FINAL REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: Protiokonazol 300 EC

Product name(s): HERA 300 EC

Chemical active substance:

prothioconazole, 300 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: Pestila Spółka z ograniczoną odpowiedzialnością

Submission date: October 2023

MS Finalisation date: March 2024; July 2024; February 2025

Version history

When	What
March 2024	ZRMs evaluated dRR submitted by Applicant.
July 2024	The final Registration Report
February 2025	Updated version

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9 Ecotoxicology (KCP 10)

9.1 Critical GAP and overall conclusions

Table 9.1-1: Table of critical GAPs

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Use-No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	**Application**				**Application rate**			PHI (days)	Remarks: e.g. g safener/ synergist per ha	**Conclusion**						
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms	Non-target plants
Zonal uses (field or outdoor uses, certain types of protected crops)																				
1	Poland	Winter wheat	F	Controlled diseases - for details please refer to dRR Part B0 and B3	broadcast spraying	BBCH 29-65 Spring, post emergence	1 a) 1 b) 2	14	0.5 – 0.65 L/ha a) 0.65 L/ha b) 1.3 L/ha	150-195 g a) 195 g b) 390 g	100-400 L/ha	35		A	A	R	A	A	A	A
2	Poland	Spring wheat	F		broadcast spraying	BBCH 29-65 Spring, post emergence	1 a) 1 b) 2	14	0.5 – 0.65 L/ha a) 0.65 L/ha b) 1.3 L/ha	150-195 g a) 195 g b) 390 g	100-400 L/ha	35		A	A	R	A	A	A	A
3	Poland	Winter triticale	F		broadcast spraying	BBCH 29-65 Spring, post emergence	1 a) 1 b) 2	14	0.5 – 0.65 L/ha a) 0.65 L/ha b) 1.3 L/ha	150-195 g a) 195 g b) 390 g	100-400 L/ha	35		A	A	R	A	A	A	A
4	Poland	Spring triticale	F		broadcast spraying	BBCH 29-65 Spring, post emergence	1 a) 1 b) 2	14	0.5 – 0.65 L/ha a) 0.65 L/ha b) 1.3 L/ha	150-195 g a) 195 g b) 390 g	100-400 L/ha	35		A	A	R	A	A	A	A
5	Poland	Spring barley	F		broadcast spraying	BBCH 29-65 Spring, post emergence	1 a) 1 b) 2	14	0.5 – 0.65 L/ha a) 0.65 L/ha b) 1.3 L/ha	150-195 g a) 195 g b) 390 g	100-400 L/ha	35		A	A	R	A	A	A	A
6	Poland	Winter barley	F		broadcast spraying	BBCH 29-65 Spring, post emergence	1 a) 1 b) 2	14	0.5 – 0.65 L/ha a) 0.65 L/ha	150-195 g a) 195 g b) 390 g	100-400 L/ha	35		A	A	R	A	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Use-No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	**Application**				**Application rate**			PHI (days)	Remarks: e.g. g safener/ synergist per ha	**Conclusion**						
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms	Non-target plants
Zonal uses (field or outdoor uses, certain types of protected crops)																				
									b) 1.3 L/ha											
7	Poland	Rye	F		broadcast spraying	BBCH 29-65 Spring, post emergence	1 a) 1 b) 2	14	0.5 – 0.65 L/ha a) 0.65 L/ha b) 1.3 L/ha	150-195 g a) 195 g b) 390 g	100-400 L/ha	35		A	A	R	A	A	A	A
8	Poland	Winter oilseed rape	F		broadcast spraying	BBCH 13-19 Autumn, post emergence	1 a) 1 b) 1	not relevant	0.5 – 0.6 L/ha a) 0.6 L/ha b) 0.6 L/ha	150-180 g a) 180 g b) 180 g	100-400 L/ha	56		A	A	R	A	A	A	A
9	Poland	Winter oilseed rape	F		broadcast spraying	BBCH 61-72 Spring, post emergence	1 a) 1 b) 2	21	0.5 – 0.6 L/ha a) 0.6 L/ha b) 1.2 L/ha	150-180 g a) 180 g b) 360 g	100-400 L/ha	56		A	A	R	A	A	A	A
Interzonal uses (use as seed treatment, in greenhouses (or other closed places of plant production), as post-harvest treatment or for treatment of empty storage rooms)																				
-	-	-	-	-	-	-	-	-	-	-	-	-	-							
Minor uses according to Article 51 (field uses)																				
10	PL	Spring oilseed rape	F	Controlled diseases - for details please refer to dRR Part B0 and B3	broadcast spraying	BBCH 16-69 Spring, post emergence	1 a) 1 b) 2	14-21	0.5 – 0.6 L/ha a) 0.6 L/ha b) 1.2 L/ha	150-180 g a) 180 g b) 360 g	100-400 L/ha	56	-	A	A	R	A	A	A	A
Minor uses according to Article 51 (interzonal uses)																				
-	-	-	-	-	-	-	-	-	-	-	-	-	-							

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

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

Explanation for column 15 – 21 “Conclusion”

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

Remarks table:

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

zRMS comments: All comments and conclusions of the zRMS are presented in grey. Minor changes are introduced directly in the text and highlighted in grey. Not agreed or not relevant information is struck through and shaded for transparency.

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

Effects on birds for Protiokonazol 300 EC were not evaluated as part of the EU review of prothioconazole. However further data on Protiokonazol 300 EC is not relevant as data on toxicity to birds for active substance are considered essential. It is possible to extrapolate from data for active substance. Therefore, all relevant data were assessed in the EU review. Risk assessments for Protiokonazol 300 EC with the proposed use pattern and EU agreed endpoints have been provided and are considered adequate.

The risk assessment for effects on birds was carried out according to the latest guidance for risk assessment for birds and mammals EFSA Journal 2009; 7(12): 1438.

The acute and reproductive risks of Protiokonazol 300 EC to birds were assessed from toxicity exposure ratios between EU agreed toxicity endpoints, estimated from studies with active substance and prothioconazole-desthio (JAU 6476-desthio) (M04), as well as SV_{90} and SV_m.

Drinking water exposure (leaf scenario) has not been estimated since Protiokonazol 300 EC is not intended to be applied on leafy vegetables forming heads or crop plants with comparable water collecting structures. Drinking water exposure (puddle scenario) has not been performed since the ratio of effective application rate to relevant endpoints does not exceed 3000 ($Koc \geq 500$ L/kg).

Exposure for earthworm-eating birds and fish-eating birds via secondary poisoning has been estimated since log P_{ow} of prothioconazole, prothioconazole-desthio (JAU 6476-desthio) (M04) and prothioconazole-S-methyl (M01) are above the trigger value of 3. The long term secondary poisoning risk of Protiokonazol 300 EC to birds were assessed from toxicity exposure ratios between EU agreed toxicity endpoints, estimated from studies with active substance and metabolites, as well as 21d PECs and PEC in worms and fishes.

The TER values where applicable exceed the trigger values of 10 for acute and 5 for reproductive and long-term risk, thus indicating no unacceptable risk to birds from the proposed use of Protiokonazol 300 EC. No risk management measures are required.

zRMS comment: Agreed.

Terrestrial vertebrates (other than birds)

Effects on mammals for Protiokonazol 300 EC were not evaluated as part of the EU review of prothioconazole. However further data on Protiokonazol 300 EC is not relevant as toxicity data to mammals for active substance are considered essential. It is possible to extrapolate from data for active substance. Therefore, all relevant data were assessed in the EU review. Risk assessments for Protiokonazol 300 EC with the proposed use pattern and EU agreed endpoints have been provided and are considered adequate.

The risk assessment for effects on terrestrial vertebrates other than birds was carried out according to the latest guidance for risk assessment for birds and mammals EFSA Journal 2009; 7(12): 1438.

The acute and reproductive risks of Protiokonazol 300 EC to mammals were assessed from toxicity exposure ratios between EU agreed toxicity endpoints, estimated from studies with active substance and prothioconazole-desthio (JAU 6476-desthio) (M04), as well as SV_{90} and SV_m.

Drinking water exposure (leaf scenario) has not been estimated since Protiokonazol 300 EC is not intended to be applied on leafy vegetables forming heads or crop plants with comparable water collecting structures. Drinking water exposure (puddle scenario) has not been performed since the ratio of effective application rate to relevant endpoints does not exceed 3000 (Koc ≥ 500 L/kg).

Exposure for earthworm-eating mammals and fish-eating mammals via secondary poisoning has been estimated since log P_{ow} of prothioconazole, prothioconazole-desthio (JAU 6476-desthio) (M04) and prothioconazole-S-methyl (M01) are above the trigger value of 3. The long term secondary poisoning risk of Protiokonazol 300 EC to mammals were assessed from toxicity exposure ratios between EU agreed toxicity endpoints, estimated from studies with active substance and metabolites, as well as 21d PECs and PEC in worms and fishes.

The TER values where applicable exceed the trigger values of 10 for acute and 5 for reproductive and long-term risk, thus indicating no unacceptable risk to mammals from the proposed use of Protiokonazol 300 EC. No risk management measures are required.

zRMS comment: Agreed.

9.1.1.2 Effects on aquatic organisms (KCP 10.2)

Effects on aquatic organisms for Protiokonazol 300 EC were not evaluated as part of the EU review of prothioconazole. The studies on effects of Protiokonazol 300 EC on *Daphnia magna* and algae were submitted in this dossier and deemed acceptable for evaluation and authorisation of Protiokonazol 300 EC.

Risk assessments for Protiokonazol 300 EC with the proposed use pattern was carried out according to the latest guidance for risk assessment for aquatic organisms in edge-of-field surface water EFSA Journal 2013; 11(7):3290.

PEC/RAC values were calculated on the basis of PECsw values as well as worst case toxicity endpoints from studies for active substance, metabolites and formulation Protiokonazol 300 EC. PECsw Step 1-3/RAC values were less than 1 for most scenarios indicating acceptable risk. In case of D2, R1, R2, R3, R4 stream, R3 stream further evaluation with Step 4 PECsw was performed.

On the basis of conducted risk assessment it was concluded that Protiokonazol 300 EC does not pose unacceptable risk provided following risk mitigations are applied for the following scenarios:

<u>Winter cereals (max. application rate 2x195 g a.s./ha, interval 14d, BBCH 29-65):</u>
- R1 stream, R4 stream – 5m buffer zone
- R3 stream – 10m buffer zone.
-

<u>Spring cereals (max. application rate 2x195 g a.s./ha, interval 14d, BBCH 29-65):</u>
- R1 stream, R2 stream, R3 stream, R4 stream – 5m buffer zone.

<u>Winter oilseed rape (max. application rate 2x180 g a.s./ha, interval 21d, BBCH 61-72):</u>
- R1 stream, R3 stream – 5m buffer zone.

<u>Winter oilseed rape (max. application rate 180 g a.s./ha, BBCH 13-19):</u>
- R3 stream – 5m buffer zone,
- D2 ditch and D2 stream - further risk refinement is necessary at national level.

Spring oilseed rape (max. application rate 2x180 g a.s./ha, interval 14-21d, BBCH 16-69):

- R1 stream, R3 stream – 5m buffer zone.

zRMS comment: Agreed.

9.1.1.3 Effects on bees (KCP 10.3.1)

Effects on bees for Protiokonazol 300 EC were not evaluated as part of the EU review of prothioconazole. The studies concerning effects of Protiokonazol 300 EC on bees were submitted in this dossier and deemed acceptable for evaluation and authorisation of Protiokonazol 300 EC.

Risk assessments for Protiokonazol 300 EC with the proposed use pattern was carried out according to the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002) and the latest Draft EFSA Guidance for risk assessment for bees EFSA Journal 2013; 11(7):3295.

The risk of Protiokonazol 300 EC to honeybees was assessed from HQ and ETR values between toxicity endpoints, estimated from acute and chronic studies with active ingredient and formulated product Protiokonazol 300 EC as well as the maximum single application rate of 195 g as/ha.

All the hazard quotients were considerably less than the respective triggers, indicating that Protiokonazol 300 EC in accordance with proposed GAP does not pose unacceptable risk to bees. No risk management measures are required.

zRMS comment: Agreed.

9.1.1.4 Effects on arthropods other than bees (KCP 10.3.2)

Effects on non-target arthropods for Protiokonazol 300 EC were not evaluated as part of the EU review of prothioconazole. The studies on effects of Protiokonazol 300 EC on arthropods were submitted in this dossier and deemed acceptable for evaluation and authorisation of Protiokonazol 300 EC.

Risk assessments for Protiokonazol 300 EC with the proposed use pattern was carried out according to the guidance for risk assessment for arthropods "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.

The in-field and off-field risk of Protiokonazol 300 EC to non-target arthropods was assessed from Hazard Quotients (HQ) between toxicity endpoints estimated from studies with the formulated product Protiokonazol 300 EC as well as in-field and off-field predicted environmental rate. No risk was determined in-field and off-field after application of Protiokonazol 300 EC in accordance with proposed GAP. No risk management measures are required.

zRMS comment: Agreed.

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

Effects on earthworms and other soil micro-organisms for Protiokonazol 300 EC were not evaluated as part of the EU review of prothioconazole. The studies on effects of Protiokonazol 300 EC on earthworms and other micro and macro-organisms were submitted in this dossier and deemed acceptable for evaluation and authorisation of Protiokonazol 300 EC.

Risk assessments for Protiokonazol 300 EC with the proposed use pattern was carried out according to the guidance for risk assessment for terrestrial ecotoxicology "Guidance Document on Terrestrial Ecotoxicology", (SANCO/10329/2002 rev.2 final, 2002).

Earthworms, collembola and *Hypoapsis*
The chronic risk of Protiokonazol 300 EC to earthworms, collembola and *Hypoapsis* was assessed from toxicity exposure ratios (TERs) between the selected toxicity endpoint for the active ingredient, metabolites and the formulated product Protiokonazol 300 EC as well as the maximum soil PECs.

The chronic TER values were greater than the trigger of 5 indicating an acceptable risk to earthworms, collembola and *Hypoapsis* following application of Protiokonazol 300 EC in accordance with proposed GAP. No risk management measures are required.

zRMS comment: Agreed.

Micro-organisms
The risk of Protiokonazol 300 EC to soil micro-organisms was evaluated by comparison of no-effect concentration in soil, derived from laboratory tests for active substance, metabolites and the formulated product Protiokonazol 300 EC with predicted application concentrations (PECs) obtained for active substances, metabolites and the formulation.

According to the performed risk assessment it was assessed that the application of Protiokonazol 300 EC in accordance with proposed GAP does not pose unacceptable risk to soil micro-organisms. No risk management measures are required.

zRMS comment: Agreed.

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

Effects on non-target terrestrial plants for Protiokonazol 300 EC were not evaluated as part of the EU review of prothioconazole. The studies on seedling emergence and vegetative vigour for Protiokonazol 300 EC were submitted in this dossier and deemed acceptable for evaluation and authorisation of Protiokonazol 300 EC.

The risk of Protiokonazol 300 EC to non-target plants was assessed from toxicity exposure ratios between toxicity endpoints for the formulation Protiokonazol 300 EC and off-field predicted environmental rate.

According to the performed risk assessment it was assessed that the application of Protiokonazol 300 EC in accordance with proposed GAP does not pose unacceptable risk to non-terrestrial plants. No risk management measures are required.

zRMS comment: Agreed.

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

Not relevant.

9.1.2 Grouping of intended uses for risk assessment

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

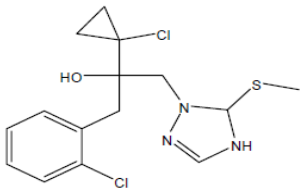
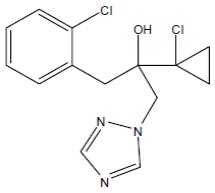
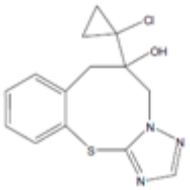
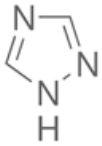
Table 9.1-2: Critical use pattern of Protiokonazol 300 EC grouped according to criterion

Grouping according to criterion			
Group	**Intended uses covered by group**	**relevant use parameters for grouping**	**relevant parameter or value for sorting**
winter cereals	winter cereals (uses no 1, 3, 6)	max. application rate: 195 g as/ha max. number of application: 2 min. interval between applications: 14 days BBCH 29-65	NR
spring cereals	spring cereals (uses no 2, 4, 5)	max. application rate: 195 g as/ha max. number of application: 2 min. interval between applications: 14 days BBCH 29-65	NR
winter oilseed rape A	winter oilseed rape (use no 7)	max. application rate: 180 g as/ha max. number of application: 1 min. interval between applications: 14 days BBCH 13-19	NR
winter oilseed rape B	winter oilseed rape (use no 8)	max. application rate: 180 g as/ha max. number of application: 2 min. interval between applications: 21 days BBCH 61-72	NR
spring oilseed	winter oilseed rape (use no 9)	max. application rate: 180 g as/ha max. number of application: 2 min. interval between applications: 14-21 days BBCH 16-69	NR

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 Protiokonazol 300 EC is indicated in the table.

Table 9.1-3 Metabolites of prothioconazole

Metabolite	Molar mass	Chemical structure	Maximum observed occurence in compartements	Risk assessment required?
M01: JAU 6476-S methyl Prothioconazole-S-Methyl CAS 178928-71-7	358.3		Soil: 14.6% (lab) Sediment: 77%	yes
M04: JAU 6476-desthio prothioconazole-desthio CAS 120983-64-4	312.2		Soil: 57.1% (field) Total system: 54.6% Water: 32.3% Sediment: 26.9%	yes
M12: Prothioconazole-thiazocine	307.8		Water: 14.1% AR, day 5 under photolysis conditions	yes
M13: 1,2,4-triazole	69.1		Soil: 0.001% Water: 37.2%	yes

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

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

Effects on birds of Protiokonazol 300 EC were not evaluated as part of the EU assessment of prothioconazole. However, the provision of further data on the Protiokonazol 300 EC is not considered essential, because it is possible to extrapolate data from the active substance. Additionally, vertebrates' studies should be avoided.

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

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

Species	Substance	Exposure System	Results	Reference
Bobwhite quail	prothioconazole	Acute	LD_{50} > 2000 mg a.s./kg bw	Yes, EFSA (2007)
Bobwhite quail	prothioconazole	5 d dietary	LC_{50} > 5000 mg a.s./kg diet **LD_{50} > 1413 mg a.s./ kg bw/day**	Yes, EFSA (2007)
Mallard duck	prothioconazole	5 d dietary	LC_{50} > 5000 mg a.s./kg diet calc. LD_{50} > 2457 mg a.s./kg bw/day	Yes, EFSA (2007)
Bobwhite quail	prothioconazole	Reproduction 22 w dietary	NOEC ≥ 1000 mg a.s./kg diet calc. NOEL ≥ 86 mg a.s./kg bw/day	Yes, EFSA (2007)
Mallard duck	prothioconazole	Reproduction 21 w dietary	NOEC = 700 mg a.s./kg diet **NOEL = 78 mg a.s./kg bw/day**	Yes, EFSA (2007)
Bobwhite quail	JAU 6476-desthio	Acute	LD_{50} > 2000 mg p.m./kg b.w.	Yes, EFSA (2007)
Bobwhite quail	JAU 6476-desthio	5 d dietary	LC_{50} = 4090 mg p.m./kg diet **LD_{50} > 297 mg p.m./kg bw/d[1]**	Yes, EFSA (2007)
Bobwhite quail	JAU 6476-desthio	Reproduction 20 w dietary	NOEC = 173 mg p.m./kg diet **NOEL = 14.8 mg p.m./kg bw/day**	Yes, EFSA (2007)
Mallard duck	JAU 6476-desthio	Reproduction 20 w dietary	NOEC ≥ 500 mg p.m./kg diet calc. NOEL = 63 mg p.m./kg bw/day	Yes, EFSA (2007)

[1] value represents the dose converted from the test group in which No Effect on mortality or food consumption was reported (1243 mg/kg diet/d multiplied by the mean daily food consumption (6.4 g/d for the 5 day exposure period) divided by the mean body-weight (26.75 g for the 5 day exposure period). A more precise conversion of the LC50 value requires reanalysis of data using the converted daily dietary doses for each test group.

9.2.1.1 Justification for new endpoints

In accordance with the Guidance EFSA/2009/1438, if the dietary LC_{50} is lower than the acute LD_{50}, the dietary value should be used in the acute risk assessment. Therefore, dietary LD_{50} values for Prothioconazole and its metabolite prothioconazole-desthio (JAU 6476-desthio) (M04) were used in the acute risk assessment as a worst case.

9.2.2 Risk assessment for spray applications

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

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the screening assessment for the use group winter cereals covers the risk for birds from all intended uses i.e. winter cereals, spring cereals, winter oilseed rape A and winter oilseed rape B (see 9.1.2).

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

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

Table 9.2-2: Screening assessment of the acute and long-term/reproductive risk for birds due to the use of Protiokonazol 300 EC (prothioconazole, worst case scenario covering use no 1-8)

Intended use	winter cereals				
Active substance	prothioconazole				
Application rate (g/ha)	2 × 195, interval: 14d				
Acute toxicity (mg/kg bw)	>1413				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
NR	Small omnivorous bird	158.8	1.2	37.16	38
Reprod. toxicity (mg/kg bw/d)	78				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
NR	Small omnivorous bird	64.8	1.4 × 0.53	9.38	8.3

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

Table 9.2-3: Screening assessment of the acute and long-term/reproductive risk for birds due to the use of Protiokonazol 300 EC (prothioconazole-desthio (JAU 6476-desthio) (M04), worst case scenario covering use no 1-8)

Intended use	winter cereals				
Active substance	prothioconazole-desthio (JAU 6476-desthio) (M04)				
Application rate (g/ha)	2 × 195[1], interval: 14d				
Acute toxicity (mg/kg bw)	>297				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
NR	Small omnivorous bird	158.8	1.2	37.16	**8.0**
Reprod. toxicity (mg/kg bw/d)	14.8				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
NR	Small omnivorous bird	64.8	1.4 × 0.53	9.38	**1.6**

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.
[1] assumed total dose of the parent and 100% conversion from parent (worst case)

Table 9.2-4: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of Protiokonazol 300 EC (prothioconazole-desthio (JAU 6476-desthio) (M04), winter cereals and spring cereals use no 1-6)

Intended use	winter cereals
Active substance	prothioconazole-desthio (JAU 6476-desthio) (M04)

Application rate (g/ha)	2 × 195[1], interval: 14d				
Acute toxicity (mg/kg bw)	>297				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Early (shoots) autumn-winter BBCH 10-29	Large herbivorous bird "goose"	30.5	1.2	7.14	41.6
BBCH 10 - 29	Small omnivorous bird "lark"	24.0	1.2	5.62	52.8
BBCH 30 -39	Small omnivorous bird "lark"	12.0	1.2	2.81	105.7
BBCH ≥ 40	Small omnivorous bird "lark"	7.2	1.2	1.68	176.8
Late season	Small granivorous/ insectivorous bird "bunting"	27.0	1.2	6.32	47.0
Reprod. toxicity (mg/kg bw/d)	14.8				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Early (shoots) autumn-winter BBCH 10-29	Large herbivorous bird "goose"	16.2	1.4 × 0.53	2.34	6.3
BBCH 10 - 29	Small omnivorous bird "lark"	10.9	1.4 × 0.53	1.58	9.4
BBCH 30 -39	Small omnivorous bird "lark"	5.4	1.4 × 0.53	0.78	19.0
BBCH ≥ 40	Small omnivorous bird "lark"	3.3	1.4 × 0.53	0.48	30.8
Late season	Small granivorous/ insectivorous bird "bunting"	12.5	1.4 × 0.53	1.81	8.2

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.
[1] assumed total dose of the parent and 100% conversion from parent (worst case)

Table 9.2-5: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of Protiokonazol 300 EC (prothioconazole-desthio (JAU 6476-desthio) (M04), winter oilseed rape use no 7)

Intended use	winter oilseed rape				
Active substance	prothioconazole-desthio (JAU 6476-desthio) (M04)				
Application rate (g/ha)	1 × 180[1]				
Acute toxicity (mg/kg bw)	>297				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
early (shoots) (BBCH 10-19)	Large herbivorous bird "goose"	39.0	1	7.02	42.3
BBCH 10 - 29	Small omnivorous bird "lark"	24.0	1	4.32	68.8

BBCH 10 - 19	medium herbivorous/granivorous bird “pigeon”	55.6	1	10.01	29.7
BBCH 10 - 19	Small insectivorous bird “wagtail”	10.9	1	1.96	151.5
Reprod. toxicity (mg/kg bw/d)	14.8				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
early (shoots) (BBCH 10-19)	Large herbivorous bird “goose”	15.9	1 × 0.53	1.52	9.7
BBCH 10 - 29	Small omnivorous bird “lark”	10.9	1 × 0.53	1.04	14.2
BBCH 10 - 19	medium herbivorous/granivorous bird “pigeon”	22.7	1 × 0.53	2.17	6.8
BBCH 10 - 19	Small insectivorous bird “wagtail”	5.9	1 × 0.53	0.56	26.4

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.
[1] assumed total dose of the parent and 100% conversion from parent (worst case)

Table 9.2-6: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of Protiokonazol 300 EC (prothioconazole-desthio (JAU 6476-desthio) (M04), winter oilseed rape use no 8)

Intended use	winter oilseed rape				
Active substance	prothioconazole-desthio (JAU 6476-desthio) (M04)				
Application rate (g/ha)	2 × 180[1], interval: 21d				
Acute toxicity (mg/kg bw)	>297				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
late – late (with seeds) BBCH 30-99	Small insectivorous bird “dunnock”	7.4	1.2[2]	1.6	185.6
BBCH ≥ 40	Small omnivorous bird “lark”	6.0	1.2[2]	1.3	228.5
BBCH ≥ 40	medium herbivorous/granivorous bird “pigeon”	2.0	1.2[2]	0.43	690.7
Reprod. toxicity (mg/kg bw/d)	14.8				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
late – late (with seeds) BBCH 30-99	Small insectivorous bird “dunnock”	2.7	1.4[2] × 0.53	0.36	41.1
BBCH ≥ 40	Small omnivorous bird “lark”	2.7	1.4[2]× 0.53	0.36	41.1
BBCH ≥ 40	medium herbivorous/granivorous bird “pigeon”	0.9	1.4[2] × 0.53	0.12	123.3

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.
[1] assumed total dose of the parent and 100% conversion from parent (worst case)

[2] MAF for 2 applications and 14d interval (worst case) since MAF for 21d interval is not provided

zRMS comment:
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). Safe use of prothiconazole and prothioconazole-desthio (M04) for birds were confirmed based on TER_A and TER_{LT} above the trigger values of 10 and 5, respectively, indicating the acute and long-term risk is acceptable for all the intended uses of **HERA 300 EC** crops. The refinement risk assessment for birds was not necessary by Applicant.

9.2.2.2 Higher-tier risk assessment

Not relevant.

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 Protiokonazol 300 EC is not intended to be applied on leafy vegetables forming heads or crop plants with comparable water collecting structures at principal growth stage 4 or later, the leaf scenario does not have to be considered.

Puddle scenario

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

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the screening assessment for the use group winter cereals covers the risk for birds from all intended uses i.e. winter cereals, spring cereals, winter oilseed rape A and winter oilseed rape B (see 9.1.2).

When multiple spray applications are considered, a MAF based on the DT_{50} in soil (single first or-der kinetics, geometric mean as used for PECgw and PECsw) is applied to achieve the effective application rate AReff.

$$AR_{eff} = AR \times MAF_m = AR \times \frac{1 - e^{-nki}}{1 - e^{-ki}}$$

where
k = ln(2)/DT50 (rate constant)
n = number of applications
i = application interval (days)

With a K(f)oc of 1765 (geomean value), prothioconazole belongs to the group of more sorptive substances. DT_{50} for prothioconazole (geomean used for PECsw and PECgw modelling) is 1.2 days. AF_{eff} equals 195 g as/ha (2x195 g as/ha, interval 14 days).

Effective application rate (g as/ha)	=	195		
Acute toxicity (mg as/kg bw)	=	1413	quotient =	0.14
Reprod. toxicity (mg as/kg bw/d)	=	78	quotient =	2.50

With a K(f)oc of 573.5 (geomean value), prothioconazole-desthio (JAU 6476-desthio) (M04) belongs to the group of more sorptive substances.

Effective application rate (g/ha)	=	195[1]		
Acute toxicity (mg/kg bw)	=	297	quotient =	0.66
Reprod. toxicity (mg/kg bw/d)	=	14.8	quotient =	13.18

[1] assumed total dose applied as a multiple application with no degradation between treatments and 100% conversion from parent (worst case)

With a K(f)oc of 2525.9 L/kg, prothioconazole-S-Methyl (M01) belongs to the group of more sorptive substances.

Effective application rate (g/ha)	=	195[1]		
Acute toxicity (mg/kg bw)	=	141.3[2]	quotient =	1.38
Reprod. toxicity (mg/kg bw/d)	=	7.8[2]	quotient =	25

[1] assumed total dose applied as a multiple application with no degradation between treatments and 100% conversion from parent (worst case)
[2] since data for metabolite not available, parent toxicity endpoints were divided by 10, LD_{50}/10 and NOEL/10

The acute and long-term risk to birds exposed to Protiokonazol 300 EC via drinking water is acceptable.

zRMS comment:
Agreed. As SIP 41061 is not intended for leafy crops forming heads, the leaf scenario does not have to be therefore considered based on the proposed uses. Evaluation of exposing for birds through the drinking water puddle scenario for the active substance and metabolite M04, demonstrate that the acceptable risk for birds for proposed use pattern of **HERA 300 EC.**

9.2.2.4 Effects of secondary poisoning

The log P_{ow} of prothioconazole, prothioconazole-desthio (JAU 6476-desthio) (M04) and prothioconazole-S-methyl (M01) is above the trigger value of 3. A risk assessment for effects due to secondary poisoning is in table below.

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 measured/predicted concentrations in soil/porewater / is based on experimental data.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the screening assessment for the use group winter cereals covers the risk for birds from all intended uses i.e. winter cereals, spring cereals, winter oilseed rape A and winter oilseed rape B (see 9.1.2).

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

Parameter	prothioconazole		Comments
PEC_{soil} (twa = 21 d) (mg/kg soil)	0.041	0.041	21-TWA PECs (dRR Part B8) (worst case, winter cereals, 2x195 g a.s./ha, interval: 14d)

Parameter	prothioconazole		Comments
log P_{ow} / P_{ow}	3.82 / 6606.93	4.05 / 11220.18	unbuffered and pH=7 EFSA (2007)
Koc	1765	1765	aged leaching study (dRR Part B8)
foc	0.02	0.02	default
BCF_{worm}	2.27	3.84	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC_{worm}	0.09	0.16	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.09	0.17	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	78	78	-
TER_{lt}	866.67	458.82	-

TER values shown in bold fall below the relevant trigger.

Table 9.2-8: Assessment of the risk for earthworm-eating birds due to exposure to prothioconazole-desthio (JAU 6476-desthio) (M04) bioaccumulation in earthworms (secondary poisoning)

Parameter	prothioconazole-desthio (JAU 6476-desthio) (M04)	Comments
PEC_{soil} (twa = 21 d) (mg/kg soil)	0.183	21-TWA PECs (dRR Part B8) (worst case, winter cereals, 2x195 g a.s./ha, interval: 14d)
log P_{ow} / P_{ow}	3.04 / 1096.48	unbuffered EFSA (2007)
Koc	573.5	geomean (dRR Part B8)
foc	0.02	default
BCF_{worm}	1.22	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC_{worm}	0.22	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.23	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	14.8	-
TER_{lt}	64.35	-

TER values shown in bold fall below the relevant trigger.

Table 9.2-9: Assessment of the risk for earthworm-eating birds due to exposure to prothioconazole-S-methyl (M01) bioaccumulation in earthworms (secondary poisoning)

Parameter	prothioconazole-S-methyl (M01)	Comments
PEC_{soil} (twa = 21 d) (mg/kg soil)	0.049	21-TWA PECs (dRR Part B8) (worst case, winter cereals, 2x195 g a.s./ha, interval: 14d)
log P_{ow} / P_{ow}	4.19 / 15488.17	EFSA (2007)
Koc	2525.9	geomean (dRR Part B8)
foc	0.02	default
BCF_{worm}	3.7	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$

Parameter	prothioconazole-S-methyl (M01)	Comments
		= (0.84 + 0.012 × P_{ow}) / foc × Koc
PEC_{worm}	0.18	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.19	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	7.8	NOELparent/10
TER_{lt}	41.05	-

TER values shown in bold fall below the relevant trigger.

The TERlt values for the assessment of the risk for earthworm-eating birds due to exposure to prothioconazole, prothioconazole-S-methyl (M01) and prothioconazole-desthio (JAU 6476-desthio) (M04) via bioaccumulation in earthworms are above the trigger TER value of 5, indicating low risk to birds.

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 / is based on the regulatory acceptable concentration for aquatic organisms as a limit value for admissible concentrations of diflufenican in water.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the screening assessment for the use group winter cereals covers the risk for birds from all intended uses i.e. winter cereals, spring cereals, winter oilseed rape A and winter oilseed rape B (see 9.1.2).

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

Parameter	prothioconazole	Comments
PEC_{sw} (twa = 21 d) (mg/L)	0.0022	worst case Step 1 21d-TWA PECsw (dRR Part B8) (winter cereals, 2x195 g a.s./ha, interval: 14d)
BCF_{fish}	19.7	EFSA (2007)
BMF	NR	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	0.043	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.007	$DDD = PEC_{fish} \times 0.159$
NOEL (mg/kg bw/d)	78	-
TER_{lt}	11142.86	-

Table 9.2-11: Assessment of the risk for fish-eating birds due to exposure to prothioconazole-desthio (JAU 6476-desthio) (M04) via bioaccumulation in fish (secondary poisoning)

Parameter	prothioconazole-desthio (JAU 6476-desthio) (M04)	Comments
PEC_{sw} (twa = 21 d) (mg/L)	0.0751	worst case Step 1 21d-TWA PECsw (dRR Part B8) (winter cereals, 2x195 g a.s./ha, interval: 14d)
BCF_{fish}	65	EFSA (2007)
BMF	NR	biomagnification factor (relevant for BCF ≥ 2000)

Parameter	prothioconazole-desthio (JAU 6476-desthio) (M04)	Comments
PEC_{fish}	4.882	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.776	$DDD = PEC_{fish} \times 0.159$
NOEL (mg/kg bw/d)	14.8	-
TER_{lt}	19.07	-

Table 9.2-12: Assessment of the risk for fish-eating birds due to exposure to prothioconazole-S-methyl (M01) via bioaccumulation in fish (secondary poisoning)

Parameter	prothioconazole-S-methyl (M01)	Comments
PEC_{sw} (twa = 21 d) (mg/L)	0.0022	Step 2 21d-TWA PECsw (dRR Part B8) (winter cereals, 2x195 g a.s./ha, interval: 14d)
BCF_{fish}	319.3 / 1995	the best / the worst scenario
BMF	NR	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	0.702 / 4.389	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.112 / 0.698	$DDD = PEC_{fish} \times 0.159$
NOEL (mg/kg bw/d)	7.8	NOELparent/10
TER_{lt}	69.64 / 11.17	-

The TERlt values for the assessment of the risk for fish-eating birds due to exposure to prothioconazole, prothioconazole-S-methyl (M01) and prothioconazole-desthio (JAU 6476-desthio) (M04) via bioaccumulation in earthworms are above the trigger TER value of 5, indicating low risk to birds.

zRMS comment:

The risk for fish-eating birds and earthworms-eating birds due to exposure to prothioconalzole and its metabolites (M04) is considered as acceptable for the worst case scenario. Since prothioconazole-S-methyl is not relevant for surface water, the risk to fish-eating birds and mammals was not necessary.

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

Not relevant. First-tier risk assessment confirmed that Protiokonazol 300 EC does not pose unacceptable acute and long term/reproductive risk to birds.

zRMS comment:

The acute and chronic risks of **HERA 300 EC** to birds were assessed from toxicity exposure ratios between toxicity endpoints, estimated from study with active substances, and maximum residues occurring on food items. For active substance all TER values exceed the relevant triggers indicating that **HERA 300 EC** does not pose an unacceptable risk to birds following applications according to recommended use pattern. Evaluation of exposing to mammals through the drinking water demonstrated the acceptable risk. The risk to earthworm - and fish-eating animals from secondary poisoning is low.

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

9.3.1 Toxicity data

Mammalian toxicity studies have been carried out with prothioconazole, metabolites and representative formulation containing prothioconazole. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on mammals of Protiokonazol 300 EC were not evaluated as part of the EU assessment of prothioconazole. However, the provision of further data on the Protiokonazol 300 EC is not considered essential, because it is possible to extrapolate data from the active substance. Additionally, vertebrates' studies should be avoided.

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

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

Species	Substance	Exposure System	Results	Reference
Rat	Prothioconazole	Oral Acute	**$LD_{50(male, female)}$ > 6200 mg a.s./kg bw/d**	Yes, EFSA (2007)
Rat	EC 250	Oral Acute	$LD_{50(male, female)}$ > 2500 mg a.s./kg bw/d	Yes, EFSA (2007)
Rat	FS 100	Oral Acute	$LD_{50(male, female)}$ > 2500 mg a.s./kg bw/d	Yes, EFSA (2007)
Rat	Prothioconazole	Long-term (2-generation), gavage	$NOEL_{parental}$ = 9.7 mg a.s./kg bw/d **$NOEL_{reproduction}$ = 95.6 mg a.s./kg bw/d**	Yes, EFSA (2007)
Rat	JAU 6476-desthio	Oral Acute	$LD_{50(female)}$ = 2506 mg p.m./kg bw/d $LD_{50(male)}$ = 2806 mg p.m./kg bw/d	Yes, EFSA (2007)
Mouse	JAU 6476-desthio	Oral Acute	$LD_{50(female)}$ = 3459 mg p.m./kg bw/d **$LD_{50(male)}$ = 2235 mg a.s./kg bw/d**	Yes, EFSA (2007)
Rat	JAU 6476-desthio	Long-term (2-generation), oral	$NOEL_{parental}$ = 2.5 mg p.m./kg bw/d **$NOEL_{reproduction}$ = 10 mg p.m./kg bw/d**	Yes, EFSA (2007)

zRMS comment: Agreed.

9.3.1.1 Justification for new endpoints

Not relevant. No new endpoints proposed.

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 Mammals and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438; hereafter referred to as EFSA/2009/1438).

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the screening assessment for the use group winter cereals covers the risk for mammals from all intended uses i.e. winter cereals, spring cereals, winter oilseed rape A and winter oilseed rape B (see 9.1.2).

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

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

Table 9.3-2: Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of Protiokonazol 300 EC (prothioconazole, worst case scenario covering use no 1-8)

Intended use	winter cereals				
Active substance	prothioconazole				
Application rate (g/ha)	2 × 195, interval: 14d				
Acute toxicity (mg/kg bw)	6200				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
NR	Small herbivorous mammal	118.4	1.2	27.7	223.8
Reprod. toxicity (mg/kg bw/d)	95.6				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
NR	Small herbivorous mammal	48.3	1.4 × 0.53	7.0	13.7

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

Table 9.3-3: Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of Protiokonazol 300 EC (prothioconazole-desthio (JAU 6476-desthio) (M04), worst case scenario covering use no 1-8)

Intended use	winter cereals
Active substance	prothioconazole-desthio (JAU 6476-desthio) (M04)
Application rate (g/ha)	2 × 195[1], interval: 14d
Acute toxicity (mg/kg bw)	2235
TER criterion	10

Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
NR	Small herbivorous mammal	118.4	1.2	27.7	80.7
Reprod. toxicity (mg/kg bw/d)	10				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
NR	Small herbivorous mammal	48.3	1.4 × 0.53	7.0	**1.4**

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.
[1] assumed total dose of the parent and 100% conversion from parent (worst case)

Table 9.3-4: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of Protiokonazol 300 EC (prothioconazole-desthio (JAU 6476-desthio) (M04), winter cereals and spring cereals use no 1-6)

Intended use	winter cereals, spring cereals				
Active substance	prothioconazole-desthio (JAU 6476-desthio) (M04)				
Application rate (g/ha)	2 × 195[1], interval: 14d				
Reprod. toxicity (mg/kg bw/d)	10				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
BBCH ≥ 20	Small insectivorous mammal "shrew"	1.9	1.4 × 0.53	0.3	33.3
BBCH ≥ 40	Small herbivorous mammal "vole"	21.7	1.4 × 0.53	3.1	**3.2**
BBCH 10-29	Small omnivorous mammal "mouse"	7.8	1.4 × 0.53	1.1	9.1
BBCH 30 - 39	Small omnivorous mammal "mouse"	3.9	1.4 × 0.53	0.6	16.7
BBCH ≥ 40	Small omnivorous mammal "mouse"	2.3	1.4 × 0.53	0.3	33.3

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.
[1] assumed total dose of the parent and 100% conversion from parent (worst case)

Table 9.3-5: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of Protiokonazol 300 EC (prothioconazole-desthio (JAU 6476-desthio) (M04), winter oilseed rape use no 7)

Intended use	winter oilseed rape				
Active substance	prothioconazole-desthio (JAU 6476-desthio) (M04)				
Application rate (g/ha)	1 × 180[1]				
Reprod. toxicity (mg/kg bw/d)	10				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
BBCH 10 - 19	Small insectivorous mammal	4.2	1 × 0.53	0.4	25

	"shrew"				
All season	Large herbivorous mammal "lagomorph"	14.3	1 × 0.53	1.4	7.1
BBCH 10-29	Small omnivorous mammal "mouse"	7.8	1 × 0.53	0.7	14.3

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.
[1] assumed total dose of the parent and 100% conversion from parent (worst case)

Table 9.3-6: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of Protiokonazol 300 EC (prothioconazole-desthio (JAU 6476-desthio) (M04), winter oilseed rape use no 8)

Intended use	winter oilseed rape				
Active substance	prothioconazole-desthio (JAU 6476-desthio) (M04)				
Application rate (g/ha)	2 × 180[1], interval: 21d				
Reprod. toxicity (mg/kg bw/d)	10				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
BBCH ≥ 20	Small insectivorous mammal "shrew"	1.9	1.4[2] × 0.53	0.3	33.3
BBCH ≥ 40	Small herbivorous mammal "vole"	18.1	1.4[2] × 0.53	2.4	**4.2**
All season	Large herbivorous mammal "lagomorph"	14.3	1.4[2] × 0.53	1.9	5.3
BBCH ≥ 40	Small omnivorous mammal "mouse"	1.9	1.4[2] × 0.53	0.3	33.3

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.
[1] assumed total dose of the parent and 100% conversion from parent (worst case)
[2] MAF for 2 applications and 14d interval (worst case) since MAF for 21d interval is not provided

The first-tier risk assessment indicates a long-term risk for small herbivorous mammal (*Common vole*) exposed to the metabolite prothioconazole-desthio (JAU 6476-desthio) (M04).

zRMS comment:

The risk assessment at first-tier performed according to Document on Risk Assessment for Birds and Mammals EFSA (EFSA Journal 2009; 7(12): 1438 was accepted. Safe use of prothioconazole for mammals were confirmed based on TER_A and TER_{LT} above the trigger values of 10 and 5, respectively. In case, prothioconazole-desthio (M04) several scenarios trigger higher-tier reproductive assessment. The refinement risk assessment is requirement for scenario:

BBCH ≥ 40	Small herbivorous mammal "vole"

The refinement risk assessment for mammals was provided by Applicant below.

9.3.2.2 Higher-tier risk assessment

In accordance with EFSA Scientific Report (2007) 106, 1-98, the refinement parameter proposed for prothioconazole-desthio (JAU 6476-desthio) (M04), was DT_{50} of 3.2 days in foliage and the maximum measured residue of 3.7 mg/kg that allowed to calculate new MAF and/or fTWA values.

Assuming first-order kinetics, the MAF and fTWA can be calculated according to the following formulas (EFSA Journal 2009; 7(12):1438):

$$MAF_m = \frac{1 - e^{-nki}}{1 - e^{-ki}}$$

With:
k = $\ln(2)/DT_{50}$ (rate constant)
n = number of applications
i = application interval (d)

$$TWA = \frac{1 - e^{-ki}}{ki}$$

With:
k = $\ln(2)/DT_{50}$ (rate constant)
i = averaging interval

The calculated MAF and fTWA values are:
- for winter and spring cereals (2 applications, 14d interval) **MAF_m = 1.05d** and **fTWA = 0.313**
- winter oilseed rape (2 applications, 21d interval) **MAFm = 1.01d** and **fTWA = 0.217**.

The refined risk assessment for small herbivorous mammal (*Common vole*) with new MAF_m and fTWA values are presented in below tables.

Table 9.3-7: Higher-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of Protiokonazol 300 EC (prothioconazole-desthio (JAU 6476-desthio) (M04), winter cereals and spring cereals use no 1-6)

Intended use	winter cereals, spring cereals
Active substance	prothioconazole-desthio (JAU 6476-desthio) (M04)
Application rate (g/ha)	2 × 195[1], interval: 14d
Reprod. toxicity (mg/kg bw/d)	10
TER criterion	5

Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
BBCH ≥ 40	Small herbivorous mammal "vole"	21.7	1.05 × 0.313	1.4	7.1

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.
[1] assumed total dose of the parent and 100% conversion from parent (worst case)

Table 9.3-8: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of Protiokonazol 300 EC (prothioconazole-desthio (JAU 6476-desthio) (M04), winter oilseed rape use no 8)

Intended use	winter oilseed rape				
Active substance	prothioconazole-desthio (JAU 6476-desthio) (M04)				
Application rate (g/ha)	2 × 180[1], interval: 21d				
Reprod. toxicity (mg/kg bw/d)	10				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
BBCH ≥ 40	Small herbivorous mammal "vole"	18.1	1.01 × 0.217	0.7	14.3

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.
[1] assumed total dose of the parent and 100% conversion from parent (worst case)
[2] MAF for 2 applications and 14d interval (worst case) since MAF for 21d interval is not provided

zRMS comment: Vole	
BBCH ≥ 40	Small herbivorous mammal "vole"

The refinement long-term risk assessment for vole was accepted by RMS. based on DT_{50} value for cereals and refined parameter of ftwa according to EFSA Conclusion 2007. Safe use of prothioconazole-desthio (M04) for vole were confirmed based on TER_{LT} above the trigger values of 5, respectively, indicating the long-term risk is acceptable for all crops.

The relevance of voles as a focal species for those crops should be consider at national level.

9.3.2.3 Drinking water exposure

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

Puddle scenario

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

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the screening assessment for the use group winter cereals covers the risk for mammals from all intended uses i.e. winter cereals, spring cereals, winter oilseed rape A and winter oilseed rape B (see 9.1.2).

When multiple spray applications are considered, a MAF based on the DT_{50} in soil (single first or-der kinetics, geometric mean as used for PECgw and PECsw) is applied to achieve the effective ap-plication rate AReff.

$$AR_{eff} = AR \times MAF_m = AR \times \frac{1 - e^{-nki}}{1 - e^{-ki}}$$

where
k = ln(2)/DT50 (rate constant)
n = number of applications
i = application interval (days)

With a K(f)oc of 1765 (geomean value), prothioconazole belongs to the group of more sorptive substances. DT_{50} for prothioconazole (geomean used for PECsw and PECgw modelling) is 1.2 days. AF_{eff} equals 195 g as/ha (2x195 g as/ha, interval 14 days).

Effective application rate (g/ha)	=	195		
Acute toxicity (mg/kg bw)	=	6200	quotient =	0.03
Reprod. toxicity (mg/kg bw/d)	=	95.6	quotient =	2.04

With a K(f)oc of 573.5 (geomean value), prothioconazole-desthio (JAU 6476-desthio) (M04) belongs to the group of more sorptive substances.

Effective application rate (g/ha)	=	195[1]		
Acute toxicity (mg/kg bw)	=	2235	quotient =	0.09
Reprod. toxicity (mg/kg bw/d)	=	10	quotient =	19.5

[1] assumed total dose applied as a multiple application with no degradation between treatments and 100% conversion from parent (worst case)

With a K(f)oc of 2525.9 L/kg, prothioconazole-S-Methyl (M01) belongs to the group of more sorptive substances.

Effective application rate (g/ha)	=	195[1]		
Acute toxicity (mg/kg bw)	=	620[2]	quotient =	0.31
Reprod. toxicity (mg/kg bw/d)	=	9.56[2]	quotient =	20.4

[1] assumed total dose applied as a multiple application with no degradation between treatments and 100% conversion from parent (worst case)
[2] since data for metabolite not available, parent toxicity endpoints were divided by 10, LD_{50}/10 and NOEL/10

The acute and long-term risk to mammals exposed to Protiokonazol 300 EC via drinking water is acceptable.

zRMS comment:
Agreed. As **HERA 300 EC** is not intended for leafy crops forming heads, the leaf scenario does not have to be therefore considered based on the proposed uses. Evaluation of exposing for mammals through the drinking water puddle scenario for the active substance and metabolite M04, demonstrate that the acceptable risk for mammals for proposed use pattern of **HERA 300 EC**.

9.3.2.4 Effects of secondary poisoning

The log P_{ow} of prothioconazole, prothioconazole-desthio (JAU 6476-desthio) (M04) and prothioconazole-S-methyl (M01) is above the trigger value of 3. A risk assessment for effects due to secondary poisoning is in table below.

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 measured/predicted concentrations in soil/porewater / is based on experimental data.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the screening assessment for the use group winter cereals covers the risk for mammals from all intended uses i.e. winter cereals, spring cereals, winter oilseed rape A and winter oilseed rape B (see 9.1.2).

Table 9.3-9: Assessment of the risk for earthworm-eating mammals due to exposure to prothioconazole via bioaccumulation in earthworms (secondary poisoning)

Parameter	prothioconazole		Comments
PEC_{soil} (twa = 21 d) (mg/kg soil)	0.041	0.041	21-TWA PECs (dRR Part B8) (worst case, winter cereals, 2x195 g a.s./ha, interval: 14d)
log P_{ow} / P_{ow}	3.82 / 6606.93	4.05 / 11220.18	unbuffered and pH=7 EFSA (2007)
Koc	1765	1765	aged leaching study (dRR Part B8)
foc	0.02	0.02	default
BCF_{worm}	2.27	3.84	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC_{worm}	0.09	0.16	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.12	0.2	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	95.6	95.6	-
TER_{lt}	796.67	478	-

TER values shown in bold fall below the relevant trigger.

Table 9.3-10: Assessment of the risk for earthworm-eating mammals due to exposure to prothioconazole-desthio (JAU 6476-desthio) (M04) via bioaccumulation in earthworms (secondary poisoning)

Parameter	prothioconazole-desthio (JAU 6476-desthio) (M04)	Comments
PEC_{soil} (twa = 21 d) (mg/kg soil)	0.183	21-TWA PECs (dRR Part B8) (worst case, winter cereals, 2x195 g a.s./ha, interval: 14d)
log P_{ow} / P_{ow}	3.04 / 1096.48	EFSA (2007)
Koc	573.5	geomean (dRR Part B8)
foc	0.02	default
BCF_{worm}	1.22	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC_{worm}	0.22	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.28	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	10	-
TER_{lt}	35.71	-

TER values shown in bold fall below the relevant trigger.

Table 9.3-11: Assessment of the risk for earthworm-eating mammals due to exposure to prothioconazole-S-methyl (M01) via bioaccumulation in earthworms (secondary poisoning)

Parameter	prothioconazole-S-methyl (M01)	Comments
PEC_{soil} (twa = 21 d) (mg/kg soil)	0.049	21-TWA PECs (dRR Part B8) (worst case, winter cereals, 2x195 g a.s./ha, interval: 14d)
log P_{ow} / P_{ow}	4.19 / 15488.17	EFSA (2007)
Koc	2525.9	geomean (dRR Part B8)
foc	0.02	default
BCF_{worm}	3.7	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC_{worm}	0.18	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.23	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	9.56	NOELparent/10
TER_{lt}	41.57	-

TER values shown in bold fall below the relevant trigger.

The TERlt values for the assessment of the risk for earthworm-eating mammals due to exposure to prothioconazole, prothioconazole-S-methyl (M01) and prothioconazole-desthio (JAU 6476-desthio) (M04) via bioaccumulation in earthworms are above the trigger TER value of 5, indicating low risk to mammals.

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 / is based on the regulatory acceptable concentration for aquatic organisms as a limit value for admissible concentrations diflufenican in water.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the screening assessment for the use group winter cereals covers the risk for mammals from all intended uses i.e. winter cereals, spring cereals, winter oilseed rape A and winter oilseed rape B (see 9.1.2).

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

Parameter	prothioconazole	Comments
PEC_{sw} (twa = 21 d) (mg/L)	0.0022	worst case Step 1 21d-TWA PECsw (dRR Part B8) (winter cereals, 2x195 g a.s./ha, interval: 14d)
BCF_{fish}	19.7	EFSA (2007)
BMF	NR	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	0.043	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.006	$DDD = PEC_{fish} \times 0.142$
NOEL (mg/kg bw/d)	95.6	-
TER_{lt}	15933.33	-

Table 9.3-13: Assessment of the risk for fish-eating mammals due to exposure to prothioconazole-desthio (JAU 6476-desthio) (M04) via bioaccumulation in fish (secondary poisoning)

Parameter	prothioconazole-desthio (JAU 6476-desthio) (M04)	Comments
PEC_{sw} (twa = 21 d) (mg/L)	0.0751	worst case Step 1 21d-TWA PECsw (dRR Part B8) (winter cereals, 2x195 g a.s./ha, interval: 14d)
BCF_{fish}	65	EFSA (2007)
BMF	NR	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	4.882	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.693	$DDD = PEC_{fish} \times 0.142$
NOEL (mg/kg bw/d)	10	-
TER_{lt}	14.43	-

Table 9.3-14: Assessment of the risk for fish-eating mammals due to exposure to prothioconazole-S-methyl (M01) via bioaccumulation in fish (secondary poisoning)

Parameter	prothioconazole-S-methyl (M01)	Comments
PEC_{sw} (twa = 21 d) (mg/L)	0.0022	Step 2 21d-TWA PECsw (dRR Part B8) (winter cereals, 2x195 g a.s./ha, interval: 14d)
BCF_{fish}	319.3 / 1995	the best / the worst scenario
BMF	NR	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	0.702 / 4.389	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.100 / 0.623	$DDD = PEC_{fish} \times 0.142$
NOEL (mg/kg bw/d)	9.56	NOELparent/10
TER_{lt}	95.6 / 15.35	-

The TERlt values for the assessment of the risk for fish-eating mammals due to exposure to prothioconazole, prothioconazole-S-methyl (M01) and prothioconazole-desthio (JAU 6476-desthio) (M04) via bioaccumulation in fish are above the trigger TER value of 5, indicating low risk to mammals.

zRMS comment:

The risk for fish-eating mammals and earthworms-eating mammals due to exposure to prothioconalzole and its metabolites (M04) is considered as acceptable for the worst case scenario. Since prothioconazole-S-methyl is not relevant for surface water, the risk to fish-eating birds and mammals was not necessary.

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

Not relevant. First-tier risk assessment confirmed that Protiokonazol 300 EC does not pose unacceptable acute and long term/reproductive risk to mammals.

zRMS comment:

Accepted.

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

Not relevant.

