint (mg test item/kg soil dry weight)								
NOEC (mortality)	556								

NOEC (biomass)	556
NOEC (reproduction)	52.9
LC ₅₀ (mortality) ¹	> 1000
EC ₁₀ (reproduction) ²	66.3 (95 % confidence limits 49.6 – 88.3)
EC ₂₀ (reproduction) ²	94.9 (95 % confidence limits 76.4 – 118)
EC ₅₀ (reproduction) ²	188 (95 % confidence limits 164 – 217)

* statistically significantly different compared to control (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm for mortality, $\alpha = 0.05$, one-sided greater and Williams-t-test, $\alpha = 0.05$, one-sided smaller for biomass and reproduction)

Negative % values for change of reproduction = increase, relative to control

¹ based on estimation of the data

² Probit analysis using linear max. likelihood regression

No significant mortality (i.e., >10%) was observed in the control group and all test concentrations except for the highest one, which showed 20% effect. Statistically significant mortality compared to the control was observed at a concentration of 1000 mg test item/kg soil d.w. (Multiple Sequentially-rejective Fisher Test after Bonferroni- Holm, $\alpha = 0.05$, one-sided greater). No pathological symptoms and no further effects on behaviour of the worms were observed during the test. The feeding activity of adult worms was reduced at a concentration of 1000 mg/kg soil d.w.

The mean weight increase of adult worms ranged between 6.8 % and 29.1 % in the treated groups and was 27.3 % in the control group. At a concentration of 1000 mg test item/kg soil d.w. the biomass increase of 6.8 %, was significantly lower (Williams-t-test, $\alpha = 0.05$, one-sided smaller) compared to the control group.

Statistically significant reduction (Williams-t-test, $\alpha = 0.05$, one-sided smaller) of the number of juveniles compared to the control group was recorded at concentrations of 95.3, 171, 309, 556 and 1000 mg test item/kg soil d.w.

The number of unhatched cocoons found at the end of the test did not show dose-dependency.

In a separate study (experimental start date 22 January 2021) with the reference item Maypon Flow (Carbendazim, SC 500) the number of juveniles was reduced by 56.5 and 99.6 % at concentrations of 5 and 10 mg product/kg soil dry weight (mean number of juveniles = 85.3 and 0.8) after 8 weeks of test duration when compared to control (mean number of juveniles = 196.1). The effects on the reduction of reproduction showed that the test system was sensitive.

Validity criteria

The test is considered valid since:

- Adult mortality was 0 % in the control treatment (must be $\leq 10\%$)
- The mean number of juveniles per control replicate was 166 to 284 (must be ≥ 30)
- The coefficient of variation for reproduction in the control treatment was 18.6 % (must be $\leq 30\%$)

Conclusion

The chronic toxicity of A23282A on *Eisenia andrei* was determined in a 56 day test. Earthworms were exposed to concentrations of 16.3, 29.4, 52.9, 95.3, 171, 309, 556, 1000 mg A23282A/kg soil d.w alongside a control. The NOEC for mortality was determined to be 556 mg test item/kg soil d.w. The LC₅₀ value for mortality was determined to be > 1000 mg test item/kg soil dry weight. The NOEC for biomass change and reproduction was determined to be 556 and 52.9 mg test item/kg soil d.w., respectively. The EC₁₀, EC₂₀ and EC₅₀ values for reproduction were calculated to be 66.3, 94.9 and 188 mg test item/kg soil d.w., respectively.

(Friedrich, 2021)

Comments of zRMS:	The report was not evaluated as it considers the chronic toxicity of cyprodinil. This study is not relevant for risk assessment of formulation A23282A.
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Reference:	KCP 10.4.1.1
Report	Taylor S. & Joyce F. (2015) Cyprodinil (A8779A) – Effects of A8779A on reproduction and growth of earthworms <i>Eisenia fetida</i> (Savigny 1826) in artificial soil. Statistical Re-analysis. Report Number CEA.1410. Cambridge Environmental Assessments, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK. (XXXX File No: A8779A_10236/ VV-28979)
Guideline(s):	OECD Guideline No. 222: Earthworm Reproduction Test (<i>Eisenia fetida</i> / <i>Eisenia andrei</i>) (2004)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Summary

Report number 10291022 (Ehlers, 2001) did not provide estimates of the EC₁₀ and EC₂₀ for the effects of A8779A on body weight change, feeding activity or reproductive toxicity to the earthworm *Eisenia fetida*. Consequently the data generated in this study have been re-analysed in order to provide these values. No EC₁₀ or EC₂₀ values for reproduction or biomass could be reliably calculated due to a lack of significant dose response relationships.

Statistical Analysis

The total amount of food provided and the number of juveniles at the end of the test were analysed. Probit analysis with linear maximum likelihood regression was used to determine the concentration response function. Chi² was used as a goodness of fit measure.

The sum of bodyweight of surviving adult earthworms were analysed as a proportion of the sum of initial body weight. Probit analysis with linear maximum likelihood regression was used to determine the concentration response function. Chi² was used as a goodness of fit measure and the proportion of variance explained by the dose/response function was determined.

All computations were carried out in the Statistical program: ToxRat Professional 2.10.05 (ToxRat Solutions GmbH, 2001-2010).

Results

There was no difference between treatments and controls and therefore no dose response in either the number of juveniles (p(F) = 0.371), feeding activity (p(F) = 0.156) or bodyweight changes (p(F) = 0.219). No EC₁₀ or EC₂₀ values could be calculated.

Conclusion

No EC₁₀ or EC₂₀ values for reproduction or biomass could be reliably calculated due to a lack of significant

concentration response relationships.

(Taylor S. & Joyce F. 2015)

Comments of zRMS:	The report was not evaluated as it considers the chronic toxicity of cyprodinil. This study is not relevant for risk assessment of formulation A23282A. The study considering the toxicity of active substance should be evaluated at EU level during substance renewal.
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Reference: KCP 10.4.1.1

Report Taylor S. & Pickering F. (2015) Cyprodinil (A8779A) – A chronic toxicity and reproduction test exposing *Eisenia fetida* to A8779A in OECD artificial soil. Statistical Re-analysis. Report Number CEA.1411. Cambridge Environmental Assessments, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK. (XXXX File No: A8779A_10237; VV-28980)

Guideline(s): OECD Guideline No. 222: Earthworm Reproduction Test (*Eisenia fetida*/*Eisenia andrei*) (2004)

Deviations: No

GLP: Yes

Acceptability: Yes

Duplication No
(if vertebrate study)

Summary

Report number 1047.094.631 (Neinstedt, 2001) did not provide estimates of the EC₁₀ and EC₂₀ for the effects of A8779A on mortality, biomass or reproduction to the earthworm *Eisenia fetida*. Consequently the data generated in this study have been re-analysed in order to provide these values.

As there were only two test item concentrations a clear dose response could not be generated and therefore no EC₁₀ or EC₂₀ values for mortality, reproduction or biomass could be reliably calculated.

Statistical Analysis

As there were only two test item concentrations no meaningful statistical analyses could be completed as there was no clear dose response.

Results

As there were only two test item concentrations no meaningful statistical analyses could be completed as there was no clear dose response and as such no EC₁₀ or EC₂₀ values could be calculated.

Conclusion

As there were only two test item concentrations a clear dose response could not be generated and therefore no EC₁₀ or EC₂₀ values for mortality, reproduction or biomass could be reliably calculated.

(Taylor S. & Pickering F. 2015)

Comments of zRMS:	The study was evaluated at the zonal level. The following endpoint of 28d-NOEC = 1.13 mg CGA249287/kg soil was accepted and used for risk assessment.
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Reference:	KCP 10.4.1.1
Report	Taylor S., Pickering F. (2015) CGA249287 – Effects of CGA249287 on the reproduction of the earthworm <i>Eisenia fetida</i> . Statistical Re-analysis. Report Number CEA.1427. Cambridge Environmental Assessments, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK. (XXXX File No: CGA249287_10008; VV-28889)
Guideline(s):	OECD Guideline No. 222: Earthworm Reproduction Test (<i>Eisenia fetida</i> / <i>Eisenia andrei</i>) (2004)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Summary

Report number L01-000120 (Pfeifle, 2001) did not provide estimates of the EC₁₀ and EC₂₀ for change in reproductive capacity or body weight of survivors of the earthworm *Eisenia fetida* treated with CGA249287. Consequently, the data generated in this study have been re-analysed in order to provide these values.

No EC₁₀ or EC₂₀ values for reproduction or body weight could be reliably calculated due to a lack of dose response.

Statistical Analysis

Mean bodyweight of surviving adult earthworms was calculated as a proportion of mean initial body weight. Probit analysis with linear maximum likelihood regression was used to determine the concentration response function. Chi² was used as a goodness of fit measure and the proportion of variance was explained by the dose/response function.

Reproduction was calculated as total number of offspring hatched per replicate. Probit analysis with linear maximum likelihood regression was used to determine the concentration response function. Chi² was used as a goodness of fit measure.

All computations were carried out in the Statistical program: ToxRat Professional 2.10.05 (ToxRat Solutions GmbH, 2001-2010).

Results

There was no significant dose response in either reproductive output ($p(F) = 0.370$) or earthworm body weight ($p(F) = 0.187$) and therefore EC₁₀ and EC₂₀ values could not be reliably determined.

Conclusion

No EC₁₀ or EC₂₀ values for reproduction or body weight could be reliably calculated due to a lack of significant dose response.

(Taylor S. & Pickering F. 2015)

Comments of zRMS:	The test was not evaluated as it considers the chronic toxicity of cyprodinil
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	metabolite. This study is not required for risk assessment of formulation A23282A. The study considering the toxicity of active substance metabolite should be evaluated at EU level during substance renewal.
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Reference:	KCP 10.4.1.1
Report	Lührs U. (2014) CGA275535 - Effects on Reproduction and Growth of Earthworms <i>Eisenia fetida</i> in Artificial Soil Report Number 92791022. Institut für Biologische Analytik und Consulting, IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany. (XXXX file No. VV-410370; CGA275535_10002)
Guideline(s):	OECD Guideline No. 222: Earthworm Reproduction Test (<i>Eisenia fetida</i> / <i>Eisenia andrei</i>) (2004)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

In a chronic toxicity test in which earthworms (*Eisenia fetida*) were exposed to CGA275535 the NOEC (based on reproduction) was determined to be 556 mg /kg soil d.w. The EC₁₀, and EC₂₀ values for reproduction were estimated to be 385 and 638 mg /kg soil d.w., respectively.

Materials

Test Material	CGA275535
Lot/Batch #:	KI 6230/3
Purity:	99% w/w
Description:	Beige solid
Stability of test compound:	Stable under standard conditions.
Reanalysis/Expiry date:	End of February 2018
Density:	Not stated
Treatments	
Test rates:	16, 29, 53, 95, 171, 309, 556 and 1000 mg CGA275535/kg soil
Control:	Untreated
Toxic standard:	Luxan Carbendazim 500 FC
Test organisms	
Species:	<i>Eisenia fetida</i> (subspecies <i>Eisenia fetida andrei</i>)
Age and weight range at test start:	Approx 8 months 300 to 600 mg
Source:	Cultured at test facility
Feeding:	5 g finely ground cattle manure each week for first 4 weeks.
Test design	
Vessels:	Plastic trays (18.3 × 13.6 × 6.4 cm) with a lid pervious to air and light.
Substrate:	Artificial soil comprising 10% sphagnum peat, 20 % kaolinite clay, 69.5 % industrial quartz sand (> 50% of the particles between 0.05 mm and 0.2 mm) and 0.5% calcium carbonate.

Replication:	Control:8 Treatment: 4
No. of worms/arena:	10
Duration of test:	8 weeks
Environmental test conditions	
Temperature:	18 to 22 °C
pH of soil:	5.6 to 5.7
Water content of soil:	51.3 to 60.4%
Photoperiod:	16 hour photoperiod (400 to 800 lux)

Study Design and Methods

Test facility: Institut für Biologische Analytik und Consulting, IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany.

Experimental dates: 15th July to 10th September 2014

Approximately 24 hours prior to test start, the artificial soil was prepared and deionised water was added to the dry components to adjust the water content to approximately 40-60% of its maximum water holding capacity (WHC). The worms were acclimatised in a separate batch of the untreated artificial substrate for approximately 24 hours before test start. The test concentrations were prepared by dispersing an exactly weighed amount of the test material in deionised water to make a stock solution. This stock solution was diluted with deionised water for each concentration and was thoroughly mixed with the artificial soil using a laboratory mixer, achieving a final nominal water content of 40-60% of WHC. The acclimatised test animals were washed, gently dried on a paper towel, weighed and randomly placed onto the test substrate (10 animals per test vessel). Worms which had not dug themselves in after 30 minutes were replaced.

After four weeks, the adult worms were removed from the test vessels, and mortality and the body weight of the surviving worms were determined. After all of the adult worms had been removed, the soil in each vessel was mixed with 5 g horse manure. Four weeks later, the number of surviving juveniles and any morphological alterations were recorded. Observations of behavioural and pathological symptoms were observed weekly.

The EC values and their 95% confidence limits were calculated by applying Probit-Analysis (Finney, 1971).

The body weight change and reproduction data were tested for normal distribution and homogeneity of variance ($\alpha = 0.05$) using the Shapiro-Wilk's test and the Levene's test, respectively. As the data for body weight changes and reproduction were normally distributed and homogeneous in both cases, Williams t-test was used to compare treatment and control values (multiple comparison, two-sided for weight and one-sided smaller for reproduction, $\alpha = 0.05$).

The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05.

Results and Discussion

Mortality and reproduction are summarised in the table below.

Table A 46: Effect of CGA275535 on mortality, growth and reproduction of *Eisenia fetida*

Endpoints	Treatment groups (mg/kg soil dry weight)								
	Control	16	29	53	95	171	309	556	1000
Mean adult mortality at 28 days (%)	0	0	0	0	0	0	0	0	0
Mean % biomass change of adults from 0-28 days	95	112	100	123	133	122	100	111	96
Mean number of juveniles after 8 weeks	312	309	355	347	333	292	273	291	199*
% difference in reproduction relative to the control	-	1.0	-13.9	-11.4	-6.8	6.3	12.3	6.6	36.0
NOEC (biomass)	1000								
NOEC (reproduction)	556								
EC ₁₀ (reproduction)	385								
EC ₂₀ (reproduction)	638								

* significantly reduced compared to the control (Williams t-test, $\alpha = 0.05$, two-sided for weight changes and one-sided smaller for reproduction)

Validity criteria

The validity criteria were met since:

- Adult mortality was 0% in the control (< 10% required)
- The mean number of juveniles for the control was 312 (> 30 required)
- The coefficient of variation for reproduction was 11.9 % (< 30% required)

Conclusions

In a chronic toxicity test in which earthworms (*Eisenia fetida*) were exposed to CGA275535 the NOEC (based on reproduction) was determined to be 556 mg /kg soil d.w. The EC₁₀, and EC₂₀ values for reproduction were estimated to be 385 and 638 mg /kg soil d.w., respectively.

(Lührs U, 2014)

Comments of zRMS:	The test was not evaluated as it considers the chronic toxicity of cyprodinil metabolite. This study is not required for risk assessment of formulation A23282A. The study considering the toxicity of active substance metabolite should be evaluated at EU level during substance renewal.
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Reference: KCP 10.4.1.1

Report Lührs U. (2015) CGA321915 – Effects on reproduction and growth of earthworms *Eisenia fetida* in Artificial Soil. Report Number 96341022.

	IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany. (XXXX file No. VV-411784; CGA321915_10012)
Guideline(s):	OECD Guideline No. 222: Earthworm Reproduction Test (<i>Eisenia fetida</i> / <i>Eisenia andrei</i>) (2004)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

In a chronic toxicity test in which earthworms (*Eisenia fetida*) were exposed to CGA321915 the NOEC (based on reproduction) was determined to be 1000 mg CGA321915/kg soil d.w. The EC₁₀, EC₂₀ and EC₅₀ could not be determined, but are assumed to be >1000 mg CGA321915/kg.

