FINAL REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: **CHR/ZF/PROTI 100 FS** Product name(s):

Gamelan 100 FS

Doraltes 100 FS

Chemical active substance:

Prothioconazole, 100 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: Innvigo Sp. z o.o.

Submission date: 05.2022

MS Finalisation date: 05/09/2022

Version history

When	What
August 2021	Submission to the Polish Ministry of Agriculture and Rural Development
October 2021	Dossier sent for evaluation
February 2022	Submission updated version of dRR
May 2022	Submission updated version of dRR
June 2022	zRMS evaluation of dRR
September 2022	Final version prepared by zRMS after Commenting period

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9 Ecotoxicology (KCP 10)

New and additional information are highlighted in yellow.

Review Comments:

This application was submitted by Innvigo Sp. z o.o. for approval of the formulation CHR/ZF/PROTI 100 FS – Gamelan 100 FS/ Doraltes 100 FS containing 100 g/L of prothioconazole for use as fungicide seed treatment on winter cereals.

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

Since this document is based on the information provided by the Applicant, all review comments, additions, and corrections have been made using commenting boxes or highlighted in grey. Any incorrect data or text not evaluated by the zRMS has been crossed out.

9.1 Critical GAP and overall conclusions

Table 9.1-1: Table of critical GAPs

GAP rev. 2022-May1 ,

date:

PPP	(product CHR/ZF/PROTI name/code)	Formulation type:	FS
Active substance 1:	prothioconazole	Conc. of as 1:	100,0 g/l
Active substance 2:	n/a	Conc. of as 2:	n/a
Active substance….:	n/a	Conc. of as ….:	n/a
Safener:	-	Conc. of safener:	conc. (c)
Synergist:	-	Conc. of synergist:	conc. (c)
Applicant: Innvigo Professional use:	Zone(s): central	Non professional use:	☒ / ☐

Verified by MS: yes/~~no~~

Field of use: Seed treatment

1	2	3	4	5	6	7	8	9	10	11	12	13	14	
UseNo. (e)	**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 per treatment**			**PHI** (days)	**Remarks:** e.g. g safener/synergist per ha (f)	
					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			
Zonal uses (field or outdoor uses, certain types of protected crops)														

1														
2														
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)														
UseNo. (e)	**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)	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	**PHI** (days)	**Remarks:** e.g. g safener/synergist per ha (f)	Sowing rate
3	PL	Winter wheat	F	*Tilletia caries, Fusarium sp.,*	winter seed	BBCH 00	a)1 b)1	n/a	a) Max. 1.0 l /t seed	a) 0.018-0.025 kg	max. 0.7	n/a	Sowing rate: 180-250 kg/ha	Sowing rate: 180-
		(TRZAW)		*Microdochium majus, Ustilago tritici*	treatment	~~n/a~~			b) Max. 1.0 l /t seed	a.s/ha b) 0.0180.025 kg a.s/ha	L/100 kg seed			250 kg/ha Weigh of 100 grains: 50 g
4	PL	Winter triticale (TTLWI)	F	*Fusarium sp., Microdochium majus*	winter seed treatment	BBCH 00 ~~n/a~~	a)1 b)1	n/a	a) Max. 1.0 l /t seed b) Max. 1.0 l /t seed	a) 0.0150.025 kg a.s/ha b) 0.0150.025 kg a.s/ha	max. 0.7 L/100 kg seed	n/a	Sowing rate: 150-250 kg/ha	Sowing rate: 150250 kg/ha Weigh of 100

														grains: 50 g
5	PL	Winter rye (SECCW)	F	*Fusarium sp., Microdochium majus, Urocystis occulta*	winter seed treatment	BBCH 00 ~~n/a~~	a)1 b)1	n/a	a) Max. 1.0 l /t seed b) Max. 1.0 l /t seed	a) 0.00950.025 kg a.s/ha b) 0.00950.025 kg a.s/ha	max. 0.7 L/100 kg seed	n/a	Sowing rate: 95250 kg/ha	Sowing rate: 95250 kg/ha Weigh of 100 grains: 50 g
Minor uses according to Article 51 (zonal uses)														
6														
7														
Minor uses according to Article 51 (interzonal uses)														
8														
9														

Remarks table
(a) e.g. wettable powder (WP), emulsifiable concentrate (EC), granule (GR)
(b) Catalogue of pesticide formulation types and international coding system CropLife International Technical Monograph n°2, 6th Edition Revised May 2008
(c)
(d) Select relevant
(e) Use number(s) in accordance with the list of all intended GAPs in Part B, Section 0 should be given in column 1
heading: g/kg or g/l
(f) No authorization possible for uses where the line is highlighted in grey, Use should be crossed out when the notifier no longer supports this use.