9.5 Effects on aquatic organisms (KCP 10.2)

9.5.1 Toxicity data

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

Effects on aquatic organisms of Protiokonazol 300 EC were not evaluated as part of the EU assessment of prothioconazole. The studies on effects of Protiokonazol 300 EC on *Daphnia magna* and algae were submitted in this dossier and deemed acceptable for evaluation and authorisation of Protiokonazol 300 EC. 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 9.5-1: Endpoints and effect values relevant for the risk assessment for aquatic organisms – prothioconazole and relevant metabolites

Species	Substance	Exposure System	Results	Reference
Fish				
Oncorhynchus mykiss	Prothioconazole	Acute	**LC_{50} = 1.83 mg a.s./L**	Yes, EFSA (2007)
Oncorhynchus mykiss	Prothioconazole (EC 250)	Acute	LC_{50} = 1.00 mg a.s./L	Yes, EFSA (2007)
Lepomis macrochirus	Prothioconazole	Acute	LC_{50} = 4.59 mg a.s./L	Yes, EFSA (2007)
Cyprinus carpio	Prothioconazole	Acute	LC_{50} = 6.91 mg a.s./L	Yes, EFSA (2007)
Cyprinus carpio	Prothioconazole (EC250)	Acute	LC_{50} = 3.72 mg a.s./L	Yes, EFSA (2007)
Oncorhynchus mykiss	Prothioconazole	Chronic,	**NOEC = 0.308 mg a.s./L**	Yes, EFSA (2007)

Species	Substance	Exposure System	Results	Reference
		ELS		
Oncorhynchus mykiss	JAU 6476-desthio	Acute	**LC_{50} = 6.63 mg p.m./L**	Yes, EFSA (2007)
Leuciscus idus melanotus	JAU 6476-desthio	Acute	LC_{50} = 13.2 mg p.m./L	Yes, EFSA (2007)
Oncorhynchus mykiss	JAU 6476-desthio	Chronic, ELS	**NOEC = 3.34 mg p.m./L**	Yes, EFSA (2007)
Oncorhynchus mykiss	JAU 6476-S-methyl	Acute	**LC_{50} = 1.8 mg p.m./L**	Yes, EFSA (2007)
Oncorhynchus mykiss	1,2,4-Triazole	Acute	**LC_{50} = 498 mg p.m./L**	Yes, EFSA (2007)
Oncorhynchus mykiss	1,2,4-Triazole	Chronic	**NOE_rC = 3.2 mg a.s./L**	Yes, EFSA (2007)
Invertebrates				
Daphnia magna	Prothioconazole	Acute	**EC_{50} = 1.3 mg a.s./L**	Yes, EFSA (2007)
Daphnia magna	Prothioconazole (EC250)	Acute	EC_{50} = 0.71 mg a.s./L	Yes, EFSA (2007)
Daphnia magna	Prothioconazole	Chronic	**NOEC = 0.56 mg a.s./L**	Yes, EFSA (2007)
Daphnia magna	JAU 6476-desthio	Acute	**EC_{50} > 10 mg p.m./L**	Yes, EFSA (2007)
Daphnia magna	JAU 6476-desthio	Chronic	**NOEC = 0.10 mg p.m./L**	Yes, EFSA (2007)
Daphnia magna	JAU 6476-S-methyl	Acute	**EC_{50} = 2.8 mg p.m./L**	Yes, EFSA (2007)
Daphnia magna	1,2,4-Triazole	Acute	**EC_{50} = 900 mg p.m./L**	Yes, EFSA (2007)
Algae				
Pseudokirchneriella subcapitata	Prothioconazole	Sub-chronic	**E_bC_{50} = 1.10 mg a.s./L** **E_rC_{50} = 2.18mg a.s./L**	Yes, EFSA (2007)
Pseudokirchneriella subcapitata	Prothioconazole (EC250)	Sub-chronic	E_bC_{50} = 2.92 mg a.s./L E_rC_{50} = 1.11 mg a.s./L	Yes, EFSA (2007)
Scenedesmus subspicatus	JAU 6476-desthio	Sub-chronic	**E_bC_{50} = 0.073 mg p.m./L** **E_rC_{50} = 0.55 mg p.m./L**	Yes, EFSA (2007)
Pseudokirchneriella subcapitata	JAU 6476-S-methyl	Sub-chronic	**E_bC_{50} = 3.77 mg p.m./L** **E_rC_{50} = 47.4 mg p.m./L**	Yes, EFSA (2007)
Pseudokirchneriella subcapitata	1,2,4-Triazole	Sub-chronic	**E_bC_{50} = 8.2 mg p.m./L*** **E_rC_{50} = 22.5 mg p.m./L***	Yes, EFSA (2007)
Sediment organisms				
Chironomus riparius	Prothioconazole	Chronic	**NOEC = 9.14 mg a.s./L**	Yes, EFSA (2007)
Chironomus riparius	JAU 6476-desthio	Chronic	**NOEC = 2.0 mg p.m../L**	Yes, EFSA (2007)
Fish, Bioconcentration				
Lepomis macrochirus	Prothioconazole	BCF_{parent} = 19.7 Clearance time (CT_{50} days): 0.8 Level of residue (%) after 14 day depuration phase: 9%		Yes, EFSA (2007)
Lepomis macrochirus	JAU 6476-desthio	BCF_{parent} = 65 Clearance time (CT_{50} days): 0.4-05 Level of residue (%) after 14 day depuration phase: 4%		Yes, EFSA (2007)
Higher-tier studies (micro- or mesocosm studies)				

Species	Substance	Exposure System	Results	Reference
Not relevant.				

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

zRMS comment:

Accepted.

Table 9.5-2: Endpoints and effect values relevant for the risk assessment for aquatic organisms – Protiokonazol 300 EC

Species	Substance	Exposure System	Results	Reference
Daphnia magna	Protiokonazol 300 EC	48 h, ss	**EC_{50} = 4.4 mg/L_{nom}** **(1.28 mg as/L_{nom} *)** NOEC = 2.07 mg/L_{nom} (0.6 mg as/L_{nom}*)	KCP 10.2.1.2/01 Mautino G /2023/ 1136.1F.SAG22
Pseudokirchneriella subcapitata	Protiokonazol 300 EC	72 h, s	grow rate **E_rC_{50} = 7.4 mg/L_{nom}** **(2.15 mg as/L_{nom}*)** NOEC = 1.0 mg/L_{nom} (0.29 mg as/L_{nom}*) yield **E_yC_{50} = 3.0 mg/L** (0.87 mg as/L_{nom}*) NOEC = 1.0 mg/L_{nom} (0.29 mg as/L_{nom}*)	KCP 10.2.1.3/01 Mautino G /2023/ 4546.1F.SAG22
Higher-tier studies (micro- or mesocosm studies)				
Not relevant.				

s: static; ss: semi-static; f: flow-through; nom: based on nominal concentrations; mm: based on mean measured concentrations
*density of Protiokonazol 300 EC is 1.031 g/mL

zRMS comment:

Accepted.

9.5.1.1 Justification for new endpoints

New endpoints are provided for the formulated product Protiokonazol 300 EC. Details of studies and results are included in Table 9.5-2. Summary of the studies is included in Appendix II. Additional studies are required according to Regulation (EC) No. 284/2013.

zRMS comment:

FISH
zRMS agree that the study for formulation **HERA 300 EC** with one active substance - prothioconzole for fish is not required in this case (primarily to limit testing on vertebrate).

Additional source of information:

The lowest fish acute endpoint for representative formulation 250 EC (with the smallest amount of the a.s./L as HERA 300 EC) in LoEP EFSA Scientific Report (2007), indicated only slight difference in the toxicity in comparison to a.s. endpoints for fish. In addition, when using the lowest fish acute endpoint presented in EFSA Scientific Report (2007) for a formulated product (expressed in a.s. units) an acceptable acute risk is concluded without risk mitigation measures.

Oncorhynchus mykiss	Prothioconazole	Acute	**LC_{50} = 1.83 mg a.s./L**	EFSA Scientific Report (2007)
Oncorhynchus mykiss	Prothioconazole (EC 250)	Acute	LC_{50} = 1.00 mg a.s./L	EFSA Scientific Report (2007)
Fish	SIP 41061	The study is not required for fish		

AQUATIC ORGANISMS SUCH AS *LEMNA GIBBA*
The Applicant haven't performed a *Lemna gibba* test with **HERA 300 EC**. However, in this case, the test with formulation *Lemna gibba* with *Lemna gibba* is not necessary. **HERA 300 EC** is not a herbicide. It is a fungicide applied at post-emergence of crops. Therefore, no toxic effects of **HERA 300 EC** formulations on aquatic plants are expected.

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, 3 and 4 PEC_{SW} for risk assessments covering the proposed use pattern and the resulting PEC/RAC ratios are presented in the table below.

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

Table 9.5-3: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prothioconazole for each organism group based on FOCUS Steps 1 and 2 calculations for the use of Protiokonazol 300 EC in winter cereals, spring cereals and winter rape

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. pro-longed**	**Algae**	**Sed. dwell. pro-longed**	**Inverteb. Acute PPP***	**Algae PPP***
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Chironomus riparius***	***Daphnia magna***	***Pseudokirchneri-ella subcapitata***
End-point		LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	NOEC	EC_{50}	E_rC_{50}
(µg/L)		1830	308	1300	560	2180 / 1100	9140	1280	2150 / 3000
AF		100	10	100	10	10	10	100	10
RAC (µg/L)		18.3	30.8	13	56	218 / 110	914	12.8	215 / 300
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**								
Step 1 winter cereals (max. application rate 2x195 g a.s./ha, interval 14d, BBCH 29-65)									
NR	21.1770	**1.16**	0.69	**1.63**	0.38	0.10 / 0.19	0.02	**1.65**	0.10 / 0.07
Step 2 winter cereals (max. application rate 2x195 g a.s./ha, interval 14d, BBCH 29-65)									
N-Europe	1.6045	0.09	0.05	0.12	0.03	0.01 / 0.02	0.00	0.13	0.01 / 0.01
S-Europe	1.6045	0.09	0.05	0.12	0.03	0.01 / 0.02	0.00	0.13	0.01 / 0.01
Step 1 spring cereals (max. application rate 2x195 g a.s./ha, interval 14d, BBCH 29-65)									
NR	21.1770	**1.16**	0.69	**1.63**	0.38	0.10 / 0.19	0.02	**1.65**	0.10 / 0.07
Step 2 spring cereals (max. application rate 2x195 g a.s./ha, interval 14d, BBCH 29-65)									
N-Europe	1.6045	0.09	0.05	0.12	0.03	0.01 / 0.02	0.00	0.13	0.01 / 0.01
S-Europe	1.6045	0.09	0.05	0.12	0.03	0.01 / 0.02	0.00	0.13	0.01 / 0.01
Step 1 winter oilseed rape (max. application rate 180 g a.s./ha, BBCH 13-19)									

NR	19.5480	**1.07**	0.63	**1.50**	0.35	0.09 / 0.18	0.02	**1.53**	0.09 / 0.07
Step 2 winter oilseed rape (max. application rate 180 g a.s./ha, BBCH 13-19)									
N-Europe	1.6554	0.09	0.05	0.13	0.03	0.01 / 0.02	0.00	0.13	0.01 / 0.01
S-Europe	1.6554	0.09	0.05	0.13	0.03	0.01 / 0.02	0.00	0.13	0.01 / 0.01
Step 1 winter oilseed rape (max. application rate 2x180 g a.s./ha, interval 21d, BBCH 61-72)									
NR	19.5480	**1.07**	0.63	**1.50**	0.35	0.09 / 0.18	0.02	**1.53**	0.09 / 0.07
Step 2 winter oilseed rape (max. application rate 2x180 g a.s./ha, interval 21d, BBCH 61-72)									
N-Europe	1.4676	0.08	0.05	0.11	0.03	0.01 / 0.02	0.00	0.13	0.01 / 0.00
S-Europe	1.4676	0.08	0.05	0.11	0.03	0.01 / 0.02	0.00	0.13	0.01 / 0.00

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
*endpoints for formulations Protiokonazol 300 EC expressed as prothioconazole

For the intended uses, calculated PEC/RAC ratios for prothioconazole indicate an acceptable risk for all groups of aquatic organisms. Therefore, no further assessment is necessary.

Table 9.5-4: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prothioconazole-desthio (JAU 6476-desthio) (M04) for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of Protiokonazol 300 EC in winter cereals (max. application rate 2x195 g a.s./ha, interval 14d, BBCH 29-65)

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Sed. dwell. prolonged**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Scenedesmus subspicatus***	***Chironomus riparius***
Endpoint (µg/L)		LC_{50} 6630	NOEC 3.34	EC_{50} 10000	NOEC 100	E_rC_{50} 550 / 73	NOEC 2000
AF		100	10	100	10	10	10
RAC (µg/L)		66.3	0.334	100	10	55 / 7.3	200
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**						
Step 1							
NR	76.4002	**1.15**	**228.74**	0.76	**7.64**	**1.39 / 10.47**	0.38
Step 2							
N-Europe	6.9715	0.11	**20.87**	0.07	0.70	0.13 / 0.096	0.03
S-Europe	12.9114	0.19	**38.66**	0.13	**1.29**	0.23 **/ 1.77**	0.06
Step 3							
D1/ditch	0.01404	0.00	0.04	0.00	0.00	0.00 / 0.00	0.00
D1/stream	0.008877	0.00	0.03	0.00	0.00	0.00 / 0.00	0.00
D2/ditch	0.09891	0.00	0.30	0.00	0.01	0.00 / 0.01	0.00
D2/stream	0.06217	0.00	0.19	0.00	0.01	0.00 / 0.01	0.00
D3/ditch	0.000001	0.00	0.00	0.00	0.00	0.00 / 0.00	0.00
D4/pond	0.001830	0.00	0.01	0.00	0.00	0.00 / 0.00	0.00
D4/stream	0.008691	0.00	0.03	0.00	0.00	0.00 / 0.00	0.00
D5/pond	0.000108	0.00	0.00	0.00	0.00	0.00 / 0.00	0.00
D5/stream	0.000881	0.00	0.00	0.00	0.00	0.00 / 0.00	0.00
D6/ditch	0.000329	0.00	0.00	0.00	0.00	0.00 / 0.00	0.00
R1/pond	0.05796	0.00	0.17	0.00	0.01	0.00 / 0.01	0.00
R1/stream	0.3619	0.01	**1.08**	0.00	0.04	0.01 / 0.05	0.00
R3/stream	0.7972	0.01	**2.39**	0.01	0.08	0.01 / 0.11	0.00
R4/stream	1.309	0.02	**3.92**	0.01	0.13	0.02 / 0.18	0.01

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

For winter cereals, calculated PEC/RAC ratios for prothioconazole-desthio (JAU 6476-desthio) (M04) did not indicate an acceptable risk for the most sensitive group of aquatic organisms (fish prolonged) in three FOCUS Step 3 scenarios. Therefore, further PEC/RAC ratios were calculated based on FOCUS

Step 4 PEC_{SW} considering reduced exposure of surface water bodies.

Table 9.5-5: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for prothioconazole-desthio (JAU 6476-desthio) (M04) based on FOCUS Step 4 calculations and toxicity data for *Oncorhynchus mykiss* with mitigation of spray drift and run-off for the use of Protiokonazol 300 EC in winter cereals (max. application rate 2x195 g a.s./ha, interval 14d, BBCH 29-65)

Intended use		winter cereals					
Active substance		prothioconazole-desthio (JAU 6476-desthio) (M04)					
Application rate (g/ha)		2x195 g a.s./ha, interval 14d, BBCH 29-65					
Nozzle reduction	**No-spray buffer (m)**	1/3	5	10	15	20	30
	Vegetated filter strip (m)	-	-	-	-	-	-
None	R1 stream	0.3619	0.04900	0.000048	-	-	-
50 %		-	-	-	-	-	-
75 %		-	-	-	-	-	-
90 %		-	-	-	-	-	-
None	R3 stream	0.7972	0.3345	0.2109	-	-	-
50 %		-	-	-	-	-	-
75 %		-	-	-	-	-	-
90 %		-	-	-	-	-	-
None	R4 stream	1.309	0.1479	0.05133	-	-	-
50 %		-	-	-	-	-	-
75 %		-	-	-	-	-	-
90 %		-	-	-	-	-	-
RAC (µg/L)							
0.334		**PEC/RAC ratio**					
None	R1 stream	**1.08**	0.15	0.00	-	-	-
50 %		-	-	-	-	-	-
75 %		-	-	-	-	-	-
90 %		-	-	-	-	-	-
None	R3 stream	**2.39**	**1.00**	0.63	-	-	-
50 %		-	-	-	-	-	-
75 %		-	-	-	-	-	-
90 %		-	-	-	-	-	-
None	R4 stream	**3.92**	0.44	0.15	-	-	-
50 %		-	-	-	-	-	-
75 %		-	-	-	-	-	-
90 %		-	-	-	-	-	-

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

For winter cereals, calculated PEC/RAC ratios indicate an acceptable risk in all FOCUS Steps 3 and 4 scenarios provided following risk mitigations are applied:

- R1 stream, R4 stream – 5m buffer zone
- R3 stream – 10m buffer zone

Table 9.5-6: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prothioconazole-desthio (JAU 6476-desthio) (M04) for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of Protiokonazol 300 EC in spring cereals (max. application rate 2x195 g a.s./ha, interval 14d, BBCH 29-65)

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Sed. dwell. prolonged**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Scenedesmus subspicatus***	***Chironomus riparius***
Endpoint (µg/L)		LC_{50} 6630	NOEC 3.34	EC_{50} 10000	NOEC 100	E_rC_{50} 550 / 73	NOEC 2000
AF		100	10	100	10	10	10
RAC (µg/L)		66.3	0.334	100	10	55 / 7.3	200
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**						
Step 1							
NR	76.4002	**1.15**	**228.74**	0.76	**7.64**	**1.39 / 10.47**	0.38
Step 2							
N-Europe	6.9715	0.11	**20.87**	0.07	0.70	0.13 / 0.96	0.03
S-Europe	12.9114	0.19	**38.66**	0.13	**1.29**	0.23 / **1.77**	0.06
Step 3							
D1/ditch	0.03250	0.00	0.10	0.00	0.00	0.00 / 0.00	0.00
D1/stream	0.02040	0.00	0.06	0.00	0.00	0.00 / 0.00	0.00
D3/ditch	0.000001	0.00	0.00	0.00	0.00	0.00 / 0.00	0.00
D4/pond	0.003576	0.00	0.01	0.00	0.00	0.00 / 0.00	0.00
D4/stream	0.01631	0.00	0.05	0.00	0.00	0.00 / 0.00	0.00
D5/pond	0.000117	0.00	0.00	0.00	0.00	0.00 / 0.00	0.00
D5/stream	0.000939	0.00	0.00	0.00	0.00	0.00 / 0.00	0.00
R4/stream	0.5127	0.01	**1.54**	0.01	0.05	0.01 / 0.07	0.00
D6/ditch*	0.001190	0.00	0.00	0.00	0.00	0.00 / 0.00	0.00
R1/pond*	0.2278	0.00	0.68	0.00	0.02	0.00 / 0.03	0.00
R1/stream*	1.114	0.02	**3.34**	0.01	0.11	0.02 / 0.15	0.01
R2/stream*	0.4041	0.01	**1.21**	0.00	0.04	0.01 / 0.06	0.00
R3/stream*	0.8274	0.01	**2.48**	0.01	0.08	0.02 / 0.11	0.00

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

For spring cereals, calculated PEC/RAC ratios for prothioconazole-desthio (JAU 6476-desthio) (M04) did not indicate an acceptable risk for the most sensitive group of aquatic organisms (fish prolonged) in four FOCUS Step 3 scenarios. Therefore, further PEC/RAC ratios were calculated based on FOCUS Step 4 PEC_{SW} considering reduced exposure of surface water bodies.

Table 9.5-7: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for prothioconazole-desthio (JAU 6476-desthio) (M04) based on FOCUS Step 4 calculations and toxicity data for *Oncorhynchus mykiss* with mitigation of spray drift and run-off for the use of Protiokonazol 300 EC in spring cereals (max. application rate 2x195 g a.s./ha, interval 14d, BBCH 29-65)

Intended use		spring cereals					
Active substance		prothioconazole-desthio (JAU 6476-desthio) (M04)					
Application rate (g/ha)		2x195 g a.s./ha, interval 14d, BBCH 29-65					
Nozzle reduction	**No-spray buffer (m)**	1/3	5	10	15	20	30
	Vegetated filter strip (m)	-	-	-	-	-	-
None	R4 stream	0.5127	0.1428	-	-	-	-
50 %		-	-	-	-	-	-
75 %		-	-	-	-	-	-
90 %		-	-	-	-	-	-
None	R1 stream*	1.114	0.1794	-	-	-	-
50 %		-	-	-	-	-	-
75 %		-	-	-	-	-	-
90 %		-	-	-	-	-	-
None	R2 stream*	0.4041	0.000765	-	-	-	-
50 %		-	-	-	-	-	-
75 %		-	-	-	-	-	-
90 %		-	-	-	-	-	-
None	R3 stream*	0.8274	0.1383	-	-	-	-
50 %		-	-	-	-	-	-
75 %		-	-	-	-	-	-
90 %		-	-	-	-	-	-
RAC (µg/L)							
0.334		**PEC/RAC ratio**					
None	R4 stream	**1.54**	0.43	-	-	-	-
50 %		-	-	-	-	-	-
75 %		-	-	-	-	-	-
90 %		-	-	-	-	-	-
None	R1 stream*	**3.34**	0.54	-	-	-	-
50 %		-	-	-	-	-	-
75 %		-	-	-	-	-	-
90 %		-	-	-	-	-	-
None	R2 stream*	**1.21**	0.00	-	-	-	-
50 %		-	-	-	-	-	-

75 %		-	-	-	-	-	-
90 %		-	-	-	-	-	-
None	R3 stream*	**2.48**	0.41	-	-	-	-
50 %		-	-	-	-	-	-
75 %		-	-	-	-	-	-
90 %		-	-	-	-	-	-

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
*maize as a surrogate scenario

For spring cereals, calculated PEC/RAC ratios indicate an acceptable risk in all FOCUS Steps 3 and 4 scenarios provided following risk mitigations are applied:

- R1 stream, R2 stream, R3 stream, R4 stream – 5m buffer zone

Table 9.5-8: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prothioconazole-desthio (JAU 6476-desthio) (M04) for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of Protiokonazol 300 EC in winter oilseed rape (max. application rate 180 g a.s./ha, BBCH 13-19)

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Sed. dwell. prolonged**
Test species		***Oncorhyn-chus mykiss***	***Oncorhyn-chus mykiss***	***Daphnia magna***	***Daphnia magna***	***Scenedesmus subspicatus***	***Chironomus riparius***
Endpoint (µg/L)		LC_{50} 6630	NOEC 3.34	EC_{50} 10000	NOEC 100	E_rC_{50} 550 / 75	NOEC 2000
AF		100	10	100	10	10	10
RAC (µg/L)		66.3	0.334	100	10	55 / 7.3	200
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**						
Step 1							
NR	35.2617	0.53	**105.57**	0.35	**3.53**	0.64 / **4.83**	0.18
Step 2							
N-Europe	5.7172	0.09	**17.12**	0.06	0.57	0.10 / 0.78	0.03
S-Europe	4.6821	0.07	**14.02**	0.05	0.47	0.09 / 0.64	0.02
Step 3							
D2/ditch	0.6636	0.01	**1.99**	0.01	0.07	0.01 / 0.09	0.00
D2/stream	0.4149	0.01	**1.24**	0.00	0.04	0.01 / 0.06	0.00
D3/ditch	0.000001	0.00	0.00	0.00	0.00	0.00 / 0.00	0.00
D4/pond	0.01029	0.00	0.03	0.00	0.00	0.00 / 0.00	0.00
D4/stream	0.06491	0.00	0.19	0.00	0.01	0.00 / 0.01	0.00
D5/pond	0.001606	0.00	0.00	0.00	0.00	0.00 / 0.00	0.00
D5/stream	0.01817	0.00	0.05	0.00	0.00	0.00 / 0.00	0.00
R1/pond	0.02771	0.00	0.08	0.00	0.00	0.00 / 0.00	0.00
R1/stream	0.3047	0.00	0.91	0.00	0.03	0.01 / 0.04	0.00
R3/stream	0.5778	0.01	**1.73**	0.01	0.06	0.01 / 0.08	0.00

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

For winter oilseed rape (single application), calculated PEC/RAC ratios for prothioconazole-desthio (JAU 6476-desthio) (M04) did not indicate an acceptable risk for the most sensitive group of aquatic organisms (fish prolonged) in three FOCUS Step 3 scenarios. Therefore, further PEC/RAC ratios were calculated based on FOCUS Step 4 PECSW considering reduced exposure of surface water bodies.

Table 9.5-9: **Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for prothioconazole-desthio (JAU 6476-desthio) (M04) based on FOCUS Step 4 calculations and toxicity data for *Oncorhynchus mykiss* with mitigation of spray drift and run-off for the use of Protiokonazol 300 EC in winter oilseed rape (max. application rate 180 g a.s./ha, BBCH 13-19)**

Intended use		winter oilseed rape					
Active substance		prothioconazole-desthio (JAU 6476-desthio) (M04)					
Application rate (g/ha)		180 g a.s./ha, BBCH 13-19					
Nozzle reduction	**No-spray buffer (m)**	1/3	5	10	15	20	30
	Vegetated filter strip (m)	-	-	-	-	-	-
None	D2 ditch	0.6636	0.6636	0.6636	-	-	-
50 %		-	-	-	-	-	-
75 %		-	-	-	-	-	-
90 %		-	-	-	-	-	-
None	D2 stream	0.4149	0.4149	0.4149	-	-	-
50 %		-	-	-	-	-	-
75 %		-	-	-	-	-	-
90 %		-	-	-	-	-	-
None	R3 stream	0.5778	0.2111	0.1401	-	-	-
50 %		-	-	-	-	-	-
75 %		-	-	-	-	-	-
90 %		-	-	-	-	-	-
RAC (µg/L)							
0.334		**PEC/RAC ratio**					
None	D2 ditch	**1.99**	**1.99**	**1.99**	-	-	-
50 %		-	-	-	-	-	-
75 %		-	-	-	-	-	-
90 %		-	-	-	-	-	-
None	D2 stream	**1.24**	**1.24**	**1.24**	-	-	-
50 %		-	-	-	-	-	-
75 %		-	-	-	-	-	-
90 %		-	-	-	-	-	-
None	R3 stream	**1.73**	0.63	0.42	-	-	-
50 %		-	-	-	-	-	-
75 %		-	-	-	-	-	-
90 %		-	-	-	-	-	-

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

For winter oilseed rape (single application), calculated PEC/RAC ratios indicate an acceptable risk in most FOCUS Steps 3 and 4 scenarios provided following risk mitigations are applied:

- R3 stream – 5m buffer zone.

In case of D2 ditch and D2 stream scenarios further risk refinement is necessary at national level.

Table 9.5-10: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prothioconazole-desthio (JAU 6476-desthio) (M04) for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of Protiokonazol 300 EC in winter oilseed rape (max. application rate 2x180 g a.s./ha, interval 21d, BBCH 61-72)

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Sed. dwell. prolonged**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Scenedesmus subspicatus***	***Chironomus riparius***
Endpoint (µg/L)		LC_{50} 6630	NOEC 3.34	EC_{50} 10000	NOEC 100	E_rC_{50} 550 73	NOEC 2000
AF		100	10	100	10	10	10
RAC (µg/L)		66.3	0.334	100	10	55 / 7.3	200
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**						
Step 1							
NR	70.5233	**1.06**	**211.15**	0.71	**7.05**	**1.28 / 9.66**	0.35
Step 2							
N-Europe	4.0051	0.06	**11.99**	0.04	0.40	0.07 / 0.55	0.02
S-Europe	7.0601	0.11	**21.14**	0.07	0.71	0.13 0.97	0.04
Step 3							
D2/ditch	0.2775	0.00	0.83	0.00	0.03	0.01 / 0.04	0.00
D2/stream	0.1800	0.00	0.54	0.00	0.02	0.00 / 0.02	0.00
D3/ditch	0.000001	0.00	0.00	0.00	0.00	0.00 / 0.00	0.00
D4/pond	0.007864	0.00	0.02	0.00	0.00	0.00 / 0.00	0.00
D4/stream	0.04939	0.00	0.15	0.00	0.00	0.00 / 0.01	0.00
D5/pond	0.000373	0.00	0.00	0.00	0.00	0.00 / 0.00	0.00
D5/stream	0.004073	0.00	0.01	0.00	0.00	0.00 / 0.00	0.00
R1/pond	0.04153	0.00	0.12	0.00	0.00	0.00 / 0.01	0.00
R1/stream	0.8306	0.01	**2.49**	0.01	0.08	0.02 / 0.11	0.00
R3/stream	0.5393	0.01	**1.61**	0.01	0.05	0.01 / 0.07	0.00

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

For winter oilseed rape (2 applications), calculated PEC/RAC ratios for prothioconazole-desthio (JAU 6476-desthio) (M04) did not indicate an acceptable risk for the most sensitive group of aquatic organisms (fish prolonged) in two FOCUS Step 3 scenarios. Therefore, further PEC/RAC ratios were calculated based on FOCUS Step 4 PECSW considering reduced exposure of surface water bodies.

Table 9.5-11: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for prothioconazole-desthio (JAU 6476-desthio) (M04) based on FOCUS Step 4 calculations and toxicity data for *Oncorhynchus mykiss* with mitigation of spray drift and run-off for the use of Protiokonazol 300 EC in winter oilseed rape (max. application rate 2x180 g a.s./ha, interval 21d, BBCH 61-72)

Intended use		winter oilseed rape					
Active substance		prothioconazole-desthio (JAU 6476-desthio) (M04)					
Application rate (g/ha)		2x180 g a.s./ha, interval 21d, BBCH 61-72					
Nozzle reduction	**No-spray buffer (m)**	1/3	5	10	15	20	30
	Vegetated filter strip (m)	-	-	-	-	-	-
None	R1 stream	0.8306	0.02609	-	-	-	-
50 %		-	-	-	-	-	-
75 %		-	-	-	-	-	-
90 %		-	-	-	-	-	-
None	R3 stream	0.5393	0.004260	-	-	-	-
50 %		-	-	-	-	-	-
75 %		-	-	-	-	-	-
90 %		-	-	-	-	-	-
RAC (µg/L)							
0.334		**PEC/RAC ratio**					
None	R1 stream	**2.49**	0.08	-	-	-	-
50 %		-	-	-	-	-	-
75 %		-	-	-	-	-	-
90 %		-	-	-	-	-	-
None	R3 stream	**1.61**	0.01	-	-	-	-
50 %		-	-	-	-	-	-
75 %		-	-	-	-	-	-
90 %		-	-	-	-	-	-

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

For winter oilseed rape (2 applications), calculated PEC/RAC ratios indicate an acceptable risk in all FOCUS Steps 3 and 4 scenarios provided following risk mitigations are applied:

- R1 stream, R3 stream – 5m buffer zone.

Table 9.5-12: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prothioconazole-S-methyl (M01) for each organism group based on FOCUS Steps 1 and 2 calculations for the use of Protiokonazol 300 EC in winter cereals, spring cereals and winter rape

Group		**Fish acute**	**Inverteb. acute**	**Algae**
Test species		***Oncorhynchus mykiss***	***Daphnia magna***	***Pseudokirchn. subcapitata***
Endpoint		LC_{50}	EC_{50}	E_rC_{50}
(µg/L)		1800	2800	47400 / 3770
AF		100	100	10
RAC (µg/L)		18	28	4740 / 377
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**			
Step 1 winter cereals (max. application rate 2x195 g a.s./ha, interval 14d, BBCH 29-65)				
NR	31.2490	**1.74**	**1.12**	0.01 / 0.08
Step 2 winter cereals (max. application rate 2x195 g a.s./ha, interval 14d, BBCH 29-65)				
N-Europe	1.6575	0.09	0.06	0.00 / 0.00
S-Europe	2.4207	0.13	0.09	0.00 / 0.01
Step 1 spring cereals (max. application rate 2x195 g a.s./ha, interval 14d, BBCH 29-65)				
NR	31.2490	**1.74**	**1.12**	0.01 / 0.08
Step 2 spring cereals (max. application rate 2x195 g a.s./ha, interval 14d, BBCH 29-65)				
N-Europe	1.6575	0.09	0.06	0.00 / 0.00
S-Europe	2.4207	0.13	0.09	0.00 / 0.01
Step 1 winter oilseed rape (max. application rate 180 g a.s./ha, BBCH 13-19)				
NR	14.4226	0.80	0.52	0.00 / 0.04
Step 1 winter oilseed rape (max. application rate 2x180 g a.s./ha, interval 21d, BBCH 61-72)				
NR	28.8452	**1.60**	**1.03**	0.01 / 0.08
Step 2 winter oilseed rape (max. application rate 2x180 g a.s./ha, interval 21d, BBCH 61-72)				
N-Europe	1.5283	0.08	0.05	0.00 / 0.00
S-Europe	1.5669	0.09	0.09	0.00 / 0.00

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

For the intended uses, calculated PEC/RAC ratios for prothioconazole-S-methyl (M01) indicate an acceptable risk for all groups of aquatic organisms. Therefore, no further assessment is necessary.

Table 9.5-13: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for 1,2,4-triazole for each organism group based on FOCUS Step 1 calculations for the use of Protiokonazol 300 EC in winter cereals, spring cereals and winter rape

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Algae**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Pseudokirchn. subcapitata***

Endpoint (µg/L)		LC_{50} 498000	NOEC 3200	EC_{50} 900000	E_rC_{50} 22500 / 8200
AF		100	10	100	10
RAC (µg/L)		4980	320	9000	2250 / 820
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**				
Step 1 winter cereals (max. application rate 2x195 g a.s./ha, interval 14d, BBCH 29-65)					
NR	9.0038	0.00	0.03	0.00	0.00 / 0.01
Step 1 spring cereals (max. application rate 2x195 g a.s./ha, interval 14d, BBCH 29-65)					
NR	9.0038	0.00	0.03	0.00	0.00 / 0.01
Step 1 winter oilseed rape (max. application rate 180 g a.s./ha, BBCH 13-19)					
NR	4.1556	0.00	0.01	0.00	0.00 / 0.01
Step 1 winter oilseed rape (max. application rate 2x180 g a.s./ha, interval 21d, BBCH 61-72)					
NR	8.3112	0.00	0.03	0.00	0.00 / 0.01

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

For the intended uses, calculated PEC/RAC ratios for 1,2,4-triazole indicate an acceptable risk for all groups of aquatic organisms. Therefore, no further assessment is necessary.

Table 9.5-14: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prothioconazole-thiazocine for each organism group based on FOCUS Steps 1 and 2 calculations for the use of Protiokonazol 300 EC in winter cereals, spring cereals and winter rape

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Sed. dwell. prolonged**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Chironomus riparius***
Endpoint (µg/L)		LC_{50} 183	NOEC 30.8	EC_{50} 130	NOEC 56	E_rC_{50} 218 / 110	NOEC 914
AF		100	10	100	10	10	10
RAC (µg/L)		1.83*	3.08*	1.3*	5.6*	21.8* / 11*	91.4*
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**						
Step 1 winter cereals (max. application rate 2x195 g a.s./ha, interval 14d, BBCH 29-65)							
NR	2.66941	**1.46**	0.87	**2.05**	0.48	0.12 / 0.24	0.03
Step 2 winter cereals (max. application rate 2x195 g a.s./ha, interval 14d, BBCH 29-65)							
N-Europe	0.20225	0.11	0.07	0.16	0.04	0.01 / 0.02	0.00
S-Europe	0.20225	0.11	0.07	0.16	0.04	0.01 / 0.02	0.00
Step 1 spring cereals (max. application rate 2x195 g a.s./ha, interval 14d, BBCH 29-65)							

NR	2.66941	**1.46**	0.87	**2.05**	0.48	0.12 / 0.24	0.03
Step 2 spring cereals (max. application rate 2x195 g a.s./ha, interval 14d, BBCH 29-65)							
N-Europe	0.20225	0.11	0.07	0.16	0.04	0.01 / 0.02	0.00
S-Europe	0.20225	0.11	0.07	0.16	0.04	0.01 / 0.02	0.00
Step 1 winter oilseed rape (max. application rate 180 g a.s./ha, BBCH 13-19)							
NR	2.46407	**1.35**	0.80	**1.90**	0.44	0.11 / 0.22	0.03
Step 2 winter oilseed rape (max. application rate 180 g a.s./ha, BBCH 13-19)							
N-Europe	0.20867	0.11	0.07	0.16	0.04	0.01 / 0.02	0.00
S-Europe	0.20867	0.11	0.07	0.16	0.04	0.01 / 0.02	0.00
Step 1 winter oilseed rape (max. application rate 2x180 g a.s./ha, interval 21d, BBCH 61-72)							
NR	2.46407	**1.35**	0.80	**1.90**	0.44	0.11 / 0.22	0.03
Step 2 winter oilseed rape (max. application rate 2x180 g a.s./ha, interval 21d, BBCH 61-72)							
N-Europe	0.18499	0.10	0.06	0.14	0.03	0.01 / 0.02	0.00
S-Europe	0.18499	0.10	0.06	0.14	0.03	0.01 / 0.02	0.00

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* it was assumed that the toxicity of the metabolite is 10 times higher than the active substance

For the intended uses, calculated PEC/RAC ratios for prothioconazole-thiazocine indicate an acceptable risk for all groups of aquatic organisms. Therefore, no further assessment is necessary.

9.5.3 Overall conclusions

PEC/RAC values were calculated on the basis of PECsw values as well as worst case toxicity endpoints from studies for active substance, metabolites and formulation Protiokonazol 300 EC. PECsw Step 1-3/RAC values were less than 1 for most scenarios indicating acceptable risk. In case of D2, R1, R2, R3, R4 stream, R3 stream further evaluation with Step 4 PECsw was performed.

On the basis of conducted risk assessment it was concluded that Protiokonazol 300 EC does not pose unacceptable risk provided following risk mitigations are applied for the following scenarios:

Winter cereals (max. application rate 2x195 g a.s./ha, interval 14d, BBCH 29-65):
- R1 stream, R4 stream – 5m buffer zone
- R3 stream – 10m buffer zone.
-

Spring cereals (max. application rate 2x195 g a.s./ha, interval 14d, BBCH 29-65):
- R1 stream, R2 stream, R3 stream, R4 stream – 5m buffer zone.

Winter oilseed rape (max. application rate 2x180 g a.s./ha, interval 21d, BBCH 61-72):
- R1 stream, R3 stream – 5m buffer zone.

Winter oilseed rape (max. application rate 180 g a.s./ha, BBCH 13-19):
- R3 stream – 5m buffer zone,
- D2 ditch and D2 stream - further risk refinement is necessary at national level.

zRMS comment:
The evaluation of the risk for aquatic 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" (EFSA Journal 2013;11(7):3290).

The formulated product **HERA 300 EC** and of the active ingredient for *D.magna* and *P.subcapitata* are comparable (they differ less than a factor of 3), the risk assessment can be based on the EU agreed endpoints from Table 9.5-1. However, additionaly risk assessment for **HERA 300 EC** and *P.subcapitata* and *Daphnia magna* was performed by zRMS.

Risk assessment for HERA 300 EC

Group		**Inverteb. acute**	**Algae**
Test species		***Daphnia magna***	***Pseudokirchn. subcapitata***
Endpoint (µg/L)		EC_{50} 1280	E_rC_{50} 2150
AF		100	10
RAC (µg/L)		12.80	215
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**		
Step 1 winter cereals (max. application rate 2x195 g a.s./ha, interval 14d, BBCH 29-65)			
NR	21.1770	**1.65**	0.098
Step 2 winter cereals (max. application rate 2x195 g a.s./ha, interval 14d, BBCH 29-65)			
N-Europe	1.6045	0.125	-
S-Europe	1.6045	0.125	-
Step 1 spring cereals (max. application rate 2x195 g a.s./ha, interval 14d, BBCH 29-65)			
NR	21.1770	1.65	0.098
Step 2 spring cereals (max. application rate 2x195 g a.s./ha, interval 14d, BBCH 29-65)			
N-Europe	1.6045	0.125	-
S-Europe	1.6045	0.125	-
Step 1 winter oilseed rape (max. application rate 180 g a.s./ha, BBCH 13-19)			
NR	19.5480	**1.53**	0.09
Step 2 winter oilseed rape (max. application rate 180 g a.s./ha, BBCH 13-19)			
N-Europe	1.6554	0.129	-
S-Europe	1.6554	0.129	-
Step 1 winter oilseed rape (max. application rate 2x180 g a.s./ha, interval 21d, BBCH 61-72)			
NR	19.5480	**1.53**	0.09
Step 2 winter oilseed rape (max. application rate 2x180 g a.s./ha, interval 21d, BBCH 61-72)			
N-Europe	1.4676	0.11	0.0068
S-Europe	1.4676	0.11	0.0068

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
*endpoints for formulations Protiokonazol 300 EC expressed as prothioconazole

For the intended uses, calculated PEC/RAC ratios for **HERA 300 EC** indicate an acceptable risk for all groups of aquatic organisms. Therefore, no further assessment is necessary.

Conclusion:

PEC/RAC values were calculated on the basis of PECsw values as well as worst case toxicity endpoints from studies for active substance, metabolites and formulation Protiokonazol 300 EC. PECsw Step 1-3/RAC values were less than 1 for most scenarios indicating acceptable risk. In case of D2, R1, R2, R3, R4 stream, R3 stream further evaluation with Step 4 PECsw was performed.

On the basis of conducted risk assessment it was concluded that Protiokonazol 300 EC does not pose unacceptable risk provided following risk mitigations are applied for the following scenarios:

Winter cereals (max. application rate 2x195 g a.s./ha, interval 14d, BBCH 29-65):
- R1 stream, R4 stream – 5m buffer zone
- R3 stream – 10m buffer zone.
-

Spring cereals (max. application rate 2x195 g a.s./ha, interval 14d, BBCH 29-65):
- R1 stream, R2 stream, R3 stream, R4 stream – 5m buffer zone.

Winter oilseed rape (max. application rate 2x180 g a.s./ha, interval 21d, BBCH 61-72):
- R1 stream, R3 stream – 5m buffer zone.

Winter oilseed rape (max. application rate 180 g a.s./ha, BBCH 13-19):
- R3 stream – 5m buffer zone,
- D2 ditch and D2 stream - further risk refinement is necessary at national level.

The risk assessment for aquatic organisms with risk mitigation measured should be considered by MSs.

Due the fact that scenarios only D3, D4, D5, R1, R3, R4 are relevant for the Central Zone calculations the remaining scenarios have not been evaluated.

9.6 Effects on bees (KCP 10.3.1)

9.6.1 Toxicity data

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

Effects on bees of Protiokonazol 300 EC were not evaluated as part of the EU assessment of prothioconazole. The studies on effects of Protiokonazol 300 EC on bees were submitted in this dossier and deemed acceptable for evaluation and authorisation of Protiokonazol 300 EC. New data submitted with this application are listed in **Błąd! Nie można odnaleźć źródła odwołania.** 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

Species	Substance	Exposure System	Results	Reference
Apis mellifera	Prothioconazole	Acute Oral	**LD_{50} > 71 µg a.s./bee**	Yes, EFSA (2007)
Apis mellifera	Prothioconazole	Acute Contact	**LD_{50} > 200 µg a.s./bee**	Yes, EFSA (2007)
Apis mellifera	Prothioconazole (EC250)	Acute Oral	LD_{50} > 48.7 µg a.s./bee	Yes, EFSA (2007)
Apis mellifera	Prothioconazole (EC250)	Acute Contact	LD_{50} > 200 µg a.s./bee	Yes, EFSA (2007)
Apis mellifera	Protiokonazol 300 EC	Acute Oral	LD50 =0.33 µL/bee (**LD50 =99.67 µg a.s./bee***)	KCP 10.3.1.1/01 Mautino G / 2023 / 1137.1F.SAG22
Apis mellifera	Protiokonazol 300 EC	Acute Contact	LD50 =0.33 µL/bee (**LD50 =98.35 µg a.s./bee***)	KCP 10.3.1.2/01 Mautino G / 2023 / 1137.1F.SAG22
Bombus spp.	Protiokonazol 300 EC	Acute Oral	LD50 >0.34 µL/bee (**LD50 > 100 µg a.s./bee***)	KCP 10.3.1.1/02 Mautino G / 2023 / 1138.1F.SAG22
Bombus spp.	Protiokonazol 300 EC	Acute Contact	LD50 >0.34 µL/bee (**LD50 > 100 µg a.s./bee***)	KCP 10.3.1.2/02 Mautino G / 2023 / 1138.1F.SAG22
Apis mellifera	Protiokonazol 300 EC	10 d, chronic	LDD50 =0.24 µg/bee/day (**LDD50 =72.38 µg as/bee/day***) NOEDD=0.012 µg/bee/day (NOEDD=3.74 µg as/bee/day*)	KCP 10.3.1.2/01 Mautino G / 2023 / 1001.1F.SAG22
Apis mellifera	Protiokonazol 300 EC	22 d, larvae chronic	LD50 =0.11 µL/larvae (LD50 = 33.21 µg as/ larvae *) NOED = 0.032 µg/larvae (**NOED =9.60 µg as/ larvae** *)	KCP 10.3.1.4/01 Mautino G / 2023 / 1002.1F.SAG22
Higher-tier studies (tunnel test, field studies)				
Not relevant.				

*density of Protiokonazol 300 EC is 1.031 g/mL

9.6.1.1 Justification for new endpoints

New endpoints are provided for the formulated product Protiokonazol 300 EC. Details of studies and results are included in Table 9.6-1. Summary of the studies is included in Appendix II. Additional studies are required according to Regulation (EC) No. 284/2013.

9.6.2 Risk assessment

9.6.2.1 Hazard quotients for bees

Risk assessment acc. to SANCO/10329/2002 rev.2 (final), October 17, 2002)

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

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the screening assessment for the use group winter cereals covers the risk for bees from all intended uses i.e. winter cereals, spring cereals, winter oilseed rape A and winter oilseed rape B (see 9.1.2).

Table 9.6-2: First-tier assessment of the risk for bees due to the use of Protiokonazol 300 EC (prothioconazole, worst case scenario covering use no 1-8)

Intended use	winter cereals		
Active substance/product	prothioconazole		
Single application rate (g/ha)	195		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	71	195	2.75
Contact toxicity	200		0.98
Product	Protiokonazol 300 EC		
Single application rate (g/ha)	195		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	99.67	195	1.96
Contact toxicity	98.35		1.98

Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure. Q_H values shown in bold breach the relevant trigger.

The acute risk to honeybees and bumblebees from use of Protiokonazol 300 EC was assessed using the maximum single application rate and the oral and contact LD_{50} values. A Hazard Quotient (HQ) of less than 50 indicates a low risk to bees.

Risk assessment acc. to EFSA Journal 2013;11(7):3295

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the screening assessment for the use group winter cereals covers the risk for bees from all intended uses i.e. winter cereals, spring cereals, winter oilseed rape A and winter oilseed rape B (see 9.1.2).

Table 9.6-3: Screening step assessment of the risk for bees due to the use Protiokonazol 300 EC (prothioconazole, worst case scenario covering use no 1-8)

Intended use	spring cereals				
Product	Protiokonazol 300 EC				
Single application rate (g/ha)	195				
Test design	**LD_{50} (µg/bee) LDD_{50} (µg/bee/day) NOED (µg/larva)**	**Single application rate (g/ha)**	**SV**	**HQ/ ETR**	**Trigger**
Honeybees					
Acute oral toxicity	99.67	195	7.6	0.01	0.2

Acute contact toxicity	98.35	195	-	2.0	42
Chronic adult oral toxicity	72.38	195	7.6	0.020	0.03
Larval development oral toxicity	9.60	195	4.4	0.09	0.2
Bumblebees					
Acute oral toxicity	> 100	195	11.2	0.02	0.036
Acute contact toxicity	> 100	195	-	2.0	7

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 are less than the trigger for downward sprays indicating that the risk to bees, bumblebees and bee larvae is acceptable.

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

Not relevant.

9.6.3 Effects on bumble bees

Not relevant.

9.6.4 Effects on solitary bees

Not relevant.

9.6.5 Overall conclusions

The acute risk of Protiokonazol 300 EC to honeybees and bumblebees was assessed from HQ and ETR values calculated between toxicity endpoints, estimated from acute oral and contact studies, chronic toxicity studies and larva toxicity with formulated product as well as the maximum single application rate. The HQ and ETR values were considerably less than the relevant triggers that means product Protiokonazol 300 EC does not pose unacceptable acute oral and contact risk to honeybees and bumble-bees. No risk mitigations are required.

zRMS comment:

Based on the acute risk assessment with the consideration SANCO/10329/2002 rev.2 (final), October 17, 2002), HQ values for adult bees from exposure of **HERA 300 EC** are < 50, indicating un acceptable risk to adult bees. the chronic studies for bees were submitted by the applicant. The studies were accepted by zRMS. The risk assessment based on these studies should be considered when GD for Bees, 2013 is implemented at EU level. The risk assessment based on studies for bees (adult and larvae) was performed in line with the EFSA 2013 guideline by Applicant. The HQ and ETR values were considerably less than the relevant triggers that means product **HERA 300 EC** does not pose unacceptable acute oral and contact risk to honeybees and bumble-bees. No risk mitigations are required.

Final decision should be taken into account at MSs level.

9.7 Effects on arthropods other than bees (KCP 10.3.2)

9.7.1 Toxicity data

Studies on the toxicity to non-target arthropods have been carried out with representative formulations containing prothioconazole. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on non-target arthropods of Protiokonazol 300 EC were not evaluated as part of the EU assessment of prothioconazole. The studies on effects of Protiokonazol 300 EC on arthropods were submitted in this dossier and deemed acceptable for evaluation and authorisation of Protiokonazol 300 EC. 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 9.7-1: Endpoints and effect values relevant for the risk assessment for non-target arthropods

Species	Substance	Exposure System	Results			Reference
Predatory mites						
Typhlodromus pyri (larvae/adults)	Prothioconazole EC250	Lab., coffin cells, 14d	LR_{50} = 18.7 g a.s./ha			Yes, EFSA (2007)
			Rate: 2 g a.s./ha 3.5 g a.s./ha 6.25 g a.s./ha 11 g a.s./ha 20 g a.s./ha	Mortality [%] 11.2 18.5 4.0 27.5 52.9	Repro [%] 4 -14 -6 19 n.a.	
Typhlodromus pyri (larvae/adults)	Prothioconazole EC250	Extended laboratory test bean leaves, 14 d	LR_{50} = 445.5 g a.s./ha			Yes, EFSA (2007)
			Rate: 100 g a.s./ha 157 g a.s./ha 245 g a.s./ha 380 g a.s./ha 600 g a.s./ha	Mortality [%] -2.3 1.5 9.1 45.1 67.8	Repro [%] 14 9 47 40 n.a.	
Typhlodromus pyri (larvae/adults)	Prothioconazole EC250	Extended laboratory test, aged residues bean leaves, Exposure 14 d	<u>Test started 1d after appl.</u> Corrected mortality: 14.5% Effect on reprod: 7.5% <u>Test started 15d after appl.</u> Corrected mortality: 6.4% Effect on reprod: -28.8%			Yes, EFSA (2007)
Parasitoids						
Aphidius rhopalosiphi (adults)	Prothioconazole EC250	Laboratory test glass plates, 14 d Control: 0.0% 63 g a.s./ha, 84 g a.s./ha, 112 g a.s./ha, 150 g a.s./ha,	LR_{50} = 139.9 g a.s./ha			Yes, EFSA (2007)
			Rate: 63 g a.s./ha 84 g a.s./ha 112 g a.s./ha 150 g a.s./ha 200 g a.s./ha	Mortality [%] 1st run: 3.5 6.9 3.5 3.5 100.0	Reprod. [%] 21 10 39 34 n.a.	

		200 g a.s./ha	Rate: 63 g a.s./ha 84 g a.s./ha 112 g a.s./ha 150 g a.s./ha 200 g a.s./ha	2nd run: 13.3 13.3 33.3 33.3 96.7	 17 36 44 53 n.a.	
Aphidius rhopalosiphi (adults)	Prothioconazole EC250	Extended laboratory test wheat plants, 14 d	48 h mortality <5% in any of test concentrations. No significant effect on reproduction in any treatment			Yes, EFSA (2007)
Foliage dwelling predators						
Coccinella septempunctata (larvae)	Prothioconazole EC250	Laboratory test glass plates, 46 d Control: 24% 25 g a.s./ha, 50 g a.s./ha, 97 g a.s./ha, 180 g a.s./ha, 375 g a.s./ha	LR_{50} = 229.8 g a.s./ha			Yes, EFSA (2007)
			Rate: 25 g a.s./ha, 50 g a.s./ha, 97 g a.s./ha, 180 g a.s./ha, 375 g a.s./ha	Mortality [%] -9.6 -5.3 25.4 30.7 73.7*	Reprod. [%] 54 0 84 549 n.a.	
			effects on reproduction are not considered to be treatment related (no adverse effects on reproduction at the highest tested treatment rate).			
Chrysoperla carnea (larvae)	Prothioconazole EC250	Laboratory test glass plates, 23 d	Rate: 200 g a.s./ha 400 g a.s./ha 600 g a.s./ha	Mortality [%] 15.2* 28.3* 41.3* No adverse efects on reproduction		Yes, EFSA (2007)
Ground dwelling predators						
Poecilus cupreus (adults)	Prothioconazole EC250	Quartz sand, 14 d,	Rate: 400 g a.s./ha 600 g a.s./ha	Mortality [%] 0.03.3 No adverse effect on feeding rate		Yes, EFSA (2007)
Aleochara bilineata (adults/larvae)	Prothioconazole EC250	Quartz sand, 87 d,	Rate: 42 g a.s./ha 200 g a.s./ha 400 g a.s./ha	Reproduction [%] 2.5 9.9 24.6		Yes, EFSA (2007)
Poecilus cupreus (adults)	Prothioconazole FS100	FS 100, ext. lab., 14 d, soil (Lufa 2.1), dressed seeds	Rate: 22.47 g a.s./ha Corrected mortality: 0% Effect on feeding rate: 5.6-9.6 %			Yes, EFSA (2007)
Aleochara bilineata (adults/larvae)	Prothioconazole FS100	FS 100, ext. lab., 82 d, soil (Lufa 2.1), dressed seeds	Rate: 19.34 g a.s./ha Effect on reproduction 11.2 %			Yes, EFSA (2007)
Pardosa spp.	Prothioconazole FS100	FS 100, ext. Lab., 14 d, soil (Lufa 2.1), dressed seeds	Rate: 22.3 g a.s./ha Corrected mortality: -3.1 % Effect on feeding rate: -18 %			Yes, EFSA (2007)

Protiokonazol 300 EC					
Typhlodromus pyri	Protiokonazol 300 EC	extended aged residue test, 14d (2D)	LR_{50} > 1300 ml/ha (>390 g as/ha) ER_{50} > 1300 ml/ha (>390 g as/ha)		KCP 10.3.2.2/01 / Mautino G / 2022 / 1020.1F.SAG22
			Rate: 650 ml/ha 1300 ml/ha	Mortality: 7.53% (0DAA) 0.00% (7 DAA) 0.00% (14DAA) 6.45% (0DAA) 6.38% (7 DAA) 6.38% (14DAA)	
			Rate: 650 ml/ha 1300 ml/ha	Reproduction: 0.99% (0DAA) 4.28% (7 DAA) 0.10% (14DAA) 0.27% (0DAA) 5.36% (7 DAA) 4.46% (14DAA)	
Aphidius rhopalosiphi	Protiokonazol 300 EC	extended aged residue test, 14d (3D)	LR_{50} > 1300 ml/ha (>390 g as/ha)		KCP 10.3.2.2/02 / Mautino G / 2022 / 1019.F1.SAG22
			Rate: 650 ml/ha 1300 ml/ha	Mortality: 7.14% (0DAA) -3.84% (7 DAA) 0.0% (14DAA) 7.14% (0DAA) 3.84% (7 DAA) 7.41% (14DAA)	
			Rate: 650 ml/ha 1300 ml/ha	Reproduction: 22.78% (0DAA) 6.77% (7 DAA) 9.83% (14DAA) 14.53%(0DAA) 22.60% (7 DAA) 24.53% (14DAA)	
Coccinella septempunctata	Protiokonazol 300 EC	extended aged residue test, 15d (2D)	LR_{50} > 1300 ml/ha (>390 g as/ha)		KCP 10.3.2.2/03 / Mautino G / 2022 / 1018.F1.SAG22
			Rate: 650 ml/ha 1300 ml/ha	Mortality: 18.42% (0DAA) 8.33% (7 DAA) 5.41% (14DAA) 26.32% (0DAA) 11.11% (7 DAA) 5.41% (14DAA)	
			Rate: 650 ml/ha 1300 ml/ha	Reproduction: 77.58% (0DAA) 76.44% (7 DAA) 82.07% (14DAA) 72.86%(0DAA) 77.40% (7 DAA) 82.27% (14DAA)	
Chrysoperla	Protiokonazol	extended aged	LR_{50} > 1300 ml/ha (>390 g as/ha)		KCP

carnea	300 EC	residue test, 14d (2D)	Rate: 650 ml/ha 1300 ml/ha	Mortality: 7.14% (0DAA) -3.84% (7 DAA) 0.0% (14DAA) 7.14% (0DAA) 3.84% (7 DAA) 7.41% (14DAA)	10.3.2.2/03 / Mautino G / 2022 / 1021.F1.SAG22
			Rate: 650 ml/ha 1300 ml/ha	Reproduction/egg hatching: 95.90% / 89.93% (0DAA) 55.82% / 85.01% (7 DAA) 64.27% / 78.66% (14DAA) 55.16% / 73.17%(0DAA) 56.20% / 85.12% (7 DAA) 61.41% / 79.24% (14DAA)	
Field or semi-field tests					
Not relevant.					