Materials

Test Material	CGA321915
Lot/Batch #:	MES 356/1
Purity:	98 % w/w
Description:	White solid
Stability of test compound:	Stable under standard conditions.
Reanalysis/Expiry date:	End of September 2016
Treatments	
Test rates:	16.3, 29.4, 52.9, 95.3, 171.5, 308.6, 555.6, 1000 mg CGA321915/kg soil d.w.
Control:	Deionised water
Toxic standard:	Carbendazim was tested at concentrations of 5 and 10 mg product/kg soil dry weight (separate study - No.: 91441022, dated July – September 2014).
Test organisms	
Species:	<i>Eisenia fetida</i> (subspecies <i>Eisenia fetida andrei</i>)
Age and weight range at test start:	Approximately 7 to 8 months with well developed clitellum; 301 – 600 mg
Source:	Bred under standard conditions at IBACON
Feeding:	Finely ground cattle manure
Test design	
Vessels:	Plastic boxes (18.3 × 13.6 × 6 cm) with a lid pervious to air and light.
Substrate:	Artificial soil comprising 10% sphagnum peat, 20 % kaolinite clay, 69.5 % industrial quartz sand (> 50% of the particles between 0.05 mm and 0.2 mm) and 0.5% calcium carbonate. 659.9 g wet weight soil, corresponding to about 500 g dry weight, of artificial soil was added to each test vessel.
Replication:	Control: 8 Treatment: 4
No. of worms/arena :	10
Duration of test:	8 weeks
Environmental conditions	
Temperature:	18 – 22 °C
pH of soil:	Test start: 5.8 – 6.0 Test end: 6.0 – 6.1

Water content of soil: Test start: 53.8 – 56.7% WHC
Test end: 54.3 – 59.7% WHC
Photoperiod: 16 h light: 8 h dark

Study Design and Methods

Test facility: IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany

Experimental dates: 22nd January to 20th March 2015

The test concentrations were prepared by dispersing an exactly weighed amount of the test material in deionised water to make a stock solution. This stock solution was diluted with deionised water for each concentration and was thoroughly mixed with the artificial soil using a laboratory mixer, achieving a final nominal water content of 40-60 % of WHC. The acclimatised test animals were washed, gently dried on a paper towel, weighed and randomly placed onto the test substrate (10 animals per test vessel). 5 g finely ground cattle manure was added each week for the first four weeks of the experiment.

After four weeks, the adult worms were removed from the test vessels, and mortality and the body weight of the surviving worms were determined. After all of the adult worms had been removed the soil in each vessel was mixed with 5 g horse manure. Four weeks later, the number of surviving juveniles and any morphological alterations were recorded. Observations of behavioural and pathological symptoms were observed at day 28.

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 2.10.05 (ToxRat Solutions GmbH). The LCx/ECx values were calculated by Probit analysis. For identifying the NOEC values Fisher's Exact Binomial Test with Bonferroni Correction and the Williams-t-test were used to compare the control with the independent test item groups.

Results and Discussion

Mortality and reproduction are summarised in the table below.

Table A 47: Effect of CGA321915 on mortality, growth and reproduction of *Eisenia fetida*

Endpoints	Treatment groups (mg CGA321915/kg soil dry weight)								
	Control	16.3	29.4	52.9	95.3	171.5	308.6	555.6	1000
Mean adult mortality at 28 days (%)	0.0	0.0	0.0	2.5	0.0	0.0	0.0	0.0	0.0
Mean % biomass change of adults from 0-28 days	43.8	46.5	48.4	42.8	43.0	46.4	42.2	42.9	36.9
Mean number of juveniles after 8 weeks	296	280	320	300	333	266	313	340	342
% difference in reproduction relative to the control	-	5.6	-8.1	-1.3	-12.4	10.2	-5.5	-14.6	-15.4
NOEC (reproduction)	1000								

Negative values indicate an increase compared to the control

Validity criteria

The test is considered valid as:

- Adult mortality was 0% in the control (< 10% required)
- The number of juveniles per control replicate was 244 to 356 (> 30 required)
- The coefficient of variation for reproduction was 15.9% (< 30% required)

Conclusions

In a chronic toxicity test in which earthworms (*Eisenia fetida*) were exposed to CGA321915 the NOEC (based on reproduction) was determined to be 1000 mg CGA321915/kg soil d.w. The EC₁₀, EC₂₀ and EC₅₀ could not be determined, but are assumed to be >1000 mg CGA321915/kg.

(Lührs U, 2015)

A 2.4.1.2 KCP 10.4.1.2 Earthworms - field studies

A 2.4.2 KCP 10.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

A 2.4.2.1 KCP 10.4.2.1 Species level testing

Comments of zRMS:	<p>The submitted study was accepted. The validity criteria were met: mean adult mortality: ≤ 20 %; observed 1.3 %; mean number of juveniles per test vessel: ≥ 100; observed: average 1055 per vessel; coefficient of variation for the mean number of juveniles: < 30 %; observed 9.0%. The following endpoints were derived: mortality: NOEC = 65.3 mg test item/kg soil d.w. LC₅₀ = 487 mg test item/kg soil d.w. reproduction: NOEC = 95.3 mg test item/kg soil d.w. EC₅₀ = 233 mg test item/kg soil d.w.</p> <p>The study results can be used in risk assessment.</p>
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Reference: KCP 10.4.2.1

Report Friedrich S, (2021), Cyprodinil/Prothioconazole EC (A23282A) - Effects on the reproduction of the collembolan *Folsomia candida*, Report Number 21 48 TCC 0029. BioChem agrar, Labor für biologische und chemische, Analytik GmbH, Kupferstraße 6, 04827 Machern OT Gerichshain, Germany (XXXX File No. VV-925508)

Guideline(s): OECD 232. Collembolan Reproduction Test in Soil (2016)

Deviations: No

GLP: Yes

Acceptability: Yes

Executive Summary

In a Collembolan reproduction study in soil the effects of A23282A on the springtail *Folsomia candida* were evaluated under laboratory test conditions using an artificial soil substrate containing 5% w/w organic matter. The NOEC for mortality of the parental collembolans was determined to be 95.3 mg A23282A/kg soil dry weight. The LC₅₀ value for mortality was calculated to be 487 mg A23282A/kg soil dry weight. The NOEC for reproduction was determined to be 95.3 mg A23282A/kg soil dry weight. The EC₁₀, EC₂₀ and EC₅₀ values for reproduction were calculated to be 92.6, 127 and 233 mg A23282A/kg soil dry weight, respectively.

Materials

Test Material	Cyprodinil/Prothioconazole EC (A23282A)
Lot/Batch #:	LCR001-021-001
Actual content of active ingredients:	Cyprodinil: 22.1 % w/w corresponding to 219 g/L Prothioconazole: 7.40 % w/w corresponding to 73.5 g/L
Description:	yellow liquid
Stability of test compound:	stable under test conditions
Recertification date:	End of September 2023
Treatments	
Test concentrations:	16.3, 29.4, 52.9, 95.3, 171, 309, 556, 1000 mg test item/kg soil dry weight (spacing factor: 1.8)
Control:	untreated substrate (deionised water only)
Toxic standard:	boric acid (separate GLP study BioChem project No.: 21 48 TCC 0035)
Application method	soil incorporation
Test organisms	
Species:	<i>Folsomia candida</i>
Age:	juvenile collembolans, 9-12 days old
Source:	originally purchased from “Biologische Bundesanstalt (BBA)”, Berlin-Dahlem. reared under ambient laboratory conditions in the test facility
Feeding:	2 mg granulated dry yeast at the start of the test and after 14 days
Test design	
Arenas:	glass container (approximately 150 mL) covered with a lid
Substrate:	artificial soil comprising 5 % sphagnum peat, 20 % kaolin clay (kaolinite content > 30 %), 74.7 % industrial quartz sand (> 50 % of the particles between 50 and 200 µm) and 0.3 % calcium carbonate. 37.5 g wet weight soil, corresponding to 30 g dry weight of artificial soil was added to each test vessel.
Replication:	8 replicates for the control group and 4 replicates for the treated groups
No. /arena :	10
Duration of test:	28 days
Environmental conditions	test
Temperature:	19.6 – 21.6 °C
pH of soil:	test start: 5.98 – 6.08 test end: 5.75 – 5.79

Water content of soil:	test start:	57.6 – 57.9 % of max. WHC
	test end:	55.8 – 56.9 % of max. WHC
Photoperiod:	16 hours light : 8 hours dark photoperiod, approximately 590 lux	

Study Design and Methods

Test facility: BioChem agrar, Labor für biologische und chemische, Analytik GmbH, Kupferstraße 6, 04827 Machern OT Gerichshain, Germany

Experimental dates: 23rd July to 20th August 2021

The test concentrations were prepared by dispersing an exactly weighed amount of the test item in deionised water to make a stock solution. This stock solution was diluted with deionised water for each test concentration and was thoroughly mixed with the artificial soil using a laboratory mixer, achieving a final nominal water content of 40-60 % of WHC. The control was treated with deionised water only.

Ten juvenile collembolans were transferred after the application to the substrate surface of each test vessel using an aspirator. Four replicates were used per test item concentration and eight replicates were used for the control. The test organisms were fed twice during the test (at the start of the test and after 14 days) with approximately 2 mg of granulated dry yeast per test vessel. Four weeks after introducing the test organisms, the surviving parental collembolans and offspring (juveniles) were counted.

All values presented throughout this report were calculated using the original raw data and were not based on rounded values.

The percentage mortality of the springtails was calculated for each treatment, both before and after correction for any control treatment losses using Abbott's formula. Step-down Cochran-Armitage test and Williams-t-test were used to compare the control with the independent test item groups. The LC_x-values for adult mortality were calculated by Weibull analysis using linear maximum likelihood regression. Confidence limits (95 %) of the LC_x values were computed by normal approximation. The EC_x values (number of juveniles) were calculated by Probit analysis using linear maximum likelihood regression. Confidence limits (95 %) of the EC_x values were computed by normal approximation.

Results

Mortality and fecundity are summarised in the table below.

Table A 48: Effects of residues of A23282A on mortality and reproduction of *Folsomia candida*

Endpoint	Treatment group (mg A23282A /kg soil dry weight)								
	Control	16.3	29.4	52.9	95.3	171	309	556	1000
% Mortality of parental collembolans after 4 weeks	1.3	2.5	2.5	0.0	2.5	15.0*	22.5*	47.5*	100*
% Corrected mortality (Abbott)	-	1.3	1.3	-1.3	1.3	13.9	21.5	46.8	100
Mean number of juveniles after 4 weeks	1055	1067	1088	1100	1079	607*	386*	138*	81*
SD	94.7	87	100.9	112.4	104.4	108.9	69.3	18.3	15.9
CV %	9.0	8.2	9.3	10.2	9.7	17.9	18.0	13.3	19.6
% Reduction of reproduction compared to control	-	-1.2	-3.2	-4.3	-2.3	42.5	63.5	86.9	92.3

	Endpoint (mg test item/kg soil dry weight)
NOEC (mortality)	95.3
NOEC (reproduction)	95.3
LC ₅₀ (mortality) ¹ (95 % C.I.)	487 (367 – 647)
EC ₁₀ (reproduction) ² (95 % C.I.)	92.6 (62.7 – 136)
EC ₂₀ (reproduction) ² (95 % C.I.)	127 (94.8 – 170)
EC ₅₀ (reproduction) ² (95 % C.I.)	233 (193 – 281)

* statistically significant different compared to the control (Step-down Cochran-Armitage test for mortality $\alpha = 0.05$, one-sided greater; Williams-t-test for reproduction, $\alpha = 0.05$, one-sided smaller)

¹ based on Weibull analysis,

² based on Probit analysis

Negative % values for reduction of reproduction = increase, relative to control

In a separate study (experimental start date 06th September 2021) with the reference item boric acid (analysed purity: 100.1 %) the EC₅₀ was determined to be 111 mg/kg soil dry weight. The LC₅₀ was determined to be 145 mg/kg soil dry weight. The NOEC for mortality and for reproduction was determined to be 44 mg/kg soil dry weight, respectively. The results of the reference test demonstrate the sensitivity of the test system given that according to OECD guideline 232 the EC₅₀ for reproduction of *Folsomia candida* with boric acid after 4 weeks should be c.a.100 mg/kg soil dry weight.

Validity criteria

The validity criteria were met since:

- Control treatment mortality was 1.3 % (must be $\leq 20\%$)
- The mean number of juveniles recorded in the control treatment was 1055 (must be ≥ 100 per replicate)
- The coefficient of variation of reproduction in the control was 9.0 % (must not be $>30\%$)

Conclusion

In a Collembola reproduction study with A23282A the NOEC for mortality of the parental collembolans was determined to be 95.3 mg A23282A/kg soil dry weight. The LC₅₀ value for mortality was calculated to be 487 mg A23282A/kg soil dry weight. The NOEC for reproduction was determined to be 95.3 mg A23282A/kg soil dry weight. The EC₁₀, EC₂₀ and EC₅₀ values for reproduction were calculated to be 92.6, 127 and 233 mg A23282A/kg soil dry weight, respectively.

(Friedrich S, 2021)

Comments of zRMS:	The test was not evaluated as it considers the chronic toxicity of cyprodinil. The study considering the toxicity of active substance or its metabolite should be evaluated at EU level during substance renewal.
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Reference: KCP 10.4.2.1

Report Lühns U. (2014) Cyprodinil EC (A14325E) - Effects on Reproduction of the Collembola *Folsomia candida* in Artificial Soil with 5% Peat. Report Number 92781016. Institut für Biologische Analytik und Consulting IBACON GmbH Arheilger Weg 17, 64380 Rossdorf, Germany. (XXXX file No. VV-

	410669; A14325E_10061)
Guideline(s):	OECD Guidelines No. 232. Collembolan Reproduction test in soil (2009) ISO 11267: Soil quality – inhibition of reproduction of Collembola (<i>Folsomia candida</i>) by soil pollutants. (1999)
Deviation(s):	No
GLP:	Yes
Acceptability:	Yes
Duplication: (if vertebrate study)	No

Executive Summary

The toxicity of A14325E to the reproduction and the parental mortality of collembola species *Folsomia candida* were determined. The NOEC was determined to be 29.4 mg/kg soil dry weight (8.67 mg cyprodinil/kg). The EC₁₀ and EC₂₀ values were estimated to be 53.2 mg and 67.7 mg test item/kg soil, respectively (15.7 and 20 mg cyprodinil/kg).

Materials

Test Material	A14325E Cyprodinil EC
Lot/Batch #:	SMO3A100
Actual content of active ingredients:	Cyprodinil: 29.1% w/w, corresponding to 295 g/L
Description:	Liquid
Stability of test compound:	Stable under standard conditions.
Reanalysis/Expiry date:	End of January 2017
Density:	1014 kg/m ³

Treatments

Test rates:	2.80, 5.04, 9.08, 16.3, 29.4, 52.9, 95.3 and 171.5 mg test item/kg soil
Control:	Untreated
Toxic standard:	Boric acid
Application method:	Mixed into artificial soil

Test organisms

Species:	<i>Folsomia candida</i>
Age:	10 – 12 day old juveniles
Source:	Culture maintained at Test Facility
Feeding:	2 mg granulated dry yeast at the start of the test and after 14 days

Test design

Arenas:	Glass containers (volume: 100 mL; diameter: 5 cm), closed tightly to avoid water evaporation, filled with 30 g artificial soil fresh weight.
Substrate:	Artificial soil comprising 5% sphagnum peat, 20% kaolinite clay, 74.7% industrial quartz sand (> 50% of the particles between 0.05 mm and 0.2 mm) and 0.3% calcium carbonate.
Replication:	Control: 8 Treatment: 4
No./arena :	10

Duration of test:	28 days
Environmental conditions	test
Temperature:	18 to 22°C
pH of soil:	Test start: 6.2 – 6.3 Test end: 6.1
Water content of soil:	Test start: 50.8% to 52.9% of WHC Test end: 46.4% to 49.7% of WHC
Photoperiod:	16 hour photoperiod (400 – 800 lux)

Study Design and Methods

Test facility: Institut für Biologische Analytik und Consulting IBACON GmbH Arheilger Weg 17, 64380 Rossdorf, Germany

Experimental dates: 1st to 30th September 2014

The test concentrations were prepared by dispersing an exactly weighed amount of the test item in deionised water to make a stock solution. This stock solution was diluted with deionised water for each test concentration and was thoroughly mixed with the artificial soil using a mixing machine, achieving a final nominal water content of 40-60% of WHC. The control was treated with deionised water only.