9.7.1.1 Justification for new endpoints

New endpoints are provided for the formulated product Protiokonazol 300 EC. Details of studies and results are included in Table 9.7-1. Summary of the studies is included in Appendix II. Additional studies are required according to Regulation (EC) No. 284/2013.

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. Additionally, recommendation included in Working document on Risk Assessment of Plant Protection Products in the Central Zone (CZSC, May 2021) was taken into account.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the screening assessment for the use group winter cereals covers the risk for arthropods from all intended uses i.e. winter cereals, spring cereals, winter oilseed rape A and winter oilseed rape B (see 9.1.2).

9.7.2.1 Risk assessment for in-field exposure

Table 9.7-2: Screening assessment of the in-field risk for non-target arthropods due to the use of Protiokonazol 300 EC (prothioconazole, worst case scenario covering use no 1-8)

Intended use	winter cereals
Product	Protiokonazol 300 EC
Application rate (ml/ha)	2 × 650*
MAF	1.7

Test species Tier I	**LR_{50} (lab.) (ml/ha)**	**$PER_{in\text{-}field}$ (g/ha)**	**$HQ_{in\text{-}field}$ criterion: HQ ≤ 2 / $PER_{in\text{-}field}$ below rate with ≤ 50 % effect?**
Typhlodromus pyri	> 1300	1105	0.85 / Yes
Aphidius rhopalosiphi	> 1300		0.85 / Yes
Coccinella septempunctata	> 1300		0.85 / Yes
Chrysoperla carnea	> 1300		0.85 / 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.
*density of Protiokonazol 300 EC is 1.031 g/mL

zRMS comment:
zRMS agrees with the Applicant's assessment with the in-field risk to non-target arthropods from the proposed use of **HERA 300 EC**, above. A low risk is demonstrated to the 2 standard first tier (HQ in-field and HQ off-field ≤ 2). Therefore, this assessment indicates that **HERA 300 EC** poses low risk in-field and off-field for non-target arthropods following application according to the proposed use patterns.

9.7.2.2 Risk assessment for off-field exposure

Table 9.7-3: Screening assessment of the off-field risk for non-target arthropods due to the use of Protiokonazol 300 EC (prothioconazole, worst case scenario covering use no 1-8)

Intended use	winter cereals				
Product	Protiokonazol 300 EC				
Application rate (ml/ha)	2 × 650*				
MAF	1.7				
VDF	5** 10# 1 (3D system)				
Test species Tier I	**LR_{50} (lab.) (ml/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (ml/ha)**	**CF**	**$HQ_{off\text{-}field}$ criterion: HQ ≤ 2 / corr. $PER_{off\text{-}field}$ below rate with ≤ 50 % effect?**
Typhlodromus pyri (2D)	> 1300	2.77%	6.12 3.06#	5 (Tier II)	0.02 / Yes 0.012 / Yes#
Aphidius rhopalosiphi (3D)	> 1300		6.12 3.06#		0.02 / Yes 0.012 / Yes#
			30.61		0.12 / Yes
Coccinella septempunctata (2D)	> 1300		6.12 3.06#		0.02 / Yes 0.012 / Yes#
Chrysoperla carnea (2D)	> 1300		6.12 3.06#		0.02 / Yes 0.012 / Yes#

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.
*density of Protiokonazol 300 EC is 1.031 g/mL
**VDF value of 5 according to the Working document on Risk Assessment of Plant Protection Products in the Central Zone (CZSC, May 2021)
#VDF=10. Some Member states require the use of VDF =10 in off-field risk assessment for non-target arthropods other than bees. The risk assessment for non-target arthropods other than bees with VDF=10 was also added by zRMS.

zRMS comment:
Some Member states require the use of VDF =10 in off-field risk assessment for non-target arthropods other than bees. The risk assessment for non-target arthropods other than bees with VDF=10 was also added by zRMS. The risk from the formulation **HERA 300 EC** indicating an acceptable off-field risk as the HQ_{values} are < 2 also based on VDF = 10. The risk assessment for non-target arthropods other than bees should be considered by MSs level.

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 of Protiokonazol 300 EC to non-target arthropods was assessed from in-field and off-field HQ between toxicity endpoints, estimated from extended laboratory studies with the formulated product Protiokonazol 300 EC as well as the maximum application rate. The HQ values were considerably less than 2, indicating that the product poses a low risk to non-target arthropods. It can be concluded that Protiokonazol 300 EC it was concluded that the application of Protiokonazol 300 EC in accordance with proposed GAP does not pose unacceptable in-field and off-field risk to non-target arthropods. No risk mitigations are required.

zRMS comment:
zRMS agrees with the Applicant's assessment with the in-field and off-field risk to non-target arthropods from the proposed use of **HERA 300 EC**, above. A low risk is demonstrated to the 2 standard first tier (HQ in-field and HQ off-field ≤ 2). Therefore, this assessment indicates that **HERA 300 EC** poses low risk in-field and off-field for non-target arthropods following application according to the proposed use patterns.

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

9.8.1 Toxicity data

Studies on the toxicity to earthworms and other non-target soil organisms (meso- and macrofauna) have been carried out with prothioconazole, its metabolites and reference formulations. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of Protiokonazol 300 EC were not evaluated as part of the EU assessment of prothioconazole. The studies on effects of Protiokonazol 300 EC on earthworms and other macro-organisms were submitted in this dossier and deemed acceptable for evaluation and authorisation of Protiokonazol 300 EC. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

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

Species	Substance	Exposure System	Results	Reference
Earthworms				
Eisenia fetida	prothioconazole	acute 10 % peat content	LC_{50} >1000 mg a.s./ kg d.wt.s (LC_{50corr} >500 mg a.s./ kg d.wt.s)	Yes, EFSA (2007)
Eisenia fetida	EC 250	acute 10 % peat content	LC_{50} >249.3 mg a.s./ kg d.wt.s (LC_{50corr} >124.7 mg a.s./ kg d.wt.s)	Yes, EFSA (2007)
Eisenia fetida	EC 250	long-term 10 % peat content	NOEC = 1.33 mg a.s./kg d.wt.s. (1000 g a.s./ha) $NOEC_{corr}$ = 0.665 mg a.s./kg d.wt.s.[1)]	Yes, EFSA (2007)
Eisenia fetida	Protiokonazol 300 EC	long-term	NOEC = 56.14 mg/kg dw (28.07 mg as/kg dw*) **$NOEC_{corr}$ = 16.34 mg/kg dw** **(8.17 mg as/kg dw*)[1)]**	KCP 10.4.1.1/01 Mautino G / 2022 / 1139.1F.SAG22
Eisenia fetida	JAU 6476-desthio (M04)	acute	LC_{50} >1000 mg/ kg d.wt.s (LC_{50corr} >500 mg/ kg d.wt.s)	Yes, EFSA (2007)
Eisenia fetida	JAU 6476-desthio (M04)	long-term	NOEC = 1.0 mg/kg d.wt.s. [2)] (**$NOEC_{corr}$ = 0.5 mg/kg d.wt.s.** [1)])	Yes, EFSA (2007)
Eisenia fetida	JAU 6476-S-methyl (M01)	acute	LC_{50} >1000 mg/kg d.wt.s (LC_{50corr} >500 mg kg d.wt.s)	Yes, EFSA (2007)
Eisenia fetida	JAU 6476-S-methyl (M01)	long-term	NOEC = 100 mg/kg d.wt.s. **NOE_{corr} = 50 mg/kg d.wt.s.** [1)]	Yes, EFSA (2007)
Collembola				
Folsomia candida	prothioconazole	long-term 10 % peat content	NOEC = 64 mg a.s./kg d.wt.s. **NOE_{corr} = 32 mg a.s./kg d.wt.s.** [1)]	Yes, EFSA (2007)
Folsomia candida	Prothioconazole FS100	seed treatment scenario long-term 10% peat con-tent	NOEC = 230 kg seeds/ha (10 g a.s./dt seeds) equivalent to 24.38 g a.s./ha	Yes, EFSA (2007)
Folsomia candida	Prothioconazole FS100	seed treatment scenario long-term 10% peat con-tent	NOEC = 1150 kg seeds/ha (10 g a.s./dt seeds) equivalent to 112 g a.s./ha	Yes, EFSA (2007)
Folsomia candida	JAU 6476-desthio (M04)	long-term	NOEC = 62.5 mg/kg d.wt.s. **NOE_{corr} = 31.25 mg/kg d.wt.s.** [1)]	Yes, EFSA (2007)
Folsomia candida	JAU 6476-S-methyl (M01)	long-term	NOEC = 31.6 mg/kg d.wt.s. **NOE_{corr} = 15.8 mg/kg d.wt.s.** [1)]	Yes, EFSA (2007)
Folsomia candida	Protiokonazol 300 EC	long-term	NOEC = 19.65 mg/kg dw (5.72 mg as/kg dw*)	KCP 10.4.2/02 Mautino G /

Species	Substance	Exposure System	Results	Reference
			$NOEC_{corr}$ = 9.83 mg/kg dw (2.86 mg as/kg dw*)[1)]	2022 / 1143.1F.SA22
Predatory mites				
Hypoaspis aculeifer	prothioconazole	long-term	NOEC = 100 mg a.s./kg d.wt.s. **$NOEC_{corr}$ = 50 mg a.s./kg d.wt.s.** [1)]	Yes, EFSA (2007)
Hypoaspis aculeifer	Protiokonazol 300 EC	long-term	NOEC = 56.14 mg/kg dw (28.07 mg as/kg dw) **$NOEC_{corr}$ = 16.34 mg/kg dw (8.17 mg as/kg dw*)[1)]**	KCP 10.4.2/01 Mautino G / 2022 / 1142.1F.SA22
Field trials				
Lumbricius terrestris, L. rubellus, L. castanea, Aporrectodea caliginosa, A. terrestris longa	EC 250	field study (grassland site)	3 × 200 g a.s./ha 5 different species identified and assessed. 46% reduction in the number of *A caliginosa* juveniles 7 weeks after first application (2 weeks after fi.nal application). No adverse effect 5 month after first application. (Maximum measured soil PEC 0.052 mg prothioconazole/kg based on soil sampling depth of 10 cm which is equivalent to a soil PEC of 0.104 mg prothioconazole/kg over the standard 5 cm depth)	Yes, EFSA (2007)
Range of species in an arable field study	FS 100 long-term	1150 kg seeds/ha (10 g a.s./dt seeds) equivalent to 122 g a.s./ha		Yes, EFSA (2007)
Litter bag test				
Field Soil Litter Degra-dation	Prothioconazole FS100	126 d, FS 100 (23.2 g a.s./ha) followed by JAU 6476 EC 250 (3 @ 200 g a.s./ha during 26 day period)	Field soil litter degradation [%] after 34 days: test item: 51.7; control: 52.1 after 95 days: test item: 74.3; control: 78.4 after 126 days test item: 92.0; control 91.2	Yes, EFSA (2007)

[1]corrected value derived by dividing the endpoint by a factor of 2 in accordance with the EPPO earthworm scheme 2002

[2] number of juveniles in control (19) less than the required number (30). Absence of a steep dose response over the 320-fold range of concentrations tested provides a weight of evidence that absolute NOEC would not differ greatly from that proposed (mid range of concentrations tested)

*density of Protiokonazol 300 EC is 1.031 g/mL

9.8.1.1 Justification for new endpoints

New endpoints are provided for the formulated product Protiokonazol 300 EC. Details of studies and results are included in Table 9.8-1. Summary of the studies is included in Appendix II. Additional studies are required according to Regulation (EC) No. 284/2013.

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.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the screening assessment for the use group winter cereals covers the risk for soil organisms from all intended uses i.e. winter cereals, spring cereals, winter oilseed rape A and winter oilseed rape B (see 9.1.2).

Table 9.8-2: 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 Protiokonazol 300 EC (worst case scenario covering use no 1-8)

Intended use	winter cereals		
Chronic effects on earthworms			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
Protiokonazol 300 EC	8.17	0.215	38.0
prothioconazole-desthio (JAU 6476-desthio) (M04)	0.5	0.202	**2.5**
prothioconazole-S-methyl (JAU 6476-S-methyl) (M01)	50	0.057	877.2
Chronic effects on *Folsomia candida*			
prothioconazole	32	0.215	148.8
Protiokonazol 300 EC	2.86	0.215	13.3
prothioconazole-desthio (JAU 6476-desthio) (M04)	31.25	0.202	154.7
prothioconazole-S-methyl (JAU 6476-S-methyl) (M01)	15.8	0.057	277.2
Chronic effects on *Hypoaspis aculeifer*			
prothioconazole	50	0.215	232.6
Protiokonazol 300 EC	8.17	0.215	38.0
prothioconazole-desthio (JAU 6476-desthio) (M04)	5[1]	0.202	252.5
prothioconazole-S-methyl (JAU 6476-S-methyl) (M01)	5[1]	0.057	894.7

[1] it was assumed that the toxicity of the metabolite is 10 times higher than the active substance
[2] PECs for cereals BBCH ≤29
[3] PECs for cereals BBCH ≥30

The long-term risk assessment for earthworms and other soil macro-organisms is acceptable except for risk to earthworms for prothioconazole-desthio (JAU 6476-desthio) (M04). In first tier risk assessment, the maximum PECs was used, so further risk assessment was performed separately for each scenario.

Table 9.8-3: 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 Protiokonazol 300 EC (use no 1-8)

Chronic effects on earthworms			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
prothioconazole-desthio (JAU 6476-desthio) (M04)			
winter and spring cereals BBCH 29, 2x195 g a.s./ha, interval: 14d	0.5	0.202	**2.5**
winter and spring cereals BBCH 30-39, 2x195 g a.s./ha, interval: 14d		0.050	10.0
winter and spring cereals BBCH 40-65, 2x195 g a.s./ha, interval: 14d		0.025	20.0
winter oilseed rape BBCH 13-19, 180 g a.s./ha		0.075	6.7
winter oilseed rape BBCH 61-72, 2x180 g a.s./ha, interval: 21d		0.045	11.1

The long term risk assessment for earthworms is acceptable except for risk for prothioconazole-desthio (JAU 6476-desthio) (M04) in cereals BBCH 29 for which further risk refinement with the existing field study conducted in Germany has been proposed below. The study has been summarised and evaluated in Draft Assessment Report (DAR) July 2005.

In the field long term study, three applications of representative formulation containing prothioconazole, at two to three week intervals, each equivalent to 200 g a.s./ha, were made to a grass field. The RMS considered the study to be representative of grassland ecosystems and acceptable for arable situations (i.e. cereals). The maximum measured level of prothioconazole-desthio recorded in the study was 0.106 mg/kg and was slightly below the maximum PECs for Protiokonazol 300 EC i.e. 0.202 mg/kg (BBCH 29). Nonetheless, RMS evaluated that, the maximum PECs for the desthio metabolite is likely to be an overestimate, hence the level of exposure in the field trial is considered to be more realistic of actual exposure levels. No significant reductions in the total number or biomass of earthworm were reported at 21 weeks after the first application, or at the final assessment at 11 months after the first application. In comparison with the control plots there was an overall increase in the number and biomass of the total earthworm population in the plots treated with prothioconazole formulation at the end of the study (11 months after first treatment). The study therefore indicates that long term impacts on earthworms populations are not to be expected from the proposed use of the reference formulation.

Results of above study allows to conclude that Protiokonazol 300 EC used in cereals BBCH 29 does not pose unacceptable risk to earthworms.

9.8.2.2 Higher-tier risk assessment

Not relevant.

9.8.3 Overall conclusions

The risk of Protiokonazol 300 EC to soil macro-organisms was evaluated by comparison of no-effect concentration in soil, derived from laboratory tests for active substance, metabolites and Protiokonazol 300 EC with appropriate predicted environmental concentrations in soil (PECs). According to the performed risk assessment as well as results of field studies, it was concluded that the application of Protiokonazol 300 EC in accordance with proposed GAP does not pose unacceptable risk to soil micro-organisms. No risk mitigations are required.

zRMS comment:
The chronic TER values for earthworms and other soil macro-organism for ppp **HERA 300 EC** were above the relevant Annex VI trigger of 5.
However in case of a.s. – prothioconazole and its metabolite M04 further refinement was needed.
Taking into account that the risk for a.s. calculated from ppp **HERA 300 EC** for earthworm was above the trigger value of 5, the risk is considered acceptable by zRMS. In addition, no adverse effects are to be expected, as proven by the results of the field study (EFSA Scientific Report, 2007). Desthio-metabolite was confirmed as being present in field study after application of Prothioconazole with a maximum concentration recorded of 0.106 mg/kg at 7 days after second application. The depth of soil from which the sample cores were taken is not stated in the study report, but is highly unlikely to have been less than 5 cm and would more typically be expected to be 10 cm. As such, the maximum PEC for prothioconazole and the metabolite JAU 6476-desthio is likely to be an overestimation, with the level of exposure in the field study being considered more realistic. In the field study, from the 5 identified earthworm species, only the number of juveniles of 1 (*Aporrectodea caliginosa*) was affected. In fact, by the end of the study, an overall increase in the number and biomass of earthworms in the treated plots was observed (11 months of exposure with 3 applications of 200g a.s./ha).

Therefore, it is concluded that HERA 300 EC and metabolites such as: M01 and M04 do not pose long-term risk to earthworms and other soil macro- and mesofauna when applied according to the proposed uses rates.

9.9 Effects on soil microbial activity (KCP 10.5)

9.9.1 Toxicity data

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

Effects on soil microorganisms of Protiokonazol 300 EC were not evaluated as part of the EU assessment of prothioconazole. The studies on effects of Protiokonazol 300 EC on microorganisms were submitted in this dossier and deemed acceptable for evaluation and authorisation of Protiokonazol 300 EC. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

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

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	prothioconazole	28 d	No influence at 2.0 kg a.s./ha (equivalent to **2.71 mg a.s./kg dry soil** according to DAR)	Yes, EFSA (2007)
N-mineralisation	JAU 6476-desthio	28 d	No influence at 1.0 kg/ha (equivalent to **1.37 mg /kg dry soil** according to DAR)	Yes, EFSA (2007)
N-mineralisation	JAU 6476-S-methyl	28 d	No influence at 1.0 kg/ha (equivalent to **2.69 mg /kg dry soil** according to DAR)	Yes, EFSA (2007)
N-mineralisation	Protiokonazol 300 EC	28 d, aerobic	**0.9 mg/kg soil dw (0.26 mg as/kg soil dw*)** < 25% deviation from control after 28 days (overall rate and interval rate) **4.5 mg/kg soil dw (1.31 mg as/kg soil dw*)** < 25% deviation from control after 28 days (overall rate) < 25% deviation from control after 42 days (interval rate)	KCP 10.5/01 Mautino G / 2022 / 545.1F.SAG22

*density of Protiokonazol 300 EC is 1.031 g/mL

9.9.1.1 Justification for new endpoints

New endpoints are provided for the formulated product Protiokonazol 300 EC. Details of studies and results are included in Table 9.9-1. Summary of the studies is included in Appendix II. Additional studies are required according to Regulation (EC) No. 284/2013.

9.9.2 Risk assessment

The evaluation of the risk for soil microorganisms was performed in accordance with the recommenda-

tions of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev 2 (final), October 17, 2002).

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

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the screening assessment for the use group winter cereals covers the risk for microorganisms from all intended uses i.e. winter cereals, spring cereals, winter oilseed rape A and winter oilseed rape B (see 9.1.2).

Table 9.9-2: Assessment of the risk for effects on soil micro-organisms due to the use of Protiokonazol 300 EC (worst case scenario covering use no 1-8)

Intended use	winter cereals		
N-mineralisation			
Product/active substance	**Max. conc. with effects ≤ 25 % (mg as/kg dw)**	**PEC_{soil} (mg as/kg dw)**	**Risk acceptable?**
prothioconazole	2.71 (at 28 d)	0.215	yes
Protiokonazol 300 EC	0.26 (at 28 d) 1.31 (at 42 d)	0.215	yes
JAU 6476-desthio (M04)	1.37 (at 28 d)	0.202	yes
JAU 6476-S-methyl (M01)	2.69 (at 28 d)	0.057	yes

9.9.3 Overall conclusions

The risk of Protiokonazol 300 EC to soil micro-organisms was evaluated by comparison of no-effect concentration in soil, derived from laboratory tests for active substance, metabolites and Protiokonazol 300 EC with appropriate predicted environmental concentrations in soil (PECs). According to the performed risk assessment it was concluded that the application of Protiokonazol 300 EC in accordance with proposed GAP does not pose unacceptable risk to soil micro-organisms. No risk mitigations are required.

zRMS comments:

The risk assessment for soil micro-organism after exposure of **HERA 300 EC** has been accepted by the zRMS. The effects on the nitrogen transformations are acceptable (<25%) at concentration which is higher than the maximum relevant PECs for the maximum application rate of **HERA 300 EC**. The results indicate no adverse effect on nitrogen transformation even at soil concentrations well higher than the ones expected following application of **HERA 300 EC.** To achieve a concise risk assessment, the risk envelope approach is applied. Here, the screening assessment for the use group winter cereals covers the risk for microorganisms from all intended uses i.e. winter cereals, spring cereals, winter oilseed rape A and winter oilseed rape B. No further risk assessment is required.

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

9.10.1 Toxicity data

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

Effects on non-target terrestrial plants of Protiokonazol 300 EC were not evaluated as part of the EU assessment of prothioconazole. The studies on seedling emergence and vegetative vigour for Protiokonazol 300 EC were submitted in this dossier and deemed acceptable for evaluation and authorisation of Protiokonazol 300 EC. 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 is in line with the results of the EU review process.

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

Species	Substance	Exposure System	Results	Reference
Most sensitive species: *Amaranthus retroflexus*	prothioconazole	Pre-emergencence 200 g a.s./ha	5% phytotoxic effect	Yes, EFSA (2007)
Most sensitive species: *Amaranthus retroflexus, Beta vulgaris*	prothioconazole	Post-emergence 250 g a.s./ha	10% phytotoxic effect	Yes, EFSA (2007)
Most sensitive species: *Amaranthus retroflexus*	JAU 6476 EC 250	Pre-emergence 200 g a.s./ha	5% phytotoxic effect	Yes, EFSA (2007)
-	JAU 6476 EC 250	Post-emergence 250 g a.s./ha	0% phytotoxic effect	Yes, EFSA (2007)
Cucumis sativus *Phaseolus vulgaris* *Solanum lycopersicum* *Beta vulgaris* *Brassica oleracea* *Zea mays* *Lolium perenne* *Allium cepa*	Protiokonazol 300 EC	21 d Seedling emergence	ER_{50} emergence >2600 ml/ha (ER_{50} emergence >780 g as/ha) ER_{50} mortality >2600 ml/ha (ER_{50} mortality >780 g as/ha) ER_{50} biomass > 2600 ml /ha (ER_{50} biomass >780 g as/ha)	KCP 10.6.2/01 Mautino G / 2023 /1140.1F.SAG22
	Protiokonazol 300 EC	21 d Vegetative vigour	ER_{50} mortality/seedling survival >2600 ml/ha (ER_{50} mortality/seedling survival >780 g as/ha) ER_{50} fresh weight/biomass >2600 ml/ha (ER_{50} mortality/seedling survival >780 g as/ha)	KCP 10.6.2/02 Mautino G / 2023 / 1141.1F.SAG22

9.10.1.1 Justification for new endpoints

New endpoints are provided for the formulated product Protiokonazol 300 EC. Details of studies and results are included in Table 9.10-1. Summary of the studies is included in Appendix II. Additional studies are required according to Regulation (EC) No. 284/2013.

9.10.2 Risk assessment

9.10.2.1 Tier-1 risk assessment (based screening data)

Not relevant.

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

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

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the screening assessment for the use group winter cereals covers the risk for non-target plants from all intended uses i.e. winter cereals, spring cereals, winter oilseed rape A and winter oilseed rape B (see 9.1.2).

Table 9.10-2: Assessment of the risk for non-target plants due to the use of Protiokonazol 300 EC (worst case scenario covering use no 1-8)

Intended use		winter cereals			
Product		Protiokonazol 300 EC			
Application rate (L/ha)		2 × 0.65, interval: 14d			
Test species	**ER_{50} (L/ha)**	**MAF**	**Drift rate (%)**	**$PER_{off\text{-}field}$ (L/ha)**	**TER criterion: TER ≥ 5**
Seedling emergence	>2.60	1	2.77	0.02	1300
Vegetative vigour		1.4[1]		0.03	86.7

MAF: Multiple application factor; PER: Predicted environmental rate; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger
[1] worst case MAFm according to EFSA Journal 2009; 7(12): 1438

9.10.2.3 Higher-tier risk assessment

Not relevant.

9.10.2.4 Risk mitigation measures

Not relevant.

9.10.3 Overall conclusions

The risk of PROTIOKONAZOL 300 EC to non-target plants was evaluated by comparison of toxicity endpoints derived from laboratory tests for the formulation PROTIOKONAZOL 300 EC. According to

the performed risk assessment it was assessed that the application of PROTIOKONAZOL 300 EC does not pose unacceptable risk to non-target plants.

zRMS comment: Accepted.

The risk assessment is based on the "Guidance Document on Terrestrial Ecotoxicology", (SANCO/10329/2002 rev.2 final, 2002). The risk assessment was performed for carrots as the worst case covering the risk assessment for other crops.

Based on the risk assessment it can be concluded that the proposed use of **HERA 300 EC** poses no unacceptable risk to non-target plants, if applied according to the recommended use pattern. No refinement risk assessment is required.

The risk assessment for non-target plants should be considered at MSs level.

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

Not available.

9.12 Monitoring data (KCP 10.8)

Not available.

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 formulation:

Table 9.13-1: Justified proposals for classification and labelling for Protiokonazol 300 EC according to Regulation (EC) No 1272/2008

Hazard class(es), categories:	Aquatic Chronic 1, H410
Hazard pictograms or Code(s) for hazard pictogram(s):	 GHS09
Signal word:	Warning
Hazard statement(s):	Very toxic to aquatic life with long lasting effects. [H410]
Precautionary statement(s):	-
Additional labelling phrases:	To avoid risks to man and the environment, comply with the instructions for use. [EUH401] Do not contaminate water with the product or its container (Do not clean application equipment near surface water/Avoid contamination via drains from farmyards and roads). [SP 1] To protect aquatic organisms, respect an 5m/10m vegetated unsprayed buffer zone of to surface water bodies. [SPe 3] Collect spillage [P391]

Table 9.13-2: Summary of evaluation of the ecotoxicological studies for Protiokonazol 300 EC

Type of test, species, model system (Guide-line)	Result	Acceptability	Classification (acc. to the criteria in Reg. 1272/2008)	Reference
Acute toxicity to aquatic organisms (lowest value)	E_yC_{50} = 3 mg/L		no classification	KCP 10.2.1.3/01 Mautino G /2023/ 4546.1F.SAG22
Chronic toxicity to aquatic organisms	no data, extrapola-tion form active substance data		Aquatic Chronic 1, H410	Please refer to dRR Part C

zRMS comment: Accepted.

Appendix 1 Lists of data considered in support of the evaluation

Tables considered not relevant can be deleted as appropriate.

MS to blacken authors of vertebrate studies in the version made available to third parties/public.

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
KCP 10.2.1.2/01	Artusio M	2023	Daphnia sp. Acute Immobilization Test (*Daphnia magna*) with PROTIOKONAZOL 300 EC (prothioconazole 300 g/L) Company Report No: 1136.1F.SAG22 Source: SAGEA Centro di Saggio s.r.l., Italy GLP Unpublished	N	Pestila Sp. z o.o.* & ProAgri Sp. z o.o.**
KCP 10.2.1.3/01	Mautino G	2023	PROTIOKONAZOL 300 EC: toxicity to green algae *Pseudokirchneriella subcapitata* in a growth inhibition study Company Report No: 4546.1F.SAG22 Source: SAGEA Centro di Saggio s.r.l., Italy GLP Unpublished	N	Pestila Sp. z o.o.* & ProAgri Sp. z o.o.**
KCP 10.3.1.1.1/01 KCP 10.3.1.1.2/01	Mautino G	2023	Effects of PROTIOKONAZOL 300 EC (prothioconazole 300 g/L) on Honeybees (*Apis mellifera* L.) in the laboratory – Acute Oral and Contact Toxicity Test Company Report No: 1137.1F.SAG22 Source: SAGEA Centro di Saggio s.r.l., Italy GLP Unpublished	N	Pestila Sp. z o.o.* & ProAgri Sp. z o.o.**
KCP 10.3.1.1.1/02 KCP	Mautino G	2023	Effects of PROTIOKONAZOL 300 EC (prothioconazole 300 g/L) on Bumblebee (Bombus terrestris L.) in the laboratory – Acute Oral and Contact Toxicity Test Company Report No: 1138.1F.SAG22	N	Pestila Sp. z o.o.* & ProAgri

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**
10.3.1.1.2/02			Source: SAGEA Centro di Saggio s.r.l., Italy GLP Unpublished		Sp. z o.o.**
KCP 10.3.1.2/01	Mautino G	2023	Effects of PROTIOKONAZOL 300 EC (prothioconazole 300 g/L) on Honeybees (*Apis mellifera* L.) in the laboratory – Chronic Oral Toxicity Test Company Report No: 1001.1F.SAG22 Source: SAGEA Centro di Saggio s.r.l., Italy GLP Unpublished	N	Pestila Sp. z o.o.* & ProAgri Sp. z o.o.**
KCP 10.3.1.4/01	Mautino G	2023	Effects of PROTIOKONAZOL 300 g/L EC (prothioconazole 300 g/L) on Honeybees (*Apis mellifera* L.) in the laboratory – Larval Toxicity Test Following Repeated Exposure Company Report No: 1002.1F.SAG22 Source: SAGEA Centro di Saggio s.r.l., Italy GLP Unpublished	N	Pestila Sp. z o.o.* & ProAgri Sp. z o.o.**
KCP 10.3.2.1/01	Mautino G	2022	Effects of PROTIOKONAZOL 300 EC (prothioconazole 300 g/L) on *Typhlodromus pyri* – Extended laboratory aged residue test – 2022 Company Report No: 1020.F.SAG22 Source: SAGEA Centro di Saggio s.r.l., Italy GLP Unpublished	N	Pestila Sp. z o.o.* & ProAgri Sp. z o.o.**
KCP 10.3.2.1/02	Mautino G	2022	Effects of PROTIOKONAZOL 300 EC (prothioconazole 300 g/L) on *Aphidius rhopalosiphi* – Extended laboratory aged residue test – 2022 Company Report No: 1019.F.SAG22 Source: SAGEA Centro di Saggio s.r.l., Italy GLP Unpublished	N	Pestila Sp. z o.o.* & ProAgri Sp. z o.o.**
KCP 10.3.2.1/03	Mautino G	2022	Effects of PROTIOKONAZOL 300 EC (prothioconazole 300 g/L) on *Coccinella septempunctata* – Extended laboratory aged residue test – 2022 Company Report No: 1018.F.SAG22	N	Pestila Sp. z o.o.* & ProAgri

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**
			Source: SAGEA Centro di Saggio s.r.l., Italy GLP Unpublished		Sp. z o.o.**
KCP 10.3.2.1/04	Mautino G	2022	Effects of PROTIOKONAZOL 300 EC (prothioconazole 300 g/L) on *Chrysoperla carnea* – Extended laboratory aged residue test – 2022 Company Report No: 1021.F.SAG22 Source: SAGEA Centro di Saggio s.r.l., Italy GLP Unpublished	N	Pestila Sp. z o.o.* & ProAgri Sp. z o.o.**
KCP 10.4.1.1/01	Mautino G	2022	Earthworm Reproduction Test (*Eisenia fetida*) with PROTIOKONAZOL 300 EC (prothioconazole 300 g/L) Company Report No: 1139.1F.SAG22 Source: SAGEA Centro di Saggio s.r.l., Italy GLP Unpublished	N	Pestila Sp. z o.o.* & ProAgri Sp. z o.o.**
KCP 10.4.2/01	Mautino G	2022	Predatory mite *Hypoaspis (Geolaelaps) aculeifer* reproduction test in soil with PROTIOKONAZOL 300 EC (prothioconazole 300 g/L) Company Report No: 1142.1F.SA22 Source: SAGEA Centro di Saggio s.r.l., Italy GLP Unpublished	N	Pestila Sp. z o.o.* & ProAgri Sp. z o.o.**
KCP 10.4.2/02	Mautino G	2022	Collembolan *Folsomia candida* reproduction test in soil with PROTIOKONAZOL 300 EC (prothioconazole 300 g/L) Company Report No: 1143.F.SA22 Source: SAGEA Centro di Saggio s.r.l., Italy GLP Unpublished	N	Pestila Sp. z o.o.* & ProAgri Sp. z o.o.**
KCP 10.5/01	Mautino G	2023	Soil Microorganisms: Nitrogen Transformation Test with PROTIOKONAZOL 300 EC (prothioconazole 300 g/L) Company Report No: 4545.1F.SAG22	N	Pestila Sp. z o.o.* & ProAgri

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**
			Source: SAGEA Centro di Saggio s.r.l., Italy GLP Unpublished		Sp. z o.o.**
KCP 10.6.2/01	Mautino G	2023	Effects of PROTIOKONAZOL 300 EC (prothioconazole 300 g/L) on terrestrial Non-target plants – Seedling Emergence and Seedling Growth Company Report No: 1140.1F.SAG22 Source: SAGEA Centro di Saggio s.r.l., Italy GLP Unpublished	N	Pestila Sp. z o.o.* & ProAgri Sp. z o.o.**
KCP 10.6.2/02	Mautino G	2023	Effects of PROTIOKONAZOL 300 EC (prothioconazole 300 g/L) on Terrestrial Plant Vegetative Vigour - OECD 227 Company Report No: 1141.1F.SAG22 Source: SAGEA Centro di Saggio s.r.l., Italy GLP Unpublished	N	Pestila Sp. z o.o.* & ProAgri Sp. z o.o.**

*Pestila Spółka z ograniczoną odpowiedzialnością (short name Pestila Sp. z o. o.)
**ProAgri Spółka z ograniczoną odpowiedzialnością (short name ProAgri Sp. z o.o.)
Please note that Pestila Sp. z o. o. and ProAgri International Sp. z o.o. are co-sponsors of the studies for Prothioconaole 300 EC and have the same rights for using data in registration processes without Letter of access issuing.

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

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

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
-	-	-	-	-	-

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

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

Appendix 2 Detailed evaluation of the new studies

A 2.1 KCP 10.1 Effects on birds and other terrestrial vertebrates

A 2.1.1 KCP 10.1.1 Effects on birds

Not relevant. No studies submitted.

A 2.1.2 KCP 10.1.2 Effects on terrestrial vertebrates other than birds

Not relevant. No studies submitted.

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

Not relevant. No studies submitted.

A 2.2 KCP 10.2 Effects on aquatic organisms

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

A 2.2.1.1 KCP 10.2.1.1 Acute toxicity to fish

Not relevant. No studies submitted.

A 2.2.1.2 KCP 10.2.1.2 Acute toxicity to aquatic invertebrates

Comments of zRMS:

Immobility of *Daphnia magna* based on nominal values.

		PROTIOKONAZOL 300 EC treatments				
	T1 Control	T2 0.43 mg f.p./L	T3 0.94 mg f.p./L	T4 2.07 mg f.p./L	T5 4.55 mg f.p./L	T6 10 mg f.p./L
	ISO standard ready-to-use	0.12 mg a.i./L	0.27 mg a.i./L	0.60 mg a.i./L	1.32 mg a.i./L	2.91 mg a.i./L
Immobility (24 hours) [mean %]	0.00	0.00	0.00	0.00	15.00	70.00
Significance [a]	-	n.s.	n.s.	n.s.	**	***
Immobility (48 hours) [mean %]	0.00	0.00	0.00	5.00	55.00	95.00
Significance [a]	-	n.s.	n.s.	n.s.	***	***

Endpoint	mg test item/L	mg a.i./L
EC_{10} (24 hours) [95% confidence intervals]	4.14 [2.37 – 5.30]	1.20 [0.69 – 1.54]
EC_{20} (24 hours) [95% confidence intervals]	5.12 [3.44 – 6.35]	1.48 [1.00 – 1.84]
EC_{50} (24 hours) [95% confidence intervals]	7.69 [6.19 – 10.18]	2.23 [1.79 – 2.95]
NOEC	2.07	0.60
LOEC	4.55	1.32
EC_{10} (48 hours) [95% confidence intervals]	2.39 [1.52 – 3.05]	0.69 [0.44 – 0.88]
EC_{20} (48 hours) [95% confidence intervals]	2.95 [2.08 – 3.64]	0.86 [0.60 – 1.06]
EC_{50} (48 hours) [95% confidence intervals]	4.40 [3.54 – 5.47]	1.28 [1.03 – 1.59]
NOEC	2.07	0.60
LOEC	4.55	1.32

a.i. = prothioconazole
-, not applicable
n.s., not significantly different compared to the control
n.d., not determined due to mathematical reasons
[a], Cochran-Armitage test procedure, $\alpha \leq 0.05$ *,0.01 **, 0.001 ***

Validity criteria of the study

Immobility in control group	At 48 hours was 0.00%, therefore, the validity criterion was met.
Dissolved oxygen concentration	Ranged from 5.01 to 6.96 mg/L at the end of the test, the validity criterion was met.

The agreed toxicity endpoints:

Immobility of *Daphnia magna* based on nominal values.

		PROTIOKONAZOL 300 EC treatments				
	T1 Control	T2 0.43 mg f.p./L	T3 0.94 mg f.p./L	T4 2.07 mg f.p./L	T5 4.55 mg f.p./L	T6 10 mg f.p./L
	ISO standard ready-to-use	0.12 mg a.i./L	0.27 mg a.i./L	0.60 mg a.i./L	1.32 mg a.i./L	2.91 mg a.i./L
Immobility (24 hours) [mean %]	0.00	0.00	0.00	0.00	15.00	70.00
Significance [a]	-	n.s.	n.s.	n.s.	**	***
Immobility (48 hours) [mean %]	0.00	0.00	0.00	5.00	55.00	95.00
Significance [a]	-	n.s.	n.s.	n.s.	***	***

Endpoint	mg test item/L	mg a.i./L
EC_{10} (24 hours) [95% confidence intervals]	4.14 [2.37 – 5.30]	1.20 [0.69 – 1.54]
EC_{20} (24 hours) [95% confidence intervals]	5.12 [3.44 – 6.35]	1.48 [1.00 – 1.84]
EC_{50} (24 hours) [95% confidence intervals]	7.69 [6.19 – 10.18]	2.23 [1.79 – 2.95]
NOEC	2.07	0.60
LOEC	4.55	1.32
EC_{10} (48 hours) [95% confidence intervals]	2.39 [1.52 – 3.05]	0.69 [0.44 – 0.88]
EC_{20} (48 hours) [95% confidence intervals]	2.95 [2.08 – 3.64]	0.86 [0.60 – 1.06]
EC_{50} (48 hours) [95% confidence intervals]	4.40 [3.54 – 5.47]	1.28 [1.03 – 1.59]
NOEC	2.07	0.60
LOEC	4.55	1.32

a.i. = prothioconazole
-, not applicable
n.s., not significantly different compared to the control
n.d., not determined due to mathematical reasons
[a], Cochran-Armitage test procedure, $\alpha \leq 0.05$ *,0.01 **, 0.001 ***

Reference:	KCP 10.2.1.2/01
Report	Daphnia sp. Acute Immobilization Test (*Daphnia magna*) with PROTIOKONAZOL 300 EC (prothioconazole 300 g/L), Artusio M.; 2023; Study code: 1136.1F.SAG22
Guideline(s):	Yes, OECD 202
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No
Validity criteria of the test:	All validity criteria of this semi-static study were met: - immobility in the control group ≤ 10%; moreover, not more than 10% of the daphnids should show signs of disease or stress or unusual behaviour such as trapping at the surface of the water - dissolved oxygen concentration in the test media ≥ 3 mg/L in all the treatments at the end of the test

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	PROTIOKONAZOL 300 EC
Formulation:	EC (prothioconazole 300 g/L)
Description (physical state):	liquid
Batch no.:	01/PRO/2022
Production date:	24 March 2022
Expiration date:	03.2025
Stability of test compound:	stability was evaluated to provide evidence that its concentration has been satisfactorily maintained preferably, at least 80% of the nominal concentration, throughout the test; For this reason, all the test solutions concentrations were analysed at the beginning (0 hours), before (24 hours - before water medium renewal) and after the renewal (24 hours - after water medium renewal) and at the end (48 hours) of the test period. Test beginning corresponded to just before the daphnids introduction in the test vessels
2. Vehicle and/or positive control:	vehicle control: ISO standard ready-to-use water medium, positive control: potassium dichromate

3. Test organism

Species:	Crustaceans *(Daphnia magna)*
Source:	purchased
Age:	daphnids less than 24-hour old
Sex:	female
Feeding:	none, 2 hours before the test start only
Test units:	multiwell plates with 30 wells of 2.9 × 2.9 × 2.5 cm, 10 mL per well, covered with a Parafilm layer to reduce the loss of water due to evaporation and to avoid the entry of dust into the test solutions

4. Environmental conditions:

Medium:	ISO standard ready-to-use water medium
pH:	6.93 to 8.30
Dissolved oxygen concentration:	5.01 to 6.96 mg/L
Medium temperature:	20.2 to 22.1 °C
Lighting:	daily cycle 24 h light

STUDY DESIGN AND METHOD

Aim of this semi-static study was to determine the 24-hour and 48-hour EC_{50} of test item PROTIOKONAZOL 300 EC (prothioconazole 300 g/L) by assessing Daphnia magna immobility for 48 hours, in comparison to a control group. All the experimental procedures were designed by following the OECD guideline method no. 202.
The ISO standard ready-to-use water medium was used as diluent to prepare test item PROTIOKONAZOL 300 EC exposure solutions at 0.43, 0.94, 2.07, 4.55, 10 mg/L concentration. Test item PROTIOKONAZOL 300 EC solutions were compared to a control group of ISO standard ready-to-use water medium only. A stock solution (treatment SM) was prepared by weighing PROTIOKONAZOL 300 EC and 1000 mL of ISO standard ready-to-use was added to reach the final water dilution content. For the test item dosages, a spacing dilution factor of 2.2 was used. Test item PROTIOKONAZOL 300 EC appearances in the dilution water was observed at the test start and after 24 and 48 hours in all dosages. No remarkable observations were noticed.

Test item PROTIOKONAZOL 300 EC (prothioconazole 300 g/L) stability was evaluated to provide evidence that its concentration has been satisfactorily maintained preferably, at least 80% of the nominal concentration, throughout the test. For this reason, all the test solutions concentrations were analysed at the beginning (0 hours), before (24 hours - before water medium renewal) and after the renewal (24 hours - after water medium renewal) and at the end (48 hours) of the test period. Test beginning corresponded to just before the daphnids introduction in the test vessels.

Immediately after the delivery, *Daphnia magna* ephippia were left to hatch in a Petri dish with ISO standard ready-to-use and kept under controlled condition for 72 hours in an incubator. Two hours before the test start, daphnids were fed with 1 mL of Spirulina powder (1 mL) as described in the DAPHTOXKIT F procedure. The following parameters will be evaluated:

- mortality (immobility) - the daphnids' mobility was visual recorded, animals were considered immobile if they were not able to swim within 15 seconds after gentle agitation of the test unit (even if they could still move their antennae)
- abnormalities (sublethal effects) - visible abnormalities concerning the animals' morphology and behavior (e.g., trapping at the surface of the water, discolored animals, etc.) were recorded.

At the end of the study, the living animals were humanely euthanized by rapid freezing.

Test design:	definitive test: control and tested concentration prepared in 4 replicates each, with 5 daphnia introduced into each replicate
Type of the exposure:	semi-static (renewal after 24 hours)
Exposure time:	48 hours
Tested concentrations, definitive test:	control (0 mg/l), 0.43, 0.94, 2.07, 4.55, 10 mg/l
Dates:	start of the study 26.08.2022 start of the experimental part: 27.09.2022 end of the experimental part: 09.12.2022 end of the study: 20.02.2022
Statistic:	Software used for statistical analysis was “ToxRatPro”, version 3.3.0 Mortality data were processed using the Cochran-Armitage test Procedure $\alpha \leq 0.05$, 0.01, 0.001, and EC_X values calculated, where possible. The No Observed Effect Rate (NOEC) and Lowest Observed Effect Rate (LOEC) values for mortality and reproduction were determined, where possible.

RESULTS

Concerning daphnids immobility, the mean immobility after 24 hours ranged from 0.00% in treatments 0.43 mg f.p./L, 0.94 mg f.p./Land 2.07 mg f.p./L to 70.00% in treatment 10 mg f.p./L. The 24-h NOEC value was 2.07 mg f.p./L and LOEC matched with the test item rate of 4.55 mg f.p./L.

The 24-h EC_{10}, EC_{20} and EC_{50} values were calculated to be 4.14 mg test item/L corresponding to 1.20 mg a.i./L, 5.12 mg test item/L corresponding to 1.48 mg a.i./L and 7.69 mg test item/L corresponding to 2.23 mg a.i/L, respectively. The EC_{10}, EC_{20} and EC_{50} actual values were calculated to be 1.02 mg a.i./L, 1.29 mg a.i./L and 2.02 mg a.i/L, respectively.

Mean mortality at 48 hours of exposure ranged from 0.00 in 0.43 mg f.p./L, 0.94 mg f.p./L and 2.07 mg f.p./L to 95.00% in 10 mg f.p./L. A slightly dose-response effect on Daphnia magna mobility was observed. The 48-h NOEC value was 2.07 mg f.p./L and LOEC matched with the test item rate of 4.55 mg f.p./L.

The 48-h EC_{10}, EC_{20} and EC_{50} values were calculated to be 2.39 mg test item/L corresponding to 0.69 mg a.i./L, 2.95 mg test item/L corresponding to 0.86 mg a.i./L and 4.40 mg test item/L corresponding to 1.28 mg a.i/L, respectively. The EC_{10}, EC_{20} and EC_{50} actual values were calculated to be 0.53 mg a.i./L, 0.67 mg a.i./L and 1.03 mg a.i/L, respectively.

Table KCP 10.2.1.2-1: Summary of results from the immortality assessment at 24 hours

Treatment number	Treatment	Rate (mg a.i./L)	Check at 24 hours		
			Immobility		p^b
			(%)	SE[a]	
T1	Control	-	0.00	± 0.00	-
T2	PROTIOKONAZOL 300 EC at 0.43 mg f.p.*/L	0.12	0.00	± 0.00	n.s.
T3	PROTIOKONAZOL 300 EC at 0.94 mg f.p.*/L	0.27	0.00	± 0.00	n.s.
T4	PROTIOKONAZOL 300 EC at 2.07 mg f.p.*/L	0.60	0.00	± 0.00	n.s.
T5	PROTIOKONAZOL 300 EC at 4.55 mg f.p.*/L	1.33	15.00	± 0.48	**
T6	PROTIOKONAZOL 300 EC at 10 mg f.p.*/L	2.91	70.00	± 0.65	***

*f.p.: formulated product
-, not applicable
n.s., not significantly different compared to the control
a, standard error from 4 replicates
b, Cochran-Armitage test procedure, α≤0.05 *,0.01 **, 0.001 ***

Table KCP 10.2.1.2-2: Summary of results from the immortality assessment at 48 hours

Treatment number	Treatment	Rate (mg a.i./L)	Check at 48 hours		
			Immobility		p^b
			(%)	SE[a]	
T1	Control	-	0.00	± 0.00	-
T2	PROTIOKONAZOL 300 EC at 0.43 mg f.p.*/L	0.12	0.00	± 0.00	n.s.
T3	PROTIOKONAZOL 300 EC at 0.94 mg f.p.*/L	0.27	0.00	± 0.00	n.s.
T4	PROTIOKONAZOL 300 EC at 2.07 mg f.p.*/L	0.60	0.00	± 0.25	n.s.
T5	PROTIOKONAZOL 300 EC at 4.55 mg f.p.*/L	1.33	55.00	± 0.85	***
T6	PROTIOKONAZOL 300 EC at 10 mg f.p.*/L	2.91	95.00	± 0.25	***

*f.p.: formulated product
-, not applicable
n.s., not significantly different compared to the control
a, standard error from 4 replicates
b, Cochran-Armitage test procedure, α≤0.05 *,0.01 **, 0.001 ***

CONCLUSION

All validity criteria of this semi-static study were met.

After 24 hours, the NOEC value was 2.07 mg f.p./L and LOEC matched with the test item rate of 4.55 mg f.p./L. The EC10, EC20 and EC50 nominal values were calculated to be 4.14 mg test item/L corresponding to 1.20 mg a.i./L, 5.12 mg test item/L corresponding to 1.48 mg a.i./L and 7.69 mg test item/L corresponding to 2.23 mg a.i/L, respectively. The EC10, EC20 and EC50 actual values were calculated to be 1.02 mg a.i./L, 1.29 mg a.i./L and 2.02 mg a.i/L, respectively.

After 48 hours, the NOEC value was 2.07 mg f.p./L and LOEC matched with the test item rate of 4.55 mg f.p./L. The EC10, EC20 and EC50 nominal values were calculated to be 2.39 mg test item/L corresponding to 0.69 mg a.i./L, 2.95 mg test item/L corresponding to 0.86 mg a.i./L and 4.40 mg test item/L corresponding to 1.28 mg a.i/L, respectively. The EC10, EC20 and EC50 actual values were calculated to be 0.53 mg a.i./L, 0.67 mg a.i./L and 1.03 mg a.i/L, respectively.

Table KCP 10.2.1.2-3: Immobility of Daphnia magna based on nominal values

		PROTIOKONAZOL 300 EC treatments				
	T1 Control	**T2 0.43 mg f.p./L**	**T3 0.94 mg f.p./L**	**T4 2.07 mg f.p./L**	**T5 4.55 mg f.p./L**	**T6 10 mg f.p./L**
	ISO standard ready-to-use	**0.12 mg a.i./L**	**0.27 mg a.i./L**	**0.60 mg a.i./L**	**1.32 mg a.i./L**	**2.91 mg a.i./L**
Immobility (24 hours) [mean %]	0.00	0.00	0.00	0.00	15.00	70.00
Significance [a]	-	n.s.	n.s.	n.s.	**	***
Immobility (48 hours) [mean %]	0.00	0.00	0.00	5.00	55.00	95.00
Significance [a]	-	n.s.	n.s.	n.s.	***	***

Endpoint	**mg test item/L**	**mg a.i./L**
EC_{10} (24 hours) [95% confidence intervals]	4.14 [2.37 – 5.30]	1.20 [0.69 – 1.54]
EC_{20} (24 hours) [95% confidence intervals]	5.12 [3.44 – 6.35]	1.48 [1.00 – 1.84]
EC_{50} (24 hours) [95% confidence intervals]	7.69 [6.19 – 10.18]	2.23 [1.79 – 2.95]
NOEC	2.07	0.60
LOEC	4.55	1.32
EC_{10} (48 hours) [95% confidence intervals]	2.39 [1.52 – 3.05]	0.69 [0.44 – 0.88]
EC_{20} (48 hours) [95% confidence intervals]	2.95 [2.08 – 3.64]	0.86 [0.60 – 1.06]
EC_{50} (48 hours) [95% confidence intervals]	4.40 [3.54 – 5.47]	1.28 [1.03 – 1.59]
NOEC	2.07	0.60
LOEC	4.55	1.32

a.i. = prothioconazole
-, not applicable
n.s., not significantly different compared to the control
n.d., not determined due to mathematical reasons
a, Cochran-Armitage test procedure, $\alpha \leq 0.05$ *,0.01 **, 0.001 ***

Table KCP 10.2.1.2-4: Immobility of Daphnia magna based on actual values

		PROTIOKONAZOL 300 EC treatments				
	T1	**T2**	**T3**	**T4**	**T5**	**T6**
24 hours	**ISO standard ready-to-use**	**0.068 mg a.i./L**	**0.22 mg a.i./L**	**0.48 mg a.i.//L**	**1.13 mg a.i./L**	**2.50 mg a.i./L**
48 hours	**ISO standard ready-to-use**	**0.060 mg a.i./L**	**0.20 mg a.i./L**	**0.45 mg a.i./L**	**1.08 mg a.i./L**	**2.44 mg a.i./L**

Endpoint	**mg a.i./L**
EC_{10} (24 hours) [95% confidence intervals]	1.02 [0.53 – 1.33]
EC_{20} (24 hours) [95% confidence intervals]	1.29 [0.83 – 1.63]
EC_{50} (24 hours) [95% confidence intervals]	2.02 [1.60 – 2.83]
NOEC	0.48
LOEC	1.13
EC_{10} (48 hours) [95% confidence intervals]	0.53 [0.33 – 0.70]
EC_{20} (48 hours) [95% confidence intervals]	0.67 [0.46 – 0.84]
EC_{50} (48 hours) [95% confidence intervals]	1.03 [0.81 – 1.30]
NOEC	0.45
LOEC	1.08

* Actual values based on the analytical analysis of the samples. Calculated by a time-weighted arithmetic mean of a series of logarithmic mean.
a.i. = prothioconazole
-, not applicable

A 2.2.1.3 KCP 10.2.1.3 Effects on aquatic algae

Comments of zRMS:

The study was accepted by zRMS.
The validity criteria was met.

Validity criteria of the study

A test is not considered acceptable if the cell density in the control cultures has not increased by a factor of at least 16 within the test period.

The coefficient of variation of daily growth rates in the control cultures must not exceed 35%.

The coefficient of variation of average growth in replicates control cultures must not exceed 7%.

In the present study the cell density in the negative control had increased on average by a factor of 161, corresponding to a daily specific growth rate equal to 1.69. The coefficient of variation of daily section-by-section growth rates was 27.1% and the coefficient of variation of average growth in negative control was 2.4%, so the study can be considered valid and acceptable.

The agreed toxicity endpoints:
Growth rate parameter
*expressed in terms of nominal test item **HERA 300 EC** concentrations*

Time (h)	**E_rC_{10} (mg/L)**	**E_rC_{20} (mg/L)**	**E_rC_{50} (mg/L)**	**NOEC (mg/L)**	**LOEC (mg/L)**
0 – 24	1.2 (0.39 – 1.9)*	1.9 (1.2 – 2.8)*	14.2 (9.6 – 21.64)*	1.0	3.1
0 – 48	1.9 (1.5 – 2.2)*	3.1 (2.3 – 3.6)*	6.5 (5.9 – 6.9)*	1.0	3.1
0 – 72	2.3 (2.0 – 2.7)*	3.6 (3.3 – 3.9)*	7.4 (7.0 – 7.7)*	1.0	3.1

* 95% confidence limits

*expressed in terms of nominal **prothioconazole** concentrations*

Time (h)	E_rC_{10} (mg/L)	E_rC_{20} (mg/L)	E_rC_{50} (mg/L)	NOEC (mg/L)	LOEC (mg/L)
0 – 24	0.36 (0.12 – 0.56)*	0.56 (0.36 – 0.83)*	4.22 (2.85 – 6.42)*	0.30	0.92
0 – 48	0.56 (0.45 – 0.65)*	0.92 (0.68 – 1.07)*	1.93 (1.75 – 2.05)*	0.30	0.92
0 – 72	0.68 (0.59 – 0.80)*	1.07 (0.98 – 1.16)*	2.20 (2.08 – 2.29)*	0.30	0.92

* 95% confidence limits

Yield parameter
*expressed in terms of nominal test item **HERA 300 EC** concentrations*

Time (h)	E_yC_{10} (mg/L)	E_yC_{20} (mg/L)	E_yC_{50} (mg/L)	NOEC (mg/L)	LOEC (mg/L)
0 – 72	1.3 (1.2 – 1.4)*	1.6 (1.5 – 1.8)*	3.0 (2.5 – 3.8)*	1.0	3.1

* 95% confidence limits

*expressed in terms of nominal **prothioconazole** concentrations*

Time (h)	E_yC_{10} (mg/L)	E_yC_{20} (mg/L)	E_yC_{50} (mg/L)	NOEC (mg/L)	LOEC (mg/L)
0 – 72	0.39 (0.36 – 0.42)*	0.48 (0.45 – 0.53)*	0.89 (0.74 – 1.13)*	0.30	0.92

* 95% confidence limits

Reference:	KCP 10.2.1.3/01
Report	PROTIOKONAZOL 300 EC: toxicity to green algae *Pseudokirchneriella subcapitata* in a growth inhibition study; Mautino G.; 2023; Study Code: 4546.1F.SAG22
Guideline(s):	Yes, OECD 201
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No
Validity criteria of the test:	All validity criteria were met: - the cell density increases by a factor of at least 16 during the whole test period - the mean coefficient of variation of daily growth rates (days 0-1, 1-2 and 2-3) must not exceed 35% - the coefficient of variation of average growth rates during the whole test period in the control replicates doesn't exceed 7%

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	PROTIOKONAZOL 300 EC
Formulation:	
Description (physical state):	EC (prothioconazole 300 g/L)
Batch no.:	liquid
Production date:	01/PRO/2022
Expiration date:	24 March 2022
Stability of test compound:	A concentration analytical check on test solutions was performed using the Analytical Method for prothioconazole determination. Samples of test media were taken, in duplicate, at time 0 and after 72 hours. Final samples were obtained by mixing the replicate test vessels media together and they were immediately centrifuged or filtered to separate the algae from the medium. Negative control samples were analysed at the same time to verify the absence of test item contamination. Samples were labelled and analysed after sampling.
2. Vehicle and/or positive control:	vehicle control: OECD medium, positive control: 3,5-dichlorophenol
3. Test organism	
Species:	*Raphidocelis subcapitata* (also known as *Pseudokirchneriella subcapitata*)
Source:	test site and originally purchased from the Institute of Plant Physiology of the University of Göttingen, Germany
Age:	3 days prior to the start of the test
Test units:	100 mL capacity conical glass flasks capped with air permeable stoppers
4. Environmental conditions:	
Medium:	OECD medium
Medium temperature:	average temperature 24.02 ºC (23.90 – 24.47 ºC);
Lighting:	light intensity ranged between 6147 and 6246 lux

STUDY DESIGN AND METHOD

The aim of the study was to determine the influence of test item PROTIOKONAZOL 300 EC on growth algae (*Pseudokirchneriella subcapitata* based on OECD 201. The algae are cultured in an environmental test chamber at 24 ± 2°C, under continuous uniform illumination of 4440-8880 Lux in the spectral range 400-700 nm. The culture medium was the same of the test medium; the stock cultures are regularly transferred to fresh medium and maintained in continuous shaking, thus ensuring the necessary amount of CO2 and keeping algae in suspension. Cultures containing deformed or abnormal cells were discarded. Only exponentially growing algal cultures were used to start the test.
In order to verify the sensitivity of the algal strain and to demonstrate that satisfactory test conditions are adopted in the laboratory, a positive control with the reference item 3,5-dichlorophenol is performed twice a year.

The test item concentrations were chosen in accordance with the results of a non-GLP pre-test (rangefinding test), whose relevant Raw Data were archived under the code of the present study. The test was performed at five test item concentrations in a geometric series, differing by a constant factor (of 3.2): 1.0, 3.1, 9.8, 31.3 and 100.0 mg test item/L, corresponding to 0.30, 0.92, 2.91, 9.30 and 29.71 mg prothioconazole/L. Additionally, a negative control (test medium without test item) was run.

Prior to the test start, a 100.0 mg test item/L stock solution (SS1) was prepared by direct weighting 0.0500 g of test item into 500 mL of algal growth medium. The stock solution was clear and transparent with the test item totally solubilized. Test item solution at 100.0 mg/L was also the highest test item tested concentration.

Algal cell density was measured every 24 hours by taking aliquots from each test concentration replicate and the negative control, diluting them in a NaCl 9 g/L solution and reading with an electronic particle counter. The measured values were used to calculate the percentage inhibition of cell growth as yield and as growth rate in comparison to the negative control.

Test design:	tested concentrations in three replicates, control in six replicates
Type of the exposure:	static
Exposure time:	72 hours
Inoculum:	10^4 cells/mL
Tested concentrations, definitive test:	control (0 mg/L), 1.0, 3.1, 9.8, 31.3 and 100.0 mg test item/L, corresponding to 0.30, 0.92, 2.91, 9.30 and 29.71 mg a.s./L
Dates:	start of the study 29.11.2022 start of the experimental part: 13.12.2022 end of the experimental part: 23.01.2023 end of the study: 21.02.2023
Statistic:	Statistical analysis was performed by test site personnel. Software used for statistical analysis was CETIS elaboration software version 1.8.7.7. The ErCx and EyCx values were determined by Linear interpolation (ICPIN) method for growth rate and yield endpoint. The NOEC and LOEC values for growth rate and yield endpoints were determined by comparison with the negative control by Bonferroni Adj t Test.

RESULTS

The calculated growth rates and the percentage of growth inhibition for the tested concentrations are reported in the following table.

Table KCP 10.2.1.3-1: Algal growth rate and percentage inhibition

Nominal test item concentration (mg/L)	0-24 h		0-48 h		0-72 h	
	Mean growth rate	Mean inhibition (%)	Mean growth rate	Mean inhibition (%)	Mean growth rate	Mean inhibition (%)
0.0 (negative control)	1.1971	-	1.4953	-	1.6914	-
1.0	1.1183	6.6	1.5066	-0.8*	1.7566	-3.9*
3.1	0.8174	31.7	1.2015	19.6	1.4819	12.4
9.8	0.6918	42.2	0.4736	68.3	0.6418	62.1
31.3	0.3949	67.0	0.3467	76.8	0.3036	82.0
100.0	0.2297	80.8	0.3768	74.8	0.2457	85.5

The calculated yield and the percentage of yield inhibition for the tested concentrations are reported in the following table.

Table KCP 10.2.1.3-2: Algal yield and percentage inhibition

Nominal test item concentration (mg/L)	Mean yield at 72h (cell/mL)	Mean yield inhibition (%)
0.0 (negative control)	1599011	-
1.0	1943244	-21.5*
3.1	844289	47.2
9.8	58632	96.3
31.3	15122	99.1
100.0	10906	99.3

CONCLUSION

Table KCP 10.2.1.3-3: Yield and Growth rate at 72 hours of exposure results assessed on the basis of nominal test item concentration

Endpoint	0 - 72 h EC_{10} (mg/L)	0 - 72 h EC_{20} (mg/L)	0 - 72 h EC_{50} (mg/L)	0 - 72 h NOEC (mg/L)	0 - 72 h LOEC (mg/L)
Growth rate	2.3 (2.0 – 2.7)*	3.6 (3.3 – 3.9)*	7.4 (7.0 – 7.7)*	1.0	3.1
Yield	1.3 (1.2 – 1.4)*	1.6 (1.5 – 1.8)*	3.0 (2.5 – 3.8)*	1.0	3.1

(*) 95% confidence limits available

Table KCP 10.2.1.3-4: Yield and Growth rate at 72 hours of exposure results assessed on the basis of nominal a.i. concentration

Endpoint	**0 - 72 h EC_{10}** (mg/L)	**0 - 72 h EC_{20}** (mg/L)	**0 - 72 h EC_{50}** (mg/L)	**0 - 72 h NOEC** (mg/L)	**0 - 72 h LOEC** (mg/L)
Growth rate	0.68 (0.59 – 0.80)*	1.07 (0.98 – 1.16)*	2.20 (2.08 – 2.29)*	0.30	0.92
Yield	0.39 (0.36 – 0.42)*	0.48 (0.45 – 0.53)*	0.89 (0.74 – 1.13)*	0.30	0.92

(*) 95% confidence limits available

A 2.2.1.4 KCP 10.2.1.4 Effects on aquatic macrophytes

Not relevant. No studies submitted.

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

Not relevant. No studies submitted.

A 2.2.3 KCP 10.2.3 Further testing on aquatic organisms

Not relevant. No studies submitted.

A 2.3 KCP 10.3 Effects on arthropods

A 2.3.1 KCP 10.3.1 Effects on bees

A 2.3.1.1 KCP 10.3.1.1 Acute toxicity to bees

A 2.3.1.1.1 KCP 10.3.1.1.1 Acute oral toxicity to bees

Comments of zRMS:	The study was accepted by zRMS. The validity criteria was met.
	Validity criteria of the study Mortality in the control group: In the control units, the mean value of dead bees was 0.00%, so the validity criterion was met. LD_{50}-24h in the reference group: The 24HAA-LD_{50} for the acute oral test was 0.16 µg a.i./bee therefore, the validity criterion was met, because in the range 0.10-0.35 µg a.i./bee. The 24HAA-LD_{50} for the acute contact test was 0.11 µg a.i./bee therefore, the validity criterion was met, because in the range 0.10-0.35 µg a.i./bee.
	The agreed toxicity endpoints:

	The NOED value at 72-HAA for Acute Oral Toxicity Test matched with test item PROTIOKONAZOL 300 EC dosage of 30.26 µg a.i./bee and the LOED value at 72-HAA was 41.19 µg a.i/bee. The LD_{50} for Acute Oral Toxicity Test was calculated on the 72-HAA mortality and correspond to 99.67 µg a.i./bee µg a.i./bee (95% confidence 92.26 µg a.i./bee – 109.57 µg a.i./bee). The NOED value at 72-HAA for Acute Contact Toxicity Test coincided to 57.60 a.i./bee and the LOED value at 72-HAA was 144.00 µg a.i/bee. The LD_{50} for Acute Contact Toxicity Test was calculated on the 72-HAA mortality and was found to be 98.35 µg a.i./bee (95% confidence limits: (18.18 - 20.85 µg a.i./bee).

Reference:	KCP 10.3.1.1.1/01
Report	Effects of PROTIOKONAZOL 300 EC (prothioconazole 300 g/L) on Honeybees (*Apis mellifera* L.) in the laboratory – Acute Oral and Contact Toxicity Test; Mautino G.; 2023; Study Code: 1137.1F.SAG22
Guideline(s):	Yes, OECD 213
Deviations:	To reach a good fit to the range finding results observed for both oral and contact acute tests and to determine an appropriate slope of the toxicity curve (dose versus mortality). A wider spacing factor equal to 2.5 has been chosen for both oral and contact Definitive tests instead 2.2. No impact.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No
Validity criteria of the test:	All validity criteria were met: - average mortality for the total number of control groups ≤ 10% at the end of the test - LD50 of the reference item meets the specified range

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	PROTIOKONAZOL 300 EC
Formulation:	EC (prothioconazole 300 g/L)
Description (physical state):	liquid
Batch no.:	01/PRO/2022
Production date:	24 March 2022
Expiration date:	03.2025
Stability of test compound:	not relevant

2. Vehicle and/or positive control: vehicle: sucrose solution in deionized water with a final concentration of 500 g/L (50% w/v).
positive control: dimethoat

3. Test organism

Species:	Bees Apis mellifera L; Insecta, Hymenoptera

Source:	Beekeeper Paolo Farinetti, Strada Montà Castino 25, Cortemilia (CN) – 12074. Three commercial beehives, queen-right, healthy (disease free) and adequately fed, with normal population of young adult worker individuals (approx. 2 weeks old) was placed at SAGEA Centro di Saggio s.r.l. Test Facility
Age:	adults (female workers)
Acclimation period:	The test units were placed into a climatic chamber, and kept under darkness at the environmental conditions of the test (25±2 ºC and 50-70% RH) for at least 4 hours, until the beginning of the test. No food or water was supplied during acclimatisation.
Diet:	Sucrose solution in water with a final concentration of 500 g/L (50% w/v) was used as food ad libitum. The syrup was administered using 2 mL syringe (deprived of the tip). One syringe was placed in each cage via an opening in the top of the test unit. If necessary, feeding solution was refilled during the test course.
Test units:	ventilated stainless steel cages 8.5 cm x 6.5 cm x 4.5 cm (length x height x width), front sidewith removable glass panel, back side perforated with 50 ventilation holes; Ø 2 mm

4. Environmental conditions:

Temperature:	23.77± 0.78ºC (23.04 – 24.84 °C)
Relative humidity:	62.0±1.9% (60.2 – 64.6%)
Photoperiod:	darkness (except during observation)

STUDY DESIGN AND METHOD

The aim of this study was to determine the acute oral toxicity of PROTIOKONAZOL 300 EC (prothioconazole 300 g/L) to honeybees *Apis mellifera*. Mortality of the bees was used as the toxic endpoint. Sublethal effects, such as changes in behaviour, were also assessed. The experiment was carried out in accordance with OECD Guideline No 213. The study comprised 9 treatments (5 concentrations of the test item with a spacing factor of 2.5, 1 control group, 3 concentrations of the reference item with a spacing factor of 2) with 3 replicates; each test unit (stainless-steel cage) contained 10 individuals. Each cage was provided with a feeder containing 2 mL of tested doses dispersed in 50% w/v sucrose solution for maximum 6 hours. The amount of treated diet consumed per group was monitored. Then, feeders were replaced with ones containing sucrose solution only *ad libitum*. Mortality was recorded after 4, 24, 48 and 72 hours after application and compared with that of control group.

Test design:	tested dose and control in three repetitions, 10 bees per repeat
Exposure time:	acute test, 72 h

Tested concentrations, definitive test:	1.28, 3.20, 8.00, 20.00, 50.00 µL test item/bee (384.00, 960.00, 2400.00, 6000.00 µg a.i./bee)
Dates:	start of the study 11.07.2022 start of the experimental part: 18.07.2022 end of the experimental part: 03.09.2022 end of the study: 15.02.2023
Statistic:	Software used for statistical analysis was "Agricultural Research Manager 2022" (ARM)". Mortality data were analysed by ANOVA test and then compared to the control group by the Student-Newman-Keuls (S-N-K), $\alpha \leq 0.05$. The LD50 was calculated where possible. The No Observed Effect Dose (NOED) and Lowest Observed Effect Dose (LOED) values for adults' emergence rate were calculated.

RESULTS

Honeybees' mortality was evaluated at 4-HAA, 24-HAA, 48-HAA and 72-HAA after a single exposure of 6 hours. Mortality was calculated in percentage at 24-HAA, 48-HAA and 72-HAA.

Table KCP 10.3.1.1.1-1: Number of dead bees over the test period (Acute Oral Toxicity Test)

Treatment no.	Treatment	Application rate (Nominal intake)	Calculated intake***	Dead bees			
				4-HAA	24-HAA	48-HAA	72-HAA
T1	Control	-	-	0	0	1	1
T2	PROTIOKONAZOL 300 EC	1.28 µL f.p.*/bee (384 µg a.i.**/bee)	0.10 µL f.p.*/bee (30.27 µg a.i.**/bee)	0	2	2	2
T3	PROTIOKONAZOL 300 EC	3.20 µL f.p./bee (960 µg a.i./bee)	0.14 µL f.p.*/bee (41.19 µg a.i.**/bee)	0	3	7	7
T4	PROTIOKONAZOL 300 EC	8 µL f.p./bee (2400 µg a.i./bee)	0.20 µL f.p.*/bee (60.31 µg a.i. **/bee)	4	7	8	8
T5	PROTIOKONAZOL 300 EC	20 µL f.p./bee (6000 µg a.i./bee)	0.30 µL f.p.*/bee (88.83 µg a.i. **/bee)	8	11	12	12
T6	PROTIOKONAZOL 300 EC	50 µL f.p./bee (15000 µg a.i./bee)	0.36 µL f.p.*/bee (106.76 µg a.i. **/bee)	11	16	18	18
T7	ROGOR L 40 ST	0.00023 µL f.p./bee (0.090 µg a.i./bee)	0.00019 µL f.p.*/bee (0.075 µg a.i. **/bee)	7	7	30	30
T8	ROGOR L 40 ST	0.00045 µL f.p./bee (0.18 µg a.i./bee)	0.00038 µL f.p.*/bee (0.15 µg a.i. **/bee)	10	10	30	30
T9	ROGOR L 40 ST	0.00088 µL f.p./bee (0.35 µg a.i./bee)	0.00072 µL f.p.*/bee (0.29 µg a.i. **/bee)	20	26	30	30

HAA = Hours After Application

*f.p.: formulated product
**a.i.: active ingredient
*** intake of µL f.p./µg a.i. calculated on the feeding consumption data obtained over the 6 hrs exposure

CONCLUSION

All study validity criteria were met. The NOED value at 72-HAA for Acute Oral Toxicity Test matched with test item PROTIOKONAZOL 300 EC dosage of 30.26 µg a.i./bee and the LOED value at 72-HAA was 41.19 µg a.i/bee. The LD_{50} for Acute Oral Toxicity Test was calculated on the 72-HAA mortality and correspond to 99.67 µg a.i./bee µg a.i./bee (95% confidence 92.26 µg a.i./bee – 109.57 µg a.i./bee).

Table KCP 10.3.1.1.1-2: Mortality at 24 HAA, 48 HAA and 72 HAA– Acute Oral Toxicity Test (Definitive test)

Endpoints	µg a.i./bee	µL f.p./bee
24-HAA LD_{50}	>106.76 µg a.i. **/bee	> 0.36 µL f.p.*/bee
24-HAA NOED	60.31 µg a.i. **/bee	0.20 µL f.p.*/bee
24-HAA LOED	88.83 µg a.i. **/bee	0.30 µL f.p.*/bee
48-HAA LD_{50}	99.67 µg a.i. **/bee	0.33 µL f.p.*/bee
48-HAA NOED	30.27 µg a.i. **/bee	0.10 µL f.p.*/bee
48-HAA LOED	41.19 µg a.i. **/bee	0.14 µL f.p.*/bee
72-HAA LD_{50}	99.67 µg a.i. **/bee	0.33 µL f.p.*/bee
72-HAA NOED	30.27 µg a.i. **/bee	0.10 µL f.p.*/bee
72-HAA LOED	41.19 µg a.i. **/bee	0.14 µL f.p.*/bee

Comments of zRMS: The study was accepted by zRMS.
The validity criteria was met.

Validity criteria of the study

Mortality in the control group	In the control units, the mean value of dead bees was 0.00%, so the validity criterion was met.
LD_{50}-24h in the reference group	The 24HAA-LD_{50} for the acute oral test was 0.16 µg a.i./bee therefore, the validity criterion was met, because in the range 0.10-0.35 µg a.i./bee. The 24HAA-LD_{50} for the acute contact test was 0.11 µg a.i./bee therefore, the validity criterion was met, because in the range 0.10-0.35 µg a.i./bee.

The agreed toxicity endpoints:

Mortality at 24 HAA and 48 HAA – Acute Contact Toxicity Test (Limit test)

Treatment number	Treatment	Test Item f.p./bumblebee (Nominal intake)	Test Item a.i/bumblebee (Nominal intake)	Mortality 24 HAA (%)	p^a	Mortality 48 HAA (%)	p^a
T1	Control	-	-	0.00	b	0.00	b
T2	PROTIOKONAZOL 300 EC	0.34 µL f.p.*/bumblebee	100 µg a.i.**/bumblebee	0.00	b	0.00	b
T3	ROGOR L 40 ST	0.025 µL f.p./ bumblebee	10 µg a.i./bumblebee	80.00	a	100.00	a

Endpoints	µg a.i./bumblebee	µL f.p./bumblebee
48-HAA LD_{50}	>100 µg a.i.**/bumblebee	>0.34 µL f.p.*/bumblebee
48-HAA NOED	>100 µg a.i.**/bumblebee	>0.34 µL f.p.*/bumblebee
48-HAA LOED	>100 µg a.i.**/bumblebee	>0.34 µL f.p.*/bumblebee

[a], S-N-K test ($P \leq 0.05$) on the data at day-8
*f.p.: formulated product
**a.i.: active ingredient

Reference: KCP 10.3.1.1.1/02

Report	Effects of PROTIOKONAZOL 300 EC (prothioconazole 300 g/L) on Bumblebee (*Bombus terrestris* L.) in the laboratory – Acute Oral and Contact Toxicity Test; Mautino G; 2023; Study Code: 1138.1F.SAG22
Guideline(s):	Yes, OECD 247
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No
Validity criteria of the test:	All validity criteria were met: - mortality in the control group: ≤ 10% at the end of the test - mortality in the toxic reference item group should be ≥ 50% at the end of the test

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	PROTIOKONAZOL 300 EC
Formulation:	EC (prothioconazole 300 g/L)
Description (physical state):	liquid
Batch no.:	01/PRO/2022
Production date:	24 March 2022
Expiration date:	03.2025
Stability of test compound:	The content of prothioconazole active ingredient was determined in the lowest concentration and in the highest concentration of the sucrose feeding solutions and in the contact water solution prepared in the biological phase of the study.

2. Vehicle and/or positive control: vehicle: Sucrose solution in deionized water with a final concentration of 500 g/L (50% w/v)
positive control: dimethoat

3. Test organism

Species:	bumbleebee (*Bombus* spp.)
Source:	Three commercial hives (Natupol, Koppert), queen-right, healthy (disease free) and adequately fed, with normal population of young adult worker individuals, purchased from Agrigonella snc, Corso Alcide de Gasperi 58, 12046 Montà (CN), Italy. The hives were kept in climatic chamber under mean controlled environmental conditions.
Age:	adult female workers

Acclimation period:	15 to 18 hours prior the start of the test with access to an untreated 50% (w/v) aqueous sucrose solution ad libitum. Moribund bumblebees during the acclimatisation period have been discarded and replaced by healthy individuals before starting the test.
Diet:	Aqueous sucrose solution 50% w/v dispensed ad libitum during acclimatization period and after the application of the test and reference items. A stock solution of 1 L was prepared once at test start of the test by mixing 500 g of sucrose and 500 mL of distilled deionized water. The stock sucrose solution was kept in refrigerator at 4°C for the whole test duration. The feeding solution was administered using a 5 mL syringe (deprived of the tip). One syringe was placed in each individual cage. If necessary, feeding solution was refilled during the test course.
Test units:	single unit plastic cages (Nicot® queen breeding system), containing one adult worker bumblebee of medium size. Each Nicot® cage had a medium internal volume of 34 cm3 (length: 7 cm; diameter 2.5 cm), adequate to bumblebee size, and was equipped with a 5 mL syringe, deprived of the tip, filled with an aqueous sucrose solution (50% w/v), needed for ad libitum feeding

4. Environmental conditions:

Temperature:	24.86 ± 0.173 °C (24.63 – 25.02 °C)
Relative humidity:	75.2 ± 1.3% (73.3 – 76.7%)
Photoperiod:	darkness condition (except during observation)

STUDY DESIGN AND METHOD

The aim of this study was to determine the acute oral toxicity of PROTIOKONAZOL 300 EC (prothioconazole 300 g/L) to bumblebees. Mortality of the bumblebees was used as the toxic endpoint. Sublethal effects (if any), such as changes in behaviour, were also be assessed.The study comprised 7 treatments (5 concentrations of the test item with a spacing factor of 2.2, 1 control group, 1 reference item) with 30 replicates; each test unit (plastic cage) contained 1 individual. Each bumblebee was provided with 40 µL of tested doses dispersed in 50% w/v sucrose solution for maximum 4 hours. The amount of treated diet consumed per group was monitored. Then, feeders were replaced with ones containing sucrose solution only *ad libitum*. Mortality was recorded at 4, 24 and 48 hours after application (HAA) and compared with that of control group. Test item was compared with an untreated control (Aqueous sucrose solution 50% w/v) and a toxic standard as recommended in the guideline. Reference item was ROGOR L 40 ST (dimethoate 400 g/L) dissolved in aqueous sucrose solution. Test and reference were dispersed in the aqueous sucrose solution on the first day of trial. Mortality was recorded at 4, 24 and 48 hours after application (HAA) and compared with that of control group.

Test design:	30 individuals from 3 different hives
Exposure time:	acute test, 48 h
Tested concentrations, definitive test:	0.015, 0.032, 0.07, 0.15 and 0.34 µg/bumblebee (4.27, 9.39, 20.66, 45.45 and 100.00 µg a.i./bumblebee)

Dates: start of the study 10.10.2022
start of the experimental part: 17.10.2022
end of the experimental part: 11.11.2022
end of the study: 15.2.2023

Statistic: Results were analyzed by the validated statistical software "Agricultural Research Manager 2022" (ARM). Levene's test was used to assess the variances' homogeneity ($P<0.05$), data were transformed in case of absence of variances' homogeneity. Correction for control mortality was carried out using the Schneider-Orelli's formula. An ANOVA and Student-Newman-Keuls (S-N-K) tests were performed on the mortality data to identify any significant differences between treatments and control groups. The LD50 values were calculated for the test item with 95% confidence interval, using a probit regression analysis. The following parameters were calculated on the 48-HAA bumblebees' mortality data: LOED/NOED and LD50.

RESULTS

Bumblebees' mortality was evaluated at 4-HAA, 24-HAA and 48-HAA after a single exposure of 4 hours. Mortality was calculated in percentage at 24-HAA and 48-HAA.

Table KCP 10.3.1.1.1-3: Number of dead bumblebees over the test period (Acute Oral Definitive Test)

Treatment no.	Treatment	Application rate (Nominal intake)	Dead bumblebees		
			4-HAA	24-HAA	48-HAA
T1	Control	-	0	0	0
T2	PROTIOKONAZOL 300 EC	0.015 µL f.p.*/bumblebee (4.27 µg a.i.**/bumblebee)	0	0	0
T3	PROTIOKONAZOL 300 EC	0.032 µL f.p.*/bumblebee (9.39 µg a.i.**/bumblebee)	0	0	0
T4	PROTIOKONAZOL 300 EC	0.07 µL f.p.*/bumblebee (20.66 µg a.i.**/bumblebee)	0	0	0
T5	PROTIOKONAZOL 300 EC	0.15 µL f.p.*/bumblebee (45.45 µg a.i.**/bumblebee)	0	0	0
T6	PROTIOKONAZOL 300 EC	0.34 µL f.p.*/bumblebee (100 µg a.i.**/bumblebee)	0	0	0
T7	ROGOR L 40 ST	0.01 µL f.p.*/bumblebee (4 µg a.i.**/bumblebee)	13	20	27

HAA = Hours After Application
*f.p.: formulated product
**a.i.: active ingredient

CONCLUSION

Concerning test item PROTIOKONAZOL 300 EC (prothioconazole 300 g/L), no significant differences were noticed between treated and control group in terms of effects on bumblebees' survival.

Table KCP 10.3.1.1.1-4: Mortality at 24 HAA and 48HAA – Acute Oral Toxicity Test

Endpoints	µg a.i./bumblebee	µL f.p./bumblebee
48-HAA LD_{50}	>100 µg a.i.**/bumblebee	>0.34 µL f.p.*/bumblebee
48-HAA NOED	>100 µg a.i.**/bumblebee	>0.34 µL f.p.*/bumblebee
48-HAA LOED	>100 µg a.i.**/bumblebee	>0.34 µL f.p.*/bumblebee

A 2.3.1.1.2 KCP 10.3.1.1.2 Acute contact toxicity to bees

Comments of zRMS: The study was accepted by zRMS.
The validity criteria was met.

Mortality in the control group	Mortality in the control group was < 10%, therefore the validity criterion was met.
Mortality in the reference group	Bumblebees' mortality was >50% at 48-HAA, therefore the validity criterion was met.

The agreed toxicity endpoints:

Mortality at 24 HAA and 48 HAA – Acute Oral Toxicity Test

Treatment number	Treatment	Test Item f.p./bumblebee (Nominal intake)	Test Item a.i/bumblebee (Nominal intake)	Mortality 24 HAA (%)	p^a	Mortality 48 HAA (%)	p^a
T1	Control	-	-	0.00	b	0.00	b
T2	PROTIOKONAZOL 300 EC	0.015 µL f.p.*/bumblebee	4.27 µg a.i.**/bumblebee	0.00	b	0.00	b
T3	PROTIOKONAZOL 300 EC	0.032 µL f.p.*/bumblebee	9.39 µg a.i.**/bumblebee	0.00	b	0.00	b
T4	PROTIOKONAZOL 300 EC	0.07 µL f.p.*/bumblebee	20.66 µg a.i.**/bumblebee	0.00	b	0.00	b
T5	PROTIOKONAZOL 300 EC	0.15 µL f.p.*/bumblebee	45.45 µg a.i.**/bumblebee	0.00	b	0.00	b
T6	PROTIOKONAZOL 300 EC	0.34 µL f.p.*/bumblebee	100 µg a.i.**/bumblebee	0.00	b	0.00	b
T7	ROGOR L 40 ST	0.01 µL f.p./bumblebee	4 µg a.i./bumblebee	66.70	a	93.30	a

Endpoints	µg a.i./bumblebee	µL f.p./bumblebee
48-HAA LD_{50}	>100 µg a.i.**/bumblebee	>0.34 µL f.p.*/bumblebee
48-HAA NOED	>100 µg a.i.**/bumblebee	>0.34 µL f.p.*/bumblebee
48-HAA LOED	>100 µg a.i.**/bumblebee	>0.34 µL f.p.*/bumblebee

[a], S-N-K test (P≤0.05) on the data at day-8
*f.p.: formulated product
**a.i.: active ingredient

Reference: KCP 10.3.1.1.2/01

Report: Effects of PROTIOKONAZOL 300 EC (prothioconazole 300 g/L) on Honeybees (*Apis mellifera* L.) in the laboratory – Acute Oral and Contact Toxicity Test; Mautino G.; 2023; Study Code: 1137.1F.SAG22

Guideline(s): Yes, OECD 214

Deviations: To reach a good fit to the range finding results observed for both oral and contact acute tests and to determine an appropriate slope of the toxicity curve (dose versus mortality). A wider spacing factor equal to 2.5 has been chosen

	for both oral and contact Definitive tests instead 2.2. No impact.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No
Validity criteria of the test:	All validity criteria were met: - average mortality for the total number of control groups ≤ 10% at the end of the test - LD50 of the reference item meets the specified range

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	PROTIOKONAZOL 300 EC
Formulation:	EC (prothioconazole 300 g/L)
Description (physical state):	liquid
Batch no.:	01/PRO/2022
Production date:	24 March 2022
Expiration date:	03.2025
Stability of test compound:	not relevant
2. Vehicle and/or positive control:	vehicle: deionized water + surfactant agent (TWEEN20 polyethylene glycol sorbitan monolaurate) positive control: dimethoat

3. Test organism

Species:	Bees Apis mellifera L; Insecta, Hymenoptera
Source:	Beekeeper Paolo Farinetti, Strada Montà Castino 25, Cortemilia (CN) – 12074. Three commercial beehives, queen-right, healthy (disease free) and adequately fed, with normal population of young adult worker individuals (approx. 2 weeks old) was placed at SAGEA Centro di Saggio s.r.l. Test Facility
Age:	adults (female workers)
Acclimation period:	The test units were placed into a climatic chamber, and kept under darkness at the environmental conditions of the test (25±2 ºC and 50-70% RH) for at least 4 hours, until the beginning of the test. No food or water was supplied during acclimatisation.
Diet:	Sucrose solution in water with a final concentration of 500 g/L (50% w/v) was used as food ad libitum. The syrup was administered using 2 mL syringe (deprived of the tip). One syringe was placed in each cage via an opening in the top of the test unit. If necessary, feeding solution was refilled during the test course.
Test units:	ventilated stainless steel cages 8.5 cm x 6.5 cm x 4.5 cm (length x height x width), front sidewith removable glass panel, back side perforated with 50 ventilation holes; Ø 2 mm

4. Environmental conditions:

Temperature:	25.71± 0.78 °C (25.25 – 26.78 °C)
Relative humidity:	3.4± 1.9% (61.1 – 65.7%)
Photoperiod:	darkness (except during observation)

STUDY DESIGN AND METHOD

The aim of this study was to determine the acute contact toxicity of PROTIOKONAZOL 300 EC (prothioconazole 300 g/L) to honeybees *Apis mellifera*. Mortality of the bees was used as the toxic endpoint. Sublethal effects, such as changes in behaviour, were also assessed. The experiment was carried out in accordance with OECD Guideline No 214. The study comprised 10 treatments (5 concentrations of the test item with a spacing factor of 2.5, 1 control group, 1 group treated with wetting agent only, 3 concentrations of the reference item with a spacing factor of 2) with 3 replicates. Each test unit (stainless-steel cage) contained 10 individuals. The tested doses were dispersed in deionized water with a wetting agent and applied once to the thorax dorsal side of the bees. The bees were then fed ad libitum with 50% sucrose solution until the end of the test. Mortality was recorded after 4, 24, 48 and 72 hours after application and compared with that of control group.

Test design:	tested dose and control in three repetitions, 10 bees per repeat
Exposure time:	acute test, 72 h
Tested concentrations, definitive test:	0.031, 0.077, 0.19, 0.48, 1.20 µL test item/bee (9.22, 23.04, 57.60, 144.00, 360.00 µg a.i./bee)
Dates:	start of the study 11.07.2022 start of the experimental part: 18.07.2022 end of the experimental part: 03.09.2022 end of the study: 15.02.2023
Statistic:	Software used for statistical analysis was “Agricultural Research Manager 2022” (ARM)”. Mortality data were analysed by ANOVA test and then compared to the control group by the Student-Newman-Keuls (S-N-K), $\alpha \leq 0.05$. The LD50 was calculated where possible. The No Observed Effect Dose (NOED) and Lowest Observed Effect Dose (LOED) values for adults’ emergence rate were calculated.

RESULTS

Honeybees' mortality was evaluated at 4-HAA, 24-HAA, 48-HAA and 72-HAA after a single exposure of 6 hours. Mortality was calculated in percentage at 24-HAA, 48-HAA and 72-HAA.

Table KCP 10.3.1.1.2-1: Number of dead bees over the test period (Acute Contact Toxicity Test)

Treatment no.	Treatment	Application rate (Nominal intake)	Dead bees			
			4-HAA	24-HAA	48-HAA	72-HAA
T1	Control	-	0	1	1	1
T2	Tween 20	1%	0	0	1	1
T3	PROTIOKONAZOL 300 EC	0.031 µL f.p./bee (9.22 µg a.i./bee) #	0	0	1	1
T4	PROTIOKONAZOL 300 EC	0.077 µL f.p./bee (23.04 µg a.i./bee) #	0	1	5	5
T5	PROTIOKONAZOL 300 EC	0.19 µL f.p./bee (57.60 µg a.i./bee) #	4	4	8	8
T6	PROTIOKONAZOL 300 EC	0.48 µL f.p./bee (144 µg a.i./bee) #	10	16	18	18
T7	PROTIOKONAZOL 300 EC	1.20 µL f.p./bee (360 µg a.i./bee) #	18	28	28	28
T8	ROGOR L 40 ST	0.00019 µL f.p./bee (0.075 µg a.i./bee) #	12	16	26	26
T9	ROGOR L 40 ST	0.00038 µL f.p./bee (0.15 µg a.i./bee) #	11	12	27	27
T10	ROGOR L 40 ST	0.00075 µL f.p./bee (0.30 µg a.i./bee) #	18	25	30	30

HAA = Hours After Application
*f.p.: formulated product
**a.i.: active ingredient
*** intake of µL f.p./µg a.i. calculated on the feeding consumption data obtained over the 6 hrs exposure

CONCLUSION

All study validity criteria were met. The NOED value at 72-HAA for Acute Contact Toxicity Test coincided to 57.60 a.i./bee and the LOED value at 72-HAA was 144.00 µg a.i/bee. The LD50 for Acute Contact Toxicity Test was calculated on the 72-HAA mortality and was found to be 98.35 µg a.i./bee (95% confidence limits: (18.18 - 20.85 µg a.i./bee).

Table KCP 10.3.1.1.1-2: Mortality at 24 HAA, 48HAA and 72 HAA – Acute Contact Toxicity Test (Definitive Test)

Endpoints	µg a.i./bee	µL f.p./bee
24-HAA LD_{50}	130.32 µg a.i.**/bee	0.43 µL f.p.*/bee
24-HAA NOED	57.60 µg a.i.**/bee	0.19 µL f.p.*/bee
24-HAA LOED	144.00 µg a.i.**/bee	0.48 µL f.p.*/bee
48-HAA LD_{50}	98.35 µg a.i.**/bee	0.33 µL f.p.*/bee
48-HAA NOED	57.60 µg a.i.**/bee	0.19 µL f.p.*/bee
48-HAA LOED	144.00 µg a.i.**/bee	0.48 µL f.p.*/bee
72-HAA LD_{50}	98.35 µg a.i.**/bee	0.33 µL f.p.*/bee
72-HAA NOED	57.60 µg a.i.**/bee	0.19 µL f.p.*/bee
72-HAA LOED	144.00 µg a.i.**/bee	0.48 µL f.p.*/bee

Comments of zRMS: The study was accepted by zRMS.
The validity criteria was met.

Validity criteria of the study

Mortality in the control group	Mortality in the control group was < 10%, therefore the validity criterion was met.
Mortality in the reference group	Bumblebees' mortality was >50% at 48-HAA, therefore the validity criterion was met.

The agreed toxicity endpoints:

Mortality at 24 HAA and 48 HAA – Acute Contact Toxicity Test (Limit test)

Treatment number	Treatment	Test Item f.p./bumblebee (Nominal intake)	Test Item a.i/bumblebee (Nominal intake)	Mortality 24 HAA (%)	p^a	Mortality 48 HAA (%)	p^a
T1	Control	-	-	0.00	b	0.00	b
T2	PROTIOKONAZOL 300 EC	0.34 µL f.p.*/bumblebee	100 µg a.i.**/bumblebee	0.00	b	0.00	b
T3	ROGOR L 40 ST	0.025 µL f.p./ bumblebee	10 µg a.i./bumblebee	80.00	a	100.00	a

Endpoints	µg a.i./bumblebee	µL f.p./bumblebee
48-HAA LD_{50}	>100 µg a.i.**/bumblebee	>0.34 µL f.p.*/bumblebee
48-HAA NOED	>100 µg a.i.**/bumblebee	>0.34 µL f.p.*/bumblebee
48-HAA LOED	>100 µg a.i.**/bumblebee	>0.34 µL f.p.*/bumblebee

[a], S-N-K test ($P \leq 0.05$) on the data at day-8
*f.p.: formulated product
**a.i.: active ingredient

Reference:	KCP 10.3.1.1.2/02
Report	Effects of PROTIOKONAZOL 300 EC (prothioconazole 300 g/L) on Bumblebee (*Bombus terrestris* L.) in the laboratory – Acute Oral and Contact Toxicity Test; Mautino G; 2023; Study Code: 1138.1F.SAG22
Guideline(s):	Yes, OECD 246
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No
Validity criteria of the test:	All validity criteria were met: - mortality in the control group: ≤ 10% at the end of the test - mortality in the toxic reference item group should be ≥ 50% at the end of the test

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	PROTIOKONAZOL 300 EC
Formulation:	EC (prothioconazole 300 g/L)
Description (physical state):	liquid

Batch no.:	01/PRO/2022
Production date:	24 March 2022
Expiration date:	03.2025
Stability of test compound:	The content of prothioconazole active ingredient in water samples was determined.
2. Vehicle and/or positive control:	deionized water + surfactant agent positive control: dimethoat
3. Test organism	
Species:	bumbleebee (*Bombus* spp.)
Source:	Three commercial hives (Natupol, Koppert), queen-right, healthy (disease free) and adequately fed, with normal population of young adult worker individuals, purchased from Agrigonella snc, Corso Alcide de Gasperi 58, 12046 Montà (CN), Italy. The hives were kept in climatic chamber under mean controlled environmental conditions.
Age:	adult female workers
Acclimation period:	15 to 18 hours prior the start of the test with access to an untreated 50% (w/v) aqueous sucrose solution ad libitum. Moribund bumblebees during the acclimatisation period have been discarded and replaced by healthy individuals before starting the test.
Diet:	Aqueous sucrose solution 50% w/v dispensed ad libitum during acclimatization period and after the application of the test and reference items. A stock solution of 1 L was prepared once at test start of the test by mixing 500 g of sucrose and 500 mL of distilled deionized water. The stock sucrose solution was kept in refrigerator at 4°C for the whole test duration. The feeding solution was administered using a 5 mL syringe (deprived of the tip). One syringe was placed in each individual cage. If necessary, feeding solution was refilled during the test course.
Test units:	single unit plastic cages (Nicot® queen breeding system), containing one adult worker bumblebee of medium size. Each Nicot® cage had a medium internal volume of 34 cm3 (length: 7 cm; diameter 2.5 cm), adequate to bumblebee size, and was equipped with a 5 mL syringe, deprived of the tip, filled with an aqueous sucrose solution (50% w/v), needed for ad libitum feeding
4. Environmental conditions:	
Temperature:	24.86 ± 0.173 °C (24.63 – 25.02 °C)
Relative humidity:	75.2 ± 1.3% (73.3 – 76.7%)
Photoperiod:	darkness condition (except during observation)

STUDY DESIGN AND METHOD

The aim of this study was to determine the acute contact toxicity of PROTIOKONAZOL 300 EC (prothioconazole 300 g/L) to bumblebees. Mortality of the bumblebees was used as the toxic endpoint. Sublethal effects (if any), such as changes in behaviour, were also be assessed. The Limit Contact toxicity test was performed using a single dose of the test item PROTIOKONAZOL 300 EC (prothioconazole 300 g/L) in a Limit Test Trial. Adult worker bumblebees were collected from three different colonies. Fifty medium size individuals were individually caged and randomly allocated in the test cages. Test item was compared with an untreated control (0.1% Triton X-100 solution used as surfactant agent) and a toxic standard as recommended in the guideline. Reference item was ROGOR L 40 ST (dimethoate 400 g/L) dissolved in. Test and reference were dispersed in an aqueous solution (0.1 % Triton X-100 solution used as surfactant agent) on the first day of trial. Mortality was recorded at 4, 24 and 48 hours after application (HAA).

Test design:	50 individuals from 3 different hives for control and test item group; 30 individuals from 3 different hives for reference item group
xposure time:	acute test, 48 h
Tested concentrations, definitive test:	0.34 µg/bumblebee (100.00 µg a.i./bumblebee) limit test
Dates:	start of the study 10.10.2022 start of the experimental part: 17.10.2022 end of the experimental part: 11.11.2022 end of the study: 15.2.2023
Statistic:	Results were analyzed by the validated statistical software "Agricultural Research Manager 2022" (ARM). Levene's test was used to assess the variances' homogeneity ($P<0.05$), data were transformed in case of absence of variances' homogeneity. Correction for control mortality was carried out using the Schneider-Orelli's formula. An ANOVA and Student-Newman-Keuls (S-N-K) tests were performed on the mortality data to identify any significant differences between treatments and control groups. The LD50 values were calculated for the test item with 95% confidence interval, using a probit regression analysis. The following parameters were calculated on the 48-HAA bumblebees' mortality data: LOED/NOED and LD50.

RESULTS

Bumblebees' mortality was evaluated at 4-HAA, 24-HAA and 48-HAA after a single exposure of 4 hours. Mortality was calculated in percentage at 24-HAA and 48-HAA.

Table KCP 10.3.1.1.2-3: Number of dead bumblebees over the test period (Acute Contact Toxicity Test – Limit test)

Treatment no.	Treatment	Application rate (Nominal intake)	Dead bumblebees		
			4-HAA	24-HAA	48-HAA
T1	Control	-	0	0	0
T2	PROTIOKONAZOL 300 EC	0.34 µL f.p.*/bumblebee (100 µg a.i.**/bumblebee)	0	0	0
T3	ROGOR L 40 ST	0.025 µL f.p./ bumblebee (10 µg a.i./ bumblebee)	8	24	28

HAA = Hours After Application
*f.p.: formulated product
**a.i.: active ingredient

CONCLUSION

It can be assumed a LD50 value >100.00 µg a.i./bumblebee for both Contact and Oral toxicity tests. The NOED and LOED values were ≥100.00 µg a.i./bumblebee and >100.00 µg a.i./bumblebee, respectively, for both Contact and Oral toxicity tests.

Table KCP 10.3.1.1.2-4: Mortality at 24 HAA and 48 HAA – Acute Contact Toxicity Test (Limit test)

Endpoints	µg a.i./bumblebee	µL f.p./bumblebee
48-HAA LD_{50}	>100 µg a.i.**/bumblebee	>0.34 µL f.p.*/bumblebee
48-HAA NOED	>100 µg a.i.**/bumblebee	>0.34 µL f.p.*/bumblebee
48-HAA LOED	>100 µg a.i.**/bumblebee	>0.34 µL f.p.*/bumblebee

A 2.3.1.2 KCP 10.3.1.2 Chronic toxicity to bees

Comments of zRMS:	The study was accepted by zRMS. The validity criteria was met. **Validity criteria of the study** Mortality in the control group: Average mortality across replicates for the control (50% w/v sucrose solution only) ≤ 15% at the end of the test (actual value was 0.00%, therefore, the validity criterion was met). Mortality in the reference group: Mortality rate at the end of the test period of 100% (actual value was 100.00%, therefore, the validity criterion was met). **The agreed toxicity endpoints:**

Mortality of young adult bees after 10 days

Treatment number	Treatment	Application rate (a.i. nominal intake)	Concentration (mg a.i./kg feeding solution)	Concentration (µg a.i./bee/day)	Mortality (%)	p^a	Survivors correction (%)[b]
T1	Control	Sucrose solution 50% w/v	-	-	0.00	e	-
T2	PROTIOKONAZOL 300 EC	23.10 µg prothioconazole/bee	92.03 mg prothioconazole/kg	3.74 µg prothioconazole /bee	6.67	e	6.67
T3	PROTIOKONAZOL 300 EC	57 µg prothioconazole/bee	227.09 mg prothioconazole/kg	8.90 µg prothioconazole /bee	20.00	d	20.00
T4	PROTIOKONAZOL 300 EC	144 µg prothioconazole/bee	573.25 mg prothioconazole/kg	27.86 µg prothioconazole /bee	33.33	c	33.33
T5	PROTIOKONAZOL 300 EC	360 µg prothioconazole/bee	1434.26 mg prothioconazole/kg	53.58 µg prothioconazole /bee	36.67	c	36.67
T6	PROTIOKONAZOL 300 EC	900 µg prothioconazole/bee	3585.66 mg prothioconazole/kg	142.69 µg prothioconazole /bee	66.67	b	66.67
T7	ROGOR L 40 ST	1 mg dimethoate/kg feeding solution		0.43 µg dimethoate /bee	100.00	a	100.00

Endpoints	mg a.i./kg feeding solution	mg f.p./kg feeding solution
LC_{10} [95% confidence intervals]	102.02 mg a.i./kg feeding solution [69.80 – 137.27]	378.36 mg f.p./kg feeding solution [272.50 – 492.34]
LC_{20} [95% confidence intervals]	294.27 mg a.i./kg feeding solution [231.89 – 358.27]	985.12 mg f.p./kg feeding solution [793.43 – 1183.88]
LC_{50} [95% confidence intervals]	1799.91 mg a.i./kg feeding solution [1511.36 – 2206.66]	6145.01 mg f.p./kg feeding solution [5146.38 – 7554.59]
NOEC	92.03 mg a.i./kg feeding solution	316.29 mg f.p./kg feeding solution
LOEC	227.09 mg a.i./kg feeding solution	780.44 mg f.p./kg feeding solution
LDD_{10}	4.45 µg a.i./bee/day [3.09 – 5.93]	0.016 µg f.p./bee/day [0.011 – 0.020]
LDD_{20}	12.46 µg a.i./bee/day [9.90 – 15.08]	0.040 µg f.p./bee/day [0.032 – 0.048]
LDD_{50}	72.38 µg a.i./bee/day [61.10 – 88.14]	0.24 µg f.p./bee/day [0.20 – 0.30]
NOEDD	3.74 µg a.i./bee/day	0.012 µg f.p./bee/day
LOEDD	8.90 µg a.i./bee/day	0.030 µg f.p./bee/day

[a], S-N-K test ($P \leq 0.05$)
[b], mean survivors corrected by Abbott's formula
-, not applicable

Reference:	KCP 10.3.1.2/01
Report	Effects of PROTIOKONAZOL 300 EC (prothioconazole 300 g/L) on Honeybees (*Apis mellifera* L.) in the laboratory – Chronic Oral Toxicity Test; Mautino G.; 2023; Study Code: 1001.1F.SAG22
Guideline(s):	Yes, OECD 245
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No
Validity criteria of the test:	All validity criteria were met: - average mortality across replicates for the control (50% w/v sucrose solution only) ≤ 15% at the end of the test; - mortality in the reference group ≥ 50% at the end of the test period

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	PROTIOKONAZOL 300 EC
Formulation:	EC (prothioconazole 300 g/L)
Description (physical state):	liquid
Batch no.:	01/PRO/2022
Production date:	24 March 2022
Expiration date:	03.2025
Stability of test compound:	The content of prothioconazole active ingredient was determined in the lowest concentration and in the highest concentration of the stock solutions and feeding solutions prepared in the biological phase of the study.
2. Vehicle and/or positive control:	vehicle: 50% sucrose solution positive control: dimethoate

3. Test organism

Species:	honeybee *Apis mellifera*
Source:	Beekeeper Paolo Farinetti, Via Montà Castino 25, 12074 Cortemilia (CN), Italy. One commercial beehive, queenright, healthy (disease free) and adequately fed, with normal population of young adult worker individuals was placed at SAGEA Centro di Saggio s.r.l. Test Facility.
Age:	max. 2-day old
Acclimation period:	The test units were placed into an incubator, and kept under darkness at the mean environmental conditions of 33±2 °C; 50-70% RH for at least 1 day, until the beginning of the test. Bees were fed ad libitum with sucrose solution only.
Diet:	Sucrose solution in water with a final concentration of 500 g/L (50% w/v) was used as food ad libitum. The syrup was administered using a 2.5 mL syringe. The syringes were inserted into the cage via an opening in the top of the test unit. Food was daily replaced by changing the feeders until the end of test. Food consumption was adjusted for the test solutions evaporation from the feeders.
Test units:	Ventilated stainless steel cages 8.5 cm x 6.5 cm x 4.5 cm (length x height x width), front side: removable glass panel, back side: perforated with 50 ventilation holes; Ø 2 mm.

4. Environmental conditions:

Temperature:	33.23 ± 0.05 °C (33.33 – 33.18 °C)

Relative humidity:	59.9 ± 0.8% (60.5 – 57.6%)
Photoperiod:	photoperiod: 0 h light: 24 h dark

STUDY DESIGN AND METHOD

The purpose of this study was to determine the chronic oral toxicity of PROTIOKONAZOL 300 EC to young adult honeybees (*Apis mellifera* L.). The study was carried out in accordance with OECD Guideline No 245. One day before test start, bees were collected from brood combs without the use of smoke and without anaesthetics. By means of a proper brush, the bees were collected in plastic containers with holes for oxygenation and immediately transported to SAGEA's laboratory. Once in the laboratory, the bees were randomly allocated to the test units (cages) after a light anaesthetisation with CO2 (2 bar for about 45 seconds). Anaesthetised bees were gently transferred to the test units by means of a plastic spoon. The study consisted of 7 treatments (5 rates of the test item, 1 control group, 1 reference item) with 3 replicates, each containing 10 bees per cage. The doses of the test and reference items were dispersed in a 50% sucrose solution in water and offered ad libitum. Feeding solutions were replaced daily by changing the feeders. Mortality was recorded daily for 10 days.

Test design:	tested dose and control in three replicates, 10 bees per replicate
Exposure time:	chronic test, 10 days
Tested concentrations, definitive test:	0.077, 0.19, 0.48, 1.20 and 3.0 µL test item/bee (23.10, 57.00, 144.00, 360.00 and 900.00 µg a.s./bee
Dates:	start of the study 11.07.2022 start of the experimental part: 14.07.2022 end of the experimental part: 11.08.2022 end of the study: 15.02.2023
Statistic:	Software used for statistical analysis was "Agricultural Research Manager 2020" (ARM)", version 2020. Mortality data were analysed by ANOVA test and subsequently, if it is significant, by S-N-K's test, $\alpha \leq 0.05$ and the LD50 calculated. On the mortality data the standard error was calculated. The No Observed Effect Dose (NOED) and Lowest Observed Effect Dose (LOED) values for mortality were calculated.

RESULTS

All study validity criteria were met.

Mortality in the control units (50% w/v sucrose solution) was 0.00% at day 10. At the end of the exposure period the cumulative mortality in the control (sucrose solution in water 50% w/v only) was 0.00% and PROTIOKONAZOL 300 EC values ranged from 6.67% in treatment 92.03 mg a.i./Kg feeding solution to 66.67% in treatment 3585.66 mg a.i./Kg feeding solution. Reference item mortality reached the 100.00%.

Table KCP 10.3.1.2-1: Average percentage of young adult bee's mortality at day 9-10

Treatment number	Treatment	Application rate (nominal intake)	Concentration (mg/kg feeding solution)	Day 9-10			
				Mortality		P[b]	Corrected survivor[c] (%)
				(%)	SE[a]		
T1	Control	Sucrose solution 50% w/v	-	0.00	±0.00	e	-
T2	PROTIOKONAZOL 300 EC	0.077 µL f.p.*/bee (23.10 µg a.i.**/bee)	316.29 mg f.p./kg feeding solution (92.03 mg a.i./kg)	6.67	±3.33	e	6.67
T3	PROTIOKONAZOL 300 EC	0.19 µL f.p./bee (57.00 µg a.i./bee)	780.44 mg f.p./kg feeding solution (227.09 mg a.i./kg)	20.00	±0.00	d	20.00
T4	PROTIOKONAZOL 300 EC	0.48 µL f.p./bee (144.00 µg a.i./bee)	1971.63 mg f.p./kg feeding solution (573.25 mg a.i./kg)	33.33	±6.67	c	33.33
T5	PROTIOKONAZOL 300 EC	1.20 µL f.p./bee (360.00 µg/bee)	4929 mg f.p./kg feeding solution (1434.26 mg a.i./kg)	36.67	±3.33	c	36.67
T6	PROTIOKONAZOL 300 EC	3 µL f.p./bee (900.00 µg a.i./bee)	12322 mg f.p./kg feeding solution (3585.66 mg a.i./kg)	66.67	±3.33	b	66.67
T7	ROGOR L 40 ST	0.0025 mg f.p./kg feeding solution (1 mg a.i./kg)		100.00	±0.00	a	100.00

a, standard error from 3 replicates
b, S-N-K test (P≤0.05)
c, mean survivor corrected by Abbott's formula
-, not applicable
*f.p.: formulated product
**a.i.: active ingredient prothioconazole

For the mean uptake of feeding solution/bee/day at the end of the test period (expressed as mean of the mean values), ranged from 49.41 mg (treatment T4) to 37.89 mg/bee/day moreover, a 141.31 mg value of mean uptake was observed for the toxic references and the control (50% w/v sucrose solution only) showed a value of 35.77 mg/bee/day.

Table KCP 10.3.1.2-2: Mean uptake of feeding solution/bee/day over the test period

Treatment no.	Mean uptake of feeding solution (mg)/bee/day											
	Day 0-1	Day 1-2	Day 2-3	Day 3-4	Day 4-5	Day 5-6	Day 6-7	Day 7-8	Day 8-9	Day 9-10	Sum[a]	Mean[b]
T1	66.80	23.20	45.33	14.63	34.57	33.33	27.83	23.03	50.30	38.67	357.70	35.77
T2	41.13	40.80	31.10	33.90	42.27	37.83	37.96	38.30	48.39	62.03	413.70	41.37
T3	44.37	34.07	36.10	27.73	41.77	32.93	30.64	31.34	58.96	60.88	398.78	39.88
T4	45.60	32.80	36.60	35.97	39.40	50.59	52.99	52.94	72.72	74.44	494.05	49.41
T5	43.30	25.73	24.67	30.50	35.70	27.95	38.17	39.90	52.89	60.10	378.91	37.89
T6	34.33	15.57	21.97	22.16	37.04	43.57	55.50	37.22	62.73	71.14	401.23	40.12
T7	67.72	134.44	221.75	-	-	-	-	-	-	-	423.92	141.31

a, sum of mean uptake feed/bee at test end over the course of the 10 days feeding period
b, mean of mean uptake feed/bee per day at test end over the course of the 10 days feeding period

Table KCP 10.3.1.2-3: Mean uptake of feeding solution/bee/day over the test period

Treatment no.	Mean uptake µg a.i./bee/day over the test period											
	Day 0-1	Day 1-2	Day 2-3	Day 3-4	Day 4-5	Day 5-6	Day 6-7	Day 7-8	Day 8-9	Day 9-10	Sum[a]	Mean[b]
T1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0	0
T2	3.80	3.69	2.70	2.91	3.79	3.59	3.44	3.61	4.27	5.59	37.37	3.74
T3	10.09	7.55	7.66	5.85	9.35	7.73	7.02	7.30	12.97	13.54	89.05	8.90
T4	26.34	18.56	19.55	18.93	22.16	29.81	30.11	31.13	40.36	41.69	278.63	27.86
T5	62.94	35.50	33.06	40.16	50.22	41.45	53.74	59.23	74.44	85.05	535.79	53.58
T6	128.65	52.46	81.06	73.03	129.18	161.19	193.68	137.68	217.28	252.70	1426.92	142.69
T7	0.06	0.12	0.19	-	-	-	-	-	-	-	0.37	0.12

[a], sum of mean uptake feed/bee at test end over the course of the 10 days feeding period
[b], mean of mean uptake feed/bee per day at test end over the course of the 10 days feeding period

By considering the mean uptake in µg of prothioconazole/bee (i.e., expressed as mean of the mean values), PROTIOKONAZOL 300 EC mean values (expressed as mean of the mean values) ranged from 3.74 to 142.69 µg a.i./bee/day on treatments 23.10 µg prothioconazole /bee and 900 µg prothioconazole /bee, respectively. ROGOR L40 ST showed a mean value of 0.43 µg a.i./bee/day.