Ten juvenile collembolans were transferred after the application to the substrate surface of each test vessel using an exhaustor. Four replicates (+ two replicates not loaded with collembolans for measurement purposes) were used per test concentration and eight were used in the control. The test organisms were fed twice during the experiment (at the start of the test and after 14 days) with approximately 2 mg of granulated dry yeast per test vessel. Four weeks after introducing the test organisms, the surviving parental collembolans and offspring (juveniles) were counted.

The results represent rounded values calculated from the exact raw data.

Mortality data were statistically analyzed using Fisher's Exact Test (multiple comparison, $\alpha = 0.05$, one-sided greater).

Reproduction data were tested for normal distribution and homogeneity of variance using Shapiro-Wilk's test and Levene's test ($\alpha = 0.05$). Since variances were not homogeneous the further statistical evaluation was performed using Bonferroni-Welch t-test (multiple comparison, $\alpha = 0.05$, one-sided smaller).

The determination of the NOEC and LOEC values was based on the results of the statistical evaluation. The EC values for reproduction were calculated by Probit Analysis (Finney 1971). The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

Results and Discussion

Mortality and fecundity are summarised in the table below.

Table A 49: Effects of residues of A14325E on mortality and reproduction of *Folsomia candida*

Endpoint	Treatment group (mg A14325E /kg soil d.w.)								
	Control	2.80	5.04	9.08	16.3	29.4	52.9	95.3	171.5
% Mortality of parental collembolans after 4 weeks	6	5	15	10	8	5	15	8	55*
Mean number of juveniles after 4 weeks	491	566	483	456	466	472	392*	337	61*
SD	73	76	31	58	43	32	32	123	30
% reduction compared to control	-	-15.3	1.5	7.1	5.0	3.9	20.1	31.3	87.6
NOEC (reproduction)	29.4 (8.67 mg cyprodinil/kg)								
EC ₁₀	53.2 (15.7 mg cyprodinil/kg)								
EC ₂₀	67.7 (20 mg/kg cyprodinil)								

* significantly reduced compared to the control (Bonferroni-Welch t-test, $\alpha = 0.05$, one-sided smaller)
negative values indicate an increased reproduction relative to the control

Validity criteria

The validity criteria are as follows:

- Control treatment mortality was 6 % (must be < 20%)
- The mean number of juvenile recorded in the control treatment was 491 (must be > 100 per replicate)
- The coefficient of variation of reproduction in the control was 14.9% (must not be > 30%)

Conclusions

In a 28-day collembola (*Folsomia candida*) study with A14325E, the NOEC for reproduction was determined to be 29.4 mg A14325E/kg soil dry weight (8.67 mg cyprodinil/kg). The EC₁₀ and EC₂₀ values were estimated to be 53.2 mg and 67.7 mg test item/kg soil, respectively (15.7 and 20 mg cyprodinil/kg).

(Lührs U, 2014)

Comments of zRMS:	The test was not evaluated as it considers the chronic toxicity of cyprodinil. This study is not required for risk assessment of formulation A23282A. The study considering the toxicity of active substance or its metabolite should be evaluated at EU level during substance renewal.
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Reference: KCP 10.4.2.1

Report Lührs U. (2014) Cyprodinil WG (A8637C) – Effects on the reproduction of the Collembola *Folsomia candida* in artificial soil with 5% peat. Report Number 92771016. Institut für Biologische Analytik und Consulting, IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany. (XXXX File No. VV-410108; A8637C_10314)

Guideline(s): OECD Guidelines No. 232. Collembolan Reproduction test in soil (2009)

ISO 11267: Soil quality – inhibition of reproduction of Collembola (*Folsomia candida*) by soil pollutants. (1999)

Deviation(s): No

GLP: Yes
Acceptability: Yes
Duplication: No
(if vertebrate study)

Executive Summary

The toxicity of A8637C to the reproduction and the parental mortality of collembola species *Folsomia candida* were determined. The NOEC for mortality and reproduction were determined to be 105 mg A8637C/kg soil d.w. (52.5 mg cyprodinil/kg), the highest concentration tested.

Materials

Test Material	Cyprodinil WG A8637C
Lot/Batch #:	SMO2C304
Actual content of active ingredients:	Cyprodinil: 50.2% w/w
Description:	Brownish granules
Stability of test compound:	Stable under standard conditions.
Reanalysis/Expiry date:	End of December 2016
Treatments	
Test concentrations:	1.72, 3.09, 5.56, 10.0, 18.0, 32.4, 58.3, 105 mg A8637C/kg d.w.
Control:	Untreated (moistened with deionised water)
Toxic standard:	Boric acid (content 100.3%) at 30.5, 48.8, 78.1, 125 and 200 mg/kg (separate study - No.: 61405016).
Application method:	A stock solution was prepared and combined with artificial soil
Test organisms	
Species:	<i>Folsomia candida</i>
Age:	10 – 12 days
Source:	Culture maintained at Test Facility
Feeding:	2 mg granulated dry yeast at the start of the test and after 14 days
Test design	
Arenas:	100 mL glass containers; diameter 5 cm; tightly closed lids
Substrate:	Artificial soil comprising 5% sphagnum peat, 20% kaolinite clay, 74.75% industrial quartz sand (> 50% of the particles between 0.05 mm and 0.2 mm) and 0.25% calcium carbonate.
Replication:	8 in the control; 4 per test item treatment; 1 additional per treatment for pH and water content checks
No./arena :	10
Duration of test:	28 days
Environmental test conditions	
Temperature:	18 – 22 °C
pH of soil:	At test start: 6.2 – 6.3 At test end: 6.0 – 6.1
Water content of soil:	At test start: 20.2 – 21.7% (49.2 – 52.9 % of MWHC) At test end: 18.9 – 20.2% (46.1 – 49.3 % of MWHC)
Photoperiod:	16:8 LD; 400 – 800 Lux

Study Design and Methods

Test facility: Institut für Biologische Analytik und Consulting, IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany

Experimental dates: 20th August to 18th September 2014

The test concentrations were prepared by dispersing an exactly weighed amount of the test item in deionised water to make a stock solution. This stock solution was diluted with deionised water for each test concentration and was thoroughly mixed with the artificial soil using a mixing machine, achieving a final nominal water content of 40-60% of MWHC. The control was treated with deionised water only.

Ten juvenile collembolans were transferred after the application to the substrate surface of each test vessel using an aspirator. There were four replicates (+ one replicate not loaded with collembolans for measurement purposes) used per test concentration and eight replicates in the control. The test organisms were fed twice during the experiment (at the start of the test and after 14 days) with approximately 2 mg of granulated dry yeast per test vessel. Four weeks after introducing the test organisms, the surviving parental collembolans and offspring (juveniles) were counted.

The 28-day mortality data for the individual test-item treatments were compared to those in the control using Fisher's Exact Test ($\alpha = 0.05$, one-sided greater). Reproductive data were tested for normal distribution and homogeneity of variance using Shapiro-Wilk's test and Levene's test ($\alpha = 0.05$). Further statistical evaluation was conducted using Williams t-test (multiple comparison, $\alpha = 0.05$, one-sided smaller). Determination of the NOEC was based on results of the statistical evaluations.

ECx values were not determined as no reduction in reproduction of more than 50% was observed.

Results and Discussion

Mortality and fecundity are summarised in the table below.

Table A 50: Effects of residues of A8637C on mortality and reproduction of *Folsomia candida*

Endpoint	Treatment group (mg A8637C/kg soil d.w.)								
	Control	1.72	3.09	5.56	10.0	18.0	32.4	58.3	105.0
% Mortality of parental collembolans after 4 weeks	13	20	23	15	28	20	23	13	18
Mean number of juveniles after 4 weeks	428	426	488	357	430	395	399	400	332
SD	77	119	147	112	38	109	85	140	58
% reduction compared to control	-	0.6	-14.1	16.6	-0.3	7.7	6.9	6.6	22.4
NOEC (reproduction)	105 mg/kg soil d.w. (52.5 mg cyprodinil/kg)								

Validity criteria

The validity criteria were met as follows:

- Control treatment mortality was 13 % (must be < 20%)
- The mean number of juveniles recorded in the control treatment was 300 to 552 (must be > 100 per replicate)
- The coefficient of variation of reproduction in the control was 18 % (must not be > 30%)

Conclusions

The toxicity of A8637C to the reproduction and the parental mortality of collembola species *Folsomia candida* were determined. The NOEC for reproduction was determined to be 105 mg A8637C/kg soil d.w.

(52.5 mg cyprodinil/kg), the highest concentration tested.

(Lührs U, 2014)

Comments of zRMS:	The test was not evaluated as it considers the chronic toxicity of cyprodinil. This study is not required for risk assessment of formulation A23282A. The study considering the toxicity of active substance or its metabolite should be evaluated at EU level during substance renewal.
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Reference:	KCP 10.4.2.1
Report	Taylor S., & Pickering, F. (2016) Cyprodinil WG (A8637C) – Effects on Reproduction of the Collembola (<i>Folsomia candida</i>) in Artificial Soil with 5% Peat - Statistical Re-analysis. Report Number CEA.1772. Cambridge Environmental Assessments, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK. (XXXX File No: VV-134367; A8637C_10368)
Guideline(s):	None
Deviation(s):	No
GLP:	No
Acceptability:	Yes
Duplication: (if vertebrate study)	No

Summary

The report from IBACON GmbH study number 92771016 (Lührs, 2014) for the reproductive toxicity test of A8637C on the collembola (*Folsomia candida*) did not provide estimates of the EC₁₀ or EC₂₀ for the response variables (adult mortality and reproduction) evaluated as part of the original study. Consequently, the data generated in this study have been re-analysed in an attempt to provide these values.

No EC₁₀ or EC₂₀ values for mortality or reproduction could be reliably calculated due to a lack of significant dose response.

Statistical Analysis

No mortality was observed after 28 days of exposure for any test concentration. In addition, there were no statistically significant differences in the feeding activity or biomass change between each of the treatment concentrations and the control in the original report. As a result, these parameters were not statistically analysed and no EC_x values could reliably be determined.

Probit analysis with linear maximum likelihood regression was used in an attempt to determine the concentration response function for reproduction. Chi² was used as a goodness of fit measure. The proportion of variance explained by the dose/ response function was determined and is presented as the coefficient of determination, r² (0 ≤ r² ≤ 1).

All computations were carried out in the Statistical program: ToxRat Professional 2.10.05 (ToxRat Solutions GmbH, 2001-2010).

Results

No statistically significant (p(F)= < 0.05) dose response was observed for either mortality or reproduction and as a result, no EC₁₀ or EC₂₀ values were reliably calculated.

Conclusion

No EC₁₀ or EC₂₀ values for mortality or reproduction could be reliably calculated due to a lack of significant dose response.

(Taylor S. & Pickering F. 2016)

Comments of zRMS:	The test was not evaluated as it considers the chronic toxicity of cyprodinil metabolite. This study is not required for risk assessment of formulation A23282A. The study considering the toxicity of active substance or its metabolite should be evaluated at EU level during substance renewal.
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Reference:	KCP 10.4.2.1
Report	Vinall S. (2012) CGA249287 – A laboratory test to determine the effects of fresh residues on the springtail <i>Folsomia candida</i> (Collembola, Isotomidae). Report Number SYN-12-40. Mambo-Tox Ltd., 2 Venture Road, Chilworth Science Park, Southampton SO16 7NP, UK. (XXXX File No. VV-402680; CGA249287_10003)
Guideline(s):	OECD Guidelines No. 232. Collembolan Reproduction test in soil (2009)
Deviation(s):	No
GLP:	Yes
Acceptability:	Yes
Duplication: (if vertebrate study)	No

Executive Summary

The effects of CGA249287 on the reproduction and the parental mortality of collembola species *Folsomia candida* were determined. Based on the mean number of F1 progeny the NOEC, EC₁₀ and EC₂₀ values were determined to be 31, 7.9 and 22.7 mg /kg soil dry weight, respectively.

Materials

Test Material	CGA249287
Lot/Batch #:	RL-2929
Purity:	100 ± 2% w/w
Description:	White solid
Stability of test compound:	Stable under standard conditions.
Reanalysis/Expiry date:	June 2018

Treatments

Test concentrations:	2.8, 5, 9, 17, 31, 56, 100, 180 and 324 mg CGA249287/kg soil dry weight
Control:	Untreated
Toxic standard:	Betosip 114 (Sipcam (114 g/L phenmedipham)
Application method:	Mixed with artificial soil

Test organisms

Species:	<i>Folsomia candida</i>
Age:	11 days old

Source:	Culture maintained at Test Facility
Feeding:	2 mg granulated dry yeast at the start of the test and after 14 days

Test design

Arenas:	Glass containers (volume: 120 mL; diameter: 4.5 cm), closed tightly to avoid water evaporation, filled with 30 g artificial soil fresh weight.
Substrate:	Artificial soil comprising 5% sphagnum peat, 20% kaolinite clay, 74. % industrial quartz sand (> 50% of the particles between 0.05 mm and 0.2 mm) and 0.2% calcium carbonate.
Replication:	Control: 8 Treatment: 4
No./arena:	10
Duration of test:	28 days

Environmental test conditions

Temperature:	19.2°C to 22.5°C
pH of soil:	Test start: 6.17 to 6.49 Test end: 5.66 to 6.23
Photoperiod:	16 hour photoperiod (440 to 510 lux)

Study Design and Methods

Experimental dates: 17th July to 26th September 2012

The test concentrations were prepared by dispersing an exactly weighed amount of the test item in deionised water to make a stock solution. This stock solution was diluted with deionised water for each test concentration and was thoroughly mixed with the artificial soil using a mixing machine, achieving a final nominal water content of 40-60 % of WHC. The control was treated with deionised water only.

Ten juvenile collembolans were transferred after the application to the substrate surface of each test vessel using an exhaustor. Four replicates (+ two replicates not loaded with collembolans for measurement purposes) were used per test concentration and eight were used for the control. The test organisms were fed twice during the experiment (at the start of the test and after 14 days) with approximately 2 mg of granulated dry yeast per test vessel. Four weeks after introducing the test organisms, the surviving parental collembolans and offspring (juveniles) were counted.

All values presented throughout this report were calculated using the original raw data and were not based on rounded values.

Mortality data were statistically analyzed using Fisher's Exact Test (multiple comparison, $\alpha = 0.05$, one-sided greater).

Probit regression analysis was performed on the data for the numbers of progeny in the test item, in order to derive the 'effect concentrations' (EC_x) for key points on the response curve (e.g. EC_{50} , EC_{20} and EC_{10}).

Results and Discussion

Mortality and fecundity are summarised in the table below.

Table A 51: Effects of residues of CGA249287 on mortality and reproduction of *Folsomia candida*

Endpoint	Control	Treatment group (mg CGA249287/kg soil d.w.)									
		2.8	5	9	17	31	56	100	180	324	Tox ref
% Mortality of parental collembolans after 4 weeks	14	15	15	15	18	13	18	20	40**	43**	62***
Mean number of juveniles after 4 weeks	624	579	636	568	552	503	401**	394**	297***	214***	85***
% reduction compared to control	-	7.3	-1.8	9.1	11.7	19.4	35.7	36.9	52.4	65.8	86.4
NOEC	31										
EC ₁₀	7.9 (95% CI = 3.3 to 13.7)										
EC ₂₀	22.7 (95% CI = 13.1 to 34.5)										

Mortality in individual test item treatments compared to the control using Fisher's Exact Test_{SEP} ($\alpha = 0.05$). Treatment means that differed significantly from the control are indicated with asterisks (*** $P < 0.001$, ** $P < 0.01$)._{SEP}

Fecundity assessment data from individual treatments were compared to the data from the control by one-way ANOVA and Dunnett's t-test ($\alpha = 0.05$). Values marked with asterisks differed significantly from the control (*** $P < 0.001$, ** $P < 0.01$)._{SEP}

Validity criteria

The validity criteria were met since:

- Control treatment mortality was 14% (must be $< 20\%$)
- The mean number of juvenile recorded in the control treatment was 624 (must be > 100 per replicate)
- The coefficient of variation of reproduction in the control was 15% (must not be $> 30\%$)

Conclusions

The NOEC based on mean number of progeny for exposure of *Folsomia candida* to CGA249287 was estimated to be 31 mg CGA249287/kg soil. EC₁₀ and EC₂₀ values were estimated to be 7.9 and 22.7 mg CGA249287/kg, respectively.