CONCLUSION

The 10-d NOEC (mortality) value corresponded to 92.03 mg prothioconazole /Kg feeding solution and 10-d LOEC (mortality) matched with the rate of 227.09 mg prothioconazole /Kg feeding solution. The LC50 was 1799.91 mg prothioconazole /Kg feeding solution. The 10-d NOEDD (mortality) value corresponded to 3.74 µg prothioconazole /bee/day and 10-d LOEDD (mortality) matched with the rate of 8.90 µg prothioconazole /bee/day. The calculated 10-day LDD50 was 72.38 µg prothioconazole /bee/day.

Table KCP 10.3.1.2-4: Mortality of young adult bees after 10 days

Endpoints	mg a.i./kg feeding solution	mg f.p./kg feeding solution
LC_{10} [95% confidence intervals]	102.02 mg a.i./kg feeding solution [69.80 – 137.27]	378.36 mg f.p./kg feeding solution [272.50 – 492.34]
LC_{20} [95% confidence intervals]	294.27 mg a.i./kg feeding solution [231.89 – 358.27]	985.12 mg f.p./kg feeding solution [793.43 – 1183.88]
LC_{50} [95% confidence intervals]	1799.91 mg a.i./kg feeding solution [1511.36 – 2206.66]	6145.01 mg f.p./kg feeding solution [5146.38 – 7554.59]
NOEC	92.03 mg a.i./kg feeding solution	316.29 mg f.p./kg feeding solution
LOEC	227.09 mg a.i./kg feeding solution	780.44 mg f.p./kg feeding solution
LDD_{10}	4.45 µg a.i./bee/day [3.09 – 5.93]	0.016 µg f.p./bee/day [0.011 – 0.020]
LDD_{20}	12.46 µg a.i./bee/day [9.90 – 15.08]	0.040 µg f.p./bee/day [0.032 – 0.048]
LDD_{50}	72.38 µg a.i./bee/day [61.10 – 88.14]	0.24 µg f.p./bee/day [0.20 – 0.30]
NOEDD	3.74 µg a.i./bee/day	0.012 µg f.p./bee/day
LOEDD	8.90 µg a.i./bee/day	0.030 µg f.p./bee/day

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

Not relevant. No studies submitted.

A 2.3.1.4 KCP 10.3.1.4 Sub-lethal effects

Comments of zRMS:

The study was accepted by zRMS.
The validity criteria was met.

Validity criteria of the study

Mortality in the control group	Cumulative larval mortality from D3 to D8 was 10.42%, therefore the validity criterion was met.
	Adult emergence at D22 was 87.50%, therefore the validity criterion was met.
Mortality in the reference group	Larval mortality was 100% at D8.

The agreed toxicity endpoints:

Adults' emergence at day-22

Treatment number	Treatment	Application rate (Nominal intake)	Test item concentration in the larval diet	Adults' emergence rate (%)	p^a	Er (%)[b]
T1	Control	-	-	87.50	a	-
T2	PROTIOKONAZOL 300 g/L EC	0.0052 µL f.p.*/larva (1.54 µg a.i.**/larva)	33.27 µL f.p./Kg of diet (9.98 mg a.i./Kg of diet)	85.42	a	2.38
T3	PROTIOKONAZOL 300 g/L EC	0.013 µL f.p./larva (3.84 µg a.i./larva)	83.20 µL f.p./Kg of diet (24.96 mg a.i./Kg of diet)	83.33	ab	4.76
T4	PROTIOKONAZOL 300 g/L EC	0.032 µL f.p./larva (9.60 µg a.i./larva)	208.00 µL f.p./Kg of diet (62.40 mg a.i./Kg of diet)	81.25	ab	7.14
T5	PROTIOKONAZOL 300 g/L EC	0.080 µL f.p./larva (24 µg a.i./larva)	520.00 µL f.p./Kg of diet (156.00 mg a.i./Kg of diet)	77.08	b	11.90
T6	PROTIOKONAZOL 300 g/L EC	0.20 µL f.p./larva (60 µg a.i./larva)	1300.00 µL f.p./Kg of diet (390.00 mg a.i./Kg of diet)	0.00	c	100.00
T7	ROGOR L 40 ST	0.018 µL f.p./larva (7.39 µg a.i./larva)	120µL f.p./Kg diet (48 mg a.i./Kg of diet)	0.00	c	100.00

Endpoints	µg a.i./larva	µL f.p./larva
ED_{50}/LD_{50} [95% confidence intervals]	33.21 µg a.i./larva (95% confidence intervals are 44.76 – 26.15 µg a.i./larva)	0.11 µL f.p./larva (95% confidence intervals are 0.15 – 0.09 µL f.p./larva)
NOED	9.60 µg a.i./larva	0.032 µL f.p./larva
LOED	24.00 µg a.i./larva	0.080 µL f.p./larva
EC_{50}/LC_{50} [95% confidence intervals]	219.46 mg a.i./kg diet (95% confidence intervals are 296.83 – 172.41 mg a.i./kg diet)	307.13 µL f.p./kg diet (95% confidence intervals are 392.63 – 252.10 µL f.p./kg diet)
NOEC	62.40 mg a.i./Kg of diet	208.00 µL f.p./Kg of diet
LOEC	156.00 mg a.i./Kg of diet	520.00 µL f.p./Kg of diet

[a], S-N-K test ($P \leq 0.05$) on the data at day-8
[b], Er = emergence % reduction in comparison to the control
-, not applicable
*f.p.: formulated product
**a.i.: active ingredient

Reference:	KCP 10.3.1.4/01
Report	Effects of PROTIOKONAZOL 300 g/L EC (prothioconazole 300 g/L) on Honeybees (*Apis mellifera* L.) in the laboratory – Larval Toxicity Test Following Repeated Exposure; Mautino G.; 2023; Study Code: 1002.1F.SAG22
Guideline(s):	Yes, OECD GD 239
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No
Validity criteria of the test:	All validity criteria were met: - in the control plate(s), cumulative larval mortality from day-3 to day-8 ≤ 15% across all replicates; - in the control plate(s), the adult emergence rate on day-22 ≥ 70% across all replicates; - test item: larval mortality ≥ 50% on day-8 across all replicates.

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	PROTIOKONAZOL 300 EC
Formulation:	EC (prothioconazole 300 g/L)
Description (physical state):	liquid
Batch no.:	01/PRO/2022
Production date:	24 March 2022
Expiration date:	03.2025
Stability of test compound:	Once during the experimental phase one aliquot of the lowest concentration and one aliquot of the highest concentration of the test item solutions were analysed.

2. Vehicle and/or positive control: vehicle: 50% sucrose solution
positive control: ROGOR L 40 ST (dimethoat)

3. Test organism

Species:	honeybee *Apis mellifera*
Source:	Beekeeper Paolo Farinetti. Three commercial beehives, queenright, healthy (disease free) and adequately fed, with normal population of young adult worker individuals (approx. 2 weeks old) was placed at SAGEA Centro di Saggio s.r.l. Test Facility.
Age:	first instar larvae

Diet:	Larval diet: The larval food was composed of the three following diets, adapted to the needs of the larvae at different stages of development: - Diet A (15 July 2022) for all theses: 50% weight of fresh royal jelly (6.600 g) + 50% weight of an aqueous solution containing 2% weight of yeast extract (0.132 g), 12% weight of glucose (0.792 g), 12% weight of fructose (0.792 g) and 4.884 g of deionized water. - Diet B (17 July 2022) for thesis T1 (untreated): 50% weight of fresh royal jelly (1.100 g) + 50% weight of an aqueous solution containing 3% weight of yeast extract (0.033 g), 15% weight of glucose (0.165 g), 15% weight of fructose (0.165 g) and 0.737 g of deionized water. - Diet B (17 July 2022) for treated theses: 50% weight of fresh royal jelly (5.500 g) + 50% weight of an aqueous solution containing 3% weight of yeast extract (0.165 g), 15% weight of glucose (0.825 g), 15% weight of fructose (0.825 g) and 2.635 g of deionized water. - Diet C (18 July 2022) for thesis T1 (untreated): 50% weight of fresh royal jelly (3.300 g) + 50% weight of an aqueous solution containing 4% weight of yeast extract (0.132 g), 18% weight of glucose (0.594 g), 18% weight of fructose (0.594 g) and 1.980 g of deionized water. - Diet C (18 July 2022) for treated theses: 50% weight of fresh royal jelly (27.500 g) + 50% weight of an aqueous solution containing 4% weight of yeast extract (1.100 g), 18% weight of glucose (4.950 g), 18% weight of fructose (4.950 g) and 11.050 g of deionized water. After preparation, both containers of diet C has been preserved in fridge well covered with parafilm at 4 °C for two days.
Test units:	Larvae were reared in crystal polystyrene grafting cells having an internal diameter of 9 mm and a depth of 8 mm. Each cell was placed into a well of a 48 multi-well plate. The top of the grafting cell was maintained at the level of the plate by placing a piece of dental roll. The plates have been sterilized before being used.
4. Environmental conditions:	
Temperature/ relative humidity:	Mean Test conditions from day-1 to day-8: temperature: 34.47 ± 0.03 °C (34.54 – 34.43 °C), relative humidity: 94.6 ± 2.10% RH (98.4 – 92.3%) RH, light: darkness (except during observation and food replacement) Mean Test conditions from day-8 to day-15: temperature: 34.63 ± 0.23 °C (34.95 – 34.32 °C), relative humidity: 85.6 ± 0.8% RH (86.7 – 84.8%) RH, light: darkness (except during observation and food replacement) Mean Test conditions from day-15 to day-22: temperature: 34.60 ± 0.01 °C (34.63 – 34.59 °C), relative humidity: 62.5 ± 0.3% RH (62.6 – 61.6%) RH, light: darkness (except during observation and food replacement)

STUDY DESIGN AND METHOD

The purpose of this study was to determine the chronic oral toxicity of the PROTIOKONAZOL 300 g/L EC (prothioconazole 300 g/L) on honeybee larvae (Apis mellifera L.) consequently to a repeated exposure under laboratory conditions, providing larvae with food added with the test item. Adults' emergence at day-22 was used as the toxic endpoint. A Range finding test was initially performed followed by the Definitive Test. The Definitive Test rates were established taking into consideration the Range finding test results. The Definitive test was performed using five doses of the test item PROTIOKONAZOL 300 g/L EC (prothioconazole 300 g/L) in a geometric series, with factor 2.5 and covering the range for ED50. Larvae were collected from three different colonies, each one representing a replicate. 16 per replicate, 48 per treatment. Test item was compared with an untreated control and a toxic standard as recommended in the guideline for a ED50 approach. Reference item was ROGOR L 40 ST (dimethoate 400 g/L). From day-3 to day-6, test and reference items were dispersed in the diet, following the OECD 239 scheme, at the suitable concentrations. Larval mortality was recorded at the time of feeding from day-4 to day-8, moreover from day-8 to day-22 pupal mortality was evaluated and on day-22, the number of emerged adults was counted.

Test design:	16 larvae x 3 colonies = 48 larvae
Exposure time:	chronic test, exposition: 4 days (from D3 to D6), duration of the test: 22 days
Tested concentrations, definitive test:	0.0052 µL/larva (1.54 µg a.i./larva) 0.013 µL/larva (3.84 µg a.i./larva) 0.032 µL/larva (9.60 µg a.i./larva) 0.080 µL/larva (24.00 µg a.i./larva) 0.20 µL/larva (60.00 µg a.i./larva)
Dates:	start of the study 11.07.2022 start of the experimental part: 15.07.2022 end of the experimental part: 06.09.2022 end of the study: 15.02.2023
Statistic:	Software used for statistical analysis was "Agricultural Research Manager" (ARM)", 2022.5. Mortality data were analysed by ANOVA test and subsequently, if it is significant, by S-N-K's test, $\alpha \leq 0.05$ and the ED50 calculated. On the mortality data the standard error was calculated. The No Observed Effect Dose (NOED) and Lowest Observed Effect Dose (LOED) values for adults' emergence rate were calculated.

RESULTS

From day-3 to day-8, larvae were exposed to the test item PROTIOKONAZOL 300 EC and reference item. The diet volume and composition were adapted on a daily basis.

Table KCP 10.3.1.4-1: Number of bee's larvae alive from day-2 to day-8

Treatment no.	Treatment	Application rate (Nominal intake)	Test item concentration in the larval diet	Bee's larvae alive						
				D2	D3	D4	D5	D6	D7	D8
T1	Control	-	-	48	48	48	45	43	43	43
T2	PROTIOKONAZOL 300 EC	0.0052 µL f.p.*/larva (1.54 µg a.i.**/larva)	33.27 µL f.p./Kg of diet (9.98 mg a.i./Kg of diet)	48	48	48	47	44	43	43
T3	PROTIOKONAZOL 300 EC	0.013 µL f.p./larva (3.84 µg a.i./larva)	83.20 µL f.p./Kg of diet (24.96 mg a.i./Kg of diet)	48	48	47	45	44	42	42
T4	PROTIOKONAZOL 300 EC	0.032 µL f.p./larva (9.60 µg a.i./larva)	208.00 µL f.p./Kg of diet (62.40 mg a.i./Kg of diet)	48	48	48	43	42	42	42
T5	PROTIOKONAZOL 300 EC	0.080 µL f.p./larva (24 µg a.i./larva)	520.00 µL f.p./Kg of diet (156.00 mg a.i./Kg of diet)	48	48	40	39	37	37	37
T6	PROTIOKONAZOL 300 EC	0.20 µL f.p./larva (60 µg a.i./larva)	1300.00 µL f.p./Kg of diet (390.00 mg a.i./Kg of diet)	48	42	35	24	12	9	7
T7	ROGOR L 40 ST	0.018 µL f.p.*/larva (7.39 µg a.i.**/larva)	120 µL f.p./Kg of diet (48 mg a.i./Kg of diet)	48	48	23	6	0	0	0

D = day
-, not applicable
*f.p.: formulated product
**a.i.: active ingredient

Table KCP 10.3.1.4-2: Cumulative mortality of bee's larvae from day-3 to day-8

Treatment		Application rate (Nominal intake)	Test item concentration in the larval diet	Cumulative %mortality					p^a
				D4	D5	D6	D7	D8	
T1	Control	-	-	0.00	6.25	10.42	10.42	10.42	d
T2	PROTIOKONAZOL 300 EC	0.0052 µL f.p.*/larva (1.54 µg a.i.**/larva)	33.27 µL f.p./Kg of diet (9.98 mg a.i./Kg of diet)	0.00	2.08	8.33	10.42	10.42	d
T3	PROTIOKONAZOL 300 EC	0.013 µL f.p./larva (3.84 µg a.i./larva)	83.20 µL f.p./Kg of diet (24.96 mg a.i./Kg of diet)	2.08	6.25	8.33	12.50	12.50	d
T4	PROTIOKONAZOL 300 EC	0.032 µL f.p./larva (9.60 µg a.i./larva)	208.00 µL f.p./Kg of diet (62.40 mg a.i./Kg of diet)	0.00	10.42	12.50	12.50	12.50	d
T5	PROTIOKONAZOL 300 EC	0.080 µL f.p./larva (24 µg a.i./larva)	520.00 µL f.p./Kg of diet (156.00 mg a.i./Kg of diet)	16.67	18.75	22.92	22.92	22.92	c
T6	PROTIOKONAZOL 300 EC	0.20 µL f.p./larva (60 µg a.i./larva)	1300.00 µL f.p./Kg of diet (390.00 mg a.i./Kg of diet)	16.67	42.86	71.43	78.57	83.33	b
T7	ROGOR L 40 ST	0.018 µL f.p.*/larva (7.39 µg a.i.**/larva)	120 µL f.p./Kg of diet (48 mg a.i./Kg of diet)	52.08	87.50	100.00	100.00	100.00	a

D = day
[a], S-N-K test (P≤0.05) on the data at day-8
-, not applicable
*f.p.: formulated product
**a.i.: active ingredient

At day-8, the cumulative mortality for PROTIOKONAZOL 300 EC ranged from 10.42% to 3.33% on treatments T2 and T6, respectively. The highest mortality was observed on treatment T7 (reference item) and the control showed a cumulative mortality of 10.42%.

Larval mortality was evaluated from day-3 to day-8 after an exposure period of 3 days (from day-3 to day-6). Pupal mortality was calculated in percentage from D8 to D22.

Table KCP 10.3.1.4-3: Percent pupal mortality at day-15 and day-22 from day-8

Treatment		Application rate (Nominal intake)	Test item concentration in the larval diet	% pupae mortality at D15	Corrected mortality at D15	% pupal mortality at D22	p^a	Corrected mortality at D22 [b]
T1	Control	-	-	2.22	-	2.22	b	-
T2	PROTIOKONAZOL 300 EC	0.0052 µL f.p.*/larva (1.54 µg a.i.**/larva)	33.27 µL f.p./Kg of diet (9.98 mg a.i./Kg of diet)	2.22	0.00	4.60	b	2.44
T3	PROTIOKONAZOL 300 EC	0.013 µL f.p./larva (3.84 µg a.i./larva)	83.20 µL f.p./Kg of diet (24.96 mg a.i./Kg of diet)	4.76	2.60	4.76	b	2.60
T4	PROTIOKONAZOL 300 EC	0.032 µL f.p./larva (9.60 µg a.i./larva)	208.00 µL f.p./Kg of diet (62.40 mg a.i./Kg of diet)	6.83	4.71	6.83	b	4.71
T5	PROTIOKONAZOL 300 EC	0.080 µL f.p./larva (24 µg a.i./larva)	520.00 µL f.p./Kg of diet (156.00 mg a.i./Kg of diet)	0.00	-2.27	0.00	b	2.27
T6	PROTIOKONAZOL 300 EC	0.20 µL f.p./larva (60 µg a.i./larva)	1300.00 µL f.p./Kg of diet (390.00 mg a.i./Kg of diet)	88.89	88.64	100.00	a	100.00
T7	ROGOR L 40 ST	0.018 µL f.p.*/larva (7.39 µg a.i.**/larva)	120 µL f.p./Kg of diet (48 mg a.i./Kg of diet)	-	-	-	-	-

D = day
a, S-N-K test (P≤0.05)
b, mean mortality corrected by Schneider-Orelli's formula
-, not applicable
*f.p.: formulated product **a.i.: active ingredient

At day-15, mortality for PROTIOKONAZOL 300 EC ranged from 0.00% (corrected value: -2.27%) to 88.89% (corrected value: 88.64%) on treatments T3 and T6. Mortality in the control corresponds to 2.22%.

At day-22, pupal mortality ranged from 0.00% to 100.00% on treatments T2 and T6, respectively. Pupal mortality in the control group corresponded to 2.22%.

Adults' emergence and percent reduction in the adults' emergence in comparison to the control were calculated at day-22.

Table KCP 10.3.1.4-4: Adults' emergence at day-22

Treatment number	Treatment	Application rate (Nominal intake)	Test item concentration in the larval diet	Adults' emergence rate (%)	p^a	Er (%)[b]
T1	Control	-	-	87.50	a	-
T2	PROTIOKONAZOL 300 g/L EC	0.0052 µL f.p.*/larva (1.54 µg a.i.**/larva)	33.27 µL f.p./Kg of diet (9.98 mg a.i./Kg of diet)	85.42	a	2.38
T3	PROTIOKONAZOL 300 g/L EC	0.013 µL f.p./larva (3.84 µg a.i./larva)	83.20 µL f.p./Kg of diet (24.96 mg a.i./Kg of diet)	83.33	ab	4.76
T4	PROTIOKONAZOL 300 g/L EC	0.032 µL f.p./larva (9.60 µg a.i./larva)	208.00 µL f.p./Kg of diet (62.40 mg a.i./Kg of diet)	81.25	ab	7.14
T5	PROTIOKONAZOL 300 g/L EC	0.080 µL f.p./larva (24 µg a.i./larva)	520.00 µL f.p./Kg of diet (156.00 mg a.i./Kg of diet)	77.08	b	11.90
T6	PROTIOKONAZOL 300 g/L EC	0.20 µL f.p./larva (60 µg a.i./larva)	1300.00 µL f.p./Kg of diet (390.00 mg a.i./Kg of diet)	0.00	c	100.00
T7	ROGOR L 40 ST	0.018 µL f.p./larva (7.39 µg a.i./larva)	120µL f.p./Kg diet (48 mg a.i./Kg of diet)	0.00	c	100.00

a, S-N-K test (P≤0.05) on the data at day-8
b, Er = emergence % reduction in comparison to the control
-, not applicable
*f.p.: formulated product
**a.i.: active ingredient

Percent reduction in emergence (Er) for the test item ranged from 2.38% to 100.00% for the lowest and highest dosages, respectively.

CONCLUSION

All study validity criteria were met.

The NOED matched with the test item PROTIOKONAZOL 300 dosage of 9.60 µg a.i./larva and NOEC was 62.40 mg a.i./kg diet. LOED value correspond to test the item dosage of 24.00 µg a.i./larva and LOEC to 156.00 mg a.i./kg diet.

The estimated ED50- days 22 for PROTIOKONAZOL 300 g/L EC was 33.21 µg a.i./larva, while the EC50 was 219.46 mg a.i./kg diet.

Table KCP 10.3.1.4-5: Results of chronic toxicity to bees

Endpoints	µg a.i./larva	µL f.p./larva
ED_{50}/LD_{50} [95% confidence intervals]	33.21 µg a.i./larva (95% confidence intervals are 44.76 – 26.15 µg a.i./larva)	0.11 µL f.p./larva (95% confidence intervals are 0.15 – 0.09 µL f.p./larva)
NOED	9.60 µg a.i./larva	0.032 µL f.p./larva
LOED	24.00 µg a.i./larva	0.080 µL f.p./larva
EC_{50}/LC_{50} [95% confidence intervals]	219.46 mg a.i./kg diet (95% confidence intervals are 296.83 – 172.41 mg a.i./kg diet)	307.13 µL f.p./kg diet (95% confidence intervals are 392.63 – 252.10 µL f.p./kg diet)
NOEC	62.40 mg a.i./Kg of diet	208.00 µL f.p./Kg of diet
LOEC	156.00 mg a.i./Kg of diet	520.00 µL f.p./Kg of diet

A 2.3.1.5 KCP 10.3.1.5 Cage and tunnel tests

Not relevant. No studies submitted.

A 2.3.1.6 KCP 10.3.1.6 Field tests with honeybees

Not relevant. No studies submitted.

A 2.3.2 KCP 10.3.2 Effects on non-target arthropods

A 2.3.2.1 KCP 10.3.2.1 Standard laboratory testing for non-target arthropods

Not relevant. No studies submitted.

A 2.3.2.2 KCP 10.3.2.2 Extended laboratory testing, aged residue studies with non-target arthropods

Comments of zRMS:	The study was accepted by zRMS. The validity criteria was met.

Validity criteria of the study

Mean mortality in the water control should be ≤20%.

The mean cumulative number of eggs per female in the water control should be ≥4.

Corrected mortality should be between 50% and 100% in the toxic reference treatment on day 7.

Mortality in the control groups on day 7	Bioassay 1 at 0 DAA	Actual value was 7.00%, therefore, the validity criterion was met.
	Bioassay 2 at 7 DAA	Actual value was 6.00%, therefore, the validity criterion was met.
	Bioassay 3 at 14 DAA	Actual value was 6.00%, therefore, the validity criterion was met.
Reproduction in the control groups	Bioassay 1 at 0 DAA	The mean cumulative number of eggs per female was 8.77, so this validity criterion was met.
	Bioassay 2 at 7 DAA	The mean cumulative number of eggs per female was 8.43, so this validity criterion was met.
	Bioassay 3 at 14 DAA	The mean cumulative number of eggs per female was 9.40, so this validity criterion was met.
Mortality in the reference group on day 7	Bioassay 1 at 0 DAA	Actual value was 78.00% (corrected value 76.34%), so the validity criterion was met.
	Bioassay 2 at 7 DAA	Actual value was 69.00% (corrected value 67.02%), so the validity criterion was met.
	Bioassay 3 at 14 DAA	Actual value was 58.00% (corrected value 55.32%), so the validity criterion was met.

The agreed toxicity endpoints:

Mortality parameter:

***Typhlodromus pyri* mortality after 7 days of exposure (Bioassay 1 at 0 DAA, 2 at 7 DAA and 3 at 14 DAA).**

	T1 Control	T2 PROTIOKONAZOL 300 EC at 650 mL test item/ha	T3 PROTIOKONAZOL 300 EC at 1300 mL test item/ha	T4 ROGOR L40 ST at 18 mL test item/ha
	Deionised water	195 g a.i./ha	390 g a.i./ha	7.2 g a.i./ha
Mortality (bioassay 1 – 0 DAA) [mean %]	7.00	14.00	13.00	78.00
Significance [a]	-	n.s.	n.s.	***
Corrected mortality [b] (bioassay 1 – 0 DAA) [%]	-	7.53	6.45	76.34
Mortality (bioassay 2 – 7 DAA) [mean %]	6.00	6.00	12.00	69.00
Significance [a]	-	n.s.	n.s.	***
Corrected mortality [b] (bioassay 2 – 7 DAA) [%]	-	0.00	6.38	67.02
Mortality (bioassay 3 – 14 DAA) [mean %]	6.00	6.00	12.00	58.00
Significance [a]	-	n.s.	n.s.	***
Corrected mortality [b] (bioassay 3 – 14 DAA) [%]	-	0.00	6.38	55.32

-, not applicable
n.s., not significantly different compared to the control
[a], Chi^2-2x2 Table test, $\alpha \leq 0.001$ ***, 0.01 **, 0.05 *

Reproduction parameter:

***Typhlodromus pyri* reproduction (Bioassay 1 at 0 DAA, 2 at 7 DAA and 3 at 14 DAA).**

	T1 Control	T2 PROTIOKONAZOL 300 EC at 650 mL test item/ha	T3 PROTIOKONAZOL 300 EC at 1300 mL test item/ha
	Deionised water	195 g a.i./ha	390 g a.i./ha
Reproduction [mean eggs/female] (bioassay 1 - 0 DAA)	8.77	8.68	8.74
Significance [a]	-	n.s.	n.s.
Effect on reproduction in the bioassay 1 at 0 DAA [%R]	-	0.99	0.27
Reproduction [mean eggs/female] (bioassay 2 - 7 DAA)	8.43	8.07	7.97
Significance [a]	-	n.s.	n.s.
Effect on reproduction in the bioassay 2 at 7 DAA [%R]	-	4.28	5.36
Reproduction [mean eggs/female] (bioassay 3 - 14 DAA)	9.40	9.39	8.98
Significance [a]	-	n.s.	n.s.
Effect on reproduction in the bioassay 3 at 14 DAA [%R]	-	0.10	4.46

-, not applicable
n.s., not significantly different compared to the control
[a], Dunnett's t-test, $\alpha \leq 0.05$ *

Reference:	KCP 10.3.2.2/01
Report	Effects of PROTIOKONAZOL 300 EC (prothioconazole 300 g/L) on Typhlodromus pyri – Extended laboratory aged residue test – 2022; Mautino G.; 2022; Study Code: 1020.1F.SAG22
Guideline(s):	Yes, IOBC, BART, EPPO
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No
Validity criteria:	Validity criteria of the test:: -mortality in the control group ≤20% on day 7; -mean cumulative number of eggs per female in the control group ≥4; -corrected mortality between 50% and 100% in the reference item on day 7.

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	PROTIOKONAZOL 300 EC
Formulation:	EC (prothioconazole 300 g/L)
Description (physical state):	liquid
Batch no.:	01/PRO/2022
Production date:	24 March 2022
Expiration date:	March 2025
Stability of test compound:	not relevant

2. Vehicle and/or positive control:	vehicle: deionised water positive control: ROGOR L 40 ST (nominally 400 g dimethoate/L)
3. Test organism	
Species:	*Typhlodromus pyri* Scheuten, Phytoseiid (Acari: Phytoseiidae)
Source:	Katz Biotech AG, Baruth, Germany
Stage at delivery:	eggs
Age at test start:	protonymphs ≤24 hours old
Acclimation period:	1 day under test conditions in an incubator
Sex:	Females and males
Diet:	pollen (100% from apple, provided by the same supplier) *ad libitum*
Test units:	one leaf disc (44-mm Ø) placed in a glass petri dish lid (54-mm Ø), with a central hole (6-mm Ø), located on a grid immersed in water. All systems were contained within a plastic container (250 × 250 × 80 mm)
Plant:	Taxonomic group: Vitaceae, Common name: grapevine, Species: Vitis vinifera L., Variety: Barbera, Source: Vivalb (Alba, CN), Transplanting date: 05 July 2022, Cultivation substrate: natural soil, Grown site: open field under a rain cover, Stage for test start: BBCH 18-19, Maintenance: automatic drip irrigation, Agrochemical and fertilizer: none
4. Environmental conditions:	
Temperature:	24.43 ± 0.63 °C (23.59 – 25.42 °C)
Relative humidity:	67.2 ± 5.5% (60.0 – 79.5%)
Photoperiod:	daily cycle 16 h day/8 h night

STUDY DESIGN AND METHOD

The study was conducted to assess the effects of the test item PROTIOKONAZOL 300 EC on mortality and reproductive of predatory mite *Typhlodromus pyri*. The aim of the study was to determine the product persistence, intended as the decline rate of residues (fresh and aged) on grapevine plants treated once with the test item PROTIOKONAZOL 300 EC (prothioconazole 300 g/L), under rain-protected field conditions.

Grapevine plants were treated with test item PROTIOKONAZOL 300 EC applied once at rates of 650 and 1300 mL test item/ha (equivalent to 195 and 390 g a.i./ha). There were 5 replicates for each treatment. Deionised water was used as diluent for the test item solutions. Test item was compared to a control group with deionised water only and reference item ROGOR L 40 ST applied once at 18 mL product/ha (7.2 g dimethoate/ha).

Application was performed in a spray chamber with a spaying surface of 2 m2 (plot length: 2 m, plot width: 1 m) and the application was carried out by simulating the good agricultural practices. After treatment, grapevine plants were left under a rain cover and at each bioassay (timing 0, 7, 14 DAA), leaf discs

were cut out from the control and treated groups' plants and transferred to the laboratory where leaf discs were obtained with a leaf puncher (44-mm Ø).

Mites were exposed to fresh and aged residue of the test item at different timings (bioassay) after application (DAA).

Three bioassays were investigated: bioassay 1, where mites were introduced immediately after the application on dried discs (0 DAA); bioassay 2, where mites were introduced 7 days after the application (7 DAA), and bioassay 3, where mites were introduced 14 days after the application (14 DAA).

At each bioassay, residual toxicity was evaluated by assessing *Typhlodromus pyri* mortality after 3 and 7 days of exposure to the test item, using grape leaf discs removed from treated grape plants. Mite reproduction was also assessed; the test units were maintained for 7 additional days, during which the number of juveniles and eggs were counted (i.e., 9, 11, and 14 days after the application). The effects of the test item were compared with a control group and a reference item. All the experimental procedures were designed by following the Blümel et al. (2000) method

To verify the sensitivity of the test system, an insecticide, i.e., ROGOR L 40 ST (nominally 400 g dimethoate/L) was used as a reference item. The control group was treated with distilled water.

Method used:	"Island method" by Joisten 2000, as described in Blumel et al (2000)
Test design:	tested concentrations, reference item and control in 5 replications, number of mites: 20 /replicate for test and reference item
Introduction of Individuals:	immediately after the test item drying (<1.5 hours from the application) for bioassay 1 (0 DAA), at 7 and 14 days after the application (DAA) for bioassay 2 and bioassay 3, respectively
Introduction Procedure:	with a fine brush, selection of the mites was randomly performed, following the spray scheme
Exposure time:	14 days (7 days of mortality phase + 7 days of fecundity test)
Tested concentrations, definitive test:	650 and 1300 ml/ha (dilution ratio: 2; volume of application was 400 L/ha) fresh residues – 0 DAA aged residues – 7 DAA aged residues – 14 DAA
Dates:	start of the study: 07.09.2022 start of the experimental part: 03.10.2022 end of the experimental part: 31.10.2022 end of the study: 17.01.2023

Statistic:	Software used for statistical analysis were "ToxRatPro" Solutions GmbH, version 3.3.0. Mortality data were processed using the Chi²-2x2 Table test, $\alpha \leq 0.05$ and the LR50 value was determined. Correction for control mortality was processed using the Schneider-Orelli formula. Reproduction data were analysed by Dunnett's t-test, $\alpha \leq 0.05$ and the ER50 value was determined where possible. The No Observed Effect Rate (NOER) and Lowest Observed Effect Rate (LOER) values for mortality and reproduction were determined, where possible.

RESULTS

All study validity criteria were met at each bioassay. Mean mortality in the water control should be ≤20%. The mean cumulative number of eggs per female in the water control should be ≥4. Corrected mortality should be between 50% and 100% in the toxic reference treatment on day 7.

Mortality assessment - Bioassay 1 (0 DAA)

For the test item PROTIOKONAZOL 300 EC, mean mortality at 3 days of exposure was 8.00% in treatment T2 (650 mL test item/ha) and 2.00% in treatment T3 (1300 mL test item/ha). The control and reference item groups showed a mortality of 3.00% and 72.00%, respectively.

Mean mortality at day 7 was 14.00% in treatment T2 (650 mL test item/ha, corrected mortality: 7.53%) and 13.00% in treatment T3 (1300 mL test item/ha, corrected mortality: 6.45%). The control and reference item groups showed a mortality of 7.00% and 78.00% (corrected 76.34%), respectively.

Given that only two test-item treatment rates were being tested, the 7-day LR50 values for mortality was estimated to be >1300 (>390 g a.i./ha), while the NOER value ≥1300 mL test item/ha (≥390 g a.i./ha) and LOER value >1300 mL test item/ha (>390 g a.i./ha).

Mortality assessment - Bioassay 2 (7 DAA)

For the test item PROTIOKONAZOL 300 EC, mean mortality at 3 days of exposure was 3.00% in treatment T2 (650 mL test item/ha) and 9.00% in treatment T3 (1300 mL test item/ha). The control group and reference item showed a mortality of 4.00 % and 65.00%, respectively.

Mean mortality at day 7 was 6.00% in treatment T2 (650 mL test item/ha, corrected mortality: 0.00%) and 12.00% in treatment T3 (1300 mL test item/ha, corrected mortality: 6.38%). The control group and reference item showed a mortality of 6.00% and 69.00% (corrected 67.02%), respectively.

Given that only two test-item treatment rates were tested, the 7-day LR50 value was estimated to be >1300 mL test item/ha (>390 g a.i./ha), while the corresponding NOER and LOER values for mortality were determined to be ≥390 and 1300 mL test item/ha (390 g a.i./ha), respectively.

Mortality assessment - Bioassay 3 (14 DAA)

For the test item PROTIOKONAZOL 300 EC, mean mortality at 3 days of exposure was 5.00% in treatment T2 (650 mL test item/ha) and 10.00 in treatment T3 (1300 mL test item/ha). The control group and reference item showed a mortality of 4.00 % and 51.00 %, respectively.

Mean mortality at day 7 was 6.00% in treatment T2 (650 mL test item/ha, corrected mortality: 0.00%) and 12.00% in treatment T3 (1300 mL test item/ha, corrected mortality: 6.38%). The control group and reference item showed a mortality of 6.00% and 58.00% (corrected 55.32%), respectively.

Given that only two test-item treatment rates were tested, the 7-day LR50 value was estimated to be

>1300 mL test item/ha (>390 g a.i./ha), while the corresponding NOER and LOER values for mortality were determined to be ≥390 and 1300 mL test item/ha (390 g a.i./ha), respectively.

Table KCP 10.3.2.2-1 Summary of results from the mortality assessment

Treatment number	Treatment Rate		Check at 3 days	Check at 7 days			
	mL test item/ha	g a.i./ha	Mean mortality (%)	Mean mortality (%)	SE[a]	*p*[b]	Corrected mean mortality[c] (%)
Mortality assessment - Bioassay 1 (0 DAA)							
T1	0 (Control)	0	3.00	7.00	±0.51	-	-
T2	650	195	8.00	14.00	±0.37	n.s.	7.53
T3	1300	390	2.00	13.00	±0.68	n.s.	6.45
T4	ROGOR L 40 ST 18 mL test item/ha	7.2	72.00	78.00	±0.51	***	76.34
Mortality assessment - Bioassay 2 (7 DAA)							
T1	0 (Control)	0	4.00	6.00	±0.58	-	-
T2	650	195	3.00	6.00	±0.73	n.s.	0.00
T3	1300	390	9.00	12.00	±1.03	n.s.	6.38
T4	ROGOR L 40 ST 18 mL test item/ha	7.2	65.00	69.00	±0.80	***	67.02
Mortality assessment - Bioassay 3 (14 DAA)							
T1	0 (Control)	0	4.00	6.00	±0.73	-	-
T2	650	195	5.00	6.00	±0.37	n.s.	0.00
T3	1300	390	10.00	12.00	±0.93	n.s.	6.38
T4	ROGOR L 40 ST 18 mL test item/ha	7.2	51.00	58.00	±0.51	***	55.32

a.i. = prothioconazole nominal content 300 g/L
-, not applicable
[a], standard error from 5 replicates
[b], Chi²-2x2 Table test, α≤0.001 ***, 0.01 **, 0.05 *
[c], mean mortality corrected by Schneider-Orelli's formula

At the end of the mortality assessment and for each bioassay, test item PROTIOKONAZOL 300 EC effect on mites' reproduction was also evaluated. Three assessments were performed starting from day 7 up to day 14 (inclusive), with a maximum interval of 3 days between each assessment (i.e. day 9, 11 and 14).

Reproduction assessment - Bioassay 1 (0 DAA)

For the test item PROTIOKONAZOL 300 EC, mean mortality at 3 days of exposure was 8.00% in treatment T2 (650 mL test item/ha) and 2.00% in treatment T3 (1300 mL test item/ha). The control and reference item groups showed a mortality of 3.00% and 72.00%, respectively.

Mean mortality at day 7 was 14.00% in treatment T2 (650 mL test item/ha, corrected mortality: 7.53%) and 13.00% in treatment T3 (1300 mL test item/ha, corrected mortality: 6.45%). The control and reference item groups showed a mortality of 7.00% and 78.00% (corrected 76.34%), respectively.

Given that only two test-item treatment rates were being tested, the 7-day LR50 values for mortality was estimated to be >1300 (>390 g a.i./ha), while the NOER value ≥1300 mL test item/ha (≥390 g a.i./ha) and LOER value >1300 mL test item/ha (>390 g a.i./ha).

Reproduction assessment - Bioassay 2 (7 DAA)

For the test item PROTIOKONAZOL 300 EC, mean mortality at 3 days of exposure was 3.00% in treat-

ment T2 (650 mL test item/ha) and 9.00% in treatment T3 (1300 mL test item/ha). The control group and reference item showed a mortality of 4.00 % and 65.00%, respectively.

Mean mortality at day 7 was 6.00% in treatment T2 (650 mL test item/ha, corrected mortality: 0.00%) and 12.00% in treatment T3 (1300 mL test item/ha, corrected mortality: 6.38%). The control group and reference item showed a mortality of 6.00% and 69.00% (corrected 67.02%), respectively.

Given that only two test-item treatment rates were tested, the 7-day LR50 value was estimated to be >1300 mL test item/ha (>390 g a.i./ha), while the corresponding NOER and LOER values for mortality were determined to be ≥390 and 1300 mL test item/ha (390 g a.i./ha), respectively.

Reproduction assessment - Bioassay 3 (14 DAA)

For the test item PROTIOKONAZOL 300 EC, mean mortality at 3 days of exposure was 5.00% in treatment T2 (650 mL test item/ha) and 10.00 in treatment T3 (1300 mL test item/ha). The control group and reference item showed a mortality of 4.00 % and 51.00 %, respectively.

Mean mortality at day 7 was 6.00% in treatment T2 (650 mL test item/ha, corrected mortality: 0.00%) and 12.00% in treatment T3 (1300 mL test item/ha, corrected mortality: 6.38%). The control group and reference item showed a mortality of 6.00% and 58.00% (corrected 55.32%), respectively.

Given that only two test-item treatment rates were tested, the 7-day LR50 value was estimated to be >1300 mL test item/ha (>390 g a.i./ha), while the corresponding NOER and LOER values for mortality were determined to be ≥390 and 1300 mL test item/ha (390 g a.i./ha), respectively.

Table KCP 10.3.2.2-2 Summary of results from the reproduction assessment

Treatment number	Treatment Rate		Mean cumulative number of eggs per female	Standard error[a]	Effect on reproduction (R%)	p^b
	mL test item/ha	g a.i./ha				
Reproduction assessment - Bioassay 1 (0 DAA)						
T1	0 (Control)	0	8.77	±0.45	-	-
T2	650	195	8.68	±0.43	0.99	n.s.
T3	1300	390	8.74	±0.64	0.27	n.s.
Reproduction assessment - Bioassay 2 (7 DAA)						
T1	0 (Control)	0	8.43	±0.32	-	-
T2	650	195	8.07	±0.38	4.28	n.s.
T3	1300	390	7.97	±0.20	5.36	n.s.
Reproduction assessment - Bioassay 3 (14 DAA)						
T1	0 (Control)	0	9.40	±0.60	-	-
T2	650	195	9.39	±0.43	0.10	n.s.
T3	1300	390	8.98	±0.87	4.46	n.s.

a.i. = prothioconazolenominal content 300 g/L
-, not applicable
n.s., not significantly different compared to the control
[a], standard error from 5 replicates
[b], Dunnett's t-test, α=0.05 *

CONCLUSION

All study validity criteria were met at each bioassay.

Bioassay 1 (0 DAA)

Given that only two test-item treatment rates were being tested, the 7-day LR_{50} values for mortality was estimated to be >1300 (>390 g a.i./ha), while the NOER value ≥1300 mL test item/ha (≥390 g a.i./ha) and LOER value >1300 mL test item/ha (>390 g a.i./ha).

The 14-day NOER (reproduction) value was estimated to be ≥1300 mL test item/ha (≥390 g a.i./ha) and the 14-day LOER (reproduction) value was determined to be >1300 mL test item/ha (>390 g a.i./ha).

The 14-day ER_{50} was estimated to be >1300 mL test item/ha (>390 g a.i./ha).

Bioassay 2 (7 DAA)

Given that only two test-item treatment rates were tested, the 7-day LR_{50} value was estimated to be >1300 mL test item/ha (>390 g a.i./ha), while the corresponding NOER and LOER values for mortality were determined to be ≥390 and 1300 mL test item/ha (390 g a.i./ha), respectively.

The 14-day NOER and LOER (reproduction) values were determined to be ≥1300 and >1300 mL test item/ha (≥390 and >390 g a.i./ha), respectively.

The 14-day ER_{50} was estimated to be >1300 mL test item/ha (>390 g a.i./ha).

Bioassay 3 (14 DAA)

Given that only two test-item treatment rates were tested, the 7-day LR_{50} value was estimated to be >1300 mL test item/ha (>390 g a.i./ha), while the corresponding NOER and LOER values for mortality were determined to be ≥390 and 1300 mL test item/ha (390 g a.i./ha), respectively.

The 14-day NOER and LOER (reproduction) values were determined to be ≥1300 and >1300 mL test item/ha (≥390 and >390 g a.i./ha), respectively.
The 14-day ER_{50} was estimated to be >1300 mL test item/ha (>390 g a.i./ha).

Table KCP 10.3.2.2-3 *Typhlodromus pyri* mortality after 7 days of exposure (Bioassay 1 at 0 DAA, 2 at 7 DAA and 3 at 14 DAA)

	T1 Control	**T2 PROTIOKONA-ZOL 300 EC at 650 mL test item/ha**	**T3 PROTIOKONA-ZOL 300 EC at 1300 mL test item/ha**	**T4 ROGOR L40 ST at 18 mL test item/ha**
	Deionised water	**195 g a.i./ha**	**390 g a.i./ha**	**7.2 g a.i./ha**
Mortality (bioassay 1 – 0 DAA) [mean %]	7.00	14.00	13.00	78.00
Significance a	-	n.s.	n.s.	***
Corrected mortality b (bio-assay 1 – 0 DAA) [%]	-	7.53	6.45	76.34
Mortality (bioassay 2 – 7 DAA) [mean %]	6.00	6.00	12.00	69.00
Significance a	-	n.s.	n.s.	***
Corrected mortality b (bio-assay 2 – 7 DAA) [%]	-	0.00	6.38	67.02
Mortality (bioassay 3 – 14 DAA) [mean %]	6.00	6.00	12.00	58.00
Significance a	-	n.s.	n.s.	***
Corrected mortality b (bio-assay 3 – 14 DAA) [%]	-	0.00	6.38	55.32

-, not applicable
n.s., not significantly different compared to the control
[a], Chi^2-2x2 Table test, α≤0.001 ***, 0.01 **, 0.05 *

Table KCP 10.3.2.2-4 Typhlodromus pyri reproduction (Bioassay 1 at 0 DAA, 2 at 7 DAA and 3 at

14 DAA)

	T1 Control	T2 PROTIOKONAZOL 300 EC at 650 mL test item/ha	T3 PROTIOKONAZOL 300 EC at 1300 mL test item/ha
	Deionised water	195 g a.i./ha	390 g a.i./ha
Reproduction [mean eggs/female] (bioassay 1 - 0 DAA)	8.77	8.68	8.74
Significance a	-	n.s.	n.s.
Effect on reproduction in the bioassay 1 at 0 DAA [%R]	-	0.99	0.27
Reproduction [mean eggs/female] (bioassay 2 - 7 DAA)	8.43	8.07	7.97
Significance a	-	n.s.	n.s.
Effect on reproduction in the bioassay 2 at 7 DAA [%R]	-	4.28	5.36
Reproduction [mean eggs/female] (bioassay 3 - 14 DAA)	9.40	9.39	8.98
Significance a	-	n.s.	n.s.
Effect on reproduction in the bioassay 3 at 14 DAA [%R]	-	0.10	4.46

-, not applicable
n.s., not significantly different compared to the control
[a], Dunnett's t-test, $\alpha \leq 0.05$ *

Table KCP 10.3.2.2-5 Endpoints for the 0-DAA, 7-DAA and 14-DAA bioassay

Endpoint	mL test item/ha	g a.i./ha[#]
0-DAA Bioassay: fresh residues		
LR_{50}[#]	>1300	>390
NOER (Mortality)	≥1300	≥390
LOER (Mortality)	>1300	>390
ER50 (Reproduction)	>1300* (95%-CLs n.d.)	>390 (95%-CLs n.d.)
NOER (Reproduction)	≥1300	≥390
LOER (Reproduction)	>1300	>390
7-DAA Bioassay: 7-day aged residues		
LR_{50}[#]	>1300	>390
NOER (Mortality)	≥1300	≥390
LOER (Mortality)	>1300	>390
ER50 (Reproduction)	>1300* (95%-CLs n.d.)	>390 (95%-CLs n.d.)
NOER (Reproduction)	≥1300	≥390
LOER (Reproduction)	>1300	>390
14-DAA Bioassay: 14-day aged residues		
LR_{50}[#]	>1300	>390
NOER (Mortality)	≥1300	≥390
LOER (Mortality)	>1300	>390
ER50 (Reproduction)	>1300* (95%-CLs n.d.)	>390 (95%-CLs n.d.)
NOER (Reproduction)	≥1300	≥390
LOER (Reproduction)	>1300	>390

#The LR_{50} could not be determined, because the number of responses was less than three. Values are estimated to be close to the highest test-item dosage. The LR_{50} values were estimated, therefore no confidence limits were provided
n.d., not determined due to mathematical reasons
95%-CLs n.d., 95% Confidence Limits not determined due to mathematical reasons
*, Since the slope of the relationship was found to be not significant, no ER and confidence limits are provided

Comments of zRMS: The study was accepted by zRMS.
The validity criteria was met.

Validity criteria of the study

Mean mortality in the water control ≤ 10% after 48 hours

The mean number of parasitized aphids (mummies) per female to be ≥ 5.

No more than two wasps producing zero mummies.

Corrected mortality > 50% in the reference item after 48 hours

Mortality in the control groups after 48 hours	Bioassay 1 at 0 DAA	Actual value was 0.00%, therefore, the validity criterion was met.
	Bioassay 2 at 7 DAA	Actual value was 6.67%, therefore, the validity criterion was met.
	Bioassay 3 at 14 DAA	Actual value was 6.67%, therefore, the validity criterion was met.
Reproduction in the control groups	Bioassay 1 at 0 DAA	The mean number of parasitized aphids per female was 33.07 and none female produced zero mummies, so this validity criteria were met.
	Bioassay 2 at 7 DAA	The mean number of parasitized aphids per female was 30.53 and none female produced zero mummies, so this validity criteria were met.
	Bioassay 3 at 14 DAA	The mean number of parasitized aphids per female was 31.87 and none female produced zero mummies, so this validity criteria were met.
Mortality in the reference item after 48 hours	Bioassay 1 at 0 DAA	Actual value was 93.33%, so the validity criterion was met.
	Bioassay 2 at 7 DAA	Actual value was 76.67% (corrected value 75%), so the validity criterion was met.
	Bioassay 3 at 14 DAA	Actual value was 66.67% (corrected value 64.29), so the validity criterion was met.

The agreed toxicity endpoints:

Mortality parameter:

Mortality of *Aphidius rhopalosiphi* (Bioassay 1 at 0 DAA, 2 at 7 DAA and 3 at 14 DAA)

	T1 Control	T2 PROTIOKONAZOL 300 EC at 650 mL test item/ha	T3 PROTIOKONAZOL 300 EC at 1300 mL test item/ha	T4 ROGOR L40 ST at 20 mL test item/ha
	Deionised water	195 g a.i./ha	390 g a.i./ha	8 g a.i./ha
Mortality (bioassay 1 – 0 DAA) [mean %]	0.00	3.33	6.67	93.33
Significance [a]	-	n.s.	n.s.	***
Mortality (bioassay 2 – 7 DAA) [mean %]	6.67	10.00	13.33	76.67
Significance [a]	-	n.s.	n.s.	***
Corrected mortality [b] (bioassay 3 – 14 DAA) [%]	-	3.57	7.14	75.00
Mortality (bioassay 3 – 14 DAA) [mean %]	6.67	6.67	10.00	66.67
Significance [a]	-	n.s.	n.s.	***
Corrected mortality [b] (bioassay 3 – 14 DAA) [%]	-	0.00	3.57	64.29

-, not applicable
n.s., not significantly different compared to the control
[a], Fisher exact test with or without (reference item) Bonferroni correction, α≤0.001 ***, 0.01 **, 0.05 *
[b], Schneider-Orelli's formula

Reproduction parameter:

Reproduction of *Aphidius rhopalosiphi* (Bioassay 1 at 0 DAA, 2 at 7 DAA and 3 at 14 DAA)

	T1 Control	T2 PROTIOKONAZOL 300 EC at 650 mL test item/ha	T3 PROTIOKONAZOL 300 EC at 1300 mL test item/ha
	Deionised water	195 g a.i./ha	390 g a.i./ha
Reproduction [mean mummies/female] (bioassay 1 - 0 DAA)	33.07	25.53	14.53
Significance [a]	-	n.s.	*
Effect on reproduction in the bioassay 1 at 0 DAA [%R]	-	22.78	56.05
Reproduction [mean mummies/female] (bioassay 2 - 7 DAA)	30.53	28.47	22.60
Significance [b]	-	n.s.	n.s.
Effect on reproduction in the bioassay 2 at 7 DAA [%R]	-	6.77	25.98
Reproduction [mean mummies/female] (bioassay 3 - 14 DAA)	31.87	28.73	24.53
Significance [b]	-	n.s.	n.s.
Effect on reproduction in the bioassay 3 at 14 DAA [%R]	-	9.83	23.01

-, not applicable
n.s., not significantly different compared to the control
[a], Williams t-test, α = 0.05 *
[b], Dunnett's t-test, α = 0.05 *

Reference:	KCP 10.3.2.1/02
Report	Effects of PROTIOKONAZOL 300 EC (prothioconazole 300 g/L) on *Aphidius rhopalosiphi* – Extended laboratory aged residue test – 2022*;* Mautino G.; 2022; Study Code: 1019.F1.SAG22
Guideline(s):	Yes, SETAC; ESCORT; IOBC/BART/EPPO
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	PROTIOKONAZOL 300 EC
Formulation:	EC (prothioconazole 300 g/L)
Description (physical state):	liquid
Batch no.:	01/PRO/2022
Production date:	24 March 2022
Expiration date:	March 2025
Stability of test compound:	not relevant

2. Vehicle and/or positive control: vehicle: deionized water
positive control: ROGOR L 40 ST (nominally 400 g dime-

	thoate/L) 20 mL product/ha (8 g dimethoate/ha).
3. Test organism	
Species:	parasitic wasp *Aphidius rhopalosiphi* (Hymenoptera, Braconidae)
Source:	Katz Biotech AG, Baruth, Germany
Acclimation period:	2 days under test conditions
Stage at delivery:	Aphid mummies
Age at test start:	adults less than 48-hour old
Sex:	female
Diet:	Cotton wool soaked in 1:3 v/v honey water solution provided ad libitum. Before the application (0 DAA) and before the test unit settlement (7 and 14 DAA), barley plants were lightly sprayed with a 10% w/w solution of sugar in water to provide both food and a foraging stimulus for the wasps; for the reproduction assessment a 1:3 v/v solution of honey and water was put on a cotton wool and provided for 24 hours.
Test units for mortality assessment:	The test unit consisted of one pot (15.0 cm Ø) with barley seedlings (Hordeum vulgare; 10 seeds per pot), confined within a clear polyacrylic and transparent cylinder (22 cm high and 10 cm Ø). The cylinder had a ventilated cap with a wasp-proof netting (0.1 x 0.5 mm mesh size) and a ventilated hole (2 cm Ø) used for wasp introduction. After the introduction of the insects, this hole was plugged up with cotton wool.
Test units for reproduction assessment:	Untreated pots (15.0 cm Ø) with barley seedlings (Hordeum vulgare; 30 seeds per pot) infested with ≥100 host aphids of all development stages (Rhopalosiphum padi; number of aphids was estimated) were enclosed within a clear polyacrylic cylinder (22 cm high and 10 cm Ø). The cylinder had a ventilated cap with a wasp-proof netting (0.1 x 0.5 mm mesh size) and a ventilated hole (2 cm Ø) used for wasp introduction. After the introduction of the insects, this hole was plugged up with a cotton wool. After the adult wasps were removed, the polyacrylic cylinders were left on the pots.
Plant:	Taxonomic group: Poaceae, Common name: barley, Species: Hordeum vulgare L., Variety: Cometa, Stage at delivery: seed, Source: Agricola Albese (Alba, CN), Cultivation substrate: artificial soil, Grown site: open field under a rain cover, Stage for test start: BBCH 12, Maintenance: bottom watering two times a week, Agrochemical and fertilizer: none, No. pots/treatment (mortality): 6, No. pots/treatment (reproduction): 15, Seeds/pot (mortality): 10, Seeds/pot (reproduction): 30

4. Environmental conditions:

Temperature:	19.97 ± 0.52 °C (19.04 – 21.32 °C)
Relative humidity:	69.8% ± 6.4% (60.5 – 79.2% RH)
Photoperiod:	16 h light:8 h dark
Light Intensity:	Mortality: 850 to 950, Reproduction: 19000 to 20000

STUDY DESIGN AND METHOD

The study was conducted to assess the effects of the test item PROTIOKONAZOL 300 EC on mortality and reproductive performance of parasitic wasp *Aphidius rhopalosiphi*. The aim of the study was to determine the product persistence, intended as the decline rate of residues (fresh and aged) on barley plants treated once with the test item PROTIOKONAZOL 300 EC (prothioconazole 300 g/L), under rain-protected field conditions.

Barley plants were treated with PROTIOKONAZOL 300 EC (prothioconazole 300 g/L) applied once at rates of 650 and 1300 mL test item/ha (equivalent to 195 and 390 g a.i./ha, when accounting for the nominal test-item purity of 300 g/L). Deionised water was used as diluent for the test item solutions. Test item was compared to a control group with deionised water only and reference item ROGOR L 40 ST.

Application was performed in a spray chamber, with a spaying surface of 2 m^2 (plot length: 2 m, plot width: 1 m) and the application was carried out by simulating the good agricultural practices. After treatment, barley plants were left under a rain cover and at each assessment timing (0, 7 and 14 days of exposure) transferred to the laboratory for the test setting.

Insects were exposed to fresh and aged residue of the test item at different timings (bioassay) after application (DAA).

Three bioassays were investigated: bioassay 1, where insects were introduced immediately after the application on dried barley plants (0 DAA); bioassay 2, where insects were introduced 7 days after the application (7 DAA), and bioassay 3, where insects were introduced 14 days after the application (14 DAA). At each bioassay, test item' repellency was recorded after 30, 60, 90, 120 and 150 minutes during the initial 3 hours after their release in the treated test units; then, residual toxicity was evaluated by assessing Aphidius rhopalosiphi mortality after 2, 24 and 48 hours of exposure. Parasitisation rate (reproduction) was evaluated after 12 days. The effects of the test item were compared with a control group and a reference item.

To verify the sensitivity of the test system and the precision of the test procedure, an insecticide, i.e., ROGOR L 40 ST (nominally 400 g dimethoate/L) was used as a reference item. The control group was treated with distilled water.

Test design:	tested concentrations, reference and control in 6 replications, number of insects: 5 females/replicate
Introduction of Individuals:	Immediately after the test item drying (<1 hours from the application) for bioassay 1 (0 DAA). At 7 and 14 days after the application (DAA) for bioassay 2 and bioassay 3, respectively.

Mortality exposure time:	48 hours + fecundity phase: 12 days
Fecundity exposure time:	parasitisation period was 24 hours, all treatment groups were evaluated 12 days after parasitisation
Tested concentrations, definitive test:	650 and 1300 ml/ha (dilution ratio: 2; volume of application was 200 L/ha) fresh residues – 0 DAA aged residues – 7 DAA aged residues – 14 DAA
Dates:	start of the study: 30.09.2022 start of the experimental part: 03.10.2022 end of the experimental part: 01.11.2022 end of the study: 22.12.2022
Statistic:	Software used for statistical analysis were "ToxRatPro" Solutions GmbH, version 3.3.0. Mortality data were processed using the Fisher's exact test with Bonferroni Correction or without (reference item), $\alpha \leq 0.05$. Correction for control mortality was processed using the Schneider-Orelli formula. For all bioassays, the 48-hours LR50 value could not be calculated, because data were not appropriate for the computation, the number of responses was less than three. Reproduction data were analysed by Williams or Dunnett's t-test, $\alpha = 0.05$ and ERx calculated. The No Observed Effect Rate (NOER) and Lowest Observed Effect Rate (LOER) values for mortality and reproduction obtained.
Validity criteria:	All validity criteria were met: -mortality in the control treatment ≤ 10% after 48 hours; -mean number of parasitized aphids per female in the control treatment ≥ 5; -no more than two surviving wasps producing zero values in the control treatment; -corrected mortality > 50% in the reference item treatment.

RESULTS

Repellence assessment - Bioassay 1 (0 DAA)

The mean percentage of wasps settled on the treated plants ranged from 32.67% in treatment T3 (1300 mL f.p./ha) to 38.67% on T2 (650 mL f.p./ha). Control group and reference item ROGOR L40 ST showed a percentage of 42.67% and 22.67%, respectively.

Concerning the mean percentage of wasps settled on cylinder, values ranged from 52.00% in treatment T2 (650 mL f.p./ha) to 56.67% in T3 (1300 mL f.p./ha). Control group and reference item showed a percentage of 48.67% and 65.33%, respectively.

Wasps settled on sand ranged from 9.33% in treatment T2 (650 mL f.p./ha) to 11.33% in treatment T3 (1300 mL f.p./ha). Control group and reference item showed a percentage of 8.00% and 11.33%, respec-

tively.

Repellence assessment - Bioassay 2 (7 DAA)

The mean percentage of wasps settled on the treated plants ranged from 27.33% in treatment T3 (1300 mL f.p./ha) to 31.33% on T2 (650 mL f.p./ha). Control group and reference item ROGOR L40 ST showed a percentage of 26.67% and 23.33%, respectively.

Concerning the mean percentage of wasps settled on cylinder, values ranged from 58.00% in treatment T2 (650 mL f.p./ha) to 64.00% in T3 (1300 mL f.p./ha). Control group and reference item showed a percentage of 66.00% and 53.33%, respectively.

Wasps settled on sand ranged from 8.67% to 10.67% for treatments T3 (1300 mL f.p./ha) and T2 (650 mL f.p./ha), respectively. Control group and reference item showed a percentage of 7.33% and 23.33%, respectively.

Repellence assessment - Bioassay 3 (14 DAA)

The mean percentage of wasps settled on the treated plants ranged from 24.00% in treatment T3 (1300 mL f.p./ha) to 30.00% on T2 (650 mL f.p./ha). Control group and reference item ROGOR L40 ST showed a percentage of 27.33% and 25.33%, respectively.

Concerning the mean percentage of wasps settled on cylinder, values ranged from 64.00% in treatment T2 (650 mL f.p./ha) to 68.00% in T3 (1300 mL f.p./ha). Control group and reference item showed a percentage of 64.00% and 62.00%, respectively.
Wasps settled on sand ranged from 6.00% in treatment T2 (650 mL f.p./ha) to 8.00% in treatment T3 (1300 mL f.p./ha). Control group and reference item showed a percentage of 8.67% and 12.67%, respectively.

Table KCP 10.3.2.2-6 Summary of results from the repellence assessment

Treatment number	Treatment rate		Mean % of wasps on treated plants	Mean % of wasps on cylinder	Mean % of wasps on sand
	mL test item/ha	g a.i./ha			
Bioassay 1 – repellence (0 DAA)					
T1	0 (Control)	0	42.67	48.67	8.00
T2	650	195	38.67	52.00	9.33
T3	1300	390	32.67	56.67	11.33
T4	ROGOR L40 ST at 20	8 dimetho-ate/ha	22.67	65.33	11.33
Bioassay 2 – repellence (7 DAA)					
T1	0 (Control)	0	26.67	66.00	7.33
T2	650	195	31.33	58.00	10.67
T3	1300	390	27.33	64.00	8.67
T4	ROGOR L40 ST at 20	8 dimetho-ate/ha	23.33	53.33	23.33
Bioassay 3 – repellence (14 DAA)					
T1	0 (Control)	0	27.33	64.00	8.67
T2	650	195	30.00	64.00	6.00
T3	1300	390	24.00	68.00	8.00
T4	ROGOR L40 ST at 20	8 dimetho-ate/ha	25.33	62.00	12.67

a.i. = prothioconazole nominal content 300 g/L

Mortality assessment - Bioassay 1 (0 DAA)

For the test item PROTIOKONAZOL 300 EC, mean mortality at 2 hours ranged from 0.00% in treatment T2 (650 mL test item/ha) to 3.33% in treatment T3 (1300 mL test item/ha). The control group and reference item showed a mortality of 0.00% and 73.33%, respectively.

Mean mortality at 24 hours ranged from 3.33% in treatment T2 (650 mL test item/ha) to 6.67% in treat-

ment T3 (1300 mL test item/ha). The control group and reference item showed a mortality value of 0.00% and 80.00%, respectively.

After 48 hours of exposure, mortality ranged from 3.33% in treatment T2 (650 mL test item/ha) to 6.67% in treatments T3 (1300 mL test item/ha, corrected mortality: 6.67%). Significant differences (93.33%) were observed when reference item ROGOR L 40 ST was applied. Control group showed a mortality value of 0.00%.

Given that only two test-item treatment rates were being tested, the 48-hours LR_{50} value for mortality could not be calculated; the LOER (mortality) value was estimated to be >1300 mL test item/ha, (>390 g a.i./ha) and NOER ≥1300 mL test item/ha, (≥390 g a.i./ha).

Mortality assessment - Bioassay 2 (7 DAA)

For the test item PROTIOKONAZOL 300 EC, mean mortality at 2 hours ranged from 3.33% to 10.00 for treatments T2 (650 mL test item/ha) and T3 (1300 mL test item/ha), respectively. The control group and reference item showed a mortality of 0.00% and 63.33%, respectively.

Mean mortality at 24 hours ranged from 6.67% in treatment T2 (650 mL test item/ha) to 13.33% in treatment T3 (1300 mL test item/ha). The control group and reference item showed a mortality value of 3.33% and 70.00%, respectively.

After 48 hours of exposure, mortality ranged from 10.00% in treatment T2 (650 mL test item/ha, corrected mortality: 3.57%) to 13.33% in treatments T3 (1300 mL test item/ha, corrected mortality: 7.14%). Significant differences (76.67%) were observed when reference item ROGOR L 40 ST was applied. Control group showed a mortality value of 6.67%.

Given that only two test-item treatment rates were being tested, the 48-hours LR_{50} value for mortality could not be calculated; the LOER (mortality) value was estimated to be >1300 mL test item/ha, (>390 g a.i./ha) and NOER ≥1300 mL test item/ha, (≥390 g a.i./ha).

Mortality assessment - Bioassay 3 (14 DAA)

For the test item PROTIOKONAZOL 300 EC, mean mortality at 2 hours ranged from 0.00% in treatment T2 (650 mL test item/ha) to 3.33% in treatment T3 (1300 mL test item/ha). The control group and reference item showed a mortality of 0.00% and 50.00%, respectively.

Mean mortality at 24 hours was 3.33% for both treatments T2 (650 mL test item/ha) and T3 (1300 mL test item/ha). The control group and reference item showed a mortality value of 3.33% and 56.67%, respectively.

After 48 hours of exposure, mortality ranged from 6.67% in treatment T2 (650 mL test item/ha) to 10.00% in treatments T3 (1300 mL test item/ha). Significant differences (66.67%) were observed when reference item ROGOR L 40 ST was applied. Control group showed a mortality value of 6.67%.

Given that only two test-item treatment rates were being tested, the 48-hours LR_{50} value for mortality could not be calculated; the LOER (mortality) value was estimated to be >1300 mL test item/ha, (>390 g a.i./ha) and NOER ≥1300 mL test item/ha, (≥390 g a.i./ha).

Table KCP 10.3.2.2-7 Summary of results from the mortality assessment

Treatment number	Treatment rate		Check at 2 hours	Check at 24 hours	Check at 48 hours			
			Mortality (%)	Mortality (%)	Mortality		p[b]	Corrected mortality[c] (%)
	mL test item/ha	g a.i./ha			(%)	SE[a]		
Mortality assessment - Bioassay 1 (0 DAA)								
T1	0 (Control)	0	0.00	0.00	0.00	±0.00	-	-
T2	650	195	0.00	3.33	3.33	±0.41	n.s.	-

T3	1300	390	3.33	6.67	6.67	±0.82	n.s.	-
T4	ROGOR L40 ST at 20	8 dimetho-ate/ha	73.33	80.00	93.33	±0.52	***	-
Mortality assessment - Bioassay 2 (7 DAA)								
T1	0 (Control)	0	0.00	3.33	6.67	±0.21	-	-
T2	650	195	3.33	6.67	10.00	±0.22	n.s.	3.57
T3	1300	390	10.00	13.33	13.33	±0.33	n.s.	7.14
T4	ROGOR L40 ST at 20	8 dimetho-ate/ha	63.33	70.00	76.67	±0.31	***	75.00
Mortality assessment - Bioassay 3 (14 DAA)								
T1	0 (Control)	0	0.00	3.33	6.67	±0.21	-	-
T2	650	195	0.00	3.33	6.67	±0.21	n.s.	0.00
T3	1300	390	3.33	3.33	10.00	±0.22	n.s.	3.57
T4	ROGOR L40 ST at 20	8 dimetho-ate/ha	50.00	56.67	66.67	±0.42	***	64.29

a.i. = prothioconazole nominal content 300 g/L
-, not applicable
n.s., not significantly different compared to the control
[a], standard error on the number of dead insects of 6 replicates
[b], Fisher test with or without (reference item only) Bonferroni Correction, α≤0.001 ***, 0.01 **, 0.05 *
[c], mean mortality corrected by Schneider-Orelli's formula

For each bioassay, at the end of the mortality assessments (48 hours of exposure), the surviving females were evaluated for reproduction after 12 days by comparing the test item treatment groups parasitisation with that of the control group.

Reproduction assessment - Bioassay 1 (0 DAA)

The mean number of mummies ranged from 14.53 in treatment T3 (1300 mL test item/ha) to 25.53 in treatment T2 (650 mL test item/ha).

Significant differences in reproduction, relative to the control group (mean of 33.07 mummies), were observed for treatment T3 (1300 mL test item/ha).

The 12-d NOER (reproduction) value was 650 mL test item/ha (195 g a.i./ha) and the 12-d LOER (reproduction) value was 1300 mL test item/ha (390 g a.i./ha).

The calculated 12-d ER_{10} of PROTIOKONAZOL 300 EC was 430 (95%-CLs 132.5 mL test item/ha – Upper limit not determined) and the 12-d ER_{20} was 603.8 (95%-CLs 184.3 mL test item/ha – Upper limit not determined). The calculated 12-d ER_{50} was 1155.9 mL test item/ha (95%-CLs 244.7 mL test item/ha – Upper Limit not determined), corresponding to 346.77 g a.i./ha (95%-CLs 69.58 – Upper limit not determined) in terms of active substance.

Reproduction assessment - Bioassay 2 (7 DAA)

The mean number of mummies ranged from 22.60 in treatment T3 (1300 mL test item/ha) to 28.47 in treatment T2 (650 mL test item/ha).

None significant differences in reproduction were observed relative to the control group (mean of 30.53 mummies).

The 12-d NOER (reproduction) value was ≥1300 mL test item/ha (≥390 g a.i./ha) and the 12-d LOER (reproduction) value was >1300 mL test item/ha (>390 g a.i./ha).

The calculated 12-d ER_{10} of PROTIOKONAZOL 300 EC was 772.6 (95%-CLs not determined) and the 12-d ER_{20} was 1106.3 (95%-CLs not determined). The 12-d ER_{50} was estimated to be >1300 mL test

item/ha (95%-CLs not determined), corresponding to a value >390 g a.i./ha (95%-CLs not determined) in terms of active substance.

Reproduction assessment - Bioassay 3 (14 DAA)

The mean number of mummies ranged from 24.53 in treatment T3 (1300 mL test item/ha) to 28.73 in treatment T2 (650 mL test item/ha).

None significant differences in reproduction were observed relative to the control group (mean of 31.87 mummies).

The 12-d NOER (reproduction) value was ≥1300 mL test item/ha (≥390 g a.i./ha) and the 12-d LOER (reproduction) value was >1300 mL test item/ha (>390 g a.i./ha).

The calculated 12-d ER_{10} of PROTIOKONAZOL 300 EC was 657.9 (95%-CLs not determined) and the 12-d ER_{20} was 1142.2 (95%-CLs not determined). The 12-d ER_{50} was estimated to be >1300 mL test item/ha (95%-CLs not determined), corresponding to a value >390 g a.i./ha (95%-CLs not determined) in terms of active substance.

Table KCP 10.3.2.2-7 Summary of results from the reproduction assessment

Treatment number	Treatment rate		Reproduction (fecundity)			
	mL test item/ha	g a.i./ha	Mean no. of mummies per female	Standard error	Reduction in reproduction (% R)	*p*[a]
Reproduction assessment - Bioassay 1 (0 DAA)						
T1	0 (Control)	0	33.07	±3.43	-	-
T2	650	195	25.53	±3.88	22.78	n.s.
T3	1300	390	14.53	±2.98	56.05	*
Reproduction assessment - Bioassay 2 (7 DAA)						
T1	0 (Control)	0	30.53	±4.97	-	-
T2	650	195	28.47	±4.13	6.77	n.s.
T3	1300	390	22.60	±3.32	25.98	n.s.
Reproduction assessment - Bioassay 3 (14 DAA)						
T1	0 (Control)	0	31.87	±3.89	-	-
T2	650	195	28.73	±3.36	9.83	n.s.
T3	1300	390	24.53	±2.72	23.01	n.s.

a.i. = prothioconazole nominal content 300 g/L
-, not applicable
n.s., not significantly different compared to the control
[a], Williams' / Dunnett's t- test, α=0.05 *

CONCLUSION

All study validity criteria were met at each bioassay.

Bioassay 1 (0 DAA)

Given that only two test-item treatment rates were being tested, the 48-hours LR_{50} value for mortality could not be determined; the LOER (mortality) value was estimated to be >1300 mL test item/ha, (>390 g a.i./ha) and NOER ≥1300 mL test item/ha, (≥390 g a.i./ha).

Concerning fecundity, the 12-d NOER (reproduction) value was 650 mL test item/ha (195 g a.i./ha) and the 12-d LOER (reproduction) value was 1300 mL test item/ha (390 g a.i./ha).

The calculated 12-d ER_{10} of PROTIOKONAZOL 300 EC was 430 (95%-CLs 132.5 mL test item/ha – Upper limit not determined) and the 12-d ER_{20} was 603.8 (95%-CLs 184.3 mL test item/ha – Upper limit

not determined). The calculated 12-d ER_{50} was 1155.9 mL test item/ha (95%-CLs 244.7 mL test item/ha – Upper Limit not determined), corresponding to 346.77 g a.i./ha (95%-CLs 69.58 – Upper limit not determined) in terms of active substance.

Bioassay 2 (7 DAA)

Given that only two test-item treatment rates were being tested, the 48-hours LR_{50} value for mortality could not be determined; the LOER (mortality) value was estimated to be >1300 mL test item/ha, (>390 g a.i./ha) and NOER ≥1300 mL test item/ha, (≥390 g a.i./ha).

Concerning fecundity, the 12-d NOER (reproduction) value estimated to be ≥1300 mL test item/ha (≥390 g a.i./ha) and the 12-d LOER (reproduction) value was >1300 mL test item/ha (>1300 g a.i./ha).

The calculated 12-d ER_{10} of PROTIOKONAZOL 300 EC was 772.6 (95%-CLs not determined) and the 12-d ER_{20} was 1106.3 (95%-CLs not determined). The 12-d ER_{50} was estimated to be >1300 mL test item/ha (95%-CLs not determined), corresponding to a value >390 g a.i./ha (95%-CLs not determined) in terms of active substance.

Bioassay 3 (14 DAA)

Given that only two test-item treatment rates were being tested, the 48-hours LR_{50} value for mortality could not be determined; the LOER (mortality) value was estimated to be >1300 mL test item/ha, (>390 g a.i./ha) and NOER ≥1300 mL test item/ha, (≥390 g a.i./ha).

Concerning fecundity, the 12-d NOER (reproduction) value estimated to be ≥1300 mL test item/ha (≥390 g a.i./ha) and the 12-d LOER (reproduction) value was >1300 mL test item/ha (>1300 g a.i./ha).

The calculated 12-d ER_{10} of PROTIOKONAZOL 300 EC was 657.9 (95%-CLs not determined) and the 12-d ER_{20} was 1142.2 (95%-CLs not determined). The 12-d ER_{50} was estimated to be >1300 mL test item/ha (95%-CLs not determined), corresponding to a value >390 g a.i./ha (95%-CLs not determined) in terms of active substance.

Table KCP 10.3.2.2-8 **Mortality of *Aphidius rhopalosiphi* (Bioassay 1 at 0 DAA, 2 at 7 DAA and 3 at 14 DAA)**

	T1 Control	**T2 PROTIOKONAZOL 300 EC at 650 mL test item/ha**	**T3 PROTIOKONAZOL 300 EC at 1300 mL test item/ha**	**T4 ROGOR L40 ST at 20 mL test item/ha**
	Deionised water	**195 g a.i./ha**	**390 g a.i./ha**	**8 g a.i./ha**
Mortality (bioassay 1 – 0 DAA) [mean %]	0.00	3.33	6.67	93.33
Significance a	-	n.s.	n.s.	***
Mortality (bioassay 2 – 7 DAA) [mean %]	6.67	10.00	13.33	76.67
Significance a	-	n.s.	n.s.	***
Corrected mortality b (bioassay 3 – 14 DAA) [%]	-	3.57	7.14	75.00
Mortality (bioassay 3 – 14 DAA) [mean %]	6.67	6.67	10.00	66.67
Significance a	-	n.s.	n.s.	***
Corrected mortality b (bioassay 3 – 14 DAA) [%]	-	0.00	3.57	64.29

-, not applicable
n.s., not significantly different compared to the control
[a], Fisher exact test with or without (reference item) Bonferroni correction, α≤0.001 ***, 0.01 **, 0.05 *
[b], Schneider-Orelli's formula

Table KCP 10.3.2.2-9 **Reproduction of *Aphidius rhopalosiphi* (Bioassay 1 at 0 DAA, 2 at 7 DAA and 3 at 14 DAA)**

	T1 Control	**T2 PROTIOKONAZOL 300 EC at 650 mL test item/ha**	**T3 PROTIOKONAZOL 300 EC at 1300 mL test item/ha**
	Deionised water	**195 g a.i./ha**	**390 g a.i./ha**
Reproduction [mean mummies/female] (bioassay 1 - 0 DAA)	33.07	25.53	14.53
Significance a	-	n.s.	*
Effect on reproduction in the bioassay 1 at 0 DAA [%R]	-	22.78	56.05
Reproduction [mean mummies/female] (bioassay 2 - 7 DAA)	30.53	28.47	22.60
Significance b	-	n.s.	n.s.
Effect on reproduction in the bioassay 2 at 7 DAA [%R]	-	6.77	25.98
Reproduction [mean mummies/female] (bioassay 3 - 14 DAA)	31.87	28.73	24.53
Significance b	-	n.s.	n.s.
Effect on reproduction in the bioassay 3 at 14 DAA [%R]	-	9.83	23.01

-, not applicable
n.s., not significantly different compared to the control
[a], Williams t-test, $\alpha = 0.05$ *
[b], Dunnett's t-test, $\alpha = 0.05$ *

Table KCP 10.3.2.2-10 Endpoints for the 0-DAA, 7-DAA and 14-DAA bioassay

Endpoint	**mL test item/ha**	**g a.i./ha**
0-DAA Bioassay: fresh residues		
LR_{50}[#]	>1300	>390
NOER (Mortality)	≥1300	≥390
LOER (Mortality)	>1300	>390
ER_{10} (Reproduction)	430.0 (132.5 – U.L. n.d.*)	129 (39.75 – U.L. n.d.*)
ER_{20} (Reproduction)	603.8 (184.3 – U.L. n.d.*)	181.14 (55.29 – U.L. n.d.*)
ER_{50} (Reproduction)	1155.9 (244.7 – U.L. n.d.*)	346.77 (73.41 – U.L. n.d.*)
NOER (Reproduction)	650	195
LOER (Reproduction)	1300	390
7-DAA Bioassay: 7-day aged residues		
LR_{50}[#]	>1300	>390
NOER (Mortality)	≥1300	≥390
LOER (Mortality)	>1300	>390
ER_{10} (Reproduction)	772.6 (95%-CLs n.d.)	(95%-CLs n.d.)
ER_{20} (Reproduction)	1106.3 (95%-CLs n.d.)	(95%-CLs n.d.)
ER_{50} (Reproduction)	>1300 (95%-CLs n.d.)	>390 (95%-CLs n.d.)
NOER (Reproduction)	≥1300	≥390
LOER (Reproduction)	>1300	>390
14-DAA Bioassay: 14-day aged residues		

Endpoint	mL test item/ha	g a.i./ha
0-DAA Bioassay: fresh residues		
LR_{50}[#]	>1300	>390
NOER (Mortality)	≥1300	≥390
LOER (Mortality)	>1300	>390
ER_{10} (Reproduction)	657.9 (95%-CLs n.d.)	197.37 (95%-CLs n.d.)
ER_{20} (Reproduction)	1142.2 (95%-CLs n.d.)	342.66 (95%-CLs n.d.)
ER_{50} (Reproduction)	>1300 (95%-CLs n.d.)	>390 (95%-CLs n.d.)
NOER (Reproduction)	≥1300	≥390
LOER (Reproduction)	>1300	>390

#The LR50 could not be determined, because the number of responses was less than three. Values are estimated to be close to the highest test-item dosage. The LR50 values were estimated, therefore no confidence limits were provided
U.L. Upper Limit
n.d., not determined due to mathematical reasons
*, Confidence Limit over the 1300 mL f.p./ha dosage is a software estimation
95%-CLs, Confidence Limits

Comments of zRMS: The study was accepted by zRMS.
The validity criteria was met.
The following criteria should be satisfied in the control for a test result to be considered valid:
-mean mortality of larvae in the water treated control should not exceed 30%;
-mean mortality of larvae in the toxic reference treatment should be higher than 40%;
-no. 2 fertile eggs per viable female per day.
The agreed toxicity endpoints:
Mortality parameter:

Coccinella septempunctata **mortality (Bioassay 1 at 0 DAA, 2 at 7 DAA and 3 at 14 DAA)**

	T1 Control	T2 PROTIOKONAZOL 300 EC at 650 mL f.p./ha	T3 PROTIOKONAZOL 300 EC at 1300 mL f.p./ha	T4 ROGOR L 40 ST at 18 mL f.p./ha
	Deionized water	195 g a.i./ha	390 g a.i./ha	7.2 g a.i./ha
Mortality (bioassay 1 – 0 DAA) [mean %]	5.00	22.50	30.00	85.00
Significance [a]	-	*	*	***
Corrected mortality [b] (bioassay 1 – 0 DAA) [%]	-	18.42	26.32	84.21
Mortality (bioassay 2 – 7 DAA) [mean %]	10.00	17.50	20.00	77.50
Significance [a]	-	n.s.	n.s.	***
Corrected mortality [b] (bioassay 2 – 7 DAA) [%]	-	8.33	11.11	75.00
Mortality (bioassay 3 – 14 DAA) [mean %]	7.50	12.50	12.50	80.00
Significance [a]	-	n.s.	n.s.	***
Corrected mortality [b] (bioassay 3 – 14 DAA) [%]	-	5.41	5.41	78.38

-, not applicable
n.s., not significantly different compared to the control
[a], Fisher's Exact Binomial test, α≤0.001 ***, 0.01 **, 0.05 *
[b], Schneider-Orelli's formula

Parameter reproduction:

***Coccinella septempunctata* reproduction (Bioassay 1 at 0 DAA, 2 at 7 DAA and 3 at 14 DAA)**

	T1 Control	T2 PROTIOKONAZOL 300 EC at 650 mL f.p./ha	T3 PROTIOKONAZOL 300 EC at 1300 mL f.p./ha
	Deionized water	195 g a.i./ha	390 g a.i./ha
Reproduction (bioassay 1 – 0 DAA) [mean no. eggs/female/day]	33.85	31.50	29.64
% egg-hatching (bioassay 1 – 0 DAA)	76.11	77.58	72.86
Reproduction (bioassay 2 – 7 DAA) [mean no. eggs/female/day]	32.00	30.38	29.55
% egg-hatching (bioassay 2 – 7 DAA)	75.57	76.44	77.40
Reproduction (bioassay 3 – 14 DAA) [mean no. eggs/female/day]	33.00	30.32	31.23
% egg-hatching (bioassay 3 – 14 DAA)	74.45	82.07	82.27

-, not applicable

Reference:	KCP 10.3.2.1/03
Report	Effects of PROTIOKONAZOL 300 EC (prothioconazole 300 g/L) on *Coccinella septempunctat* – Extended laboratory aged residue test – 2022; Mautino G.; 2022; Study Code: 1018.F1.SAG22
Guideline(s):	Yes, SETAC; ESCORT; IOBC/BART/EPPO
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	PROTIOKONAZOL 300 EC
Formulation:	EC (prothioconazole 300 g/L)
Description (physical state):	liquid
Batch no.:	01/PRO/2022
Production date:	24 March 2022
Expiration date:	March 2025
Stability of test compound:	not relevant

2. Vehicle and/or positive control: vehicle: deionized water
positive control: ROGOR L 40 ST (nominally 400 g dime-

	thoate/L) 18 mL product/ha (7.2 g dimethoate/ha).
3. Test organism	
Species:	Coleoptera, Coccinellidae, *Coccinella septempunctata* L.
Source:	Katz Biotech AG, Baruth, Germany
Acclimation period:	2-3 days under test conditions
Stage at delivery:	eggs
Age at test start:	first instar larvae, 3-day old
Sex:	mix (females and males)
Diet:	During the mortality assessments larvae were fed ad libitum with aphids (Acyrthosiphon pisum Mordv.) of mixed stages. During the fecundity evaluation, ladybirds were fed daily with honey and 3-4 aphid infested (Acyrthosiphon pisum) broad bean stems. Plastic tubes filled with water and closed with a cotton wool were also provided for water supply. Water was provided permanently in a reservoir and replaced at least once a week.
Test units for mortality assessment:	Cylinder (45.8 mm Ø x 50 mm) of transparent plastic provided with PTFE (politetrafluoroetilene), a perforated lid (28 mm Ø) and with an insect proof net. The cylinder was inserted into a plate provided with a leaf disc of bean (50 mm Ø) leaning on two filter paper layers and blocked to it by rubbers, arranged in a cross design.
Test units for reproduction assessment:	Transparent plastic boxes (145 × 135 × 85 mm) with a perforated lid provided with an insect proof net for aeration. At the box bottom one layer of filter paper, while inside, three pieces of bubble wrap (PE) and a dark plastic cylinder, as oviposition substrate. Egg clutches were stored in individually labelled plastic containers (60 mL in volume) until larval hatch, over a wet layer of filter paper.
Plant:	Taxonomic group: Fabaceae, Species: Phaseolus vulgaris L., Variety: Bingo, , Source: Agricola Albese (Alba, CN), Sowing date: 01 Sep 2022, Emergence date: 04 Sep 2022, Cultivation substrate: natural soil, Grown site: open field under a rain cover, Stage for test start: BBCH 18-19, Maintenance: automatic drip irrigation, three irrigation events per day, Agrochemical and fertilizer: none
4. Environmental conditions:	
Temperature:	24.66 ± 0.53 °C (25.50 – 23.59 °C)
Relative humidity:	68.4 ± 6.2% (79.9 – 60.0%) RH
Photoperiod:	16 h light:8 h dark
Light Intensity:	1800 - 2200

STUDY DESIGN AND METHOD

The aim of the study was to determine the product persistence, intended as the rate of decline of residues (fresh and aged) on bean leaves of plants growing under rain-protected field conditions treated with test item PROTIOKONAZOL 300 EC (prothioconazole 300 g/L). Residual toxicity was evaluated observing the effects on *Coccinella septempunctata* mortality over time and to evaluate the sub-lethal effects on insect fecundity subsequent to their exposure to the test item at different timings (bioassay) after application (DAA).

Three bioassays were investigated: bioassay 1, where insects were introduced immediately after the application on dried discs (0 DAA); bioassay 2, where insects were introduced 7 days after the application (7 DAA), and bioassay 3, where insects were introduced 14 days after the application (14 DAA).

Bean plants were treated with PROTIOKONAZOL 300 EC applied once at 1300 and 650 mL f.p./ha (equivalent to 390 and 195 g a.i./ha, respectively); There were 40 replicates for each treatment. Deionised water was used as diluent for the test item solutions. PROTIOKONAZOL 300 EC was compared to a control group with deionised water only and reference item ROGOR L 40 ST applied once at 18 mL product/ha (7.2 g dimethoate/ha).

Application was performed in a spray chamber, with a spraying surface of 2 m2 (length: 2 m, width: 1 m). After the application, spray residues were left to dry 30-40 minutes before the insects' introduction in the test units. Then, treated leaves were cut out from the bean plants of the control and treated groups and immediately transferred to the laboratory where leaf discs were obtained with a leaf puncher (50-mm Ø).

Insects were exposed to fresh and aged residue of the test item at different timings (bioassay) after application (DAA).

The larvae were exposed to fresh and aged residue on bean leaf discs at three different times: 0, 7, and 14 days after application (DAA). At the end of this period, the observations consisted in recording percent mortality; when the survived pupae were hatch in the control, females and males (adults) were sexed, assessed for their reproductive performance and transferred to the mass-rearing units. The reproduction test started one week after the first egg laying observation. Insects' oviposition was checked daily for up to 15 days. The eggs-hatching was assessed. Alive beetles were recorded.

To verify the sensitivity of the test system and the precision of the test procedure, an insecticide, i.e., ROGOR L 40 ST (nominally 400 g dimethoate/L) was used as a reference item. The control group was treated with distilled water.

Test design:	tested concentrations, reference and control in 40 replications, 1 larva/replicate
Introduction of Individuals:	Immediately after the test item drying for bioassay 1 (0 DAA). At 7 and 14 days after the application (DAA) for bioassay 2 and bioassay 3, respectively.
Introduction Procedure	With a fine brush, selection of the individuals was impartially performed, following the spray scheme.
Mortality exposure time:	until hatching of adult ladybugs
Fecundity exposure time:	15 days (starting one week after the first egg-laying)

Tested concentrations, definitive test:	650 and 1300 ml/ha (dilution ratio: 2; volume of application was 400 L/ha) fresh residues – 0 DAA aged residues – 7 DAA aged residues – 14 DAA
Dates:	start of the study: 06.09.2022 start of the experimental part: 21.09.2022 end of the experimental part: 21.11.2022 end of the study: 17.01.2023
Statistic:	Software used for statistical analysis were "ToxRatPro" Solutions GmbH, version 3.3.0 and "RStudio", version 3.0.2. Mortality data were processed using the Fisher's exact test, $\alpha \leq 0.05$ and the LR50 value was determined. Correction for control mortality was processed using the Schneider-Orelli formula. The No Observed Effect Rate (NOER) and Lowest Observed Effect Rate (LOER) values for mortality and reproduction were determined, where possible.
Validity criteria:	The following criteria should be satisfied in the control for a test result to be considered valid: -mean mortality of larvae in the water treated control should not exceed 30%; -mean mortality of larvae in the toxic reference treatment should be higher than 40%; -no. 2 fertile eggs per viable female per day.

RESULTS

Mortality assessment - Bioassay 1 (0 DAA)

Given that only two test-item treatment rates were tested, the LR50 value was estimated to be >1300 mL test item/ha (390 g a.s./ha), while the corresponding NOER and LOER values for mortality were determined to be <650 and 650 mL test item/ha (195 g a.s./ha), respectively.

Mortality assessment - Bioassay 2 (7 DAA)

Given that only two test-item treatment rates were tested, the LR_{50} value was estimated to be >1300 mL test item/ha (390 g a.s./ha), while the corresponding NOER and LOER values for mortality were determined to be ≥ 1300 test item/ha (390 g a.s./ha).

Mortality assessment - Bioassay 3 (14 DAA)

Given that only two test-item treatment rates were tested, the LR_{50} value was estimated to be >1300 mL test item/ha (390 g a.s./ha), while the corresponding NOER and LOER values for mortality were determined to be ≥ 1300 test item/ha (390 g a.s./ha).

Table KCP 10.3.2.2-11 Average percentage of *Coccinella septempunctata* mortality (Bioassay 1 – 0 DAA)

	Treatment	Rate (g a.i./ha)	Mortality (%) ±SE[a]	*P*[b]	Corrected mortality[c] (%)
Mortality assessment - Bioassay 1 (0 DAA)					

T1	Control	-	5.00	± 0.03	-	-
T2	PROTIOKONAZOL 300 EC at 650 mL f.p./ha	195	22.50	± 0.07	*	18.42
T3	PROTIOKONAZOL 300 EC at 1300 mL f.p./ha	390	30.00	±0.07	*	26.32
T4	ROGOR L 40 ST at 18 mL f.p./ha	7.2	85.00	±0.06	***	84.21
Mortality assessment - Bioassay 2 (7 DAA)						
T1	Control	-	10.00	± 0.05	-	-
T2	PROTIOKONAZOL 300 EC at 650 mL f.p./ha	195	17.50	± 0.06	n.s	8.33
T3	PROTIOKONAZOL 300 EC at 1300 mL f.p./ha	390	20.00	±0.06	n.s	11.11
T4	ROGOR L 40 ST at 18 mL f.p./ha	7.2	77.50	±0.07	***	75.00
Mortality assessment - Bioassay 3 (14 DAA)						
T1	Control	-	7.50	±0.04	-	-
T2	PROTIOKONAZOL 300 EC at 650 mL f.p./ha	195	12.50	± 0.05	n.s	5.41
T3	PROTIOKONAZOL 300 EC at 1300 mL f.p./ha	390	12.50	±0.05	n.s	5.41
T4	ROGOR L 40 ST at 18 mL f.p./ha	7.2	80.00	±0.06	***	78.38

a, standard error from 40 replicates
b, Fisher's Exact Binomial test, α≤0.001 ***, 0.01 **, 0.05 *
c, mean mortality corrected by Schneider-Orelli's formula
-, not applicable
n.s., not significantly different compared to the control

The mean number of eggs laid per female per day and the egg hatching rate were assessed.

Table KCP 10.3.2.2-12 Mean number of eggs per female per day

Treatment name	Treatment	Mean number of females	Mean no. of eggs	Mean no. of hatched eggs	Mean number of eggs per female per day (RrX)	SE[a]	Egg-hatching (%)
Fecundity assessment - Bioassay 1 (0 DAA)							
T1	Control	4.98	169.05	128.61	33.85	±0.87	76.11[b]
T2	PROTIOKONAZOL 300 EC at 650 mL f.p./ha	4.11	130.18	100.59	31.50	±0.66	77.58[b]
T3	PROTIOKONAZOL 300 EC at 1300 mL f.p./ha	3.11	92.52	66.05	29.64	±2.47	72.86[b]
Fecundity assessment - Bioassay 2 (7 DAA)							
T1	Control	3.98	127.80	97.05	32.00	±0.60	75.57[b]
T2	PROTIOKONAZOL 300 EC at 650 mL f.p./ha	4.05	121.39	91.95	30.38	±1.95	76.44[b]
T3	PROTIOKONAZOL 300 EC at 1300 mL f.p./ha	3.48	101.41	78.57	29.55	±1.42	77.40[b]
Fecundity assessment - Bioassay 3 (14 DAA)							

T1	Control	4.48	148.43	110.55	33.00	±0.93	74.45[b]
T2	PROTIOKONAZOL 300 EC at 650 mL f.p./ha	4.02	119.55	98.07	30.32	±1.47	82.07[b]
T3	PROTIOKONAZOL 300 EC at 1300 mL f.p./ha	3.66	109.73	90.45	31.23	±1.24	82.27[b]

[a], standard error (SE) from the number of breeding boxes.
[b], mean values from four breeding boxes.

CONCLUSION

All study validity criteria were met at each bioassay.

For all bioassays, the LR_{50} values could not be determined, because data were not appropriate for the computation, the number of responses was less than three.

Bioassay 1 (0 DAA)

For the 0-DAA bioassay, the mortality percentage for the test item was 22.50% in T2 (PROTIOKONAZOL 300 EC at 650 mL f.p./ha, corrected mortality: 18.42%) and 30.00% in T3 (PROTIOKONAZOL 300 EC at 1300 mL f.p./ha, corrected mortality: 26.32%). Significant differences were noticed between the treatment T2, T3 and the control, showing a 5.00% of mortality. The reference item ROGOR L 40 ST was significantly different with a mortality value of 85.00% (corrected mortality: 84.21%).

The LR_{50} value was estimated to be >1300 mL test item/ha (390 g a.s./ha), while the corresponding NOER and LOER values for mortality were determined to be <650 and 650 mL test item/ha (195 g a.s./ha), respectively.

For reproduction, the mean number of eggs per female per day was 31.50 in T2 (PROTIOKONAZOL 300 EC at 650 mL f.p./ha) and 29.64 in T3 (PROTIOKONAZOL 300 EC at 1300 mL f.p./ha). The control, showing a mean number of eggs per female per day of 33.85.

The hatching rate was 77.58% in treatment T2 (PROTIOKONAZOL 300 EC at 650 mL f.p./ha) and 72.86% in treatment T3 (PROTIOKONAZOL 300 EC at 1300 mL f.p./ha), while in the control it was equal to 76.11%.

Bioassay 2 (7 DAA)

For the 7-DAA bioassay, the mortality percentage for the test item was 17.50% in T2 (PROTIOKONAZOL 300 EC at 650 mL f.p./ha, corrected mortality: 8.33%) and 20.00% in T3 (PROTIOKONAZOL 300 EC at 1300 mL f.p./ha, corrected mortality: 11.11%). No significant differences were noticed between the treatments and the control, showing a 10.00% of mortality. The reference item ROGOR L 40 ST was significantly different with a mortality value of 77.50% (corrected mortality: 75.00%).

The LR_{50} value was estimated to be >1300 mL test item/ha (390 g a.s./ha), while the corresponding NOER and LOER values for mortality were determined to be ≥ 1300 test item/ha (390 g a.s./ha).

For reproduction, the mean number of eggs per female per day was 30.38 in T2 (PROTIOKONAZOL 300 EC at 650 mL f.p./ha) and 29.55 in T3 (PROTIOKONAZOL 300 EC at 1300 mL f.p./ha). The control, showing a mean number of eggs per female per day of 32.00.

The hatching rate was 76.44% in treatment T2 (PROTIOKONAZOL 300 EC at 650 mL f.p./ha) and 77.40% in treatment T3 (PROTIOKONAZOL 300 EC at 1300 mL f.p./ha), while in the control it was equal to 75.57%.

Bioassay 3 (14 DAA)

For the 14-DAA bioassay, the mortality percentage for the test item was 12.50% in T2 (PROTIOKONAZOL 300 EC at 650 mL f.p./ha, corrected mortality: 5.41%) and 12.50% in T3 (PROTIOKONAZOL 300 EC at 1300 mL f.p./ha, corrected mortality: 5.41%). No significant differences were noticed between the treatments and the control, showing a 7.50% of mortality. The reference item ROGOR L 40 ST was significantly different with a mortality value of 80.00% (corrected mortality: 78.38%).

The LR_{50} value was estimated to be >1300 mL test item/ha (390 g a.s./ha), while the corresponding NOER and LOER values for mortality were determined to be ≥ 1300 test item/ha (390 g a.s./ha).

For reproduction, the mean number of eggs per female per day was 30.32 in T2 (PROTIOKONAZOL 300 EC at 650 mL f.p./ha) and 31.23 in T3 (PROTIOKONAZOL 300 EC at 1300 mL f.p./ha). The control, showing a mean number of eggs per female per day of 33.00.

The hatching rate was 82.07% in treatment T2 (PROTIOKONAZOL 300 EC at 650 mL f.p./ha) and 82.27% in treatment T3 (PROTIOKONAZOL 300 EC at 1300 mL f.p./ha), while in the control it was equal to 74.45%.

Table KCP 10.3.2.2-13 **Mortality of *Coccinella septempunctata* (Bioassay 1 at 0 DAA, 2 at 7 DAA and 3 at 14 DAA)**

	T1 Control	**T2 PROTIOKONAZOL 300 EC at 650 mL test item/ha**	**T3 PROTIOKONAZOL 300 EC at 1300 mL test item/ha**	**T4 ROGOR L40 ST at 18 mL test item/ha**
	Deionised water	**195 g a.i./ha**	**390 g a.i./ha**	**7.2 g a.i./ha**
Mortality (bioassay 1 – 0 DAA) [mean %]	5.00	22.50	30.00	85.00
Significance [a]	-	*	*	***
Corrected mortality [b] (bioassay 1 – 0 DAA) [%]	-	18.42	26.32	84.21
Mortality (bioassay 2 – 7 DAA) [mean %]	10.00	17.50	20.00	77.50
Significance [a]	-	n.s.	n.s.	***
Corrected mortality [b] (bioassay 2 – 7 DAA) [%]	-	8.33	11.11	75.00
Mortality (bioassay 3 – 14 DAA) [mean %]	7.50	12.50	12.50	80.00
Significance [a]	-	n.s.	n.s.	***
Corrected mortality [b] (bioassay 3 – 14 DAA) [%]	-	5.41	5.41	78.38

-, not applicable
n.s., not significantly different compared to the control
[a], Fisher exact test with or without (reference item) Bonferroni correction, α≤0.001 ***, 0.01 **, 0.05 *
[b], Schneider-Orelli's formula

Table KCP 10.3.2.2-14 **Reproduction of *Coccinella septempunctata* (Bioassay 1 at 0 DAA, 2 at 7 DAA and 3 at 14 DAA)**

	T1 Control	**T2 PROTIOKONAZOL 300 EC at 650 mL test item/ha**	**T3 PROTIOKONAZOL 300 EC at 1300 mL test item/ha**
	Deionised water	**195 g a.i./ha**	**390 g a.i./ha**
Reproduction (bioassay 1 – 0 DAA) [mean no. eggs/female/day]	33.85	31.50	29.64
% egg-hatching (bioassay 1 – 0 DAA)	76.11	77.58	72.86
Reproduction (bioassay 2 – 7 DAA) [mean no. eggs/female/day]	32.00	30.38	29.55
% egg-hatching (bioassay 2 – 7 DAA)	75.57	76.44	77.40
Reproduction (bioassay 3 – 14 DAA) [mean no. eggs/female/day]	33.00	30.32	31.23
% egg-hatching (bioassay 3 – 14	74.45	82.07	82.27

DAA)			

-, not applicable

Table KCP 10.3.2.2-15 Endpoints for the 0-DAA, 7-DAA and 14-DAA bioassay

Endpoint	mL test item/ha	g a.i./ha[#]
0-DAA Bioassay: fresh residues		
LR_{50}	>1300	>390
NOER (Mortality)	<650	<195
LOER (Mortality)	650	195
7-DAA Bioassay: 7-day aged residues		
LR_{50}	>1300	>390
NOER (Mortality)	≥1300	≥390
LOER (Mortality)	≥1300	≥390
14-DAA Bioassay: 14-day aged residues		
LR_{50}	>1300	>390
NOER (Mortality)	≥1300	≥390
LOER (Mortality)	≥1300	≥390

*The LR50 could not be determined, but is estimated to be close to the highest test-item treatment group of 1300 mL test item/ha (400 g a.s./ha)
Based on a nominal active substance (prothioconazole) content of 300 g/L with the following formula: (mL test item/ha / 1000) × 300 g/L
Note: the values were estimated, therefore no confidence limits were provided

Comments of zRMS: The study was accepted by zRMS.
The validity criteria was met.

Validity criteria of the study

Mortality in control check	Bioassay 1 at 0 DAA	Mortality was 6.67%, therefore, the validity criterion was met.
	Bioassay 2 at 7 DAA	Mortality was 13.33%, therefore, the validity criterion was met.
	Bioassay 3 at 14 DAA	Mortality was 10.00%, therefore, the validity criterion was met.
Reproduction in control check	Bioassay 1 at 0 DAA	The mean number of eggs per female per day was 58.62, so this validity criterion was met.
	Bioassay 2 at 7 DAA	The mean number of eggs per female per day was 60.01, so this validity criterion was met.
	Bioassay 3 at 14 DAA	The mean number of eggs per female per day was 67.45, so this validity criterion was met.
Hatching rate in control check	Bioassay 1 at 0 DAA	The hatching rate was 84.34% and so the validity criterion was met.
	Bioassay 2 at 7 DAA	The hatching rate was 85.94% and so the validity criterion was met.
	Bioassay 3 at 14 DAA	The hatching rate was 79.64% and so the validity criterion was met.
Mortality in reference	Bioassay 1 at 0 DAA	Mortality was 82.14% in the toxic reference treatment and so the validity criterion was met.
	Bioassay 2 at 7 DAA	Mortality was 73.08% in the toxic reference treatment and so the validity criterion was met.
	Bioassay 3 at 14 DAA	Mortality was 74.08% in the toxic reference treatment and so the validity criterion was met.

The agreed toxicity endpoints:

Endpoints for the 0 DAA, 7 DAA and 14 DAA bioassay.

Endpoints	mL test item/ha	g a.i./ha#
0-DAA Bioassay: fresh residues		
LR_{50}*	>1300 (95%-CLs n.d.)	>390 (95%-CLs n.d.)
NOER (Mortality)	≥1300	≥390
LOER (Mortality)	≥1300	≥390
7-DAA Bioassay: 7-day aged residues		
LR_{50}*	>1300 (95%-CLs n.d.)	>390 (95%-CLs n.d.)
NOER (Mortality)	≥1300	≥390
LOER (Mortality)	≥1300	≥390
14-DAA Bioassay: 14-day aged residues		
LR_{50}*	>1300 (95%-CLs n.d.)	>390 (95%-CLs n.d.)
NOER (Mortality)	≥1300	≥390
LOER (Mortality)	≥1300	≥390

*The LR_{50} could not be determined, because the number of responses was less than three. Values are estimated to be close to the highest test-item dosage. The LR_{50} values were estimated, therefore no confidence limits were provided

Based on a nominal prothioconazole content of 300 g/L

95%-CLs n.d., Confidence Limits not determined due to mathematical reasons

Reference:	KCP 10.3.2.1/04
Report	Effects of PROTIOKONAZOL 300 EC (prothioconazole 300 g/L) on *Chrysoperla carnea* – Extended laboratory aged residue test – 2022; Mautino G.; 2022; Study Code: 1021.F1.SAG22
Guideline(s):	Yes, SETAC; ESCORT; IOBC/BART/EPPO
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No
Validity criteria:	All validity criteria were met: -mortality in the water control ≤ 20% (dead larvae, pupae and adults during emergence); -mean number of eggs per female per day in the water control ≥ 15; -mean hatching rate ≥ 70; -mortality in the reference group ≥ 50 %.

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	PROTIOKONAZOL 300 EC
Formulation:	EC (prothioconazole 300 g/L)
Description (physical state):	liquid
Batch no.:	01/PRO/2022
Production date:	24 March 2022
Expiration date:	March 2025

Stability of test compound:	not relevant
2. Vehicle and/or positive control:	vehicle: deionized water positive control: ROGOR L 40 ST (nominally 400 g dimethoate/L) 18 mL product/ha (7.2 g dimethoate/ha).
3. Test organism	
Species:	Neuroptera, Chrysopidae *Chrysoperla carnea* Stephens
Source:	Katz Biotech AG, Baruth, Germany
Acclimation period:	2 days under test conditions (same feeding)
Stage at delivery:	eggs
Age at test start:	first instar larvae
Sex:	mix (females and males)
Diet:	During mortality evaluation, Sitotroga sp.'s eggs were put into the test units the same day of test units setting after the application and then, replaced 3 times a week. During the fecundity evaluation, the adult diet consisted of mixture of 15 mL condensed milk, 1 egg, 1 egg yolk, 30 g honey, 20 g fructose, 30 g dried brewer's yeast, 50 g wheatgerm and 45 mL drinking water. This food was available continuously and replaced at least twice, preferably three times per week. Water was provided permanently in a reservoir and replaced at least once a week.
Test units for mortality assessment:	Cylinder (45.8 mm Ø x 50 mm) of transparent plastic provided with PTFE (politetrafluoroetilene), a perforated lid (28 mm Ø) and with an insect proof net. The cylinder was inserted into a plate (57 mm Ø) provided with a leaf disc of bean (50 mm Ø) leaning on two filter paper layers and blocked to it by rubbers, arranged in a cross design.
Test units for reproduction assessment:	Glass cylinder (5000 cc, 16 cm Ø, 29 cm) provided with an insect proof net, blocked to it by rubbers.
Egg-hatching assessment	Containers of transparent plastic (18 x 13 x 4 cm) provided with a perforated lid with an insect proof net.
Plant:	Taxonomic group: Vitaceae, Common name: Common grapevine, Species: Vitis vinifera L, Variety: Moscato, Cultivation substrate: artificial soil, Grown site: Potted plants in field conditions under a cover for rain protection, Maintenance: automatic drip irrigation, three irrigation events per day, none for agrochemical and fertilized, Transplant date: 16 May 2022
4. Environmental conditions:	
Temperature:	25.77 ± 0.92°C (26.92 – 23.04°C)
Relative humidity:	62.9 ± 1.7% (67.0 – 62.2%)
Photoperiod:	16 h light:8 h dark

Light Intensity:	1900-2500 lux

STUDY DESIGN AND METHOD

The aim of the study was to determine the product persistence, intended as the rate of decline of residues (fresh and aged) on grapevine leaves of plants growing under rain-protected field conditions treated with test item PROTIOKONAZOL 300 EC (prothioconazole 300 g/L). Residual toxicity was evaluated observing the effects on Chrysoperla carnea mortality over time and to evaluate the sub-lethal effects on insect fecundity subsequent to their exposure to the test item at different timings (bioassay) after application (DAA).

Grapevine plants were treated with PROTIOKONAZOL 300 EC applied once at 1300 and 650 mL f.p./ha (equivalent to 390 and 195 g a.i./ha, respectively); There were 30 replicates for each treatment. Deionised water was used as diluent for the test item solutions. PROTIOKONAZOL 300 EC was compared to a control group with deionised water only and reference item ROGOR L 40 ST applied once at 18 mL product/ha (7.2 g dimethoate/ha). Application was performed in a spray chamber, with a spraying surface of 2 m2 (length: 2 m, width: 1 m). After the application, spray residues were left to dry 30-40 minutes before the insects' introduction in the test units. Then, treated leaves were cut out from the grapevine plants of the control and treated groups and immediately transferred to the laboratory where leaf discs were obtained with a leaf puncher (50-mm Ø).

Insects were exposed to fresh and aged residue of the test item at different timings (bioassay) after application (DAA).

Three bioassays were investigated: bioassay 1, where insects were introduced immediately after the application on dried discs (0 DAA); bioassay 2, where insects were introduced 7 days after the application (7 DAA), and bioassay 3, where insects were introduced 14 days after the application (14 DAA). The effects of the test item were compared with a control group and a reference item.

The experimental procedures were designed by following the “Laboratory method to test effects of plant protection products on larvae of *Chrysoperla carnea* (Neuroptera, Chrysopidae)” by Vogt et al. (2000).

To verify the sensitivity of the test system and the precision of the test procedure, an insecticide, i.e., ROGOR L 40 ST (nominally 400 g dimethoate/L) was used as a reference item. The control group was treated with distilled water.

Test design:	tested concentrations, reference and control in 30 replications, 1 larvae/replicate
Introduction of Individuals:	Immediately after the test item drying for bioassay 1 (0 DAA). At 7 and 14 days after the application (DAA) for bioassay 2 and bioassay 3, respectively.
Introduction Procedure:	With a fine brush, selection of the individuals was impartially performed, following the spray scheme.
Mortality exposure time:	until hatching of adult lacewings
Fecundity exposure time:	until egg-hatching

Tested concentrations, definitive test:	650 and 1300 ml/ha (dilution ratio: 2; volume of application was 400 L/ha) fresh residues – 0 DAA aged residues – 7 DAA aged residues – 14 DAA
Dates:	start of the study: 29.07.2022 start of the experimental part: 03.08.2022 end of the experimental part: 22.09.2022 end of the study: 22.12.2022
Statistic:	Software used for statistical analysis were "ToxRatPro" Solutions GmbH, version 3.3.0 and "RStudio", version 3.0.2. Mortality data were processed using the Fisher's exact test, $\alpha \leq 0.05$ and the LR50 value was determined. Correction for control mortality was processed using the Schneider-Orelli formula. The No Observed Effect Rate (NOER) and Lowest Observed Effect Rate (LOER) values for mortality and reproduction were determined, where possible.
Validity criteria:	The following criteria should be satisfied in the control for a test result to be considered valid: -mortality in the water control ≤ 20% (dead larvae, pupae and adults during emergence); -mean number of eggs per female per day in the water control ≥ 15; -mean hatching rate ≥ 70; -mortality in the reference group ≥ 50 %.

RESULTS

Mortality assessment - Bioassay 1 (0 DAA)

The mortality percentage for the test item was 13.33% in T2 (PROTIOKONAZOL 300 EC 650 mL f.p./ha, corrected mortality: 7.14%) and 13.33% in T3 (PROTIOKONAZOL 300 EC at 1300 mL f.p./ha, corrected mortality: 7.14%). No significant differences were noticed between the treatments and the control, showing a 6.67% of mortality.

The reference item ROGOR L 40 ST was significantly different with a mortality value of 83.33% (corrected mortality: 82.14%).