(Vinall S, 2012)

Comments of zRMS:	<p>The test was not evaluated as it considers the chronic toxicity of cyprodinil metabolite. This study is not required for risk assessment of formulation A23282A.</p> <p>The study considering the toxicity of active substance or its metabolite should be evaluated at EU level during substance renewal.</p>
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Reference: KCP 10.4.2.1

Report Lührs U. (2014) CGA275535 - Effects on Reproduction of the Collembola *Folsomia candida* in Artificial Soil with 5%. Report Number 92791016. Institut für Biologische Analytik und Consulting IBACON GmbH Arheilger Weg 17 64380 Rossdorf, Germany. (XXXX File No. VV-410751; CGA275535_10004)

Guideline(s): OECD Guidelines No. 232. Collembolan Reproduction test in soil (2009)

ISO 11267: Soil quality – inhibition of reproduction of Collembola

(*Folsomia candida*) by soil pollutants. (1999)

Deviation(s): No
GLP: Yes
Acceptability: Yes
Duplication: No
(if vertebrate study)

Executive Summary

The effects of CGA275535 on the reproduction and the parental mortality of collembola species *Folsomia candida* were determined. The NOEC based on number of F1 progeny was determined to be 171.5 mg /kg soil dry weight.

Materials

Test Material CGA275535
Lot/Batch #: K1 6230/3
Purity: 99% w/w
Description: Beige solid
Stability of test compound: Stable under standard conditions.
Reanalysis/Expiry date: End of February 2018
Density: Not stated

Treatments

Test rates: 16.3, 29.4, 52.9, 95.3, 171.5, 308.6, 555.6 and 1000 mg CGA275535/kg soil dry weight
Control: Untreated
Toxic standard: Boric acid tested once a year
Application method: Mixed with artificial soil

Test organisms

Species: *Folsomia candida*
Age: 10 – 12 days
Source: Culture maintained at Test Facility
Feeding: 2 mg granulated dry yeast at the start of the test and after 14 days

Test design

Arenas: Glass containers (volume: 100 mL; diameter: 5 cm), closed tightly to avoid water evaporation, filled with 30 g artificial soil fresh weight.
Substrate: Artificial soil comprising 5% sphagnum peat, 20% kaolinite clay, 74.8% industrial quartz sand (> 50% of the particles between 0.05 mm and 0.2 mm) and 0.2% calcium carbonate.
Replication: Control: 8
Treatment: 4
No./arena : 10
Duration of test: 28 days

Environmental test conditions

Temperature: 18°C to 22°C
pH of soil: Test start: 6.1 to 6.3
Test end: 5.8 to 5.9
Water content of soil: Test start: 51.2% to 53.2% of WHC
Test end: 47.3% to 49.7% of WHC
Photoperiod: 16 hour photoperiod (400 to 800 lux)

Study Design and Methods

Test facility: Institut für Biologische Analytik und Consulting, IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany

Experimental dates: 8th August to 8th September 2014

The test concentrations were prepared by dispersing an exactly weighed amount of the test item in deionised water to make a stock solution. This stock solution was diluted with deionised water for each test concentration and was thoroughly mixed with the artificial soil using a mixing machine, achieving a final nominal water content of 40-60% of WHC. The control was treated with deionised water only.

Ten juvenile collembolans were transferred after the application to the substrate surface of each test vessel using an exhaustor. Four replicates (+ two replicates not loaded with collembolans for measurement purposes) were used per test concentration and eight were used for the control. The test organisms were fed twice during the experiment (at the start of the test and after 14 days) with approximately 2 mg of granulated dry yeast per test vessel. Four weeks after introducing the test organisms, the surviving parental collembolans and offspring (juveniles) were counted.

All values presented throughout this report were calculated using the original raw data and were not based on rounded values.

Mortality data were statistically analyzed using Fisher's Exact Test (multiple comparison, $\alpha = 0.05$, one-sided greater).

Reproduction data were tested for normal distribution and homogeneity of variance using Shapiro-Wilk's test and Levene's test ($\alpha = 0.05$). Further statistical evaluation was performed using Williams t-test (multiple comparison, $\alpha = 0.05$, one-sided smaller).

The determination of the NOEC and LOEC values was based on the results of the statistical evaluation. EC values and their 95% confidence limits at day 28 were not determined by statistical analysis as no reduction of reproduction above 50% was observed. The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

Results and Discussion

Mortality and fecundity are summarised in the table below.

Table A 52: Effects of residues of CGA275535 on mortality and reproduction of *Folsomia candida*

Endpoint	Treatment group (mg CGA275535/kg soil d.w.)								
	Control	16.3	29.4	52.9	95.3	171.5	308.6	555.6	1000
% Mortality of parental collembolans after 4 weeks	11	20	18	8	13	5	20	8	10
Mean number of juveniles after 4 weeks	553	517	479	459	488	582	443*	470*	452*
SD	68	98	87	44	92	109	49	77	47
% reduction compared to control	-	6.6	13.4	17.1	11.9	-5.2	20.0	15.1	18.3
NOEC (reproduction)	171.5								

* significantly different compared to the control (Williams t-test, $\alpha = 0.05$, one-sided smaller)

Validity criteria

The validity criteria were met as follows:

- Control treatment mortality was 11% (must be <20%)
- The mean number of juveniles recorded in the control treatment was 553 (must be >100 per replicate)
- The coefficient of variation of reproduction in the control was 12.3 % (must not be >30%)

Conclusions

The effects of CGA275535 on the reproduction and the parental mortality of collembola species *Folsomia candida* were determined. The NOEC based on number of F1 progeny was determined to be 171.5 mg/kg soil dry weight.

(Lührs U, 2014)

Comments of zRMS:	The test was not evaluated as it considers the chronic toxicity of cyprodinil metabolite. This study is not required for risk assessment of formulation A23282A. The study considering the toxicity of active substance or its metabolite should be evaluated at EU level during substance renewal.
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Reference: KCP 10.4.2.1

Report Lührs U. (2015) CGA321915 – Effects on Reproduction of the Collembola *Folsomia candida* in Artificial Soil with 5 % Peat. Report Number 96341016. Institut für Biologische Analytik und Consulting, IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany. (XXXX file No. VV-412025; CGA321915_10010).

Guideline(s): OECD Guidelines No. 232. Collembolan Reproduction test in soil (2009)

ISO 11267: Soil quality – inhibition of reproduction of Collembola (*Folsomia candida*) by soil pollutants. (1999)

Deviation(s): No

GLP: Yes

Acceptability: Yes

Duplication: No
(if vertebrate study)

Executive Summary

The effects of CGA321915 on the reproduction and the parental mortality of collembola species *Folsomia candida* were determined. The NOEC based on number of F1 progeny was determined to be 1000 mg CGA321915/kg soil dry weight.

Materials

Test Material	CGA321915
Parent:	CGA219417 (Cyprodinil)
Lot/Batch #:	MES 356/1
Purity:	98% w/w ± 2 %
Description:	White solid
Stability of test compound:	Stable under test conditions
Reanalysis/Expiry date:	30 September 2016

Density:	Not applicable
Treatments	
Test concentrations:	7.813, 15.63, 31.25, 62.5, 125, 250, 500 and 1000 mg CGA321915/kg soil dry weight
Control:	Deionised water
Toxic standard:	Boric Acid (purity: 100.3 %) at 30.5, 48.8, 78.1, 125 and 200 mg boric acid/kg dry soil weight (separate study - No.: 61405016, date: June to August 2014)
Application method:	CGA321915 dispersed in deionised water was mixed into artificial soil prior to introduction of collembolans
Test organisms	
Species:	<i>Folsomia candida</i> (Willem)
Age:	Juveniles, 10 to 12 days old
Source:	Culture maintained at Test Facility (origin not reported)
Feeding:	Approximately 2 mg granulated dried yeast at the start of the test and after 14 days
Test design	
Arenas:	Glass containers (100 mL capacity, 5 cm diameter) with close-fitting lids
Substrate:	Artificial soil comprising 5% sphagnum peat, 20% kaolinite clay, 74.8% fine quartz sand (> 50% of the particles between 0.05 mm and 0.2 mm) and 0.2% calcium carbonate. 30 g \pm 1.0 g fresh weight of artificial soil was added to each test vessel
Replication:	Treated groups 4, control group 8, plus an additional jar per treatment for measurement purposes
No./arena :	10
Duration of test:	28 days
Environmental conditions	test
Temperature:	18 to 22 °C
pH of soil:	Test start: 6.0 to 6.1 Test end: 5.9 to 6.1
Water content of soil:	Test start: 50.4 to 51.7% of WHC Test end: 47.3 to 50.9% of WHC
Photoperiod:	16 hours light and 8 hours dark at 400 to 800 lux

Study Design and Methods

Experimental dates: 16th February to 17th March 2015

The test concentrations were prepared by dispersing an exactly weighed amount of the test item in deionised water to make a solution of the highest test concentration. This solution was then serially diluted to prepare the lower test concentrations and the treatments thoroughly mixed with the artificial soil using a laboratory mixer, achieving a final nominal water content of 40 - 60 % of WHC. The control was treated with deionised water only.

Ten juvenile collembolans were transferred after the application to the substrate surface of each test vessel using an aspirator. Four replicates were used per test concentration and eight replicates were used for the control (+ one replicate per treatment not loaded with collembolans for measurement purposes). The test organisms were fed twice during the experiment (at the start of the test and after 14 days) with approximately 2 mg of granulated dried yeast per test vessel. Four weeks after introducing the test organisms, the surviving parental collembolans and offspring (juveniles) were counted.

All values presented throughout this report were calculated using the original raw data and were not based on rounded values.

The percentage mortality of the springtails was calculated for each treatment. The 28-day mortality data for

the individual test-item treatments were compared to those for the control using Fisher's Exact Binomial Test (multiple comparison, with Bonferroni Correction, $\alpha = 0.05$, one-sided greater). The percentage reduction in reproductive performance in the test item treatment groups, compared to the control group, was calculated.

The data from the test-item treatments were compared to the control data by Williams t-test (multiple comparison, $\alpha = 0.05$, one-sided smaller). The results were used to determine the NOEC with respect to reproduction. The ECx values could not be determined due to the outcome of the test.

Results and Discussion

Mortality and fecundity are summarised in the table below.

Table A 53: Effects of residues of CGA321915 on mortality and reproduction of *Folsomia candida*

Endpoint	Treatment group (mg CGA321915/kg soil d.w.)								
	Control	7.813	15.63	31.25	62.5	125	250	500	1000
Mean mortality of parental collembolans after 4 weeks ^a (%)	6	10	5	13	5	5	15	5	10
Mean number of juveniles after 4 weeks ^b	469	488	480	505	544	516	461	447	408
SD	33	98	55	28	69	84	67	50	45
% reduction compared to control ^c	-	-4	-2	-8	-16	-10	2	5	13
NOEC (reproduction)	1000								

^a Mean mortality amongst springtails originally introduced. Individual treatments compared to the control data using Fisher's Exact Binomial Test (multiple comparison, with Bonferroni Correction, $\alpha = 0.05$, one-sided greater). No statistically significant differences were determined.

^b Fecundity data were compared to the control by Williams t-test (multiple comparison, $\alpha = 0.05$, one-sided smaller). No statistically significant differences were determined.

^c A positive value indicates a decrease in reproduction compared to the control

Validity criteria

The validity criteria were met as follows:

- Control treatment mortality (mean) was 6% (must be < 20%)
- The mean number of juveniles recorded in the control treatment was 469 (must be > 100 per replicate)
- The coefficient of variation of reproduction in the control was 7% (must not be > 30%)

Conclusions

The effects of CGA321915 on the reproduction and the parental mortality of collembola species *Folsomia candida* were determined. The NOEC based on number of F1 progeny was determined to be 1000 mg CGA321915/kg soil dry weight.

It was not possible to derive EC₁₀ or EC₂₀ values from the data

(Lührs U, 2015)

Comments of zRMS:	<p>The submitted study was accepted.</p> <p>The validity criteria were met:</p> <ul style="list-style-type: none">• mean mortality of adult females: $\leq 20\%$; observed 6.3%;• mean number of juveniles per replicate: ≥ 50; observed 323.1;• coefficient of variation (mean number of juveniles per replicate): $\leq 30\%$; observed 11.2 %. <p>The following endpoints were derived:</p> <ul style="list-style-type: none">• mortality: NOEC = 309 mg test item/kg soil d.w. LC₅₀ = 381.9 mg test item/kg soil d.w.• reproduction: NOEC = 171 mg test item/kg soil d.w. (corrected 26.5 mg kg dw soil) EC₅₀ = 507.4 mg test item/kg soil d.w. <p>The study results can be used in risk assessment.</p>
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Reference: KCP 10.4.2.1

Report Schulz, L., (2021), Cyprodinil/Prothioconazole EC (A23282A) - Effects on the reproduction of the predatory mite *Hypoaspis aculeifer*, Report number 21 48 THC 0030. BioChem agrar Labor für biologische und chemische, Analytik GmbH, Kupferstraße 6, 04827 Machern OT Gerichshain, Germany (XXXX File No. VV-931773).

Guideline(s): OECD 226. Predatory mite (*Hypoaspis (Geolaps) aculeifer*) reproduction test in soil, (2016)

Deviations: No

GLP: Yes

Acceptability: Yes

Executive Summary

The effects of A23282A on the mortality and reproductive output of the soil mite species *Hypoaspis aculeifer* were determined during a 14-day test.

The LC₅₀ value for mortality was calculated to be 382 mg test item/kg soil dry weight. The EC₁₀, EC₂₀ and EC₅₀ values for reproduction were calculated to be 257, 325 and 507 mg test item/kg soil dry weight, respectively. The NOEC for mortality and for reproduction were determined to be 309 and 171 mg test item/kg soil dry weight, respectively.

Materials

Test Material	Cyprodinil/Prothioconazole EC (A23282A)		
Lot/Batch #:	LCR001-021-001		
Other Batch ID:	1160912		
Actual content of active ingredients:	cyprodinil	22.1 % w/w corresponding to 219 g/L	

	prothioconazole	7.40 % w/w corresponding to 73.5 g/L
Description:	yellow liquid	
Stability of test compound:	Stable under the given conditions	
Reanalysis/Expiry date:	End of September 2023	
Density:	993 kg/m³	
Treatments		
Test concentrations:	16.3, 29.4, 52.9, 95.3, 171, 309, 556, 1000 mg test item/kg soil dry weight (spacing factor: 1.8)	
Control:	Deionised water	
Toxic standard:	Dimethoate 400 EC (400 g/L, nominal) (separate GLP study)	
Test organisms		
Species	<i>Hypoaspis aculeifer</i> (CANESTRINI)	
Source:	Obtained synchronised from “Katz Biotech AG”, Baruth, Germany, 1 day before study start and kept in the test facility under ambient laboratory (20 ± 2 °C) conditions until test start	
Food:	<i>Tyrophagus putrescentiae</i> (SCHRANK) provided every 2-3 days	
Age at test start:	Adult (28 - 30 days old)	
Test design		
Vessels:	160 mL WECK-jar with glass lid	
Substrate:	<ul style="list-style-type: none">- 5 % sphagnum peat; origin: Torfwerk Moorkultur Ramsloh, 26683 Saterland, Germany, classified according to DIN 11540 (as close to pH 5.5-6.0 as possible, no visible plant remains, finely ground, dried to measured moisture content)- 20 % kaolin clay (kaolinite content > 30 %); type: Kaolin W, origin: ERBSLÖH Lohrheim GmbH, 65558 Lohrheim, Germany- 0.225 % calcium carbonate; origin: MERCK KGaA, 64271 Darmstadt, Germany- 74.775 % industrial quartz sand; type: Millisil W3, origin: Quarzwerke GmbH, 50207 Frechen, Germany (predominantly fine sand with more than 50 % of the particles between 50 and 200 µm)- deionised water	
Replication:	control: 8 (+ 2 replicates for determination of water content and pH-value; without predatory mites) treated group: 4 (+ 2 replicates for determination of water content and pH-value; without predatory mites)	
No. of mites/arena:	10 adult females	
Duration of test:	14 days	
Environmental test conditions		
Temperature:	20.4 - 21.2 °C	
pH:	test start: 6.1 - 6.2 test end: 5.7 - 5.9	
Water content of soil:	test start: 18.09 - 18.68 (equivalent to 47.18 - 48.71 % of maximum WHC) test end: 18.30 - 18.94 (equivalent to 47.74 - 49.40 % of maximum WHC)	
Photoperiod:	duration: light : dark = 16 h : 8 h	

intensity: 478 lux

Study Design and Methods

Test facility: BioChem agrar GmbH, Kupferstraße 6, 04827 Machern OT Gerichshain, Germany

Experimental dates: 17th September to 12th October 2021

Adult females of the soil mite *Hypoaspis aculeifer* were exposed to different concentrations of Cyprodinil/Prothioconazole EC (A23282A) incorporated into the test soil. An exact weighed amount of the test item was mixed with deionised water to make a stock solution, and appropriate volumes of this stock solution were further diluted with deionised water to obtain the test concentrations such that, when added to pre-moistened artificial soil, a final moisture content value of approximately 50% WHC was achieved. Ten adult females were transferred to the test vessels which contained untreated (control), or test item treated artificial soil. As a source of food, cheese mites (*Tyrophagus putrescentiae*) were added to the soil surface every 2-3 days. The test was carried out under a controlled light-dark cycle. Fourteen days after introducing the test organisms, the surviving adult mites and the juveniles of *Hypoaspis aculeifer* were extracted by heat/light extraction and counted. From these data the mortality of the adult females and the reproductive output were calculated.