Given that only two test-item treatment rates were tested, the LR_{50} value was estimated to be >1300 mL test item/ha (390 g a.s./ha), while the corresponding NOER and LOER values for mortality were determined to be ≥ 1300 test item/ha (390 g a.s./ha).

Mortality assessment - Bioassay 2 (7 DAA)

The mortality percentage for the test item was 10.00% in T2 (PROTIOKONAZOL 300 EC at 650 mL f.p./ha, corrected mortality: -3.84%) and 16.67% in T3 (PROTIOKONAZOL 300 EC at 1300 mL f.p./ha, corrected mortality: 3.85%). No significant differences were noticed between the treatments and the control, showing a 13.33% of mortality.

The reference item ROGOR L 40 ST was significantly different with a mortality value of 76.67% (corrected mortality: 73.08%).

Given that only two test-item treatment rates were tested, the LR_{50} value was estimated to be >1300 mL

test item/ha (390 g a.s./ha), while the corresponding NOER and LOER values for mortality were determined to be ≥ 1300 test item/ha (390 g a.s./ha).

Mortality assessment - Bioassay 3 (14 DAA)

The mortality percentage for the test item was 10.00% in T2 (PROTIOKONAZOL 300 EC at 650 mL f.p./ha, corrected mortality: 0.00%) and 16.67% in T3 (PROTIOKONAZOL 300 EC at 1300 mL f.p./ha, corrected mortality: 7.41%). No significant differences were noticed between the treatments and the control, showing a 10.00% of mortality.

The reference item ROGOR L 40 ST was significantly different with a mortality value of 76.67% (corrected mortality: 74.07%).

Given that only two test-item treatment rates were tested, the LR_{50} value was estimated to be >1300 mL test item/ha (390 g a.s./ha), while the corresponding NOER and LOER values for mortality were determined to be ≥ 1300 test item/ha (390 g a.s./ha).

Table KCP 10.3.2.2-16 Average percentage of *Chrysoperla carnea* mortality (Bioassay 1 – 0DAA)

	Treatment	Rate (g a.i./ha)	Mortality (%) ±SE[b]		*p*[a]	Corrected mortality[c] (%)
Mortality assessment - Bioassay 1 (0 DAA)						
T1	Control	-	6.67	± 4.63	-	-
T2	PROTIOKONAZOL 300 EC at 650 mL f.p./ha	195	13.33	± 6.31	n.s	7.14
T3	PROTIOKONAZOL 300 EC at 1300 mL f.p./ha	390	13.33	±6.31	n.s	7.14
T4	ROGOR L 40 ST at 18 mL f.p./ha	7.2	83.33	±6.92	***	82.14
Mortality assessment - Bioassay 2 (7 DAA)						
T1	Control	-	13.33	± 6.31	-	-
T2	PROTIOKONAZOL 300 EC at 650 mL f.p./ha	195	10.00	± 5.57	n.s	-3.84
T3	PROTIOKONAZOL 300 EC at 1300 mL f.p./ha	390	16.67	±6.92	n.s	3.85
T4	ROGOR L 40 ST at 18 mL f.p./ha	7.2	76.67	±7.85	***	73.08
Mortality assessment - Bioassay 3 (14 DAA)						
T1	Control	-	10.00	± 5.57	-	-
T2	PROTIOKONAZOL 300 EC at 650 mL f.p./ha	195	10.00	± 5.57	n.s	0.00
T3	PROTIOKONAZOL 300 EC at 1300 mL f.p./ha	390	16.67	±6.92	n.s	7.41
T4	ROGOR L 40 ST at 18 mL f.p./ha	7.2	76.67	±7.85	***	74.08

-, not applicable
n.s., not significantly different compared to the control
[a], Fisher's Exact Binomial test, α≤0.001 ***, 0.01 **, 0.05 *
[b], standard error from 30 replicates
[c], mean mortality corrected by Schneider-Orelli's formula

One week after the first egg laying had been observed, the mean number of eggs laid per female per day and the hatching rate of eggs was assessed.

Fecundity assessment - Bioassay 1 (0 DAA)

The mean number of eggs per female per day was 59.90 in T2 (PROTIOKONAZOL 300 EC at 650 mL f.p./ha) and 55.16 in T3 (PROTIOKONAZOL 300 EC at 1300 mL f.p./ha). The control, showing a mean number of eggs per female per day of 58.62.

The hatching rate was 89.93% in treatment T2 (PROTIOKONAZOL 300 EC at 650 mL f.p./ha) and 73.17% in treatment T3 (PROTIOKONAZOL 300 EC at 1300 mL f.p./ha), while in the control it was equal to 84.34%.

Fecundity assessment - Bioassay 2 (7 DAA)

The mean number of eggs per female per day was 55.82 in T2 (PROTIOKONAZOL 300 EC at 650 mL f.p./ha) and 56.20 in T3 (PROTIOKONAZOL 300 EC at 1300 mL f.p./ha). The control, showing a mean number of eggs per female per day of 60.01.

The hatching rate was 85.01% in treatment T2 (PROTIOKONAZOL 300 EC at 650 mL f.p./ha) and 85.12% in treatment T3 (PROTIOKONAZOL 300 EC at 1300 mL f.p./ha), while in the control it was equal to 85.94%.

Fecundity assessment - Bioassay 3 (14 DAA)

The mean number of eggs per female per day was 64.27 in T2 (PROTIOKONAZOL 300 EC at 650 mL f.p./ha) and 61.41 in T3 (PROTIOKONAZOL 300 EC at 1300 mL f.p./ha). The control, showing a mean number of eggs per female per day of 67.45.

The hatching rate was 78.66% in treatment T2 (PROTIOKONAZOL 300 EC at 650 mL f.p./ha) and 79.24% in treatment T3 (PROTIOKONAZOL 300 EC at 1300 mL f.p./ha), while in the control it was equal to 79.64%.

Table KCP 10.3.2.2-17 Mean number of eggs per female per day

Treatment number	Treatment	Replicate	First oviposition		Second oviposition		Mean number of eggs per female per day	SE
			Female	Eggs	Femals	Eggs		
Fecundity assessment - Bioassay 1 (0 DAA)								
T1	Control	I	6	188	6	208	58.62	±7.38
		II	7	171	6	162		
T2	PROTIOKONAZOL 300 EC at 650 mL f.p./ha	I	5	154	5	150	59.90	±0.90
		II	5	186	5	109		
T3	PROTIOKONAZOL 300 EC at 650 mL f.p./ha	I	6	146	6	181	55.16	±0.66
		II	6	138	5	169		
Fecundity assessment - Bioassay 2 (7 DAA)								
T1	Control	I	7	149	6	195	60.01	±7.08
		II	6	186	6	183		
T2	PROTIOKONAZOL 300 EC at 650 mL f.p./ha	I	6	191	5	154	55.82	±6.91
		II	6	100	5	169		
T3	PROTIOKONAZOL 300 EC at 650 mL f.p./ha	I	6	154	6	134	56.20	±8.20
		II	5	150	5	172		
Fecundity assessment - Bioassay 3 (14 DAA)								
T1	Control	I	6	227	5	120	67.45	±4.36
		II	6	209	5	186		
T2	PROTIOKONAZOL 300	I	6	197	6	200	64.27	±1.90

	EC at 650 mL f.p./ha	II	6	180	5	163		
T3	PROTIOKONAZOL 300 EC at 650 mL f.p./ha	I	5	137	5	258	61.41	±10.41
		II	6	131	5	124		

Table KCP 10.3.2.2-18 Average percentage of egg hatching

Treatment number	Treatment	Replicate	First oviposition		Second oviposition		% egg-hatching
			Eggs	Larvae	Eggs	Larvae	
Fecundity assessment - Bioassay 1 (0 DAA)							
T1	Control	I	188	185	208	169	84.34
		II	171	167	162	97	
T2	PROTIOKONAZOL 300 EC at 650 mL f.p./ha	I	154	154	150	97	89.93
		II	186	183	109	104	
T3	PROTIOKONAZOL 300 EC at 650 mL f.p./ha	I	146	122	181	135	73.17
		II	138	100	169	108	
Fecundity assessment - Bioassay 2 (7 DAA)							
T1	Control	I	149	134	195	172	85.94
		II	186	165	183	141	
T2	PROTIOKONAZOL 300 EC at 650 mL f.p./ha	I	191	185	154	113	85.01
		II	100	90	169	135	
T3	PROTIOKONAZOL 300 EC at 650 mL f.p./ha	I	154	132	134	132	85.12
		II	150	113	172	140	
Fecundity assessment - Bioassay 3 (14 DAA)							
T1	Control	I	227	200	120	105	79.64
		II	209	174	186	108	
T2	PROTIOKONAZOL 300 EC at 650 mL f.p./ha	I	197	193	200	148	78.66
		II	180	157	163	88	
T3	PROTIOKONAZOL 300 EC at 650 mL f.p./ha	I	137	82	258	197	79.24
		II	131	80	124	144	

CONCLUSION

All study validity criteria were met at each bioassay. For all bioassays, the LR_{50} values could not be determined, because data were not appropriate for the computation, the number of responses was less than three.

Bioassay 1 (0 DAA)

For the 0-DAA bioassay, the mortality percentage for the test item was 13.33% in T2 (PROTIOKONAZOL 300 EC 650 mL f.p./ha, corrected mortality: 7.14%) and 13.33% in T3 (PROTIOKONAZOL 300 EC at 1300 mL f.p./ha, corrected mortality: 7.14%). No significant differences were noticed between the treatments and the control, showing a 6.67% of mortality. The reference item ROGOR L 40 ST was significantly different with a mortality value of 83.33% (corrected mortality: 82.14%).

The LR_{50} value was estimated to be >1300 mL test item/ha (390 g a.s./ha), while the corresponding NOER and LOER values for mortality were determined to be ≥ 1300 test item/ha (390 g a.s./ha).

For reproduction, the mean number of eggs per female per day was 59.90 in T2 (PROTIOKONAZOL 300 EC at 650 mL f.p./ha) and 55.16 in T3 (PROTIOKONAZOL 300 EC at 1300 mL f.p./ha). The control, showing a mean number of eggs per female per day of 58.62.

The hatching rate was 89.93% in treatment T2 (PROTIOKONAZOL 300 EC at 650 mL f.p./ha) and 73.17% in treatment T3 (PROTIOKONAZOL 300 EC at 1300 mL f.p./ha), while in the control it was equal to 84.34%.

Bioassay 2 (7 DAA)

For the 7-DAA bioassay, the mortality percentage for the test item was 10.00% in T2 (PROTIOKONAZOL 300 EC at 650 mL f.p./ha, corrected mortality: -3.84%) and 16.67% in T3 (PROTIOKONAZOL 300 EC at 1300 mL f.p./ha, corrected mortality: 3.85%). No significant differences were noticed between the treatments and the control, showing a 13.33% of mortality. The reference item ROGOR L 40 ST was significantly different with a mortality value of 76.67% (corrected mortality: 73.08%).

The LR_{50} value was estimated to be >1300 mL test item/ha (390 g a.s./ha), while the corresponding NOER and LOER values for mortality were determined to be ≥ 1300 test item/ha (390 g a.s./ha).

For reproduction, the mean number of eggs per female per day was 55.82 in T2 (PROTIOKONAZOL 300 EC at 650 mL f.p./ha) and 56.20 in T3 (PROTIOKONAZOL 300 EC at 1300 mL f.p./ha). The control, showing a mean number of eggs per female per day of 60.01.

The hatching rate was 85.01% in treatment T2 (PROTIOKONAZOL 300 EC at 650 mL f.p./ha) and 85.12% in treatment T3 (PROTIOKONAZOL 300 EC at 1300 mL f.p./ha), while in the control it was equal to 85.94%.

Bioassay 3 (14 DAA)

For the 14-DAA bioassay, the mortality percentage for the test item was 10.00% in T2 (PROTIOKONAZOL 300 EC at 650 mL f.p./ha, corrected mortality: 0.00%) and 16.67% in T3 (PROTIOKONAZOL 300 EC at 1300 mL f.p./ha, corrected mortality: 7.41%). No significant differences were noticed between the treatments and the control, showing a 10.00% of mortality. The reference item ROGOR L 40 ST was significantly different with a mortality value of 76.67% (corrected mortality: 74.07%).

The LR_{50} value was estimated to be >1300 mL test item/ha (390 g a.s./ha), while the corresponding NOER and LOER values for mortality were determined to be ≥ 1300 test item/ha (390 g a.s./ha).

For reproduction, the mean number of eggs per female per day was 64.27 in T2 (PROTIOKONAZOL 300 EC at 650 mL f.p./ha) and 61.41 in T3 (PROTIOKONAZOL 300 EC at 1300 mL f.p./ha). The control, showing a mean number of eggs per female per day of 67.45.

The hatching rate was 78.66% in treatment T2 (PROTIOKONAZOL 300 EC at 650 mL f.p./ha) and 79.24% in treatment T3 (PROTIOKONAZOL 300 EC at 1300 mL f.p./ha), while in the control it was equal to 79.64%.

Table KCP 10.3.2.2-19 **Mortality of *Chrysoperla carnea* (Bioassay 1 at 0 DAA, 2 at 7 DAA and 3 at 14 DAA)**

	T1 Control	**T2 PROTIOKONAZOL 300 EC at 650 mL test item/ha**	**T3 PROTIOKONAZOL 300 EC at 1300 mL test item/ha**	**T4 ROGOR L40 ST at 18 mL test item/ha**
	Deionised water	**195 g a.i./ha**	**390 g a.i./ha**	**7.2 g a.i./ha**
Mortality (bioassay 1 – 0 DAA) [mean %]	6.67	13.33	13.33	83.33
Significance [a]	-	n.s.	n.s.	***

Corrected mortality [b] (bioassay 1 – 0 DAA) [%]	-	7.14	7.14	82.14
Mortality (bioassay 2 – 7 DAA) [mean %]	13.33	10.00	16.67	76.67
Significance [a]	-	n.s.	n.s.	***
Corrected mortality [b] (bioassay 2 – 7 DAA) [%]	-	-3.84	3.85	73.08
Mortality (bioassay 3 – 14 DAA) [mean %]	10.00	10.00	16.67	76.67
Significance [a]	-	n.s.	n.s.	***
Corrected mortality [b] (bioassay 3 – 14 DAA) [%]	-	0.00	7.41	74.08

-, not applicable
n.s., not significantly different compared to the control
a, Fisher's Exact Binomial test, $\alpha \leq 0.001$ ***, 0.01 **, 0.05 *
b, Schneider-Orelli's formula

Table KCP 10.3.2.2-20 Fecundity of *Chrysoperla carnea* (Bioassay 1 at 0 DAA, 2 at 7 DAA and 3 at 14 DAA)

	T1 Control	**T2 PROTIOKONAZOL 300 EC at 650 mL test item/ha**	**T3 PROTIOKONAZOL 300 EC at 1300 mL test item/ha**
	Deionised water	**195 g a.i./ha**	**390 g a.i./ha**
Reproduction (bioassay 1 – 0 DAA) [mean no. eggs/female/day]	58.62	59.90	55.16
% egg-hatching (bioassay 1 – 0 DAA)	84.34	89.93	73.17
Reproduction (bioassay 2 – 7 DAA) [mean no. eggs/female/day]	60.01	55.82	56.20
% egg-hatching (bioassay 2 – 7 DAA)	85.94	85.01	85.12
Reproduction (bioassay 3 – 14 DAA) [mean no. eggs/female/day]	67.45	64.27	61.41
% egg-hatching (bioassay 3 – 14 DAA)	79.64	78.66	79.24

-, not applicable

Table KCP 10.3.2.2-21 Endpoints for the 0-DAA, 7-DAA and 14-DAA bioassay

Endpoint	**mL test item/ha**	**g a.i./ha**
0-DAA Bioassay: fresh residues		
LR_{50}	>1300	>390
NOER (Mortality)	≥1300	≥390
LOER (Mortality)	≥1300	≥390
7-DAA Bioassay: 7-day aged residues		
LR_{50}	>1300	>390
NOER (Mortality)	≥1300	≥390
LOER (Mortality)	≥1300	≥390
14-DAA Bioassay: 14-day aged residues		
LR_{50}	>1300	>390

NOER (Mortality)	≥1300	≥390
LOER (Mortality)	≥1300	≥390

*The LR50 could not be determined, but is estimated to be close to the highest test-item treatment group of 1300 mL test item/ha 390 g a.s./ha)
Based on a nominal active substance (prothioconazole) content of 300 g/L with the following formula: (mL test item/ha / 1000) × 300 g/L
Note: the values were estimated, therefore no confidence limits were provided

A 2.4 KCP 10.4 Effects on non-target soil meso- and macrofauna

A 2.4.1 KCP 10.4.1 Earthworms

A 2.4.1.1 KCP 10.4.1.1 Earthworms - sub-lethal effects

Comments of zRMS:

The study was accepted by zRMS.
The validity criteria was met.

Validity criteria of the study

Adult mortality in control	Adult mortality to be ≤ 10% (actual mortality was 0.00%, so the validity criterion was met).
Reproduction of control	Each replicate to have produced ≥ 30 juveniles (actual values was ranged from 45 to 73, so the validity criterion was met).
Coefficient of variation of reproduction in control	The coefficient of variation to be ≤ 30% (actual value was 17.04%, so the validity criterion was met).

The agreed toxicity endpoints:

Body change and mortality of *Eisenia fetida* after 28 days of exposure

	PROTIKONAZOL 300 EC								
	T1 Control	T2 56.14 mg test item/kg soil d.w.	T3 101.05 mg test item/kg soil d.w.	T4 181.89 mg test item/kg soil d.w.	T5 327.41 mg test item/kg soil d.w.	T6 589.33 mg test item/kg soil d.w.	T7 1060.80 mg test item/kg soil d.w.	T8 1909.44 mg test item/kg soil d.w.	T9 3437 mg test item/kg soil d.w.
	Deionised water	16.34 mg a.i./kg soil d.w.	29.40 mg a.i./kg soil d.w.	52.93 mg a.i./kg soil d.w.	95.27 mg a.i./kg soil d.w.	171.48 mg a.i./kg soil d.w.	308.67 mg a.i./kg soil d.w.	555.61 mg a.i./kg soil d.w.	1000.10 mg a.i./kg soil d.w.
Effect on body weight change [%R]	-	-0.8	2.1	0.4	1.1	1.7	22.5	#	#
Significance [a]	-	n.s.	n.s.	n.s.	n.s.	n.s.	*	*	*
Mortality [mean %]	0.00	0.00	0.00	0.00	0.00	2.50	72.50	100.00	100.00
Significance [b]	-	n.s.	n.s.	n.s.	n.s.	n.s.	***	***	***

Endpoint	mg test item/kg soil d.w.	mg a.i./kg soil d.w.
EC_{10} [95% confidence intervals]	864.11 [754.13 – 990.11]	251.44 [219.44 – 288.10]
EC_{20} [95% confidence intervals]	1029.68 [876.12 – 1206.77]	299.62 [254.93 – 351.15]
EC_{50} [95% confidence intervals]	1440.00 [1031.03 – 1983.61]	419.01 [300.01 – 577.19]
NOEC (Biomass)	589.33	171.48
LOEC (Biomass)	1060.80	308.67
LC_{10} [95% confidence intervals]	690.44 [566.53 – 771.67]	200.90 [164.85 – 224.54]
LC_{20} [95% confidence intervals]	763.11 [653.77 – 839.10]	222.05 [190.23 – 244.16]
LC_{50} [95% confidence intervals]	924.10 [840.66 – 1007.42]	268.89 [244.61 – 293.14]
NOEC (Mortality)	589.33	171.48
LOEC (Mortality)	1060.80	308.67

d.w.= dry weight soil
a.i. = prothioconazole
-, not applicable
#, not applicable due to total mortality and statistical analysis not performed
a, Williams t-test, α=0.05 *
n.s., not significantly different compared to the control
b, Cochran-Armitage Test, α=0.001 ***

Fecundity of *Eisenia fetida* after 56 days of exposure

	PROTIKONAZOL 300 EC								
	T1 Control	T2 56.14 mg test item/kg soil d.w.	T3 101.05 mg test item/kg soil d.w.	T4 181.89 mg test item/kg soil d.w.	T5 327.41 mg test item/kg soil d.w.	T6 589.33 mg test item/kg soil d.w.	T7 1060.80 mg test item/kg soil d.w.	T8 1909.44 mg test item/kg soil d.w.	T9 3437 mg test item/kg soil d.w.
	Deionised water	16.34 mg a.i./kg soil d.w.	29.40 mg a.i./kg soil d.w.	52.93 mg a.i./kg soil d.w.	95.27 mg a.i./kg soil d.w.	171.48 mg a.i./kg soil d.w.	308.67 mg a.i./kg soil d.w.	555.61 mg a.i./kg soil d.w.	1000.10 mg a.i./kg soil d.w.
Fecundity [no. juveniles]	60.50	54.00	38.25	38.50	30.25	20.75	6.00	0.00	0.00
Effect on fecundity [%R]	-	10.7	36.8	36.4	50.0	65.7	90.1	100.0	100.0
Significance [a]	-	n.s.	***	***	***	***	***	***	***

Endpoint	mg test item/kg soil d.w.	mg a.i./kg soil d.w.
EC_{10} [95% confidence intervals]	47.28 [23.39 – 95.56]	13.76 [6.81 – 27.81]
EC_{20} [95% confidence intervals]	n.d.	n.d.
EC_{50} [95% confidence intervals]	270.08 [119.89 – 613.50]	78.59 [34.89 – 178.52]
NOEC (Fecundity)	56.14	16.34
LOEC (Fecundity)	101.05	29.40

d.w.= dry weight soil
a.i. = prothioconazole
-, not applicable
a, Welsh-t-test after Bonferroni-Holm correction, $\alpha \leq 0.001$ ***
n.s., not significantly different compared to the control
n.d., not determined due to mathematical reasons

Reference:	KCP 10.4.1.1/01
Report	Earthworm Reproduction Test (*Eisenia fetida*) with PROTIOKONAZOL 300 EC (prothioconazole 300 g/L); Mautino G.; 2022; Study Code: 1139.1F.SAG22
Guideline(s):	Yes, OECD 222
Deviations:	1) To add the Dosages of the Definitive test. No study impact. 2) To add the Analytical Phase plan. To exclude the test item presence in the control group, treatment T1 (Deionised water) will be add to the Specimens code table in paragraph Sampling. No study impact.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No
Validity criteria:	The validity criteria were met: - each replicate (containing 10 adults) to have produced ≥ 30 juveniles by the end of the test; - the reproductions coefficient of variation to be ≤ 30%; - adult mortality over the initial 4 weeks of the test to be ≤ 10%.

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	PROTIOKONAZOL 300 EC
Formulation:	EC (prothioconazole 300 g/L)
Description (physical state):	liquid
Batch no.:	01/PRO/2022

Production date:	24 March 2022
Expiration date:	March 2025
Stability of test compound:	Test item PROTIOKONAZOL 300 EC (prothioconazole 300 g/L) stability was evaluated to provide evidence that its concentration has been satisfactorily maintained.
2. Vehicle and/or positive control:	vehicle: deionized water positive control: boric acid
3. Test organism	
Species:	Oligochaeta (Lumbricide, Lumbricinae) *Eisenia fetida* (Savigny, 1826)
Source:	Rino Carli – Lombri & Co, Lessona (BI), Italy
Age:	adults, between 9 to 10 months old, with well-developed clitellum, age range between test individuals not differing by more than 4 weeks
Body weight:	300 to 600 mg
Acclimation period:	1 day in artificial soil under test condition
Diet:	finely ground cow manure was used as food during first 28 days of the test, and food withheld for the last 28 days of the test
Test units:	plastic box (145 × 135 × 85 mm) with perforated and transparent lid with 600 g soil/treatment
4. Environmental conditions:	
Temperature:	20.26 ± 0.625 °C (19.12 – 21.22 °C)
Soil:	artificial soil; Sphagnum-peat 10%, Kaolin clay 6.66%, Quartz-sand 83.34%, $CaCO_3$ 0%
WHC:	70.63%
pH:	5.77
Humidity:	63.0 ± 2.9% (60.2 – 74.3%)
Photoperiod:	photoperiod: 16 h light: 8 h dark; light intensity: 600 lux

STUDY DESIGN AND METHOD

The aim of the study was to determine the effect of the test item PROTIOKONAZOL 300 EC on Eisenia fetida mortality and biomass (acute effects) and to evaluate the sub-lethal effects on earthworm reproduction (fecundity) subsequent to their exposure to the test item applied once in the artificial soil. The study comprised 9 treatments (8 concentration of the test item, 1 control group) with 8 replicates for the control group and 4 replicates for treated group; each test unit contained 10 worms. The earthworms were exposed on soil previously treated with test item and observed for 28 (adult mortality). At the end of this period, the percent mortality and mean weight of the survivors were determined. The adults were removed from the soil and the test units were maintained for 28 additional days. After that, the number of offspring were assessed (juvenile reproduction).

Test design:	8 replicates for the control group and 4 replicates for treated group; each test unit contained 10 worms
Exposure time:	56 days
Tested concentrations, definitive test:	56.14 mg/kg soil d.w. (16.34 mg as/ kg soil d.w.) 101.05 mg/kg soil d.w. (29.40 mg as / kg soil d.w.) 181.89 mg/kg soil d.w. (52.93 mg as / kg soil d.w.) 327.41 mg/kg soil d.w. (95.27 mg as /kg soil d.w.) 589.33 mg/kg soil d.w. (171.48 mg as /kg soil d.w.) 1060.80 mg/kg soil d.w. (308.67 mg as /kg soil d.w.) 1909.44 mg/kg soil d.w. (555.61 mg as /kg soil d.w.) 3437 mg/kg soil d.w. (1000.10 mg as /kg soil d.w.)
Dates:	start of the study: 15.07.2022 start of the experimental part: 15.07.2022 end of the experimental part: 18.10.2022 end of the study: 15.02.2023
Statistic:	Software used for statistical analysis were "ToxRatPro" Solutions GmbH, version 3.3.0. Mortality data were processed using the Cochran-Armitage Test $\alpha \leq 0.05$. Fecundity data were analysed using the Welsh-t-test after Bonferroni-Holm correction $\alpha \leq 0.05$. Biomass data were analysed using Williams t-test $\alpha = 0.05$. The No Observed Effect Rate (NOEC) and Lowest Observed Effect Rate (LOEC) values for mortality, biomass and fecundity were determined where possible.

RESULTS

The average biomass (compared to the earthworms' weight at the beginning of the test) in the control group increased by 16.22 ± 0.00%, while biomass increase in the treated groups ranged between 13.04 ± 0.01% (T6 – 589.33 mg test item/kg soil d.w.) to 16.61 ± 0.01% (T4 – 181.89 mg test item/kg soil d.w.). In T7 (1060.80 mg test item/kg soil d.w.) there was a decrease of the biomass of -4.54 ± 0.01. Regarding T8 (1909.44 mg test item/kg soil d.w.) end T9 (3437 mg test item/kg soil d.w.) could not be estimated the biomass change due to total mortality.

The effect on the body weight change ranged between -0.8 in T2 (56.14 mg test item/kg soil d.w.) to 22.5 in T7 (1060.80 mg test item/kg soil d.w.).

A significant difference in terms of body weight reduction was observed for the treatment T7 (1060.80 mg test item/kg soil d.w.) in comparison to the control group; the calculated NOEC (biomass) value was of 589.33 mg test item/kg soil d.w. (171.48 mg a.i./ kg soil d.w.) and a LOEC (biomass) value of 1060.80 mg test item/kg soil d.w. (308.67 mg a.i./ kg soil d.w.).

The 28-day EC10 value was of 864.11 mg test item/kg soil d.w. (95%-CL: 754.13 – 990.11), EC20 value was of 1029.68 mg test item/kg soil d.w. (95%-CL: 876.12 – 1206.77) and EC50 value was of 1440.00 mg test item/kg soil d.w. (95%-CL: 1031.03 – 1983.61) corresponding to 251.44 mg a.i./kg soil d.w. (95%-CL: 219.44 – 288.10); 299.62 mg a.i./kg soil d.w. (95%-CL: 254.93 – 351.15) and 419.01 mg a.i./kg soil d.w. (95%-CL: 300.01 – 577.19), in terms of active ingredient.

Table KCP 10.4.1.1-1: Summary of results of the biomass assessments

Treatment name	Treatment number	Concentration (mg a.i./kg soil d.w.)	Check 28 days					
			Mean body weight (mg)		Body weight change (% ±SE[a])		Effect on body weight change [% R] [b]	P[c]
			Start	End				
Control	T1	-	417.11	484.80	16.22	±0.00	-	-
PROTIOKONAZOL 300 EC at 56.14 mg test item/kg soil d.w.	T2	16.34	424.00	488.83	15.29	±0.01	-0.8	n.s.
PROTIOKONAZOL 300 EC at 101.05 mg test item/kg soil d.w.	T3	29.40	407.58	474.68	16.50	±0.01	2.1	n.s.
PROTIOKONAZOL 300 EC at 181.89 mg test item/kg soil d.w.	T4	52.93	414.00	482.73	16.61	±0.01	0.4	n.s.
PROTIOKONAZOL 300 EC at 327.41 mg test item/kg soil d.w.	T5	95.27	418.65	479.33	14.50	±0.01	1.1	n.s.
PROTIOKONAZOL 300 EC at 589.33 mg test item/kg soil d.w.	T6	171.48	421.75	476.60	13.04	±0.01	1.7	n.s.
PROTIOKONAZOL 300 EC at 1060.80 mg test item/kg soil d.w.	T7	308.67	393.65	375.63	-4.54	±0.01	22.5	*
PROTIOKONAZOL 300 EC at 1909.44 mg test item/kg soil d.w.	T8	555.61	413.63	#	#	#	#	*
PROTIOKONAZOL 300 EC at 3437 mg test item/kg soil d.w.	T9	1000.10	404.73	#	#	#	#	*

a.i. = prothioconazole
d.w.= dry weight soil
a, standard error from 8 replicates (control group) and from 4 replicates (treated groups)
b, weight reduction
c, the p values from Williams t-test, α=0.05*
-, not applicable
n.s., not significantly different compared to the control

Mean adult mortality after 28 days was ranged between 2.50% in T6 (589.33 mg test item/kg soil d.w.) to 100% in T9 (3437 mg test item/kg soil d.w.); compared to 0.00% in the control group.

A significant difference in terms of survival was observed for the treatment T7 (1060.80 mg test item/kg soil d.w.), T8 (1909.44 mg test item/kg soil d.w.) and T9 (3437 mg test item/kg soil d.w) in comparison to the control group; the calculated NOEC (mortality) and LOEC (mortality) values were of 589.33 mg test item/kg soil d.w. (171.48 mg a.i./kg soil d.w.) and 1060.80 mg test item/kg soil d.w. (308.67 mg a.i./kg soil d.w.) respectively. The 28-day LC10 value was of 690.44 mg test item/kg soil d.w. (95%-CL: 566.53 – 771.67) corresponding to 200.90 mg a.i./kg soil d.w. (95%-CL: 164.85 – 224.54) active ingredient. The 28-d (mortality) LC20 value was of 763.11 mg test item/kg soil d.w. (95%-CL: 653.77 – 839.10) corresponding to 222.05 mg a.i./kg soil d.w. (95%-CL: 190.23 – 244.16) active ingredient. The 28-day LC50 value was of 924.10 mg test item/kg soil d.w. (95%-CL: 840.66 – 1007.42) corresponding to 268.89 mg a.i./kg soil d.w. (95%-CL: 244.61 – 293.14) in terms of active ingredient.

Concerning morphological alterations, no effects were noticed as consequence of the treatment.

Table KCP 10.4.1.1-2: Summary of results of the mortality assessments

Treatment name	Treatment number	Concentration (mg a.i./kg soil d.w.)	Check 28 days Mortality		
			Mean (%)	±SE[a]	p^b
Control	T1	-	0.00	±0.00	-
PROTIOKONAZOL 300 EC at 56.14 mg test item/kg soil d.w.	T2	16.34	0.00	±0.00	n.s.
PROTIOKONAZOL 300 EC at 101.05 mg test item/kg soil d.w.	T3	29.40	0.00	±0.00	n.s.
PROTIOKONAZOL 300 EC at 181.89 mg test item/kg soil d.w.	T4	52.93	0.00	±0.00	n.s.
PROTIOKONAZOL 300 EC at 327.41 mg test item/kg soil d.w.	T5	95.27	0.00	±0.00	n.s.
PROTIOKONAZOL 300 EC at 589.33 mg test item/kg soil d.w.	T6	171.48	2.50	±0.25	n.s.
PROTIOKONAZOL 300 EC at 1060.80 mg test item/kg soil d.w.	T7	308.67	72.50	±0.48	***
PROTIOKONAZOL 300 EC at 1909.44 mg test item/kg soil d.w.	T8	555.61	100.00	±0.00	***
PROTIOKONAZOL 300 EC at 3437 mg test item/kg soil d.w.	T9	1000.10	100.00	±0.00	***

a.i. = prothioconazole
d.w.= dry weight soil
a, standard error from 8 replicates (control group) and from 4 replicates (treated groups)
b, Cochran-Armitage Test $\alpha \leq 0.001$***
-, not applicable
n.s., not significantly different compared to the control

In the control group, the number of juvenile worms per replicate ranged from 45 to 73, with a CV of 17.04%. The mean number of juvenile worms was 60.50 in the control group and in the treated group ranged between 0.00 in T9 (3437 mg test item/kg soil d.w.) to 54.00 in T2 (56.14 mg test item /kg soil d.w.). The effect on fecundity ranged between 10.7% in T2 (56.14 mg test item /kg soil d.w.) to 100.0% in T9 (3437 mg test item/kg soil d.w.).

Significant differences were observed in terms of fecundity reduction for the treatments T3 (101.05 mg test item/kg soil d.w.), T4 (181.89 mg test item/kg soil d.w.), T5 (327.41 mg test item/kg soil d.w.), T6 (589.33 mg test item/kg soil d.w.), T7 (1060.80 mg test item/kg soil d.w.), T8 (1909.44 mg test item/kg

soil d.w) and T9 (3437 mg test item/kg soil d.w in comparison to the control.

The NOEC (fecundity) and LOEC (fecundity) values were 56.14 mg test item/kg soil d.w. (16.34 mg a.i./kg soil d.w.) and 101.05 mg test item/kg soil d.w. (29.40 mg a.i./kg soil d.w.) respectively. The 56-d (fecundity) EC10 value observed was of 47.28 mg test item/kg soil d.w. (95%-CL: 23.39 – 95.56) corresponding to 13.76 mg a.i./kg soil d.w. (95%-CL: 6.81 – 27.81 mg a.i./kg soil d.w.), active substance. The 56-d (fecundity) EC50 value observed was of 270.08 mg test item/kg soil d.w. (95%- CL: 119.89 – 613.50) corresponding to 78.59 mg a.i./kg soil d.w. (95%-CL: 34.89 – 178.52) in terms of active ingredient. The 56-d (fecundity) EC20 value could not be estimated due to mathematical reasons.

Table KCP 10.4.1.1-3: Summary of results of the fecundity assessments

Treatment name	Treatment number	Concentration (mg a.i./kg soil d.w.)	Check 56 days			
			Mean number of juvenile earthworms (%±SE[a])		p^b	Fecundity inhibition (% control)
Control	T1	-	60.50	±3.64	-	-
PROTIOKONAZOL 300 EC at 56.14 mg test item/kg soil d.w.	T2	16.34	54.00	±5.52	n.s.	10.7
PROTIOKONAZOL 300 EC at 101.05 mg test item/kg soil d.w.	T3	29.40	38.25	±6.29	***	36.8
PROTIOKONAZOL 300 EC at 181.89 mg test item/kg soil d.w.	T4	52.93	38.50	±3.18	***	36.4
PROTIOKONAZOL 300 EC at 327.41 mg test item/kg soil d.w.	T5	95.27	30.25	±1.11	***	50.0
PROTIOKONAZOL 300 EC at 589.33 mg test item/kg soil d.w.	T6	171.48	20.75	±2.43	***	65.7
PROTIOKONAZOL 300 EC at 1060.80 mg test item/kg soil d.w.	T7	308.67	6.00	±1.83	***	90.1
PROTIOKONAZOL 300 EC at 1909.44 mg test item/kg soil d.w.	T8	555.61	0.00	±0.00	***	100.0
PROTIOKONAZOL 300 EC at 3437 mg test item/kg soil d.w.	T9	1000.10	0.00	±0.00	***	100.0

a.i. = prothioconazole
d.w.= dry weight soil
a, standard error from 8 replicates (control group) and from 4 replicates (treated groups)
b, the p values from Welsh-t-test after Bonferroni-Holm $\alpha \leq 0.001$***
-, not applicable
n.s., not significantly different compared to the control

Feeding activity resulted in 25 g of consumed mature cow manure for both control and treated groups. No behavioural abnormalities were observed, and all worms burrowed into the soil within 15 minutes after

introduction.

Table KCP 10.4.1.1-4: Feeding activity of adult earthworms

Treatment name	Treatment number	Concentration (mg a.i./kg soil d.w.)	Check 28 days Total of added food (g)	
Control	T1	-	25	±0.00[a]
PROTIOKONAZOL 300 EC at 56.14 mg test item/kg soil d.w.	T2	16.34	25	±0.00[a]
PROTIOKONAZOL 300 EC at 101.05 mg test item/kg soil d.w.	T3	29.40	25	±0.00[a]
PROTIOKONAZOL 300 EC at 181.89 mg test item/kg soil d.w.	T4	52.93	25	±0.00[a]
PROTIOKONAZOL 300 EC at 327.41 mg test item/kg soil d.w.	T5	95.27	25	±0.00[a]
PROTIOKONAZOL 300 EC at 589.33 mg test item/kg soil d.w.	T6	171.48	25	±0.00[a]
PROTIOKONAZOL 300 EC at 1060.80 mg test item/kg soil d.w.	T7	308.67	25	±0.00[a]
PROTIOKONAZOL 300 EC at 1909.44 mg test item/kg soil d.w.	T8	555.61	25	±0.00[a]
PROTIOKONAZOL 300 EC at 3437 mg test item/kg soil d.w.	T9	1000.10	25	±0.00[a]

a.i. = prothioconazole
d.w.= dry weight soil
-, not applicable
a, standard error from 8 replicates (control group) and from 4 replicates (treated groups)

CONCLUSION

All study validity criteria were met.

The 28-day NOEC (biomass) observed was of 589.33 mg test item/kg soil d.w. (171.48 mg a.i./ kg soil d.w.) and a LOEC (biomass) value of 1060.80 mg test item/kg soil d.w. (308.67 mg a.i./ kg soil d.w.). The 28-day EC50 (biomass) observed was of 1440.00 mg test item/kg soil d.w. (1031.03 – 1983.61); corresponding to 419.01 mg a.i./kg soil d.w. (300.01 – 577.19) active ingredient.

The 28-day NOEC (mortality) and LOEC (mortality) observed were of 589.33 mg test item/kg soil d.w. (171.48 mg a.i./ kg soil d.w.) and 1060.80 mg test item/kg soil d.w. (308.67 mg a.i./ kg soil d.w.) respectively. The 28-day LC50 observed was of 924.10 mg test item/kg soil d.w. (95%-CL: 840.66 – 1007.42) corresponding to 268.89 mg a.i./kg soil d.w. (95%-CL: 244.61 – 293.14) active ingredient.

The 56-day NOEC (fecundity) and LOEC (fecundity) values were of 56.14 mg test item/kg soil d.w. (16.34 mg a.i./ kg soil d.w.) and 101.05 mg test item/kg soil d.w. (29.40 mg a.i./ kg soil d.w.) respectively. The 56-d (fecundity) EC50 observed was of 270.08 mg test item/kg soil d.w. (95%-CL: 119.89 – 613.50) corresponding to 78.59 mg a.i./kg soil d.w. (95%-CL: 34.89 – 178.52) active ingredient.

A 2.4.1.2 KCP 10.4.1.2 Earthworms - field studies

Not relevant. No studies submitted.

A 2.4.2 KCP 10.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

Comments of zRMS:

The study was accepted by zRMS.
The validity criteria was met.

Validity criteria of the study

Mortality in untreated control	Control mean mortality to be ≤ 20% at the end of the test (actual value was 0.00%, so the validity criterion was met).
Reproduction in untreated control	The mean number of juveniles per test unit to be ≥ 50 at the end of the test (actual value was 230.50, so this validity criterion was met).
Coefficient of Variation of reproduction in untreated control	The coefficient of variation of reproduction in control to be ≤ 30% at the end of the test (actual value was 13.96%, so the validity criterion was met).

The agreed toxicity endpoints:

Mortality of soil mite *Hypoaspis* (*Geolaelaps*) *aculeifer*

	PROTIKONAZOL 300 EC								
	T1 Control	T2 56.14 mg test item/kg soil d.w.	T3 101.05 mg test item/kg soil d.w.	T4 181.89 mg test item/kg soil d.w.	T5 327.41 mg test item/kg soil d.w.	T6 589.33 mg test item/kg soil d.w.	T7 1060.80 mg test item/kg soil d.w.	T8 1909.44 mg test item/kg soil d.w.	T9 3437 mg test item/kg soil d.w.
	Deionised water	16.34 mg a.i./kg soil d.w.	29.40 mg a.i./kg soil d.w.	52.93 mg a.i./kg soil d.w.	95.27 mg a.i./kg soil d.w.	171.48 mg a.i./kg soil d.w.	308.67 mg a.i./kg soil d.w.	555.61 mg a.i./kg soil d.w.	1000.10 mg a.i./kg soil d.w.
Mortality [mean %]	0.00	0.00	0.00	0.00	10.00	22.50	65.00	67.50	87.50
Significance [a]	-	n.s.	n.s.	n.s.	***	***	***	***	***

Endpoint	mg test item/kg soil d.w.	mg a.i./kg soil d.w.
LC_{10} [95% confidence intervals]	345.06 (251.65 – 435.05)	100.41 (73.23 – 126.59)
LC_{20} [95% confidence intervals]	510.42 (400.22 – 618.50)	148.52 (116.46 – 179.97)
LC_{50} [95% confidence intervals]	1079.49 (908.12 – 1298.08)	314.11 (264.24 – 377.71)
NOEC (Mortality)	181.89	52.93
LOEC (Mortality)	327.41	95.27

d.w.= dry weight soil
a.i. = prothioconazole
*, Cochran-Armitage Test, α≤0.001 ***
-, not applicable
n.s., not significantly different compared to the control

Reproduction of soil mite *Hypoaspis (Geolaelaps) aculeifer*

	PROTIKONAZOL 300 EC								
	T1 Control	T2 56.14 mg test item/kg soil d.w.	T3 101.05 mg test item/kg soil d.w.	T4 181.89 mg test item/kg soil d.w.	T5 327.41 mg test item/kg soil d.w.	T6 589.33 mg test item/kg soil d.w.	T7 1060.80 mg test item/kg soil d.w.	T8 1909.44 mg test item/kg soil d.w.	T9 3437 mg test item/kg soil d.w.
	Deionised water	16.34 mg a.i./kg soil d.w.	29.40 mg a.i./kg soil d.w.	52.93 mg a.i./kg soil d.w.	95.27 mg a.i./kg soil d.w.	171.48 mg a.i./kg soil d.w.	308.67 mg a.i./kg soil d.w.	555.61 mg a.i./kg soil d.w.	1000.10 mg a.i./kg soil d.w.
Fecundity [no. juveniles]	230.50	218.25	189.00	141.00	149.50	129.00	31.25	0.75	0.00
Effect on fecundity [%R]	-	5.3	18.0	38.8	35.1	44.0	86.4	99.7	100.0
Significance [a]	-	n.s.	*	*	*	*	*	*	*

Endpoint	mg test item/kg soil d.w.	mg a.i./kg soil d.w.
EC_{10} [95% confidence intervals]	92.00 (77.31 – 109.48)	26.77 (22.50 – 31.86)
EC_{20} [95% confidence intervals]	156.01 (131.56 – 185.37)	45.40 (38.28 – 53.94)
EC_{50} [95% confidence intervals]	428.51 (345.94 – 527.48)	124.69 (100.66 – 153.49)
NOEC (Fecundity)	56.14	16.34
LOEC (Fecundity)	101.05	29.40

d.w.= dry weight soil
a.i. = prothioconazole
*, Williams t-test, α=0.05 *
-, not applicable
n.s., not significantly different compared to the control

Reference: KCP 10.4.2/01

Report: Predatory mite *Hypoaspis (Geolaelaps) aculeifer* reproduction test in soil with PROTIOKONAZOL 300 EC (prothioconazole 300 g/L); Mautino G.; 2022; Study Code: 1142.1F.SA22

Guideline(s): Yes, OECD 226

Deviations:

1) To add the Dosages of the Definitive test 2) To add the Analytical Phase plan deadlines 3) To modify the Specimens code table in paragraph Sampling 4) To modify the paragraph number in Specimens shipment and Analytical Phase 5) To add the Analytical Phase plan. No study impact.

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study): No

Validity criteria: The validity criteria were met:
- mean adult mortality ≤20% at the end of the test;
- mean number of juveniles per replicate at least 50 at the end of the test
- coefficient of variation calculated for the number of juvenile mites per replicate ≤30% at the end of the Definitive test.

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	PROTIOKONAZOL 300 EC
Formulation:	EC (prothioconazole 300 g/L)
Description (physical state):	liquid
Batch no.:	01/PRO/2022
Production date:	24 March 2022
Expiration date:	March 2025
Stability of test compound:	Test item PROTIOKONAZOL 300 EC (prothioconazole 300 g/L) stability was evaluated to provide evidence that its concentration has been satisfactorily maintained.
2. Vehicle and/or positive control:	vehicle: deionized water positive control: ROGOR L 40 ST (nominally 400 g dimethoate/L)

3. Test organism

Species:	Predatory mite (Acari, Laelapidae), Hypoaspis (Geolaelaps) aculeifer Canestrini
Source:	Katz Biotech AG, Baruth, Germany
Age:	ca. 7-14 days after becoming adults, 28-35 days after the start of the egg-laying period
Sex:	female
Diet:	during all the tests, cheese mites (*Tyrophagus putrescentiae*, Schrank) were provided ad libitum, three times a week, as food source
Test units:	inert plastic (non-toxic) box (diameter: 4.6 cm), partly transparent, with a cross-sectional area that allows the actual soil depth within it of 1.5 cm with polyethylene lid and 20 g of soil/treatment

4. Environmental conditions:

Temperature:	20.32 ± 0.410°C (19.07 – 20.68 °C)
Soil:	artificial soil; Sphagnum-peat 5%, Kaolin clay 7%, Quartz-sand 88%, $CaCO_3$ 0%
WHC:	46.35%
pH:	5.95
Humidity:	72.4 ± 3.7% (64.6 – 76.6%) RH
Photoperiod:	photoperiod: 16 h light: 8 h dark

STUDY DESIGN AND METHOD

The aim of the study was to determine the effect of PROTIOKONAZOL 300 EC (prothioconazole 300 g/L) on the vitality and reproduction of predatory mite under laboratory conditions in an artificial soil substrate. All the experimental procedures were designed by following the OECD Guidelines for testing of chemicals no. 226 (2016). For this reason, *Hypoaspis (Geolaelaps) aculeifer* has been selected to investigate the effect of the test item applied alone on this non-target organism following its application into the soil. Endpoints were mite mortality, No Observed Effect Concentration (NOEC) and the reproductive capacity for survivors.

Test design:	8 for the control; 4 per test item; each test unit contained 10 females
Exposure time:	14 days
Tested concentrations, definitive test:	56.14 mg/kg soil d.w. (16.34 mg as/ kg soil d.w.) 101.05 mg/kg soil d.w. (29.40 mg as / kg soil d.w.) 181.89 mg/kg soil d.w. (52.93 mg as / kg soil d.w.) 327.41 mg/kg soil d.w. (95.27 mg as /kg soil d.w.) 589.33 mg/kg soil d.w. (171.48 mg as /kg soil d.w.) 1060.80 mg/kg soil d.w. (308.67 mg as /kg soil d.w.) 1909.44 mg/kg soil d.w. (555.61 mg as /kg soil d.w.) 3437 mg/kg soil d.w. (1000.10 mg as /kg soil d.w.)
Dates:	start of the study: 24.08.2022 start of the experimental part: 24.08.2022 end of the experimental part: 14.11.2022 end of the study: 15.02.2013
Statistic:	Software used for statistical analysis was “ToxRatPro” Solutions GmbH, version 3.3.0. Mortality data were processed using the Cochran-Armitage Test $\alpha \leq 0.05$. Reproduction data were analysed with a Williams t-test $\alpha=0.05$. The No Observed Effect Concentration (NOEC) and Lowest Observed Effect Concentration (LOEC) values for mortality, and fecundity were obtained.

RESULTS

Table KCP 10.4.2-1: Mortality of soil mite *Hypoaspis (Geolaelaps) aculeifer*

	PROTIKONAZOL 300 EC								
	T1 Control	T2 56.14 mg test item/kg soil d.w.	T3 101.05 mg test item/kg soil d.w.	T4 181.89 mg test item/kg soil d.w.	T5 327.41 mg test item/kg soil d.w.	T6 589.33 mg test item/kg soil d.w.	T7 1060.80 mg test item/kg soil d.w.	T8 1909.44 mg test item/kg soil d.w.	T9 3437 mg test item/kg soil d.w.
	Deionised water	16.34 mg a.i./kg soil d.w.	29.40 mg a.i./kg soil d.w.	52.93 mg a.i./kg soil d.w.	95.27 mg a.i./kg soil d.w.	171.48 mg a.i./kg soil d.w.	308.67 mg a.i./kg soil d.w.	555.61 mg a.i./kg soil d.w.	1000.10 mg a.i./kg soil d.w.
Mortality [mean %]	0.00	0.00	0.00	0.00	10.00	22.50	65.00	67.50	87.50
Significance [a]	-	n.s.	n.s.	n.s.	***	***	***	***	***

d.w.= dry weight soil
a.i. = prothioconazole
[a], Cochran-Armitage Test, $\alpha \leq 0.001$ ***
-, not applicable
n.s., not significantly different compared to the control

Table KCP 10.4.2-2: Reproduction of soil mite *Hypoaspis (Geolaelaps) aculeifer*

	PROTIKONAZOL 300 EC								
	T1 Control	T2 56.14 mg test item/kg soil d.w.	T3 101.05 mg test item/kg soil d.w.	T4 181.89 mg test item/kg soil d.w.	T5 327.41 mg test item/kg soil d.w.	T6 589.33 mg test item/kg soil d.w.	T7 1060.80 mg test item/kg soil d.w.	T8 1909.44 mg test item/kg soil d.w.	T9 3437 mg test item/kg soil d.w.
	Deionised water	16.34 mg a.i./kg soil d.w.	29.40 mg a.i./kg soil d.w.	52.93 mg a.i./kg soil d.w.	95.27 mg a.i./kg soil d.w.	171.48 mg a.i./kg soil d.w.	308.67 mg a.i./kg soil d.w.	555.61 mg a.i./kg soil d.w.	1000.10 mg a.i./kg soil d.w.
Fecundity [no. juveniles]	230.50	218.25	189.00	141.00	149.50	129.00	31.25	0.75	0.00
Effect on fecundity [%R]	-	5.3	18.0	38.8	35.1	44.0	86.4	99.7	100.0
Significance [a]	-	n.s.	*	*	*	*	*	*	*

d.w.= dry weight soil
a.i. = prothioconazole
[a], Williams t-test, $\alpha=0.05$ *
-, not applicable
n.s., not significantly different compared to the control

CONCLUSION

The NOEC (mortality) and LOEC (mortality) observed were of 181.89 mg test item/kg soil d.w. (52.93 mg a.i./ kg soil d.w.) and 327.41 mg test item/kg soil d.w. (95.27 mg a.i./kg soil d.w.) respectively. The LC50 observed was of 1079.49 mg test item/kg soil d.w. corresponding to 314.11 mg a.i./kg soil d.w.

Table KCP 10.4.2-3: *Hypoaspis* (*Geolaelaps*) *aculeifer* test– final results for mortality

Endpoint	mg test item/kg soil d.w.	mg a.i./kg soil d.w.
LC_{10} [95% confidence intervals]	345.06 (251.65 – 435.05)	100.41 (73.23 – 126.59)
LC_{20} [95% confidence intervals]	510.42 (400.22 – 618.50)	148.52 (116.46 – 179.97)
LC_{50} [95% confidence intervals]	1079.49 (908.12 – 1298.08)	314.11 (264.24 – 377.71)
NOEC (Mortality)	181.89	52.93
LOEC (Mortality)	327.41	95.27

The NOEC (fecundity) and LOEC (fecundity) values were of 56.14 mg test item/kg soil d.w. (16.34 mg a.i./ kg soil d.w.) and 101.05 mg test item/kg soil d.w. (29.40 mg a.i./ kg soil d.w.) respectively. The EC50 observed was of 428.51 mg test item/kg soil d.w. corresponding to 124.69 mg a.i./kg soil d.w. active ingredient.

Table KCP 10.4.2-4: *Hypoaspis* (*Geolaelaps*) *aculeifer* test– final results for reproduction

Endpoint	mg test item/kg soil d.w.	mg a.i./kg soil d.w.
EC_{10} [95% confidence intervals]	92.00 (77.31 – 109.48)	26.77 (22.50 – 31.86)
EC_{20} [95% confidence intervals]	156.01 (131.56 – 185.37)	45.40 (38.28 – 53.94)
EC_{50} [95% confidence intervals]	428.51 (345.94 – 527.48)	124.69 (100.66 – 153.49)
NOEC (Fecundity)	56.14	16.34
LOEC (Fecundity)	101.05	29.40

Comments of zRMS:	The study was accepted by zRMS. The validity criteria was met.

Validity criteria of the study

All the validity criteria were met, as following detailed, therefore the study can be considered valid:

Adult mortality in control	Mean adult mortality should not exceed 20% at the end of the test (actual value was 6.25%, so the validity criterion was met).
Reproduction of control	Mean number of juveniles per vessel should be at least 100 at the end of the test (actual values ranged from 311 to 410, so this validity criterion was met).
Coefficient of variation of reproduction in control	The coefficient of variation calculated for the number of juveniles should be less than 30% (actual value was 8.26%, so the validity criterion was met).

The agreed toxicity endpoints:

Mortality of *Folsomia candida* after 28 days

	Protikonazol 300 EC								
	T1 Control	T2 19.65 mg test item/kg soil d.w.	T3 35.37 mg test item/kg soil d.w.	T4 63.67 mg test item/kg soil d.w.	T5 114.60 mg test item/kg soil d.w.	T6 206.28 mg test item/kg soil d.w.	T7 371.30 mg test item/kg soil d.w.	T8 668.33 mg test item/kg soil d.w.	T9 1203 mg test item/kg soil d.w.
	Deionised water	5.72 mg a.i./kg soil d.w.	10.29 mg a.i./kg soil d.w.	18.53 mg a.i./kg soil d.w.	33.35 mg a.i./kg soil d.w.	60.022 mg a.i./kg soil d.w.	108.040 mg a.i./kg soil d.w.	194.47 mg a.i./kg soil d.w.	350.050 mg a.i./kg soil d.w.
Mortality [mean %]	6.25	7.50	10.00	12.50	22.50	27.50	42.50	77.50	100.00
Significance [a]	-	n.s.	n.s.	n.s.	**	***	***	***	***
Corrected mortality[b] [%]	-	1.33	4.00	6.67	17.33	22.67	38.67	76.00	100.00

Endpoint	mg test item/kg soil d.w.	mg a.i./kg soil d.w.
LC_{10} [95% confidence intervals]	50.23 [13.38 – 93.42]	14.62 [3.89 – 27.18]
LC_{50} [95% confidence intervals]	282.43 [169.72 – 533.35]	82.18 [49.39 – 155.19]
NOEC (Mortality)	63.67	18.53
LOEC (Mortality)	114.60	33.35

d.w. = dry weight
a.i. = prothioconazole
[a], Cochran-Armitage test, α≤0.001 ***, 0.01 **, 0.05 *
-, not applicable;
n.s., not significantly different compared to the control
[b], Schneider-Orelli formula

Fecundity of *Folsomia candida* after 28 days

	Protikonazol 300 EC								
	T1 Control	T2 19.65 mg test item/kg soil d.w.	T3 35.37 mg test item/kg soil d.w.	T4 63.67 mg test item/kg soil d.w.	T5 114.60 mg test item/kg soil d.w.	T6 206.28 mg test item/kg soil d.w.	T7 371.30 mg test item/kg soil d.w.	T8 668.33 mg test item/kg soil d.w.	T9 1203 mg test item/kg soil d.w.
	Deionised water	5.72 mg a.i./kg soil d.w.	10.29 mg a.i./kg soil d.w.	18.53 mg a.i./kg soil d.w.	33.35 mg a.i./kg soil d.w.	60.022 mg a.i./kg soil d.w.	108.040 mg a.i./kg soil d.w.	194.47 mg a.i./kg soil d.w.	350.050 mg a.i./kg soil d.w.
Fecundity [no. juveniles]	380.63	351.75	341.25	252.25	271.50	220.50	185.25	70.75	0.00
Significance [c]	-	n.s.	*	*	*	*	*	*	*
Effect on reproduction [%R]	-	7.6	10.3	33.7	28.7	42.1	51.3	81.4	100.0

Endpoint	mg test item/kg soil d.w.	mg a.i./kg soil d.w.
EC_{10} [95% confidence intervals]	39.05 [20.81 – 73.25]	11.36 [6.06 – 21.31]
EC_{50} [95% confidence intervals]	240.63 [114.81 – 502.64]	70.02 [33.41 – 146.26]
NOEC (Fecundity)	19.65	5.72
LOEC (Fecundity)	35.37	10.29

d.w. = dry weight
a.i. = prothioconazole
-, not applicable;
n.s., not significantly different compared to the control
[c], Williams t-test, α=0.05 *

Reference: KCP 10.4.2/02

Report	Collembolan *Folsomia candida* reproduction test in soil with PROTIOKONAZOL 300 EC (prothioconazole 300 g/L); Mautino G.; 2022; Study Code: 1143.1F.SA22
Guideline(s):	Yes, OECD 232
Deviations:	1) To add the Dosages of the Definitive test. 2) To add the Analytical Phase plan. No impact.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No
Validity criteria:	The validity criteria were met: - mean adult mortality should not exceed 20% at the end of the test; - the mean number of juveniles per vessel should be at least 100 at the end of the test; - the coefficient of variation calculated for the number of juveniles should be less than 30% at the end of the Definitive test.