Percentage mortality (number of dead adults) for the treatment groups was calculated. Missing mites were counted as dead. Observations in the treatment group were expressed relative to the control group. The mortality in the treatment groups was calculated according to Abbott (1925).

The reduction of reproductive output (Rr) for the treatment groups was calculated in comparison to the control group.

The statistical analysis was performed with the software ToxRat Professional 3.3.0 (RATTE, 2018). The Step-down Rao-Scott-Cochran-Armitage Test Procedure and Multiple Sequentially-rejective Welch-t-test after Bonferroni-Holm were used to compare the control with the independent test item groups. Logit analysis using linear maximum likelihood regression was used in LC_x calculation. Probit analysis using linear maximum likelihood regression was used in EC_x calculation.

Results

Mortality and fecundity are summarised in the table below.

Table A 54: Effects of A23282A on mortality and reproduction of *Hypoaspis aculeifer*

Endpoint	Treatment group (mg test item/kg soil d.w.)								
	Control	16.3	29.4	52.9	95.3	171	309	556	1000
	Mortality of adult mites after 14 days								
Mean mortality (%)	6.3	7.5	0.0	2.5	0.0	5.0	27.5	87.5*	100.0*
Corrected mortality (Abbott) (%)	-	1.3	-6.7	-4.0	-6.7	-1.3	22.7	86.7	100.0
	Number of juveniles after 14 days								
Mean no. juveniles	323.1	303.0	321.3	321.0	332.3	305.3	261.0*	151.0*	18.0*
Standard deviation	36.3	29.8	28.7	11.3	18.6	11.9	29.3	63.8	4.5
Coefficient of variation (%)	11.2	9.8	8.9	3.5	5.6	3.9	11.2	42.2	25.3
Reduction of reproduction compared to control (%)	-	6.2	0.6	0.7	-2.8	5.5	19.2	53.3	94.4

	Endpoint (mg test item/kg soil d.w.)
NOEC (mortality)	309
LC ₅₀ (mortality) ¹⁾	381.9 (95 % confidence limit 275.0 - 559.3)
NOEC (reproduction)	171
EC ₁₀ (reproduction) ²⁾	257.0 (95 % confidence limit 209.2 - 315.7)
EC ₂₀ (reproduction) ²⁾	324.6 (95 % confidence limit 278.3 - 378.7)
EC ₅₀ (reproduction) ²⁾	507.4 (95 % confidence limit 459.8 - 559.9)

* statistically significant compared to control (Step-down Rao-Scott-Cochran-Armitage Test Procedure for mortality, $\alpha = 0.05$, one-sided greater and Multiple Sequentially-rejective Welch-t-test after Bonferroni-Holm for reproduction, $\alpha = 0.05$, one-sided smaller)

Negative % values for reduction of reproduction = increase, relative to control

¹⁾ based on Logit analysis using linear maximum likelihood regression

²⁾ based on Probit analysis using linear maximum likelihood regression

In a separate study (experimental start date 12th May 2021), the EC₅₀ (reproduction) of the reference item Dimethoate 400 EC (400 g/L, nominal) was calculated to be 3.66 mg a.s./kg soil d.w. The results of the reference test demonstrate the sensitivity of the test system.

Validity Criteria

The validity criteria for the control group were met:

- Mean mortality of adult females: $\leq 20\%$ (observed: 6.3 %)
- Mean number of juveniles per replicate: ≥ 50 (calculated: 323.1)
- Coefficient of variation (mean number of juveniles per replicate): $\leq 30\%$ (calculated: 11.2 %)

Conclusion

The effects of A23282A on the mortality and reproductive output of the soil mite species *Hypoaspis aculeifer* were determined during a 14-day test.

The LC₅₀ value for mortality was calculated to be 382 mg test item/kg soil dry weight. The EC₁₀, EC₂₀ and EC₅₀ values for reproduction were calculated to be 257, 325 and 507 mg test item/kg soil dry weight, respectively. The NOEC for mortality and for reproduction were determined to be 309 and 171 mg test item/kg soil dry weight, respectively.

(Schulz, 2021)

Comments of zRMS:	The test was not evaluated as it considers the chronic toxicity of cyprodinil. The study considering the toxicity of active substance or its metabolite should be evaluated at EU level during substance renewal.
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Reference:	KCP 10.4.2.1
Report	Lühns U. (2014) Cyprodinil EC (A14325E) - Effects on Reproduction of the Predatory Mite <i>Hypoaspis aculeifer</i> in Artificial Soil with 5% Peat. Report Number 92781089. Institut für Biologische Analytik und Consulting IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany. (XXXX file No. VV-410670; A14325E_10062)
Guideline(s):	OECD Guideline 226: Predatory mite (<i>Hypoaspis</i> (<i>Geolaelaps</i>) <i>aculeifer</i>) reproduction test in soil. (2008)
Deviation(s):	No

GLP: Yes
Acceptability: Yes
Duplication: No
(if vertebrate study)

Executive Summary

In a test conducted with A14325E no statistically significant effects on reproduction of *Hypoaspis aculeifer* up to and including the concentration of 1000 mg test item/kg soil were observed.

Therefore, the overall NOEC was 1000 mg/kg soil dry weight (295 mg cyprodinil/kg), the highest concentration tested.

Materials

Test Material	A14325E
	Cyprodinil EC
Lot/Batch #:	SMO3A100
Actual content of active ingredients:	Cyprodinil: 29.1% w/w, corresponding to 295 g/L
Description:	Light yellow Liquid
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	End of January 2017
Density:	1014 kg/m ³
Treatments	
Test concentrations:	16.3, 29.4, 52.9, 95.3, 171.5, 308.6, 555.6 and 1000 mg test item/kg soil
Control:	Untreated
Toxic standard:	BAS 152 11 I Perfekthion (nominally 400 g dimethoate/L, analysed 400.9 g dimethoate/L)
Test organisms	
Species	<i>Hypoaspis aculeifer</i> (CANESTRINI)
Source:	Cultured in test facility
Food:	Cheese mites, <i>Tyrophagus putrescentiae</i> at 2 spatulas on day 0, 2 and 4, 1 spatula on day 7 and 9, ½ spatulas on day 11.
Age at test start:	7 days after reaching adulthood
Test design	
Vessels:	Glass containers (volume: 100 mL; diameter: 5 cm), tight screw top closure to avoid water evaporation, filled with approximately 20 g artificial soil dry weight.
Substrate:	Artificial soil comprising 5% sphagnum peat, 20 % kaolinite clay, 74.7% industrial quartz sand and 0.3% calcium carbonate.
Replication:	Control group: 8 Treated group: 4
No. of mites/arena :	10
Duration of test:	14 days
Environmental test conditions	
Temperature:	18 to 22°C
pH:	Test start: 6.2 Test end: 6.1 – 6.2
Water content of soil:	Test start: 50.4% to 53.1% of maximum WHC Test end: 49.4% to 51.9% of maximum WHC
Photoperiod:	16 h light: 8 h dark, 400 to 800 lux

Study Design and Methods

Test facility: Institut für Biologische Analytik und Consulting, IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany

Experimental dates: 1st to 17th September 2014

Adult females of the soil mite *Hypoaspis aculeifer* were exposed to concentrations of A14325E incorporated into the test soil. An exactly weighed amount of the test item was mixed with purified water to make a stock solution, and appropriate volumes of this stock solution were further diluted with purified water to obtain the test concentrations such that, when added to pre-moistened artificial soil, a final moisture content value of 50% WHC was achieved. Adult females were transferred to the test vessels which contained untreated (control), reference item or test item treated artificial soil. Ten adult females were introduced to each test vessel. As a source of food, cheese mites (*Tyrophagus putrescentiae*) were added to the soil surface throughout the test. The test was carried out under controlled light-dark cycle. Fourteen days after introducing the test organisms, the surviving mites and the juveniles of *Hypoaspis aculeifer* were extracted by heat/light extraction. From these data the mortality of the adult females and the reproductive output were calculated.

The mean number of dead adult female mites for each treatment, the mean number of juvenile mites for each treatment, the NOEC, the LOEC values, and the LC₅₀ at day 14 were not able to be determined by statistical analysis as no mortality above 50% was observed.

Mortality data were statistically analysed using Fisher's Exact test (multiple comparison, $\alpha = 0.05$, one-sided greater).

Reproduction data were tested for normal distribution and homogeneity of variance using Shapiro-Wilk's test and Levene's test ($\alpha = 0.05$). As data were normally distributed and homogeneous, the further statistical evaluation was performed using Williams t-test (multiple comparison, $\alpha = 0.05$, one-sided smaller).

The determination of the NOEC and LOEC values was based on the results of the statistical evaluation. The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

Results and Discussion

Mortality and fecundity are summarised in the table below.

Table A 55: Effects of residues of A14325E on mortality and reproduction of *Hypoaspis aculeifer*

Endpoint	Treatment group (mg A14325E/kg soil d.w.)								
	Control	16.3	29.4	52.9	95.3	171.5	308.6	555.6	1000
	Mortality of adult mites after 14 days								
% mortality	8	13	5	5	8	5	0	8	0
	Number of juveniles after 14 days								
Mean no. progeny per replicate ^c	258	271	291	285	257	277	280	254	266
standard deviation	24	30	28	23	39	12	21	38	37
% reduction compared to control	-	-5.0	-12.7	-10.6	0.3	-7.5	-8.6	1.6	-3.0

The results represent rounded values calculated from the exact raw data
Negative values indicate an increase relative to the control.

Validity Criteria

The validity criteria for the control group were met:

- Mean mortality of adult females: $\leq 20\%$ (observed: 8%)
- Mean number of juveniles per replicate: ≥ 50 (calculated: 258)
- Coefficient of variation (mean number of juveniles per replicate: $\leq 30\%$)

Conclusions

In a test conducted with A14325E no statistically significant effects on reproduction of *Hypoaspis aculeifer* up to and including the concentration of 1000 mg test item/kg soil were observed.

Therefore, the overall NOEC was 1000 mg/kg soil dry weight (295 mg cyprodinil/kg), the highest concentration tested.

(Lührs U, 2014)

Comments of zRMS:	The test was not evaluated as it considers the chronic toxicity of cyprodinil metabolite. This study is not required for risk assessment of formulation A23282A. The study considering the toxicity of active substance or its metabolite should be evaluated at EU level during substance renewal.
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Reference:	KCP 10.4.2.1
Report	Lührs U. (2014) Cyprodinil WG (A8637C) - Effects on Reproduction of the Predatory Mite <i>Hypoaspis aculeifer</i> in Artificial Soil with 5% Peat Report Number 92771089. Institut für Biologische Analytik und Consulting, IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany. (XXXX file No. VV-410371; A8637C_10312)
Guideline(s):	OECD (2008). OECD Guideline for Testing of Chemicals, Section 2 – Effects on Biotic Systems, Method 226 (adopted 3 October 2008): Predatory mite (<i>Hypoaspis</i> (<i>Geolaelaps</i>) <i>aculeifer</i>) reproduction test in soil.
Deviation(s):	No
GLP:	Yes
Acceptability:	Yes
Duplication: (if vertebrate study)	No

Executive Summary

The potential effects of A8637C on the mortality and reproductive success of the soil mite species *Hypoaspis aculeifer* (Canestrini) were determined during a 14-day test. The NOEC was determined to be 555.6 mg /kg soil dry weight (277.8 mg cyprodinil/kg soil).

Materials

Test Material	A8637C Cyprodinil WG
Lot/Batch #:	SMO2C304
Actual content of active ingredients:	50.2% w/w
Description:	Brownish solid

Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	End of December 2016
Density:	Not stated
Treatments	
Test concentrations:	16.3, 29.4, 52.9, 95.3, 171.5, 308.6, 555.6 and 1000 mg A8637C/kg soil dry weight
Control:	Untreated substrate, i.e. purified water only
Toxic standard:	BAS 152 11 I Perfekthion (nominally 400 g dimethoate/L, analysed 400.9 g dimethoate/L)
Test organisms	
Species	<i>Hypoaspis aculeifer</i> (CANESTRINI)
Source:	Cultured in test facility
Food:	Cheese mites, <i>Tyrophagus putrescentiae</i> at 2 spatulas on day 0, 1 and 5, 1 spatula on day 7 and 9, ½ a spatula on day 12.
Age at test start:	12 days after reaching adult stage
Test design	
Vessels:	Glass containers (volume: 100 mL; diameter: 5 cm), tight screw top closure to avoid water evaporation, filled with approx 20 g soil d.w.
Substrate:	Artificial soil comprising 5% sphagnum peat, 20 % kaolinite clay, 74.75% industrial quartz sand and 0.25% calcium carbonate.
Replication:	Control group: 8 Treated group: 4
No. of mites/arena :	10 females
Duration of test:	14 days
Environmental test conditions	
Temperature:	18 to 22 °C
pH:	Test start: 6.1 - 6.2 Test end: 6.0 – 6.1
Water content of soil:	50.4 to 53.2% of maximum WHC
Photoperiod:	16 h light : 8 h dark, 400 to 800 lux

Study Design and Methods

Experimental dates: 20th August to 4th September 2014

Adult females of the soil mite *Hypoaspis aculeifer* were exposed to concentrations of A8637C incorporated into the test soil. An exactly weighed amount of the test item was mixed with purified water to make a stock solution, and appropriate volumes of this stock solution were further diluted with purified water to obtain the test concentrations such that, when added to pre-moistened artificial soil, a final moisture content value of 50% WHC was achieved. Adult females were transferred to the test vessels which contained untreated (control), reference item or test item treated artificial soil. Ten adult females were introduced to each test vessel. As a source of food, cheese mites (*Tyrophagus putrescentiae*) were added to the soil surface throughout the test. The test was carried out under controlled light-dark cycle. Fourteen days after introducing the test organisms, the surviving mites and the juveniles of *Hypoaspis aculeifer* were extracted by heat/light extraction. From these data the mortality of the adult females and the reproductive output were calculated.

The mean number of dead adult female mites for each treatment, the mean number of juvenile mites for each treatment, the NOEC, the LOEC values, and the LC₅₀ at day 14 were not able to be determined by statistical analysis as no mortality above 50% was observed.

Mortality data were statistically analysed using Fisher's Exact test (multiple comparison, $\alpha = 0.05$, one-

sided greater). Reproduction data were tested for normal distribution and homogeneity of variance using Shapiro-Wilk's test and Levene's test ($\alpha = 0.05$). As data were normally distributed and homogeneous, the further statistical evaluation was performed using Williams t-test (multiple comparison, $\alpha = 0.05$, one-sided smaller).