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	PROTIOKONAZOL 300 EC
Formulation:	EC (prothioconazole 300 g/L)
Description (physical state):	liquid
Batch no.:	01/PRO/2022
Production date:	24 March 2022
Expiration date:	March 2025
Stability of test compound:	Test item PROTIOKONAZOL 300 EC (prothioconazole 300 g/L) stability was evaluated to provide evidence that its concentration has been satisfactorily maintained.

2. Vehicle and/or positive control: vehicle: deionized water
positive control: boric acid

3. Test organism

Species:	Collembola (*Folsomia candida*)
Source:	University of Parma
Age:	9-12-day-old juveniles
Sex:	female
Diet:	At the beginning of the test and after 14 days with dried baker's yeast

Test units:	Inert plastic (non-toxic) box (diameter: 4.6 cm), partly transparent, with a cross-sectional area that allows the actual soil depth within it of 2-4 cm, polyethylene lid, 30 g of soil/test unit

4. Environmental conditions:

Temperature:	20.29 ± 0.408 °C (19.07 – 20.68 °C)
Soil:	artificial soil
WHC:	50%
Humidity:	71.4 ± 4.5% (64.5 – 76.6%)
Photoperiod:	photoperiod: 16 h light: 8 h dark, 580 lux

STUDY DESIGN AND METHOD

Aim of the study was to determine the 28-day LCx and 28-day ECx for test item PROTIOKONAZOL 300 EC (prothioconazole 300 g/L) by assessing Collembolan mortality and reproduction under laboratory conditions in an artificial soil substrate. All the experimental procedures were designed by following the OECD Guidelines for testing of chemicals no.232. The study (definitive test) encompassed 9 treatments (8 concentrations of the test item, 1 control group) with 8 replicates for the control group and 4 replicates for the treatment groups; each test unit contained 10 collembola. Collembola were exposed to soil previously treated with the test item and observed for 28 days. Assessment of adult mortality, behavioural effects and reproduction rate (mean number of juveniles produced per vessel over the test period) were performed after a 28-day exposure in treated and control groups soils.

Test design:	8 for the control; 4 per test item; 10 collembolans per replicate
Exposure time:	28 days
Tested concentrations, definitive test:	19.65 mg /kg soil d.w. (5.72 mg a.s./ kg soil d.w.) 35.37 mg /kg soil d.w. (10.29 mg a.s./ kg soil d.w.) 63.67 mg /kg soil d.w. (18.53 mg a.s./ kg soil d.w.) 114.60 mg /kg soil d.w. (33.35 mg a.s./kg soil d.w.) 206.28 mg /kg soil d.w. (60.022 mg a.s./kg soil d.w.) 371.30 mg / kg soil d.w. (108.040 mg a.s./kg soil d.w.) 668.33 mg / kg soil d.w. (194.47 mg a.s./kg soil d.w.) 1203 mg / kg soil d.w. (350.050 mg a.s./kg soil d.w.)
Dates:	start of the study: 16.08.2022 start of the experimental part: 21.08.2022 end of the experimental part: 23.11.2022 end of the study: 15.02.2013

Statistic:

Software used for statistical analysis was "ToxRatPro" Solutions GmbH, version 3.3.0. Mortality data were processed using the Cochran-Armitage test, $\alpha \leq 0.05$ and LCx was estimated. Correction for control mortality was processed using the Schneider-Orelli formula. Fecundity data were analysed by Williams t-test, $\alpha = 0.05$ and ECx estimated. The No Observed Effect Rate (NOEC) and Lowest Observed Effect Rate (LOEC) values for mortality, biomass and reproduction were obtained.

RESULTS

All study validity criteria were met.

Table KCP 10.4.2-5: Mortality of *Folsomia candida*

	Protikonazol 300 EC								
	T1 Control	T2 19.65 mg test item/kg soil d.w.	T3 35.37 mg test item/kg soil d.w.	T4 63.67 mg test item/kg soil d.w.	T5 114.60 mg test item/kg soil d.w.	T6 206.28 mg test item/kg soil d.w.	T7 371.30 mg test item/kg soil d.w.	T8 668.33 mg test item/kg soil d.w.	T9 1203 mg test item/kg soil d.w.
	Deionised water	5.72 mg a.i./kg soil d.w.	10.29 mg a.i./kg soil d.w.	18.53 mg a.i./kg soil d.w.	33.35 mg a.i./kg soil d.w.	60.022 mg a.i./kg soil d.w.	108.040 mg a.i./kg soil d.w.	194.47 mg a.i./kg soil d.w.	350.050 mg a.i./kg soil d.w.
Mortality [mean %]	6.25	7.50	10.00	12.50	22.50	27.50	42.50	77.50	100.00
Significance [a]	-	n.s.	n.s.	n.s.	**	***	***	***	***
Corrected mortality [b] [%]	-	1.33	4.00	6.67	17.33	22.67	38.67	76.00	100.00

d.w. = dry weight
a.i. = prothioconazole
[a], Cochran-Armitage test, $\alpha \leq 0.001$ ***, 0.01 **, 0.05 *
-, not applicable;
n.s., not significantly different compared to the control
[b], Schneider-Orelli formula

Table KCP 10.4.2-6: Reproduction of *Folsomia candida*

	Protikonazol 300 EC								
	T1 Control	T2 19.65 mg test item/kg soil d.w.	T3 35.37 mg test item/kg soil d.w.	T4 63.67 mg test item/kg soil d.w.	T5 114.60 mg test item/kg soil d.w.	T6 206.28 mg test item/kg soil d.w.	T7 371.30 mg test item/kg soil d.w.	T8 668.33 mg test item/kg soil d.w.	T9 1203 mg test item/kg soil d.w.
	Deionised water	5.72 mg a.i./kg soil d.w.	10.29 mg a.i./kg soil d.w.	18.53 mg a.i./kg soil d.w.	33.35 mg a.i./kg soil d.w.	60.022 mg a.i./kg soil d.w.	108.040 mg a.i./kg soil d.w.	194.47 mg a.i./kg soil d.w.	350.050 mg a.i./kg soil d.w.
Fecundity [no. juveniles]	380.63	351.75	341.25	252.25	271.50	220.50	185.25	70.75	0.00
Significance[c]	-	n.s.	*	*	*	*	*	*	*
Effect on reproduction [%R]	-	7.6	10.3	33.7	28.7	42.1	51.3	81.4	100.0

d.w. = dry weight
a.i. = prothioconazole
-, not applicable;
n.s., not significantly different compared to the control
c, Williams t-test, α=0.05 *

CONCLUSION

The NOEC (mortality) and LOEC (mortality) observed were of 63.67 mg test item/kg soil d.w. (18.53 mg a.i./ kg soil d.w.) and 114.60 mg test item/kg soil d.w. (33.35 mg a.i./kg soil d.w.) respectively. The LC50 observed was of 282.43 mg test item/kg soil d.w. corresponding to 82.18 mg a.i./kg soil d.w. active ingredient.

Table KCP 10.4.2-7: *Folsomia candida* test– final results for mortality

Endpoint	mg test item/kg soil d.w.	mg a.i./kg soil d.w.
LC_{10} [95% confidence intervals]	50.23 [13.38 – 93.42]	14.62 [3.89 – 27.18]
LC_{50} [95% confidence intervals]	282.43 [169.72 – 533.35]	82.18 [49.39 – 155.19]
NOEC (Mortality)	63.67	18.53
LOEC (Mortality)	114.60	33.35

The NOEC (fecundity) and LOEC (fecundity) values were of 19.65 mg test item/kg soil d.w. (5.72 mg a.i./ kg soil d.w.) and 35.37 mg test item/kg soil d.w. (10.29 mg a.i./ kg soil d.w.) respectively. The EC50 observed was of 240.63 mg test item/kg soil d.w. corresponding to 70.02 mg a.i./kg soil d.w. active ingredient.

Table KCP 10.4.2-8: *Folsomia candida* test– final results for reproduction

Endpoint	mg test item/kg soil d.w.	mg a.i./kg soil d.w.
EC_{10} [95% confidence intervals]	39.05 [20.81 – 73.25]	11.36 [6.06 – 21.31]
EC_{50} [95% confidence intervals]	240.63 [114.81 – 502.64]	70.02 [33.41 – 146.26]
NOEC (Fecundity)	19.65	5.72
LOEC (Fecundity)	35.37	10.29

A 2.4.2.1 KCP 10.4.2.2 Higher tier testing

Not relevant. No studies submitted.

A 2.5 KCP 10.5 Effects on soil nitrogen transformation

Comments of zRMS:	The study was accepted by zRMS. The validity criteria was met.
	Validity criteria of the study Validity criteria of the negative control: The coefficient of variation of nitrate concentration between the replicates ranged from 4.5% to 10.1%, which is in accordance with the validity criterion of the test as required by OECD guideline No. 216, *i.e.*, a maximum variation of 15 %.
	The agreed toxicity endpoints:

Interval rate - Mean nitrate formation rate and percentage deviation from control in untreated and treated soil after 7, 14, 28 and 42 days

Interval rate (mg nitrate/kg soil d.w./day)					
	Time / Replicate	**0 – 7**	**7 - 14**	**14 - 28**	**28 - 42**
Control (0.0 mg/kg soil d.w.)	A	0.83	7.62	4.23	1.31
	B	1.94	5.83	4.20	2.89
	C	1.06	5.57	3.13	3.06
	Mean	**1.28**	**6.34**	**3.85**	**2.42**
0.9 mg/kg soil d.w. (2)	A	1.39	5.47	3.18	n.d.
	B	1.60	4.60	3.41	n.d.
	C	1.40	5.13	3.46	n.d.
	Mean	**1.46**	**5.07**	**3.35**	**n.d.**
	Deviation from Control % (1)	**-14.6**	**20.1**	**13.1*[1]**	**n.d.**
4.5 mg/kg soil d.w. (2)	A	2.76	4.49	2.74	2.43
	B	2.96	5.33	2.68	2.63
	C	3.29	5.79	2.75	2.18
	Mean	**3.01**	**5.21**	**2.73**	**2.42**
	Deviation from Control % (1)	**-135.4**	**17.9**	**29.3*[2]**	**0.2*[3]**

(1) a positive percentage indicates that nitrate formation rate in the treated soil is lower than in the control soil. A negative percentage indicates that nitrate formation rate in the treated soil is higher than in the control soil.
(2) concentrations expressed as test item.
n.d. not determined
*[1] Deviation from control at 28th day in lowest treatment was assessed as not significant by Dunnett Test.
*[2] Deviation from control at 28th day in highest treatment was assessed as significant by Dunnett Test.
*[3] Deviation from control at 42nd day in highest treatment was assessed as not significant by Equal variance t two-sample Test

Reference:	KCP 10.5/01
Report	Soil Microorganisms: Nitrogen Transformation Test with PROTIOKONAZOL 300 EC (prothioconazole 300 g/L); Mautino G.; 2023; Study Code: 4545.1F.SAG22
Guideline(s):	Yes, OECD 216
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No
Validity criteria of the test:	All validity criteria were met. - the variation between replicate control samples should be less than ± 15% (from test start until 42nd day it was in the range 4.5% – 10.1 %)

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):

Formulation:	PROTIOKONAZOL 300 EC
EC (prothioconazole 300 g/L)	
Description (physical state):	liquid

Batch no.:	01/PRO/2022
Production date:	24 March 2022

2. Vehicle and/or positive control: vehicle: deionized water
positive control: Nitrate Standard

3. Test organism

Soil:	Soil type (code 2.3, sandy loam soil), purchased from the.
Source:	research institute LUFA (Landwirtschaftliche Untersuchungs- und Forschungsanstalt Speyer) in Germany
Soil preparation:	pre-incubated for 21 days in the same conditions in which the test will be conducted (20 ± 2 °C, in dark conditions)
Test units:	2000 mL capacity glass vessel covered with perforated plastic film was used, to assure a sufficient head space and to allow gas exchange, the test vessels were incubated in an environmental test chamber, in dark conditions

4. Environmental conditions:

Temperature:	20.0°C – 20.1°C with a mean value of 20.0°C and a standard deviation of 0.02°C.
Photoperiod:	dark
Sand content:	60.7% according to German norm (DIN)*; 0.063-2.0 mm 63.7% according to USDA norm*; 0.05-2.0 mm
pH:	5.9 ± 0.4
Organic carbon content:	0.64± 0.07
Microbial biomass:	3.9% of the total soil organic carbon
Water Holding Capacity:	35.4 % ± 2.3 %
Weight per volume:	1297 ± 48 g / 1000 mL
Moisture content:	6.9%
Nitrogen (% N):	0.08 ± 0.02
Nitrate (mg/kg dry weight):	56.5 mg/kg d.w.
Cation exchange capacity:	5.7 ± 0.5 meq/100 g

STUDY DESIGN AND METHOD

The aim of this study was to assess adverse effects of the test item PROTIOKONAZOL 300 EC on the process of nitrogen transformation of aerobic soil microorganisms. The study encompassed 3 treatments (2 rates of the test item, 1 control group) with 3 replicates for the control group and for each test item concentration were tested. The soils of treated groups and control were incubated for 28 days. Soil samples were taken from each replicate on days 0, 7, 14 and 28. Nitrate formation rate in each treatment was calculated both as overall rate and as interval rate and those values were compared with that in the control and percent deviation from the control was calculated.

Test design:	concentrations and control in 3 replicates
Exposure time:	28 days for the lowest treatment and 42 days for the highest treatment
Tested concentrations, definitive test:	1PEC – 0.9 mg/kg soil (0.27 mg a.s./kg soil) 5PEC – 4.5 mg/kg soil (1.35 mg a.s./kg soil)
Dates:	start of the study 22.11.202022 start of the experimental part: 22.11.2022 end of the experimental part: 16.01.2023 end of the study: 20.02.2023
Statistic:	Statistical analysis was performed by test site personnel. Software used for statistical analysis was CETIS elaboration software v. 1.8.7.7. The Dunnett´s test was used for analysis.

RESULTS

Results of the study are summarised in below table.

Table KCP 10.5.-1: Interval rate - Mean nitrate formation rate and percentage deviation from control in untreated and treated soil after 7, 14, 28 and 42 days.

Interval rate (mg nitrate/kg soil d.w./day)					
	Time / Replicate	**0 – 7**	**7 - 14**	**14 - 28**	**28 - 42**
Control (0.0 mg/kg soil d.w.)	A	0.83	7.62	4.23	1.31
	B	1.94	5.83	4.20	2.89
	C	1.06	5.57	3.13	3.06
	Mean	**1.28**	**6.34**	**3.85**	**2.42**
0.9 mg/kg soil d.w. (2)	A	1.39	5.47	3.18	n.d.
	B	1.60	4.60	3.41	n.d.
	C	1.40	5.13	3.46	n.d.
	Mean	**1.46**	**5.07**	**3.35**	**n.d.**
	Deviation from Control % (1)	**-14.6**	**20.1**	**13.1*[1]**	**n.d.**
4.5 mg/kg soil d.w. (2)	A	2.76	4.49	2.74	2.43
	B	2.96	5.33	2.68	2.63
	C	3.29	5.79	2.75	2.18
	Mean	**3.01**	**5.21**	**2.73**	**2.42**
	Deviation from Control % (1)	**-135.4**	**17.9**	**29.3*[2]**	**0.2*[3]**

(1) a positive percentage indicates that nitrate formation rate in the treated soil is lower than in the control soil. A negative percentage indicates that nitrate formation rate in the treated soil is higher than in the control soil.
(2) concentrations expressed as test item.
n.d. not determined
*1 Deviation from control at 28th day in lowest treatment was assessed as not significant by Dunnett Test.
*2 Deviation from control at 28th day in highest treatment was assessed as significant by Dunnett Test.
*3 Deviation from control at 42nd day in highest treatment was assessed as not significant by Equal variance t two-sample Test

Biological results in terms of **Overall rate**	On 28th day the difference (mean value) between nitrogen transformation rate, expressed as overall rate, in untreated soil and treated soil was lower than 25%.		
Test item concentration [mg/kg soil d.w.] (*)	Overall rate - Deviation from control % (1)		
	0-7	0-14	0-28
0.9	-14.6	14.3	13.7
4.5	-135.4	-7.8	10.8

(1) positive percentage indicates that nitrate formation rate in the treated soil was lower than in control soil.
(*) corresponding to 0.27 mg prothioconazole/kg d.w. and 1.35 mg prothioconazole/kg d.w., calculated considering nominal active ingredient content, 29.71% w/w.

Biological results in terms of **Interval rate**	On 28th day the difference (mean value) between nitrogen transformation rate, expressed as interval rate, in untreated soil and treated soil was lower than 25% for treatment at 0.9 mg/kg d.w., but greater than 25% as absolute value for treatment at 4.5 mg/kg d.w. Therefore, for the highest treatment an additional determination after 14 days (day 42) was performed.

Test item concentration [mg/kg soil d.w.] (*)	Interval rate - Deviation from control % (1)			
	0-7	7-14	14-28	28-42
0.9	-14.6	20.1	13.1	Not determ.
4.5	-135.4	17.9	29.3	0.2

(1) positive percentage indicates that nitrate formation rate in the treated soil was lower than in control soil.
(*) corresponding to 0.27 mg prothioconazole/kg d.w. and 1.35 mg prothioconazole/kg d.w., calculated considering nominal active ingredient content, 29.71% w/w.

CONCLUSION

For lower treatment, after 28 days, differences from the control were lower than 25% both if calculated in terms of overall rate and interval rate.

For higher treatment, differences from the control were lower than 25% after 28 days, if calculated in terms of overall rate and after 42 days, if calculated in terms of interval rate.

According to these results, the test item can be evaluated as having no long-term influence on nitrogen transformation in soils.

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

Not relevant. No studies submitted.

A 2.6.2 KCP 10.6.2 Testing on non-target plants

Com-ments of zRMS:	The study was accepted by zRMS. The validity criteria was met.

Validity criteria of the study

Criteria	Results
Seedling emergence ≥ 70% in the control group;	Actual values ranged from 86.67% to 100% in the control group.
Seedlings without visible phytotoxic effects (e.g.: chlorosis, necrosis, wilting, leaf and stem deformations) and the plant species exhibit only normal variation in growth and morphology in the control group;	Actual result: plants did not exhibit phytotoxicity symptoms, only normal variation in their growth and morphology were observed in the control group, so the validity criterion was met.
Mean survival of emerged control seedlings ≥ 90% for the duration of the study in the control group;	Actual mortality was 0.00% for all the plant species in the control group, therefore, the validity criterion was met.
Identical environmental conditions for all the selected plant species and growing media with the same amount of substrate from the same source.	Actual result: identical environmental conditions and growing media with the same amount of soil matrix, support media, or substrate was used, so the validity criterion was met.

The agreed toxicity endpoints:

Endpoints for the Non target plant species emergence.

	Endpoints (mL f.p./ha)			Endpoints (g a.i./ha)		
	NOEC	**LOEC**	**EC_{50}**	**NOEC**	**LOEC**	**EC_{50}**
Cucumis sativus	≥ 2600	> 2600	> 2600 (95%-CLs n.d.)	≥ 780	> 780	> 780 (95%-CLs n.d.)
Phaseolus vulgaris	≥ 2600	> 2600	> 2600 (95%-CLs n.d.)	≥ 780	> 780	> 780 (95%-CLs n.d.)
Solanum lycopersicum	≥ 2600	> 2600	> 2600 (95%-CLs n.d.)	≥ 780	> 780	> 780 (95%-CLs n.d.)
Beta vulgaris	≥ 2600	> 2600	> 2600 (95%-CLs n.d.)	≥ 780	> 780	> 780 (95%-CLs n.d.)
Brassica oleracea	≥ 2600	> 2600	> 2600 (95%-CLs n.d.)	≥ 780	> 780	> 780 (95%-CLs n.d.)
Zea mays	≥ 2600	> 2600	> 2600 (95%-CLs n.d.)	≥ 780	> 780	> 780 (95%-CLs n.d.)
Lolium perenne	≥ 2600	> 2600	> 2600 (95%-CLs n.d.)	≥ 780	> 780	> 780 (95%-CLs n.d.)
Allium cepa	≥ 2600	> 2600	> 2600 (95%-CLs n.d.)	≥ 780	> 780	> 780 (95%-CLs n.d.)

f.p.: formulated product
a.i.: active ingredient
n.d.: not determined due to mathematical reasons
95%-CLs, Confidence Limits

Endpoints for the Non target plant species post-emergence mortality.

	Endpoints (mL f.p./ha)			Endpoints (g a.i./ha)		
	NOEC	**LOEC**	**LC_{50}**	**NOEC**	**LOEC**	**LC_{50}**
Cucumis sativus	≥ 2600	> 2600	> 2600 (95%-CLs n.d.)	≥ 780	> 780	> 780 (95%-CLs n.d.)
Phaseolus vulgar-	≥ 2600	> 2600	> 2600 (95%-	≥ 780	> 780	> 780 (95%-

is			CLs n.d.)			CLs n.d.)
Solanum lycoper-sicum	≥ 2600	> 2600	> 2600 (95%-CLs n.d.)	≥ 780	> 780	> 780 (95%-CLs n.d.)
Beta vulgaris	≥ 2600	> 2600	> 2600 (95%-CLs n.d.)	≥ 780	> 780	> 780 (95%-CLs n.d.)
Brassica oleracea	≥ 2600	> 2600	> 2600 (95%-CLs n.d.)	≥ 780	> 780	> 780 (95%-CLs n.d.)
Zea mays	≥ 2600	> 2600	> 2600 (95%-CLs n.d.)	≥ 780	> 780	> 780 (95%-CLs n.d.)
Lolium perenne	≥ 2600	> 2600	> 2600 (95%-CLs n.d.)	≥ 780	> 780	> 780 (95%-CLs n.d.)
Allium cepa	≥ 2600	> 2600	> 2600 (95%-CLs n.d.)	≥ 780	> 780	> 780 (95%-CLs n.d.)

f.p.: formulated product
a.i.: active ingredient
n.d.: not determined due to mathematical reasons
95%-CLs, Confidence Limits

Endpoints for the Non target plant species shoot fresh weight (*i.e.*, biomass).

	Endpoints (mL f.p./ha)				**Endpoints (g a.i./ha)**			
	NOEC	**LOEC**	**EC_{25}**	**EC_{50}**	**NOEC**	**LOEC**	**EC_{25}**	**EC_{50}**
Cucumis sativus	650	1300	2064.89 (790.20 – U.L. n.d.*)	> 2600 (95%-CLs n.d.)	195	390	619.47 (237.06 – U.L. n.d.**)	> 780 (95%-CLs n.d.)
Phaseolus vulgaris	1300	2600	> 2600 (2377.85 – U.L. n.d.*)	> 2600 (U.L. n.d.*)	390	780	> 780 (713.36 – U.L. n.d.**)	> 780 (U.L. n.d.**)
Solanum lycopersi-cum	1300	2600	> 2600 (0.00. – n.d.)	> 2600 (95%-CLs n.d.)	390	780	> 780 (0.00. – n.d.)	> 780 (95%-CLs n.d.)
Beta vulgar-is	1300	2600	2588.77 (1817.27 – 2599.23	> 2600 (2578.27 – U.L. n.d.*)	390	780	776.63 (545.18 – 779.77)	> 780 (773.48 – U.L. n.d.**)
Brassica oleracea	650	1300	> 2600 (880.38 – U.L. n.d.*)	> 2600 (95%-CLs n.d.)	195	390	> 780 (264.11 – U.L. n.d.**)	> 780 (95%-CLs n.d.)
Zea mays	1300	2600	2237.21 (1430.27 – U.L. n.d.*)	> 2600 (1880.68 – U.L. n.d.*)	390	780	671.16 (429.08 – U.L.	> 780 (564.20 – U.L.

							n.d.**)	n.d.**)
Lolium perenne	≥ 2600	> 2600	n.d.	> 2600 (95%-CLs n.d.)	≥ 780	> 780	n.d.	> 780 (95%-CLs n.d.)
Allium cepa	≥ 2600	> 2600	n.d.	> 2600 (95%-CLs n.d.)	≥ 780	> 780	n.d.	> 780 (95%-CLs n.d.)

f.p.: formulated product
a.i.: active ingredient
95%-CLs n.d., Confidence Limits not determined due to mathematical reasons
n.d., not determined due to mathematical reasons
U.L., Upper Limit
*, Confidence Limit over the 2600 mL f.p./ha dosage is a software estimation
**, Confidence Limit over the 780 g a.i../ha dosage is a software estimation

Phytotoxicity parameter:

Concerning the phytotoxicity, for cucumber the NOEC value was 650 mL f.p./ha. The estimated EC_{50} was > 2600 mL f.p./ha

For bean the NOEC (phytotoxicity) value was 2600 mL f.p./ha. The estimated EC_{50} was > 2600 mL f.p./ha.

For tomato the NOEC (phytotoxicity) value was 325 mL f.p./ha. The estimated EC_{50} was > 2600 mL f.p./ha.

For sugar beet the NOEC (phytotoxicity) value was 162.50 mL f.p./ha. The estimated EC_{50} was > 2600 mL f.p./ha.

For cabbage the NOEC (phytotoxicity) value was 325 mL f.p./ha. The estimated EC_{50} was > 2600 mL f.p./ha.

For corn the NOEC (phytotoxicity) value was 2600 mL f.p./ha. The estimated EC_{50} was > 2600 mL f.p./ha.

For perennial ryegrass the NOEC (phytotoxicity) value was 650 mL f.p./ha. The estimated EC_{50} was > 2600 mL f.p./ha.

For onion the NOEC (phytotoxicity) value was 325 mL f.p./ha. The estimated EC_{50} was > 2600 mL f.p./ha.

Reference:	KCP 10.6.2/01
Report	Effects of PROTIOKONAZOL 300 EC (prothioconazole 300 g/L) on terrestrial Non-target plants – Seedling Emergence and Seedling Growth; Mautino G.; 2023; Study Code: 1140.1F.SAG22
Guideline(s):	Yes, OECD 208
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No
Validity criteria of the test:	All validity criteria of this study were met: - seedling emergence ≥ 70%; - the seedlings do not exhibit visible phytotoxic effects and the plants exhibit only normal variation in growth and morphology for that particular species;

- mean survival of emergence control seedlings ≥ 90% for the duration of the study;
- identical environmental conditions for a particular species and growing media with the same amount of soil matrix, support media or substrate.

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	PROTIOKONAZOL 300 EC
Formulation:	EC
Description (physical state):	liquid
Batch no.:	01/PRO/2022
Production date:	24 March 2022
Expiration date:	March 2025
Stability of test compound:	In order to confirm the concentration of test item active substance in the applied solution, two samples of 200 ml (one for shipment and one as retain sample) of the remaining spray solution were collected immediately after application on treatment 2600 mL f.p./ha (780 g a.i./ha) and placed separately in plastic containers for storage. One sample was sent to the RENOLAB s.r.l. laboratory for the analytical verification and a second one stored as a retained sample.
2. Vehicle and/or positive control:	vehicle control: deionised water positive control: not relevant
3. Test plants:	eight non-target plant species were tested, five dicotyledonous and three monocotyledonous species, representing seven different plant families: Cucumber *Cucumis sativus*, Bean *Phaseolus vulgaris*, Tomato *Solanum lycopersicum*, Sugar beet *Beta vulgaris*, Cabbage *Brassica oleracea*, Corn *Zea mays*, Perennial ryegrass *Lolium perenne* and Onion *Allium cepa*
Test containers:	commercial non-porous plastic, 15.1 cm in diameter, approx. 2L
Seed distribution	manually into the soil one day before application
Irrigation	tap watering after sowing and at least once a week
Soil:	sandy loam, particle size: all particles ≤ 2 mm, C_{org}: 1.6%, pH: 6.97
4. Environmental conditions:	
Temperature:	24.95 ± 0.224°C (26.60 – 25.47°C)
Relative humidity:	72.6 ± 4.8% (60.7 – 79.5 %)

Photoperiod:	controlled light – dark cycles (16h:8h), light intensity: 18000 – 20000 lux

STUDY DESIGN AND METHODS

The aim of the study was to determine the 21-day ECx of the test item PROTIOKONAZOL 300 EC (prothioconazole 300 g/L) for different non-target plant species by assessing their emergence, post-emergence survival (21-day LCx) and fresh weight, subsequent to the test item application on the bare soil surface. Experimental procedures were designed by following OECD Guidelines for Testing of Chemicals No. 208 "Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test". Eight non-target plant species were tested, five dicotyledonous and three monocotyledonous species, representing seven different plant families: *Cucumis sativus, Phaseolus vulgaris, Solanum lycopersicum, Beta vulgaris, Brassica oleracea, Zea mays, Lolium perenne* and *Allium cepa*. After sowing, the maximum test item dosage was sprayed onto the soil surface, simulating a typical spray application by using a water volume of 400 L/ha. The study encompassed 6 treatments (5 test item dosage and the control group). 30 plants were sown per treatment and crop species. Immediately after the application, two samples of 200 ml of stock solution were collected for analytical determination of active substance content. Growth stages at 7, 14 and 21 days after 50% seedling emergence in the control group was reported according to BBCH scale. The following parameters were estimated or calculated:

Seedling emergence

Germination was checked weekly (i.e., at 7, 14 and 21 days) after 50% seedling emergence in the control group. Percent emergence of survived plants was determined until 21 days after 50% seedling emergence in the control group.

Phytotoxicity

Plants were visually checked for possible phytotoxic symptoms at 7, 14 and 21 days after 50% seedling emergence in the control group. A scoring system for visual injury comparison between treated and untreated pots was used. Any observed symptom of phytotoxicity was assessed following the criteria outlined in EPPO standard PP 1/135(3).

Seedling mortality

The number of living and dead plants was recorded at 7, 14 and 21 days after 50% seedling emergence in the control plants. A plant was considered dead if it had emerged, but no living tissues were observable at the time of the assessments. Percent mortality will be calculated on the number of emerged seedlings in each pot.

Fresh weight

The fresh shoot weight (biomass: all portions of the plant above the soil surface, excluding roots) was determined at 21 days after 50% seedlings emergence in the control group.

Test design:	Each pot represents a replicate, 3 seeds per pot for cucumber, bean, tomato, sugar beet, cabbage and corn and 5 seeds per pot perennial ryegrass and onion, 30 seeds were sown per treatment
Exposure time:	21 days after the spraying
Tested concentrations, definitive test:	162.50, 325, 650, 1300 and 2600 ml/ha (48.75, 97.50, 195, 390 and 780 g a.s./ha) + 400 mL/ha
Dates:	start of the study 17.10.2022 start of the experimental part: 21.10.2022 end of the experimental part: 14.12.2022 end of the study: 06.03.2023

Statistic:

Software used for statistical analysis was "ToxRatPro", version 3.3.0

Plants' emergence data were processed using Fisher test, and Chi2 2x2 t-test, with Bonferroni Correction. The EC_{25} and EC_{50} were calculated with probit (normal sigmoid) where possible.

Post-emergence mortality data were analysed using Rao-Scott-Cochran-Armitage test. The LC_{25} and LC_{50} were calculated with probit (normal sigmoid) where possible.

Shoot fresh weight at 21 days was evaluatedusing different tests: Welsh-t-test after Bonferroni-Holm, Williams multiple t-test, Jonckheere-Terpstra test, U-test with Bonferroni Correction, and Dunnett' t-test. The EC_{25} and EC_{50} were calculated with a 3-parameter non-linear regression where possible.

CONCLUSION

The results are summarised in following tables:

Table KCP 10.6.2-1: Endpoints for the Non target plant species emergence.

	Endpoints (mL f.p./ha)			**Endpoints (g a.i./ha)**		
	NOEC	**LOEC**	**EC_{50}**	**NOEC**	**LOEC**	**EC_{50}**
Cucumis sativus	≥ 2600	> 2600	> 2600 (95%-CLs n.d.)	≥ 780	> 780	> 780 (95%-CLs n.d.)
Phaseolus vulgaris	≥ 2600	> 2600	> 2600 (95%-CLs n.d.)	≥ 780	> 780	> 780 (95%-CLs n.d.)
Solanum lycopersicum	≥ 2600	> 2600	> 2600 (95%-CLs n.d.)	≥ 780	> 780	> 780 (95%-CLs n.d.)
Beta vulgaris	≥ 2600	> 2600	> 2600 (95%-CLs n.d.)	≥ 780	> 780	> 780 (95%-CLs n.d.)
Brassica oleracea	≥ 2600	> 2600	> 2600 (95%-CLs n.d.)	≥ 780	> 780	> 780 (95%-CLs n.d.)
Zea mays	≥ 2600	> 2600	> 2600 (95%-CLs n.d.)	≥ 780	> 780	> 780 (95%-CLs n.d.)
Lolium perenne	≥ 2600	> 2600	> 2600 (95%-CLs n.d.)	≥ 780	> 780	> 780 (95%-CLs n.d.)
Allium cepa	≥ 2600	> 2600	> 2600 (95%-CLs n.d.)	≥ 780	> 780	> 780 (95%-CLs n.d.)

f.p.: formulated product
a.i.: active ingredient
n.d.: not determined due to mathematical reasons
95%-CLs, Confidence Limits

Table KCP 10.6.2-2: Endpoints for the Non target plant species post-emergence mortality.

	Endpoints (mL f.p./ha)			Endpoints (g a.i./ha)		
	NOEC	**LOEC**	**LC_{50}**	**NOEC**	**LOEC**	**LC_{50}**
Cucumis sativus	≥ 2600	> 2600	> 2600 (95%-CLs n.d.)	≥ 780	> 780	> 780 (95%-CLs n.d.)
Phaseolus vulgaris	≥ 2600	> 2600	> 2600 (95%-CLs n.d.)	≥ 780	> 780	> 780 (95%-CLs n.d.)
Solanum lycopersicum	≥ 2600	> 2600	> 2600 (95%-CLs n.d.)	≥ 780	> 780	> 780 (95%-CLs n.d.)
Beta vulgaris	≥ 2600	> 2600	> 2600 (95%-CLs n.d.)	≥ 780	> 780	> 780 (95%-CLs n.d.)
Brassica oleracea	≥ 2600	> 2600	> 2600 (95%-CLs n.d.)	≥ 780	> 780	> 780 (95%-CLs n.d.)
Zea mays	≥ 2600	> 2600	> 2600 (95%-CLs n.d.)	≥ 780	> 780	> 780 (95%-CLs n.d.)
Lolium perenne	≥ 2600	> 2600	> 2600 (95%-CLs n.d.)	≥ 780	> 780	> 780 (95%-CLs n.d.)
Allium cepa	≥ 2600	> 2600	> 2600 (95%-CLs n.d.)	≥ 780	> 780	> 780 (95%-CLs n.d.)

f.p.: formulated product
a.i.: active ingredient
n.d.: not determined due to mathematical reasons
95%-CLs, Confidence Limits

Table KCP 10.6.2-3: Endpoints for the Non target plant species shoot fresh weight (*i.e.*, biomass).

	Endpoints (mL f.p./ha)				Endpoints (g a.i./ha)			
	NOEC	**LOEC**	**EC_{25}**	**EC_{50}**	**NOEC**	**LOEC**	**EC_{25}**	**EC_{50}**
Cucumis sativus	650	1300	2064.89 (790.20 – U.L. n.d.*)	> 2600 (95%-CLs n.d.)	195	390	619.47 (237.06 – U.L. n.d.**)	> 780 (95%-CLs n.d.)
Phaseolus vulgaris	1300	2600	> 2600 (2377.85 – U.L. n.d.*)	> 2600 (U.L. n.d.*)	390	780	> 780 (713.36 – U.L. n.d.**)	> 780 (U.L. n.d.**)
Solanum lycopersicum	1300	2600	> 2600 (0.00. – n.d.)	> 2600 (95%-CLs n.d.)	390	780	> 780 (0.00. – n.d.)	> 780 (95%-CLs n.d.)
Beta vulgaris	1300	2600	2588.77 (1817.27 – 2599.23	> 2600 (2578.27 – U.L. n.d.*)	390	780	776.63 (545.18 – 779.77)	> 780 (773.48 – U.L. n.d.**)
Brassica oleracea	650	1300	> 2600 (880.38 – U.L. n.d.*)	> 2600 (95%-CLs n.d.)	195	390	> 780 (264.11 – U.L. n.d.**)	> 780 (95%-CLs n.d.)

Zea mays	1300	2600	2237.21 (1430.27 – U.L. n.d.*)	> 2600 (1880.68 – U.L. n.d.*)	390	780	671.16 (429.08 – U.L. n.d.**)	> 780 (564.20 – U.L. n.d.**)
Lolium perenne	≥ 2600	> 2600	n.d.	> 2600 (95%-CLs n.d.)	≥ 780	> 780	n.d.	> 780 (95%-CLs n.d.)
Allium cepa	≥ 2600	> 2600	n.d.	> 2600 (95%-CLs n.d.)	≥ 780	> 780	n.d.	> 780 (95%-CLs n.d.)

f.p.: formulated product
a.i.: active ingredient
95%-CLs n.d., Confidence Limits not determined due to mathematical reasons
n.d., not determined due to mathematical reasons
U.L., Upper Limit
*, Confidence Limit over the 2600 mL f.p./ha dosage is a software estimation
**, Confidence Limit over the 780 g a.i../ha dosage is a software estimation

Comments of zRMS:

The study was accepted by zRMS.
The validity criteria was met.

Validity criteria of the study

Criteria	Results
Seedling emergence ≥ 70% in the control:	Plants emergence on day 21 was 100.00% for each species therefore, the validity criterion was met.
The plants do not exhibit visible phytotoxic effects (*e.g.* chlorosis, necrosis, wilting, leaf and stem deformations) and the plants exhibit only normal variation in growth and morphology for that particular species	Plants in the control group do not exhibit visible phytotoxic effects and only normal variation in species growth and morphology were observed, so the validity criterion was met.
The mean plant survival is at least 90% for the duration of the study in the control group	Mean survival of emerged plants was 100.00% at the end of the study for all the tested species, therefore, the validity criterion was met.
Identical environmental conditions for a particular species and growing media with the same amount of soil matrix, support media or substrate.	Met for all the plants' species.

The agreed toxicity endpoints:
Endpoints for the Non target plant species mortality (seedling survival at 21 days)

	Endpoints (mL f.p./ha)			Endpoints (g a.i./ha)		
	NOEC	**LOEC**	**LC_{50}**	**NOEC**	**LOEC**	**LC_{50}**
Cucumis sativus	≥ 2600	> 2600	> 2600 (95%-CLs n.d.)	≥ 780	> 780	> 780 (95%-CLs n.d.)
Phaseolus vulgaris	≥ 2600	> 2600	> 2600 (95%-CLs n.d.)	≥ 780	> 780	> 780 (95%-CLs n.d.)
Solanum lyco-persicon	≥ 2600	> 2600	> 2600 (95%-CLs n.d.)	≥ 780	> 780	> 780 (95%-CLs n.d.)
Beta vulgaris	≥ 2600	> 2600	> 2600 (95%-CLs n.d.)	≥ 780	> 780	> 780 (95%-CLs n.d.)
Brassica oleracea	≥ 2600	> 2600	> 2600 (95%-CLs n.d.)	≥ 780	> 780	> 780 (95%-CLs n.d.)
Zea mays	≥ 2600	> 2600	> 2600 (95%-CLs n.d.)	≥ 780	> 780	> 780 (95%-CLs n.d.)
Lolium perenne	≥ 2600	> 2600	> 2600 (95%-CLs n.d.)	≥ 780	> 780	> 780 (95%-CLs n.d.)
Allium cepa	≥ 2600	> 2600	> 2600 (95%-CLs n.d.)	≥ 780	> 780	> 780 (95%-CLs n.d.)

f.p.: formulated product
a.i.: active ingredient
n.d.: not determined due to mathematical reasons
95%-CLs, Confidence Limits

Endpoints for the non-target plant species shoot fresh weight (biomass at 21 days)

	Endpoints (mL f.p./ha)	Endpoints (g a.i./ha)

	NOEC	LOEC	EC_{25}	EC_{50}	NOEC	LOEC	EC_{25}	EC_{50}
Cucumis sativus	≥ 2600	> 2600	n.d.	> 2600 (95%-CLs n.d.)	≥ 780	> 780	n.d.	> 780 (95%-CLs n.d.)
Phaseolus vulgaris	≥ 2600	> 2600	n.d.	> 2600 (95%-CLs n.d.)	≥ 780	> 780	n.d.	> 780 (95%-CLs n.d.)
Solanum lycopersicon	≥ 2600	> 2600	n.d.	> 2600 (95%-CLs n.d.)	≥ 780	> 780	n.d.	> 780 (95%-CLs n.d.)
Beta vulgaris	≥ 2600	> 2600	n.d.	> 2600 (95%-CLs n.d.)	≥ 780	> 780	n.d.	> 780 (95%-CLs n.d.)
Brassica oleracea	≥ 2600	> 2600	n.d.	> 2600 (95%-CLs n.d.)	≥ 780	> 780	n.d.	> 780 (95%-CLs n.d.)
Zea mays	≥ 2600	> 2600	n.d.	> 2600 (95%-CLs n.d.)	≥ 780	> 780	n.d.	> 780 (95%-CLs n.d.)
Lolium perenne	1300	2600	> 2600 (U.L. n.d.*)	> 2600 (U.L. n.d.* – n.d.)	390	780	> 780 (U.L. n.d.**)	> 780 (CL n.d.** – n.d.)
Allium cepa	≥ 2600	> 2600	n.d.	> 2600 (95%-CLs n.d.)	≥ 780	> 780	n.d.	> 780 (95%-CLs n.d.)

f.p.: formulated product
a.i.: active ingredient
95%-CLs n.d., Confidence Limits not determined due to mathematical reasons
n.d., not determined due to mathematical reasons
U.L., Upper Limit
*, Confidence Limit over the 2600 mL f.p./ha dosage is a software estimation
**, Confidence Limit over the 780 g a.i../ha dosage is a software estimation

Phytotoxicity

At each assessment, plants were visually checked for possible phytotoxic symptoms at 7, 14 and 21 days after the application. Any observed symptom of phytotoxicity was assessed following the EPPO standard PP 1/135(3) guideline criteria. Percent visual injury was reported using the qualitative description of the phytotoxic symptoms (e.g. chlorosis, necrosis, wilting, deformation, etc.).

Concerning the phytotoxicity, for cucumber the NOEC value was estimated to be ≥ 2600 mL f.p./ha. The estimated EC_{50} was > 2600 mL f.p./ha.

For bean the NOEC (phytotoxicity) value was estimated to be ≥ 2600 mL f.p./ha. The estimated EC_{50} was > 2600 mL f.p./ha.

For tomato the NOEC (phytotoxicity) value was estimated to be ≥ 2600 mL f.p./ha. The estimated EC_{50} was > 2600 mL f.p./ha.

For sugar beet the NOEC (phytotoxicity) value was 325 mL f.p./ha. The estimated EC_{50} was > 2600 mL f.p./ha.

For cabbage the NOEC (phytotoxicity) value was 650 mL f.p./ha. The estimated EC_{50} was > 2600 mL f.p./ha.

For corn the NOEC (phytotoxicity) value was estimated to be ≥ 2600 mL f.p./ha. The estimated EC_{50} was > 2600 mL f.p./ha.

For perennial ryegrass the NOEC (phytotoxicity) value was estimated to be ≥ 2600 mL f.p./ha. The estimated EC_{50} was > 2600 mL f.p./ha.

For onion the NOEC (phytotoxicity) value was estimated to be ≥ 2600 mL f.p./ha. The estimated EC_{50} was > 2600 mL f.p./ha.

Endpoints for the Non target plant species phytotoxicity (phytotoxicity at 21 days)

	Endpoints (mL f.p./ha)		Endpoints (g a.i./ha)	
	NOEC	EC_{50}	NOEC	EC_{50}
Cucumis sativus	≥ 2600	> 2600 (95%-CLs n.d.)	≥ 780	> 780 (95%-CLs n.d.)
Phaseolus vulgaris	≥ 2600	> 2600 (95%-CLs n.d.)	≥ 780	> 780 (95%-CLs n.d.)
Solanum lycopersicon	≥ 2600	> 2600 (95%-CLs n.d.)	≥ 780	> 780 (95%-CLs n.d.)
Beta vulgaris	325	> 2600 (95%-CLs n.d.)	97.50	> 780 (95%-CLs n.d.)
Brassica oleracea	650	> 2600 (95%-CLs n.d.)	195	> 780 (95%-CLs n.d.)
Zea mays	≥ 2600	> 2600 (95%-CLs n.d.)	≥ 780	> 780 (95%-CLs n.d.)
Lolium perenne	≥ 2600	> 2600 (95%-CLs n.d.)	≥ 780	> 780 (95%-CLs n.d.)
Allium cepa	≥ 2600	> 2600 (95%-CLs n.d.)	≥ 780	> 780 (95%-CLs n.d.)

f.p.: formulated product
a.i.: active ingredient
95%-CLs n.d., Confidence Limits not determined due to mathematical reasons

Reference:	KCP 10.6.2/02
Report	Effects of PROTIOKONAZOL 300 EC (prothioconazole 300 g/L) on Terrestrial Plant Vegetative Vigour - OECD 227; Mautino G.; 2023; Study Code: 1141.1F.SAG22
Guideline(s):	Yes, OECD 227
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No
Validity criteria of the test:	All validity criteria of this study were met: - seedling emergence ≥ 70%; - the plants do not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis, wilting, leaf and stem deformations) and the plants exhibit only normal variation in growth and morphology for that particular species; - mean plant survival is ≥ 90% for the duration of the study; - identical environmental conditions for a particular species and growing media with the same amount of soil matrix, support media or substrate.

MATERIALS AND METHODS

1. Test material

Test item (chemical/other name):	PROTIOKONAZOL 300 EC
Formulation:	EC
Description (physical state):	liquid
Batch no.:	01/PRO/2022

Production date:	24 March 2022
Expiration date:	March 2025
Stability of test compound:	In order to confirm the concentration of test item active substance in the applied solution, two samples of 200 ml (one for shipment and one as retain sample) of the remaining spray solution were collected immediately after application on treatment 2600 mL f.p./ha (780 g a.i./ha) and placed separately in plastic containers for storage. One sample was sent to the RENOLAB s.r.l. laboratory for the analytical verification and a second one stored as a retained sample.
2. Vehicle and/or positive control:	vehicle control: deionised water positive control: not relevant
3. Test plants:	eight non-target plant species were tested, five dicotyledonous and three monocotyledonous species, representing seven different plant families: Cucumber *Cucumis sativus,* Bean *Phaseolus vulgaris,* Tomato *Solanum lycopersicum,* Sugar beet *Beta vulgaris,* Cabbage *Brassica oleracea,* Corn *Zea mays,* Perennial ryegrass *Lolium perenne* and Onion *Allium cepa*
Test containers:	commercial non-porous plastic, 15.1 cm in diameter, approx. 2L
Seed distribution	manually into the soil one day before application
Irrigation	tap watering after sowing and at least once a week
Soil:	sandy loam, particle size: all particles ≤ 2 mm, C_{org}: 1.6%, pH: 6.97
4. Environmental conditions:	
Temperature:	24.46 ± 0.550 °C (23.70– 25.89 °C)
Relative humidity:	63.9 ± 8.5% (55.3 – 83.0%)
Photoperiod:	controlled light – dark cycles (16h:8h), light intensity: 18900-20000 lux

STUDY DESIGN AND METHODS

Aim of the study was to assess the potential effects of PROTIOKONAZOL 300 EC (prothioconazole 300 g/L) on vegetative vigour of no. 8 different non-target plant species by assessing their emergence, seedling survival, phytotoxicity and shoots' fresh weight of the survived plants at the end of the trial, subsequent to the Test item application on the leaves and above-ground portions of plants. All the experimental procedures were designed by following the OECD No. 227 (2006) test guideline. Eight plants' species were tested, five dicotyledonous and three monocotyledonous species, representing seven plant families: Cucumis sativus, Phaseolus vulgaris, Solanum lycopersicum, Beta vulgaris, Brassica oleracea, Zea mays, Lolium perenne and Allium cepa. Plants were grown from seed until they reached the 2- to 4- true leaf stage. Then, the Test item was sprayed on the leaves and above-ground portions of plants. The study encompassed 6 treatments (no. 5 Test item dosages and one control group). The Test item rate was sprayed by using a water volume of 400 L/ha, simulating typical spray applications. No. 30 plants were sown per plant species and treatment. Immediately after the application, two samples of 200 ml of stock solution were collected for analytical determination of active substance content. Growth stages of plants at 7, 14

and 21 days after the application were recorded according to the BBCH scale. The following parameters were estimated or calculated:

Mortality - seedling survival

At 7, 14 and 21 days after the application the number of living and dead plants were recorded. A plant was considered dead if no living tissues (shoots) were observed at the soil surface. Percent mortality was calculated on the number of survived seedlings in each pot.

Plants fresh weight (biomass)

After 21 days from the application, the effects on plants development were evaluated. The final biomass was measured by weighing the plants' fresh shoots. Biomass was defined as all portions of the plant above the soil surface, without roots. The percent reduction in plants' biomass was compared to that of the control group.

Phytotoxicity

At each assessment, plants were visually checked for possible phytotoxic symptoms at 7, 14 and 21 days after the application. Any observed symptom of phytotoxicity was assessed following the EPPO standard PP 1/135(3) guideline criteria. Percent visual injury was reported using the qualitative description of the phytotoxic symptoms (e.g. chlorosis, necrosis, wilting, deformation, etc.).

Test design:	30 plants were sown per treatment and crop species
Exposure time:	21 days after the spraying
Tested concentrations, definitive test:	162.50, 325, 650, 1300 and 2600 ml/ha (48.75, 97.50, 195, 390 and 780 g a.s./ha) + 400 mL/ha
Dates:	start of the study 18.10.2022 start of the experimental part: 01.12.2022 end of the experimental part: 22.12.2022 end of the study: 06.03.2023
Statistic:	Software used for statistical analysis was “ToxRatPro”, version 3.3.0. Shoot fresh weight at 21 days was evaluated using Dunnett‘s t-test; Bonferroni-Holm U-test and Williams t-test. The EC25 and EC50 were calculated where possible. The No Observed Effect Concentration (NOEC) and Lowest Observed Effect Concentration (LOEC) values for plants' seedling survival and shoot fresh weight were provided.

CONCLUSION

The results are summarised in following tables:

Table KCP 10.6.2-4: Endpoints for the Non target plant species mortality (seedling survival at 21 days)

	Endpoints (mL f.p./ha)			Endpoints (g a.i./ha)		
	NOEC	**LOEC**	**LC_{50}**	**NOEC**	**LOEC**	**LC_{50}**
Cucumis sativus	≥ 2600	> 2600	> 2600 (95%-CLs n.d.)	≥ 780	> 780	> 780 (95%-CLs n.d.)
Phaseolus vulgaris	≥ 2600	> 2600	> 2600 (95%-CLs n.d.)	≥ 780	> 780	> 780 (95%-CLs n.d.)
Solanum lyco-	≥ 2600	> 2600	> 2600 (95%-CLs	≥ 780	> 780	> 780 (95%-CLs n.d.)

persicon			n.d.)			
Beta vulgaris	≥ 2600	> 2600	> 2600 (95%-CLs n.d.)	≥ 780	> 780	> 780 (95%-CLs n.d.)
Brassica oleracea	≥ 2600	> 2600	> 2600 (95%-CLs n.d.)	≥ 780	> 780	> 780 (95%-CLs n.d.)
Zea mays	≥ 2600	> 2600	> 2600 (95%-CLs n.d.)	≥ 780	> 780	> 780 (95%-CLs n.d.)
Lolium perenne	≥ 2600	> 2600	> 2600 (95%-CLs n.d.)	≥ 780	> 780	> 780 (95%-CLs n.d.)
Allium cepa	≥ 2600	> 2600	> 2600 (95%-CLs n.d.)	≥ 780	> 780	> 780 (95%-CLs n.d.)

f.p.: formulated product
a.i.: active ingredient
n.d.: not determined due to mathematical reasons
95%-CLs, Confidence Limits

Table KCP 10.6.2-5: Endpoints for the Non target plant species shoot fresh weight (biomass at 21 days)

	Endpoints (mL f.p./ha)				Endpoints (g a.i./ha)			
	NOEC	**LOEC**	**EC_{25}**	**EC_{50}**	**NOEC**	**LOEC**	**EC_{25}**	**EC_{50}**
Cucumis sativus	≥ 2600	> 2600	n.d.	> 2600 (95%-CLs n.d.)	≥ 780	> 780	n.d.	> 780 (95%-CLs n.d.)
Phaseolus vulgaris	≥ 2600	> 2600	n.d.	> 2600 (95%-CLs n.d.)	≥ 780	> 780	n.d.	> 780 (95%-CLs n.d.)
Solanum lycopersicon	≥ 2600	> 2600	n.d.	> 2600 (95%-CLs n.d.)	≥ 780	> 780	n.d.	> 780 (95%-CLs n.d.)
Beta vulgar-is	≥ 2600	> 2600	n.d.	> 2600 (95%-CLs n.d.)	≥ 780	> 780	n.d.	> 780 (95%-CLs n.d.)
Brassica oleracea	≥ 2600	> 2600	n.d.	> 2600 (95%-CLs n.d.)	≥ 780	> 780	n.d.	> 780 (95%-CLs n.d.)
Zea mays	≥ 2600	> 2600	n.d.	> 2600 (95%-CLs n.d.)	≥ 780	> 780	n.d.	> 780 (95%-CLs n.d.)
Lolium perenne	1300	2600	> 2600 (U.L. n.d.*)	> 2600 (U.L. n.d.* – n.d.)	390	780	> 780 (U.L. n.d.**)	> 780 (U.L. n.d.** – n.d.)
Allium cepa	≥ 2600	> 2600	n.d.	> 2600 (95%-CLs n.d.)	≥ 780	> 780	n.d.	> 780 (95%-CLs n.d.)

f.p.: formulated product
a.i.: active ingredient
95%-CLs n.d., Confidence Limits not determined due to mathematical reasons
n.d., not determined due to mathematical reasons
U.L., Upper Limit
*, Confidence Limit over the 2600 mL f.p./ha dosage is a software estimation
**, Confidence Limit over the 780 g a.i../ha dosage is a software estimation

A 2.6.3 KCP 10.6.3 Extended laboratory studies on non-target plants

Not relevant. No studies submitted.

A 2.7 KCP 10.7 Effects on other terrestrial organisms (flora and fauna)

Not relevant. No studies submitted.

A 2.8 KCP 10.8 Monitoring data

Not relevant. No studies submitted.