The determination of the NOEC and LOEC values was based on the results of the statistical evaluation. The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05.

Results and Discussion

Mortality and fecundity are summarised in the table below.

Table A 56: Effects of residues of A8637C on mortality and reproduction of *Hypoaspis aculeifer*

Endpoint	Treatment group (mg A8637C/kg soil d.w.)								
	Control	16.3	29.4	52.9	95.3	171.5	308.6	555.6	1000
Mortality of adult mites after 14 days									
% mortality	5	3	3	3	10	0	0	5	8
Number of juveniles after 14 days									
Mean no. progeny per replicate	257	247	237	236	259	250	227	236	218*
standard deviation	16	25	16	22	32	39	35	19	13
% reduction compared to control	-	3.9	7.8	8.3	-0.8	2.7	11.5	8.2	15.0

* significantly reduced compared to the control (Williams t-test, $\alpha = 0.05$, one-sided smaller)

The results represent rounded values calculated from the exact raw data

Validity Criteria

The validity criteria for the control group were met:

- Mean mortality of adult females: $\leq 20\%$ (observed: 5%)
- Mean number of juveniles per replicate: ≥ 50 (calculated: 257)
- Coefficient of variation (mean number of juveniles per replicate): $\leq 30\%$ (calculated: 6.2%)

Conclusions

In a test conducted with A8637C reproduction was not reduced compared to the control up to and including the concentration of 555.6 mg test item/kg soil but was reduced at 1000 mg test item/kg soil. Therefore, the overall No Observed Effect Concentration (NOEC) was determined to be 555.6 mg test item/kg soil (277.8 mg cyprodinil/kg soil).

(Lührs U, 2014)

Comments of zRMS:	<p>The test was not evaluated as it considers the chronic toxicity of cyprodinil metabolite.</p> <p>This study is not required for risk assessment of formulation A23282A.</p> <p>The study considering the toxicity of active substance or its metabolite should be evaluated at EU level during substance renewal.</p>
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Reference:	KCP 10.4.2.1
Report	Lühns U. (2014) CGA275535 - Effects on Reproduction of the Predatory Mite <i>Hypoaspis aculeifer</i> in Artificial Soil with 5% Peat. Report Number 92791089. Institut für Biologische Analytik und Consulting, IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany. (XXXX file No. VV-410172; CGA275535_10000)
Guideline(s):	OECD Guidelines No. 226 (2016)
Deviation(s):	No
GLP:	Yes
Acceptability:	Yes
Duplication: (if vertebrate study)	No

Executive Summary

In a *Hypoaspis aculeifer* test conducted with CGA275535, the NOEC for reproduction was determined to be 171.5 mg test item/kg soil dry weight. EC₁₀ and EC₂₀ values were estimated to be 104.6 and 272.5 mg CGA249287/kg, respectively.

Materials

Test Material	CGA275535
Lot/Batch #:	KI 6230/3
Actual content of active ingredients:	CGA275535 99% w/w
Description:	Solid
Stability of test compound:	Stable under standard conditions.
Reanalysis/Expiry date:	End of February 2018
Density:	Not stated

Treatments

Test concentrations:	16.3, 29.4, 52.9, 95.3, 171.5, 308.6, 555.6 and 1000 mg test item/kg soil
Control:	Untreated
Toxic standard:	Dimethoate: 400.0 g/L (nominal), 400.9 g/L (analysed)
Application method:	Mixed with artificial soil.

Test organisms

Species:	<i>Hypoaspis aculeifer</i>
Age:	Adults, approximately 11 days after reaching the adult stage.
Source:	Culture maintained at Test Facility.
Feeding:	Two spatulas of cheese mites (<i>Tyrophagus putrescentiae</i> cultured by IBACON) at experimental start, 1-2 spatulas on day 3, 5, 7 and 10, ½ spatula on day 12.

Test design

Arenas:	Glass containers (volume: 100 mL; diameter: 5 cm), tight screw top closure to avoid water evaporation, filled with approximately 20 g ± 1.0 g artificial soil dry weight.
Replication:	Control: 8 Treatment: 4
No. of mites/arena:	10

Duration of test:	14 days
Environmental test conditions	
Temperature:	18°C to 22°C
Humidity:	Not stated
Photoperiod:	16 h photoperiod (400 to 800 lux).

Study Design and Methods

Test facility: Institut für Biologische Analytik und Consulting, IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany

Experimental dates: 8th to 25th August 2014

A stock solution was prepared by dissolving 481.5 mg of CGA275535 in 30 mL acetone. A dilution series was prepared from the stock solution by adding 12 mL of acetone to 15 mL of the second stock solution or the respective dilution. The test item blended sand was added to artificial soil with reduced sand fraction to result in a final net weight of 260 g. This resulted in the following nominal concentrations: 16.3, 29.4, 52.9, 95.3, 171.5, 308.6, 555.6 and 1000 mg test item/kg soil.

Mortality data were statistically analysed using Fisher's Exact test (multiple comparison, $\alpha = 0.05$, one-sided greater).

Reproduction data were tested for normal distribution and homogeneity of variance using Shapiro-Wilk's test and Levene's test ($\alpha = 0.05$). As data were normally distributed and homogeneous, the further statistical evaluation was performed using Williams t-test (multiple comparison, $\alpha = 0.05$, one-sided smaller).

The determination of the NOEC and LOEC values was based on the results of the statistical evaluation. The EC values were calculated by Probit Analysis. The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

Results and Discussion

Mortality and reproduction are summarised in the table below.

Table A 57: Effects of residues of CGA275535 on mortality and reproduction of *Hypoaspis aculeifer*

Endpoint	Treatment group (mg CGA275535/kg soil d.w.)								
	Control	16.3	29.4	52.9	95.3	171.5	308.6	555.6	1000
	Mortality of adult mites after 14 days								
% mortality ^a	1	3	3	5	5	5	8	3	3
	Number of juveniles after 14 days								
Mean no. progeny per replicate	226	223	202	223	219	228	143	162	140
Reduction as % of the control	-	99	89	99	97	101	63*	72*	62*

The results represent rounded values calculated from the exact raw data

* Significantly reduced compared to the control (Williams t-test, $\alpha = 0.05$, one-sided smaller)

Validity criteria

The validity criteria were met since:

- Control mortality was 1% (should be < 20%)
- Control reproduction was 183 to 274 (should be >50 juveniles per test unit)
- Coefficient of Variation (CV) of the control reproduction was 12.4% (should be < 30%)

Conclusions

In a *Hypoaspis aculeifer* test conducted with CGA275535, the NOEC for reproduction was determined to be 171.5 mg test item/kg soil dry weight. EC₁₀ and EC₂₀ values were estimated to be 104.6 and 272.5 mg CGA249287/kg, respectively.

(Lührs U, 2014)

Comments of zRMS:	The test was not evaluated as it considers the chronic toxicity of cyprodinil metabolite. This study is not required for risk assessment of formulation A23282A. The study considering the toxicity of active substance or its metabolite should be evaluated at EU level during substance renewal.
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Reference:	KCP 10.4.2.1
Report	Lührs U. (2015), CGA321915 - Effects on Reproduction of the Predatory Mite <i>Hypoaspis aculeifer</i> in Artificial Soil with 5 % Peat. Report Number 96341089. Institut für Biologische Analytik und Consulting, IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany. (XXXX file No. VV-412028; CGA321915_10011)
Guideline(s):	OECD Guideline 226: Predatory mite (<i>Hypoaspis (Geolaelaps) aculeifer</i>) reproduction test in soil. (2008)
Deviation(s):	No
GLP:	Yes
Acceptability:	Yes
Duplication: (if vertebrate study)	No

Executive Summary

In a *Hypoaspis aculeifer* test conducted with CGA321915, the NOEC for reproduction was determined to be 1000 mg test item/kg soil dry weight, the highest concentration tested.

Materials

Test Material	CGA321915
Parent:	CGA219417 (Cyprodinil)
Lot/Batch #:	MES 356/1
Purity:	98% w/w (estimated error: ± 2 %)
Description:	White solid
Stability of test compound:	Stable under test conditions
Reanalysis/Expiry date:	30 September 2016
Density:	Not applicable

Treatments

Test concentrations:	62.5, 125, 250, 500 and 1000 mg CGA321915/kg soil dry weight
Control:	Deionised water
Toxic standard:	BAS 152 11 I Perfekthion (nominally 400 g dimethoate/L, analysed 400.9 g dimethoate/L) applied at a rate of 1.3, 2.0, 3.0, 4.5 and 6.8 mg dimethoate/kg soil (separate study, Project 93461089, performed June 2014)

Test organisms

Species	<i>Hypoaspis aculeifer</i> (CANESTRINI)
Source:	Cultured in test facility (origin not reported)
Food:	Cheese mites, <i>Tyrophagus putrescentiae</i> at 2 spatulas on day 0, 2 and 4, 1 spatula on day 7 and 9, ½ a spatula on day 11
Age at test start:	Adults. approximately 7 days after reaching the adult stage

Test design

Vessels:	Glass containers (volume: 100 mL; diameter: 5 cm), tight screw top closure to avoid water evaporation, filled with approximately 20 g ± 1.0 g soil d.w.
Substrate:	Artificial soil comprising 5% sphagnum peat, 20% kaolinite clay, 74.8% fine quartz sand and 0.2% calcium carbonate
Replication:	Control group: 8 Treated group: 4 1 additional replicate per treatment for measurement purposes
No. of mites/arena :	10
Duration of test:	14 days

Environmental test conditions

Temperature:	18 to 22 °C
pH:	Test start: 6.0 to 6.1 Test end: 5.9 to 6.0
Water content of soil:	Test start: 50.4 to 51.7% of maximum WHC Test end: 48.1 to 51.2% of maximum WHC
Photoperiod:	16 h light : 8 h dark, 400 to 800 lux

Study Design and Methods

Test facility: Institut für Biologische Analytik und Consulting, IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany

Experimental dates: 16th February to 4th March 2015

Adult females of the soil mite *Hypoaspis aculeifer* were exposed to concentrations of CGA321915 incorporated into the test soil. An exactly weighed amount of the test item was mixed with deionised water to make a stock solution of the highest test concentration, and the lower test concentrations were obtained using a series of dilutions such that, when added to pre-moistened artificial soil, a final moisture content value of 40 to 60% WHC was achieved. Adult females were transferred to the test vessels which contained untreated (control) or test item treated artificial soil. Ten adult females were introduced to each test vessel. As a source of food, cheese mites (*Tyrophagus putrescentiae*) were added to the soil surface throughout the test. The test was carried out under controlled light-dark cycle. Fourteen days after introducing the test organisms, the surviving mites and the juveniles of *Hypoaspis aculeifer* were extracted by heat/light extraction. From these data the mortality of the adult females and the reproductive output were calculated.

The mean number of dead adult female mites for each treatment, the mean number of juvenile mites for each treatment, the NOEC, the LOEC values, and the LC₅₀ at day 14 were not able to be determined by

statistical analysis as no mortality above 50% was observed.

Mortality data were statistically analysed using Fisher's Exact Binomial test (multiple comparison, with Bonferroni Correction, $\alpha = 0.05$, one-sided greater). Reproduction data were tested for normal distribution and homogeneity of variance using Shapiro-Wilk's test and Levene's test ($\alpha = 0.05$). As data were normally distributed and homogeneous, the further statistical evaluation was performed using Williams t-test (multiple comparison, $\alpha = 0.05$, one-sided smaller).

The determination of the NOEC and LOEC values was based on the results of the statistical evaluation. The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05.

Results and Discussion

Mortality and fecundity are summarised in the table below.

Table A 58: Effects of residues of CGA321915 on mortality and reproduction of *Hypoaspis aculeifer*

Endpoint	Treatment group (mg CGA321915/kg soil d.w.)					
	Control	62.5	125	250	500	1000
	Mortality of adult mites after 14 days					
Mean mortality (%)	6	3	5	3	3	0
	Number of juveniles after 14 days					
Mean no. progeny per replicate	212	222	197	217	212	202
standard deviation	12	25	23	22	8	33
% reduction compared to control ^a	-	-5	7	-3	0	5

The results represent rounded values calculated from the exact raw data

^a Negative values indicate an increased reproduction relative to the control

Validity Criteria

The validity criteria for the control group were met:

- Mean mortality of adult females in control: $\leq 20\%$ (observed: 6%)
- Mean number of juveniles per replicate: ≥ 50 (calculated: 212)
- Coefficient of variation (mean number of juveniles per replicate): $\leq 30\%$ (calculated: 5.7%)

Conclusions

The effects of CGA321915 on the mortality and reproductive output of the soil mite species *Hypoaspis aculeifer* were determined during a 14-day test. The NOEC was determined to be 1000 mg /kg soil dry weight, the highest concentration tested.

It was not possible to derive EC₁₀ or EC₂₀ values from the data

(Lührs U, 2015)

Comments of zRMS:	<p>The test was not evaluated as it considers the chronic toxicity of cyprodinil metabolite.</p> <p>This study is not required for risk assessment of formulation A23282A.</p> <p>The study considering the toxicity of active substance or its metabolite should be evaluated at EU level during substance renewal.</p>
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Reference:	KCP 10.4.2.1
Report	Schulz L. (2014) CGA249287 - Effects on the Reproduction of the Predatory Mite <i>Hypoaspis aculeifer</i> . Report Number 14 10 48 194 S. BioChem agrar Labor für biologische und chemische Analytik GmbH Kupferstraße 6, 04827 Gerichshain, Germany. (XXXX file No. VV-410243; CGA249287_10005)
Guideline(s):	OECD (2008). Effects on Biotic Systems, Method 226 Predatory mite (<i>Hypoaspis</i> (Geolaelaps) <i>aculeifer</i>) reproduction test in soil.
Deviation(s):	No
GLP:	Yes
Acceptability:	Yes
Duplication: (if vertebrate study)	No

Executive Summary

In a 14-day *Hypoaspis aculeifer* study with CGA249287 the NOEC for reproduction was determined to be 74 mg test item/kg soil dry weight. EC₁₀ and EC₂₀ values were estimated to be 70.5 and 321.3 mg CGA249287/kg, respectively.

Materials

Test Material	CGA249287
Lot/Batch #:	RL-2929
Actual content of active ingredients:	100% w/w
Description:	White solid
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	End of June 2018
Density:	Not stated
Treatments	
Test concentrations:	23, 41, 74, 133, 240, 432, 778, 1400 mg test item/kg soil d.w. (spacing factor: 1.8)
Control:	Untreated substrate, i.e. purified water only
Toxic standard:	Dimethoate (analysed purity: 99.8%, tolerance ± 1.0%)
Test organisms	
Species	<i>Hypoaspis aculeifer</i>
Source:	Originally obtained from Bayer CropScience AG and maintained at the Test Facility.
Food:	Before and during test every 2-3 days, Cheese mites, <i>Tyrophagus putrescentiae</i> (SCHRANK)
Age at test start:	Adults with an age difference of 3 days
Test design	
Vessels:	100 mL SCHOTT-bottles with screw cap (4 cm diameter, 11 cm high)
Substrate:	Artificial soil comprising 5% sphagnum peat, 20 % kaolinite clay, 74.8% industrial quartz sand and 0.2% calcium carbonate. 300g soil d.w. in control. 200g in treatment.
Replication:	Control group: 8 Treated group: 4
No. of mites/arena :	10 females
Duration of test:	14 days

Environmental conditions	test
Temperature:	19.7 - 21.4°C
pH:	5.5 – 6.0
Water content of soil:	46.39 - 51.14% of WHC
Photoperiod:	16 h light: 8 h dark (513 lux)

Study Design and Methods

Test facility: BioChem agrar Labor für biologische und chemische Analytik GmbH Kupferstraße 6, 04827 Gerichshain, Germany

Experimental dates: 8th to 27th August 2014

Adult females of the soil mite *Hypoaspis aculeifer* were exposed to concentrations of CGA249287 incorporated into the test soil. An exactly weighed amount of the test item was mixed with purified water to make a stock solution, and appropriate volumes of this stock solution were further diluted with purified water to obtain the test concentrations such that, when added to pre-moistened artificial soil, a final moisture content value of 50% WHC was achieved. Adult females were transferred to the test vessels which contained untreated (control), reference item or test item treated artificial soil. Ten adult females were introduced to each test vessel. As a source of food, cheese mites (*Tyrophagus putrescentiae*) were added to the soil surface of each test arena at the beginning of the test, and cheese mites ad libitum (every 2-3 days) throughout the test. The test was carried out under controlled light-dark cycle. Fourteen days after introducing the test organisms, the surviving mites and the juveniles of *Hypoaspis aculeifer* were extracted by heat/light extraction. From these data the mortality of the adult females and the reproductive output were calculated.

Mortality (number of dead adults) in % for the treatment groups was calculated. Missing mites were counted as dead. Observations in the treatment group were expressed relative to the control group. The corrected mortality in the treatment groups was calculated using Abbott's formula.

The reduction of reproductive output (Rr) for the treatment groups was calculated in comparison to the control.

$$Rr (\%) = (1 - Rt / Rc) * 100 \%$$

Rt and Rc are the absolute values observed in the treatment and control groups.

The statistical analysis was performed with the software ToxRat Professional 2.10.05. Fisher's Exact Binomial Test with Bonferroni Correction and the Williams-t-test were used to compare the control with the independent test item groups. Probit analysis was used for ECx calculation.

Results and Discussion

Mortality and fecundity are summarised in the table below.

Table A 59: Effects of residues of CGA249287 on mortality and reproduction of *Hypoaspis aculeifer*

Endpoint	Treatment group (mg CGA249287 /kg soil d.w.)								
	Control	23	41	74	133	240	432	778	1400
Mortality of adult mites after 14 days									
% mortality ^a	1.3	0	0	0	7.5	2.5	0	0	0
Number of juveniles after 14 days									

Mean no. progeny per replicate ^c	251.9	242.8	248.3	228.5	195.8*	205.3*	190.0*	196.8*	162.8*
standard deviation	35.1	47.1	17.8	29.8	17.3	9.6	21.8	18.7	47.0
coefficient of variation %	13.9	19.4	7.2	13.1	8.9	4.7	11.5	9.5	28.9
% reduction compared to control ^d	-	3.6	1.4	9.3	22.3	18.5	24.6	21.9	35.4

^a Mortality amongst mites originally introduced. Individual test-item treatments compared to the control using Fisher's Exact Test ($\alpha = 0.05$). Treatments that differed significantly from the control are indicated with an asterisk (*).

^b Calculated using Abbott's formula for corrected mortality (Abbott, 1925): $M (\%) = (1 - t/c) * 100\%$

^c The results for each treatment were individually compared to the control by one-way ANOVA and Dunnett's t-test ($\alpha = 0.05$). Values marked with an asterisk (*) differed significantly from the control.

^d Percent reduction: $(1 - R_t/R_c) * 100\%$, where R_t = mean number of juvenile mites observed in the treated group(s) and R_c = mean number of juvenile mites observed in the control group. A positive value indicates a decrease and a negative value indicates an increase in reproduction, relative to the control.

Validity Criteria

The validity criteria for the control group were met:

- Mean mortality of adult females: $\leq 20\%$ (observed: 1.3%)
- Mean number of juvenile per replicate: ≥ 50 (calculated: 251.9)
- Coefficient of variation (mean number of juveniles per replicate): $\leq 30\%$ (calculated: 13.9%)

Conclusions

In a 14-day *Hypoaspis aculeifer* study with CGA249287 the NOEC for reproduction was determined to be 74 mg test item/kg soil dry weight. EC_{10} and EC_{20} values were estimated to be 70.5 and 321.3 mg CGA249287/kg, respectively.

(Schulz L, 2014)

A 2.4.2.2 KCP 10.4.2.2 Higher tier testing

A 2.5 KCP 10.5 Effects on soil nitrogen transformation

Comments of zRMS:	<p>The submitted study was accepted.</p> <p>The validity criteria were met.</p> <p>No adverse effects on soil nitrogen transformation (measured as NO_3-N-production) and on soil carbon transformation (measured as O_2-consumption) at the end of the 28-day incubation period were observed.</p> <p>The effect less than 25% was observed at application rate of 2.65 and 13.24 mg A23282A/kg dw soil.</p>
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Reference: KCP 10.5

Report Schulz, L., (2021), Cyprodinil/Prothioconazole EC (A23282A) - Effects on the activity of soil microflora (nitrogen and carbon transformation tests), Report number 21 48 SMO 0018. BioChem agrar, Labor für biologische und

chemische, Analytik GmbH, Kupferstraße 6, 04827 Machern OT Gerichshain, Germany. (XXXX File No. VV-933827)

Guideline(s):	OECD guidelines 216, Soil Microorganisms: Nitrogen Transformation Test (2000) OECD guidelines 217, Soil Microorganisms: Carbon Transformation Test (2000)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Executive Summary

The test item A23282A (tested at 2.65 mg/kg soil dry weight, corresponding to 2 L test item/ha and 13.24 mg/kg soil dry weight, corresponding to 10 L test item/ha) caused no adverse effects (deviation from control < 25 %, OECD 216/217) on soil nitrogen transformation (measured as NO₃-N-production) and on soil carbon transformation (measured as O₂-consumption) at the end of the 28-day incubation period.

Materials

Test Material	Cyprodinil/Prothioconazole EC (A23282A)
Lot/Batch #:	LCR001-021-001 (1160912)
Actual content of active ingredients:	cyprodinil 22.1 % w/w corresponding to 219 g/L prothioconazole 7.40 % w/w corresponding to 73.5 g/L
Description:	yellow liquid
Stability of test compound:	Stable under the given conditions
Reanalysis/Expiry date:	End of September 2023
Density:	993 kg/m ³

Treatments

Test concentrations:	2.65 mg test item/kg soil dry weight (2 L test item/ha) 13.24 mg test item/kg soil dry weight (10 L test item/ha)
Control:	Deionised water
Toxic standard:	Dinoterb (tested in separate GLP study)

Test design

Soil type:	loamy sand (DIN ISO 11277; DIN 4220)
Test units:	Nitrogen transformation test: wide-mouth glass flasks (500 mL) Carbon transformation test: steel vessels (4 L)
Replication:	3
Sampling intervals :	0 (3 hours after application), 7, 14 and 28 days
Duration of test:	28 days

Environmental test conditions

Temperature:	19.4 - 20.5 °C
pH of soil:	Nitrogen transformation test: 5.7 - 5.8, Carbon transformation test: 5.7 - 5.8
Soil moisture content:	Approximately 45 % of maximum water holding capacity

Photoperiod: Continuous darkness

Study Design and Methods

Test facility: BioChem agrar GmbH, Kupferstraße 6, 04827 Machern OT Gerichshain, Germany

Experimental dates: 1st to 29th September 2021.

Soil samples were treated with A23282A at two doses, 2.65 and 13.24 mg A23282A/kg dry soil (corresponding to 2 L test item/ha and 10 L test item/ha, respectively). Test concentrations related to a soil depth of 5 cm and a soil density of 1.5 g/cm³.

The test item was mixed with deionised water and the test solution was subsequently mixed with the soil (carbon transformation test: laboratory mixer, nitrogen transformation test: hand stirrer). Water was added to the soil to achieve a water content of approximately 45 % of WHC. The water content of the soil in each test vessel was determined at test start (after application) and adjusted once a week to the required range of 40 - 50 % of WHC.

Three replicate soil samples were prepared for each treatment rate and the control for the nitrogen transformation test and carbon transformation test.

Mean nitrogen content (mg NO₃/kg soil d.w.), standard deviation and coefficient of variation as well as the mean nitrogen content/day (mg NO₃/kg soil d.w./day) were calculated for each treatment group and sampling date.

For the evaluation of the results the relative deviations (%) of the test item treatment groups from the control were calculated (based on the mean nitrogen content/day) for each sampling date.

The cumulative O₂ consumption after 12 hours was calculated (using regression analysis; the goodness of fit (R²) was > 0.99 in all replicates and on all days). Furthermore, standard deviation and coefficient of variation were calculated for each treatment group and sampling dates.

For evaluation of the results the relative deviations (%) of the test item treatment groups from the control were calculated for each sampling date. A 2-sided Student-t-test at 5 % significance level was performed for statistical evaluation.

Results

Results from the Nitrogen transformation test and the Carbon transformation test are summarised in the tables below.

Table A 60: Effects on Nitrogen Transformation in Soil after Treatment with A23282A

Time Interval (days)	Control		2.65 mg test item/kg soil dry weight			13.24 mg test item/kg soil dry weight		
	NO ₃ -N [mg/kg soil d.w.]	NO ₃ -N [mg/kg soil d.w./day]	NO ₃ -N [mg/kg soil d.w.]	NO ₃ -N [mg/kg soil d.w./day]	Deviation from control [%] ¹⁾	NO ₃ -N [mg/kg soil d.w.]	NO ₃ -N [mg/kg soil d.w./day]	Deviation from control [%] ¹⁾
0 - 7	47.9	2.50	46.5	2.35	-5.7	47.5	2.47	-1.0
0 - 14	72.3	2.99	68.3	2.74*	-8.6	69.8	2.83	-5.6
0 - 28	86.6	2.01	86.2	2.01	+0.1	85.4	1.97	-1.7

¹⁾ based on NO₃-nitrogen-production; - = inhibition; + = stimulation

* = statistically significant differences between the control and the test item treatments were calculated (Student-t-test for homogeneous variances, 2-sided, α = 0.05).

The calculations were performed with non-rounded values.

Table A 61: Effects on Carbon Transformation in Soil after Treatment with A23282A

Days after application	Control	2.65 mg test item/kg soil dry weight			13.24 mg test item/kg soil dry weight		
	O ₂ -consumption [mg/kg soil d.w./h]	O ₂ -consumption [mg/kg soil d.w./h]	CV [%]	Deviation from control [%] ¹⁾	O ₂ -consumption [mg/kg soil d.w./h]	CV [%]	Deviation from control [%] ¹⁾
0	8.61	8.79	+2.1	+2.1	8.91	+3.4	+3.4
7	8.07	8.23	+1.9	+1.9	7.98	-1.1	-1.1
14	8.17	8.17	0.0	±0.0	8.06	-1.4	-1.4
28	7.69	7.56	-1.6	-1.6	7.55	-1.8	-1.8

¹⁾ based on O₂-consumption; - = inhibition; + = stimulation

Not statistically significantly different to control (Student-t-test for homogeneous variances, 2-sided, $\alpha = 0.05$)

The calculations were performed with non-rounded values.

In a separate GLP study the reference item was tested at concentrations of 6.80, 13.60 and 27.20 mg/kg dry soil. (start date: 5th January 2021) the reference item Dinoterb caused a stimulation of nitrogen transformation of +26.9 %, +43.2 % and +27.2 % at 6.80 mg, 13.60 mg and 27.20 mg Dinoterb/kg soil dry weight, respectively, and an inhibition of carbon transformation of -25.4 %, -42.4 % and -48.3 % at 6.80 mg, 13.60 mg and 27.20 mg Dinoterb per kg soil dry weight, respectively, 28 days after application.

Validity criteria

The validity criteria are listed below:

- The coefficient of variation in the control group of the nitrogen and carbon transformation tests (for the whole test duration) were at maximum 5.2 and 3.6 % respectively (must be ≤ 15 %)
- The toxic standard caused effects of +26.9 %, +43.2 % and +27.2 % at concentrations 6.80, 13.60 and 27.20 mg Dinoterb/kg soil d.w. in the Nitrogen transformation test after a 28 day exposure, demonstrating the sensitivity of the test system (must be ≥ 25 %)
- The toxic standard caused effects of -25.4 %, -42.4 % and -48.3 % at concentrations 6.80, 13.60 and 27.20 mg Dinoterb/kg soil d.w., respectively in the Carbon transformation test after a 28 day exposure, demonstrating the sensitivity of the test system (must be ≥ 25 %)

Conclusion

The test item A23282A (tested at 2.65 mg/kg soil dry weight, corresponding to 2 L test item/ha, and 13.24 mg/kg soil dry weight, corresponding to 10 L test item/ha) caused no adverse effects (deviation from control < 25 %, OECD 216/217) on soil nitrogen transformation (measured as NO₃-N-production) and on soil carbon transformation (measured as O₂-consumption) at the end of the 28-day incubation period.

(Schulz L., 2021)

During the review of the B9 the expert from the NL made this comment:

It is not clear from the study evaluation whether the total nitrate formation is reported over 0-7, 0-14, 0-28 h, etc. or nitrate formation rates over each interval 0-7, 7-14, 14-28 h, etc. The OECD guidance states that the results should be based on nitrogen formation rate, "After 0, 7, 14 days and 28 days of incubation, samples of treated and control soils are extracted with an appropriate solvent, and the quantities of nitrate in the extracts are determined. The rate of nitrogen formation in treated samples is compared with the rate in the controls, and the percent deviation of the treated from the control is calculated."

Although the guidance does not specify that this should be done per interval, this is the only way the test would yield useful information. For instance, if total nitrogen or nitrogen formation rates at d 0-28 are considered, sampling at day 7 and 14 would be meaningless. The zRMS is requested to clarify this.

It is not clear to the Applicant what the expert is referring to. The results in Table A60 are a fair reflection of the values presented in the report, and this is the way that the data on nitrate formation rates are always presented by Contract Research Organisations across the EU. As the zRMS stated:

The submitted study was accepted.

The validity criteria were met.

No adverse effects on soil nitrogen transformation (measured as NO₃-N-production) and on soil carbon transformation (measured as O₂-consumption) at the end of the 28-day incubation period were observed.

The effect less than 25% was observed at application rate of 2.65 and 13.24 mg A23282A/kg dw soil.

Comments of zRMS:	The test was not evaluated. The study considering the toxicity of active substance or its metabolite should be evaluated at EU level during substance renewal.
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Reference:	KCP 10.5
Report	Hammesfahr U. (2015) CGA321915 - Effects on Activity of Soil Microflora (Carbon and Nitrogen Transformation) in the Laboratory, Report Number 96341080, Institut für Biologische Analytik und Consulting, iBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany. (XXXX file No. VV-412058; CGA321915_10008).
Guideline(s):	OECD guidelines 216, Soil Microorganisms: Nitrogen Transformation Test (2000) OECD guidelines 217, Soil Microorganisms: Carbon Transformation Test (2000)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

CGA321915 was applied to the soil at concentrations of 1.53 mg CGA321915/kg soil d.w. and 5.10 mg CGA321915/kg soil d.w. The test item CGA321915 caused no adverse effects on soil nitrogen transformation (measured as NO₃-N-production) or on soil carbon transformation (measured as CO₂-production) at the end of the 28-day incubation period.

Materials

Test Material	CGA321915 CSAA400257
Parent:	CGA219417 (Cyprodinil)
Lot/Batch #:	MES 356/1
Purity:	98 % w/w ± 2 %

Description:	White solid
Stability of test compound:	Stable under test conditions
Reanalysis/Expiry date:	30 September 2016
Density:	Not applicable

Treatments

Test concentrations:	1.53 and 5.10 mg CGA321915/kg soil d.w.
Control:	Deionised water only
Toxic standard:	Sodium chloride (99.9 % purity) at a concentration of 16.0 g/kg (Separate study – IBACON study code: 30699080, date May 2014 to August 2014)

Test design

Soil type:	Silty sand soil collected from a site in the district of Darmstadt-Dieburg, Germany; 7.5 % clay, 29.3 % silt and 63.2 % sand
Test units:	Nitrogen transformation test: 250 to 500 g soil dry weight in approximately 0.5 L plastic boxes (dimensions 0.10 m width x 0.10 m depth x 0.065 m height) filled up to 6 cm, covered with perforated lids Carbon transformation test: 750 to 1000 g soil dry weight in approximately 1 L plastic boxes (dimensions 0.12 m width x 0.165 m depth x 0.065 m height) filled up to 6 cm, covered with perforated lids
Replication:	3 per treatment rate and control
Sampling intervals:	≤ 6 hours, and 7, 14 and 28 days after application
Duration of test:	28 days
Environmental test conditions	
Temperature:	20 ± 2 °C
pH of soil:	7.0 – 7.2
Soil moisture content:	48 to 55 % of WHC
Photoperiod:	Constant darkness

Study Design and Methods

Experimental dates: 27th January to 27th February 2015

Soil samples were treated with CGA321915 at two doses, 1.53 and 5.10 mg CGA321915/kg dry soil. Test concentrations related to a soil depth of 5 cm and a soil density of 1.5 g/cm³.

The test item was mixed with deionised water and the test solution was subsequently mixed with the soil in the laboratory mixer. Water was added to the soil to achieve a water content of 49 - 52 % of WHC. The water content of the soil in one replicate of each treatment group was determined at each sampling date, and water losses were compensated by adding pure water.

Three replicate soil samples were prepared for each treatment rate and the control for the nitrogen transformation test and carbon transformation test.

Mean nitrogen content (mg NO₃--N/kg soil d.w), standard deviation and coefficient of variation as well as the mean nitrate nitrogen content/day (mg NO₃/kg soil d.w./day) were calculated for each treatment group and sampling date.

For the evaluation of the results the relative deviations (%) of the test item treatment groups from the control were calculated for each sampling date. A Student t-test (pair wise comparison, two-sided, $\alpha = 0.05$) was performed for statistical evaluation.

Mean O₂ consumption after 12 hours was calculated using regression analysis. Furthermore, standard

deviation and coefficient of variation were calculated for each treatment group and sampling date.

For the evaluation of the results the relative deviations (%) of the test item treatment groups from the control were calculated for each sampling date. A Student t-test (pair wise comparison, two-sided, $\alpha = 0.05$) was performed for statistical evaluation.

Results and Discussion

Results from the Nitrogen transformation test and the Carbon transformation test are summarised in the tables below.

Table A 62: Effects on nitrogen transformation in soil after treatment with CGA321915

Time Interval (days)	Control			1.53 mg CGA321915/kg soil dry weight				5.10 mg CGA321915/kg soil dry weight			
	NO ₃ -N [mg/kg soil d.w.]	NO ₃ -N [mg/kg soil d.w./day]	% CV	NO ₃ -N [mg/kg soil d.w.]	NO ₃ -N [mg/kg soil d.w./day]	Deviation from control [%] ¹⁾	% CV	NO ₃ -N [mg/kg soil d.w.]	NO ₃ -N [mg/kg soil d.w./day]	Deviation from control [%] ¹⁾	% CV
0 - 7	14.336	-0.275	-32.36	14.244	-0.279	1.45	-14.70	15.000	-0.204	-25.82	-25.98
0 - 14	19.869	0.258	5.43	19.563	0.240	-6.98	12.08	20.421	0.285	10.47	6.67
0 - 28	35.772	0.697	2.30	35.135	0.676	-3.01	0.89	37.189	0.741	6.31	4.72

The calculations were performed with non-rounded values

CV [%] = Coefficient of Variation

¹⁾ based on NO₃-nitrogen production; - = inhibition; + = stimulation

No statistically significant differences between the control and the test item treatments were calculated

Table A 63: Effects on carbon transformation in soil after treatment with CGA321915

Days after application	Control		1.53 mg CGA321915/kg soil dry weight			5.10 mg CGA321915/kg soil dry weight		
	CO ₂ – production [mg CO ₂ /kg soil d.w./h]	CV [%]	CO ₂ –production [mg CO ₂ /kg soil d.w./h]	CV [%]	Deviation from control [%] ¹⁾	CO ₂ –production [mg CO ₂ /kg soil d.w./h]	CV [%]	Deviation from control [%] ¹⁾
0	12.184	2.05	12.598	3.24	3.40	12.201	1.94	0.14
7	13.055	1.03	12.902	2.14	-1.17	12.862	0.83	-1.48
14	14.671	0.66	15.231	3.17	3.82	15.080	0.90	2.79
28	8.864	3.77	9.057	1.93	2.18	8.835	4.04	-0.33

The calculations were performed with non-rounded values

CV [%] = Coefficient of Variation

¹⁾ based on CO₂ production; - = inhibition; + = stimulation

No statistically significant differences between the control and the test item treatments were calculated

Validity criteria

The validity criteria are listed below:

- The coefficient of variation in the control replicates of the nitrogen and carbon transformation tests after 28 days were 2.30 % and 3.77 %, respectively (must be ≤ 15 %)
- The toxic standard caused an effect of -83.45 % at a concentration of 16 g/kg soil d.w. in the Nitrogen transformation test at Day 28, demonstrating the sensitivity of the test system (must be ≥ 25 %)

- The toxic standard caused an effect of -67.21 % at a concentration of 16 g/kg soil d.w. in the Carbon transformation test at Day 28, demonstrating the sensitivity of the test system (must be ≥ 25 %)

Conclusions

CGA321915 was applied to the soil at concentrations of 1.53 mg CGA321915/kg soil d.w. and 5.10 mg CGA321915/kg soil d.w. The test item CGA321915 caused no adverse effects on soil nitrogen transformation (measured as NO₃-N-production) and on soil carbon transformation (measured as CO₂-production) at the end of the 28-day incubation period.

(Hammesfahr U, 2015)

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 submitted study was accepted The validity criteria were met. A minor deviation had no impact on final results.</p> <p>The following biological observations were noted:</p> <ul style="list-style-type: none"> • seedling emergence: none of the tested species showed any phytotoxic effects up to and including the top treatment rate of 2 L A23282A /ha; • vegetative vigour: Onion and wheat did not show any phytotoxic effects up to and including the top treatment rate 2000 mL A23282A/ha. Sugar beet was the most sensitive species showing phytotoxic effects starting at 250 mL A23282A up to and including 2000 mL A23282A. Soybean showed slight phytotoxic effects at 500, 1000 and 2000 mL A23282A/ha. Cucumber showed phytotoxic effects at 1000 and 2000 mL A23282A/ha. Oilseed rape showed slight phytotoxic effects at the top rate of 2000 mL A23282A. <p>The ER₅₀ for all species tested are >2000 mL A23282A/ha.</p> <p>The study was accepted as a supplementary one</p>
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Reference:	KCP 10.6.1
Report	Jones K., 2021, Cyprodinil/prothioconazole EC (A23282A) - Phytotoxicity to non-target plants screening test, Report No. ACE-21-258. AgroChemex Environmental Ltd., Aldhams Farm Research Station, Dead Lane, Lawford, Manningtree, Essex, CO11 2NF, United Kingdom, ACE-21-25. (XXXX File No. VV-921642)
Guideline(s):	<ul style="list-style-type: none"> - Study was carried out following a standardised study protocol based on XXXX herbicide profiling test. - Study design based on: OECD 208 and OECD 227
Deviations:	No. None that had an impact on the study results.
GLP:	Yes. No claim of GLP was made for the soil analysis.
Acceptability:	Yes

Executive Summary

The effects of A23282A on the seedling emergence and vegetative vigour of six non-target plant species (onion, wheat, sugar beet, oilseed rape, cucumber and soybean) were assessed following a range of applications, 0 (deionised water only), 62.5, 125, 250, 500, 1000 and 2000 mL A23282A/ha.

For seedling emergence, none of the tested species showed any phytotoxic effects up to and including the top treatment rate of 2000 mL A23282A /ha.

For vegetative vigour, onion and wheat did not show any phytotoxic effects up to and including the top treatment rate of 2000 mL A23282A/ha. Sugar beet was the most sensitive species showing slight phytotoxic effects starting at 250 mL A23282A up to and including 2000 mL A23282A. Soybean showed slight phytotoxic effects at 500, 1000 and 2000 mL A23282A/ha. Cucumber showed slight phytotoxic effects at 1000 and 2000 mL A23282A/ha. Oilseed rape showed slight phytotoxic effects at the top treatment rate of 2000 mL A23282A.

Materials

Test material	A23282A
Lot/Batch #:	LCR001-021-001
Actual content of active ingredients:	Cyprodinil: 22.1% w/w corresponding to 219 g/L Prothioconazole: 7.40% w/w corresponding to 73.5 g/L
Description:	Yellow liquid
Stability of test compound:	Stable under normal conditions
Recertification date:	End of September 2023
Density:	993 kg/m ³ (0.993 g/mL)

Treatments

Test concentrations:	62.5, 125, 250, 500, 1000 and 2000 mL A23282A/ha
Control:	Deionised water
Spray volume:	200 L/ha ± 10%
Application method:	Mardrive cabinet track sprayer

Test organisms

Species:	Onion (<i>Allium cepa</i>), Wheat (<i>Triticum aestivum</i>), Sugar beet (<i>Beta vulgaris</i>), Oilseed rape (<i>Brassica napus</i>), Cucumber (<i>Cucumis sativus</i>), Soybean (<i>Glycine max</i>).
Test soil:	The soil was mixed in a ratio of approximately 20 L sterile loam, 10 L sand and 4 L grit (granite chippings 2-6 mm). 25 g Osmocote® Start and 100 g Osmocote® Pro (slow-release fertilisers) were incorporated into 34 litres of soil mix prior to study start. Organic carbon content 1.3 %.

Test design

Test vessels:	Non-porous plastic pots were used (9 x 9 x10 cm; W x D x H).
Sampling interval:	Seedling emergence: Visual phytotoxicity assessment undertaken 28 days after application of the test item. Vegetative vigour: Visual phytotoxicity assessment undertaken 21 days after application of the test item.
Replication:	Three pots per treatment with four seeds/plants per pot (six seeds/plants for onion).
Duration:	28 days for seedling emergence test; 21 days for vegetative vigour test.

Environmental conditions

Test temperature:	Min: 19.0°C – Max: 32.5°C (Mean: 22.9°C)
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Humidity:	Min: 37.5% – Max: 77.9% (Mean: 56.9%)
Soil pH:	7.9
Lighting:	Min: 0.4 kilolux – Max: 80.1 kilolux (Mean: 15.8 kilolux)

Study Design and Methods

Test facility: AgroChemex Environmental Ltd., Aldhams Farm Research Station, Dead Lane, Lawford, Manningtree, Essex, CO11 2NF, United Kingdom.

Experimental dates: 15th June 2021 (Date of first application) to 13th July 2021 (Final assessment – glass house phase).

Foliage of plants of two monocot species (*Allium cepa*, and *Triticum aestivum*) and four dicot species (*Beta vulgaris*, *Brassica napus*, *Cucumis sativus* and *Glycine max*) were sprayed with A23282A to assess the vegetative vigour. For the seedling emergence test, A23282A was applied directly to the soil.

For the vegetative vigour, seeds were germinated in seed trays of Levington F1 compost and four seedlings (six for onion) were transplanted shortly after emergence at BBCH Growth Stage 10 into plastic pots with the test soil. After planting, the pots were placed in the glasshouse where the seedlings were allowed to develop into plants with two to four true leaves (BBCH growth 12 -14) before being used in the study.

For the seedling emergence, four seeds (six for onion) were sown in plastic pot with a depth of 1-2 cm prior to application.

On the day of application, a primary stock solution was prepared by diluting 2.9804 g of A23282A into 300 mL deionised water. This solution was mixed by swirling and inversion. This solution served as the spray mixture for the 2000 mL A23282A/ha application rate. Lower dose rates (1000, 500, 250, 125 and 62.5 mL A23282A/ha) were prepared by serial dilution.

The spray solutions were not analysed to determine the active ingredient concentration and no statistical analysis was required for this study.

At the final assessments for seedling emergence and vegetative vigour the level of phytotoxicity was recorded using a visual scale of 0 to 100%:

Table A 64: Assessment of Injury Scale

Nominal % effect	Description of effects
0	Vigorous healthy plants, emergence of normal amount of seeds, indistinguishable from control
10	Vigorous, but with slight discoloration, malformation or stunting – slightly impaired emergence, growth or development
20	Less vigorous, with discoloration, malformation or stunting – slightly impaired growth and development, recovery likely, rate of emergence slightly reduced
30	Less vigorous, with obvious discoloration, malformation or stunting – impaired growth and development, recovery likely, rate of emergence reduced
40	Less vigorous, with more pronounced discoloration, malformation or stunting – recovery possible, clear reduction of rate of emergence
50	Poor vigour due to discoloration, malformation or stunting – recovery possible, emergence rate only about half of the control
60	Poor vigour due to discoloration, malformation or stunting and senescence – recovery doubtful, emergence of only a minor part of the seeds
70	Very poor vigour due to discoloration, malformation, stunting or senescence – still growing but recovery unlikely, emergence of few seeds only
80	Very poor vigour due to severe discoloration, malformation, stunting or senescence – recovery

	unlikely, emergence of very few seeds only
90	Very poor vigour – not all tissue dead but further growth unlikely, only some germination
100	Complete destruction of plant parts above ground, complete inhibition of germination

Results

The results of the seedling emergence test are summarised in the table below:

Table A 65: Emergence in the seedling emergence test

Test species	Total number of seeds (day 0)	Emergence, Total number (mean % of emerged seedlings)						
		Application rate (mL A23282A/ha)						
		0 (deionised water only)	62.5	125	250	500	1000	2000
Onion	18	17 (94)	18 (100)	17 (94)	16 (89)	16 (89)	16* (89)	17 (94)
Wheat	12	11 (92)	12 (100)	11 (92)	11 (92)	11 (92)	12 (100)	11 (92)
Sugar beet	12	11 (92)	12 (100)	12 (100)	12 (100)	12 (100)	12 (100)	12 (100)
Oilseed rape	12	10 (83)	11 (92)	10 (83)	11 (92)	10 (83)	9 (75)	10 (83)
Cucumber	12	12 (100)	12 (100)	12 (100)	12 (100)	12 (100)	12 (100)	12 (100)
Soybean	12	12 (100)	12 (100)	11 (92)	10 (83)	12 (100)	12 (100)	12 (100)

*In one of the onion replicates at treatment rate 1000 mL A23282A/ha, five plants had emerged, but one subsequently died. All remaining plants in the replicate did not show any phytotoxic effects.

Table A 66: Visual Observation of phytotoxicity in the seedling emergence test

Application rate (mL A23282A /ha)	0 (deionised water only)	62.5	125	250	500	1000	2000
Onion	0	0	0	0	0	0	0
Wheat	0	0	0	0	0	0	0
Sugar beet	0	0	0	0	0	0	0
Oilseed rape	0	0	0	0	0	0	0
Cucumber	0	0	0	0	0	0	0
Soybean	0	0	0	0	0	0	0

The results of the visual observation of phytotoxicity in the vegetative vigour are given in the table below.

Table A 67: Visual observation of phytotoxicity in the vegetative vigour test

Application rate (mL A23282A /ha)	Phytotoxicity scale from 0 – 100%						
	0 (deionised water only)	62.5	125	250	500	1000	2000
Onion	0	0	0	0	0	0	0
Wheat	0	0	0	0	0	0	0

Sugar beet	0	0	0	5	10	22	32
Oilseed rape	0	0	0	0	0	0	12
Cucumber	0	0	0	0	0	7	25
Soybean	0	0	0	0	5	23	30

Validity criteria

The test was considered valid since;

- The seedling emergence in the controls was 83-100% (must be $\geq 70\%$)
- The control plants did not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis, and wilting, leaf and stem deformation) and the plants exhibited only normal variation in growth and morphology for that particular species.
- Environmental conditions for a particular species were identical and growing media contained the same amount of soil matrix, support media or substrate from the same source.

Conclusion

The effects of A23282A on the seedling emergence and vegetative vigour of six non-target plant species were assessed.

Seedling emergence and growth:

None of the tested species showed any phytotoxic effects up to and including the top treatment rate of 2000 mL A23282A /ha.

Vegetative vigour:

Onion and wheat did not show any phytotoxic effects up to and including the top treatment rate 2000 mL A23282A/ha.

Sugar beet was the most sensitive species showing phytotoxic effects starting at 250 mL A23282A up to and including 2000 mL A23282A. Soybean showed slight phytotoxic effects at 500, 1000 and 2000 mL A23282A/ha. Cucumber showed phytotoxic effects at 1000 and 2000 mL A23282A/ha. Oilseed rape showed slight phytotoxic effects at the top rate of 2000 mL A23282A.

(Jones K, 2021)

A 2.6.2 KCP 10.6.2 Testing on non-target plants

A 2.6.3 KCP 10.6.3 Extended laboratory studies on non-target plants

A 2.6.4 KCP 10.6.4 Semi-field and field tests on non-target plants

A 2.7 KCP 10.7 Effects on other terrestrial organisms (flora and fauna)

A 2.8 KCP 10.8 Monitoring data