Remarks columns:

1 Numeration necessary to allow references
2 Use official codes/nomenclatures of EU Member States
3 For crops, the EU and Codex classifications (both) should be used; when 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 nonprofessional field use, G: professional greenhouse use, Gn: non-professional greenhouse use, Gpn: professional and non-professional greenhouse use, I: indoor application Scientific names and EPPO-Codes of target pests/diseases/ weeds or, when relevant, the common names of the pest groups (e.g. biting and sucking insects, soil born insects, foliar fungi, weeds) and the developmental stages of the pests and pest groups at the moment of application must be named.
5 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.
6 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
7 The maximum number of application possible under practical conditions of use must be provided.
8 Minimum interval (in days) between applications of the same product
9 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.
10 The dimension (g, kg) must be clearly specified. (Maximum) dose of a.s. per treatment (usually g, kg or L product / ha).
11 If water volume range depends on application equipments (e.g. ULVA or LVA) it should be mentioned under “application: method/kind”.
12 PHI - minimum pre-harvest interval
13 Remarks may include: Extent of use/economic importance/restrictions

Column 15: zRMS conclusion.

A	Acceptable
R	Acceptable with further restriction
C	To be confirmed by cMS
N	Not acceptable / evaluation not possible
n.r.	Not relevant for section 3

Review Comments:

GAP presented in the Table 9.1-1 of this document is revised with consideration of the outcome of the evaluation performed in area of ecotoxicology.

9.1.1 Overall conclusions

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

The 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.

CHR/ZF/PROTI 100 FS pose no unacceptable risk to birds and mammals used according to the label.

Birds

Acute risk due to the use of CHR/ZF/PROTI in cereals is acceptable.

Long-term risk for use of the product in cereals showed unacceptable risk at TIER 1. Higher risk assessment was performed.

For use in cereals the refinement based on DT_{50} for prothioconazole and its relevant metabolite Prothioconazole-desthio (M4) was provided and accepted.

The acceptability of the refinement should be considered at the MS level.

Mammals

Acute risk due to the use of CHR/ZF/PROTI in cereals is acceptable.

Chronic/reproductive risk due to the use of CHR/ZF/PROTI in cereals was not acceptable for both granivorous and omnivorous birds at Tier 1, therefore further risk refinement is necessary.

For use in cereals the refinement based on DT_{50} for prothioconazole and its relevant metabolite Prothioconazole-desthio (M4) was provided and accepted. Wood mouse was chosen as a focal species. And refinement of PT was also accepted, taking to consideration BBCH and type of crop.

The acceptability of the refinement should be considered at the MS level.

Since CHR/ZF/PROTI is a seed treatment used in cereals (i.e. seeds attractive to birds and mammals as a food source) to minimise the exposure for birds and mammals inclusion of following safety phrases on the label should be respected:

SPe5: To protect birds/wild mammals the treated seeds should be entirely incorporated in the soil; ensure that the treated seeds are also fully incorporated at the end of rows. SPe6: To protect birds/wild mammals remove spillages.

9.1.1.2 Effects on aquatic organisms (KCP 10.2)

CHR/ZF/PROTI 100 FS pose no unacceptable risk according to the label No mitigation measures are required. According to calculation method of ecotoxicological classification and data obtained from acute aquatic toxicity tests, product CHR/ZF/PROTIO 100 FS is classified as Aquatic Chronic 2, H411.

~~with appropriate buffer zone.~~

9.1.1.3 Effects on bees (KCP 10.3.1)

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

CHR/ZF/PROTI 100 FS pose no unacceptable risk to bees according to the label.

Concerned Member States must decide on the consideration of data requirements of the EFSA Bee guidance (2013) on national level.

9.1.1.4 Effects on arthropods other than bees (KCP 10.3.2)

The risk to non-target arthropods is assessed using the approach recommended in the published ESCORT 2 *document* (Candolfi *et al.* 2001)[1] and the *EC Guidance Document on Terrestrial Ecotoxicology.*

CHR/ZF/PROTI 100 FS pose no unacceptable risk to non-target arthropods other than bees according to the label

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

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

CHR/ZF/PROTI 100 FS pose no unacceptable risk to earthworms and other non-target soil organisms according to the label

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

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.

CHR/ZF/PROTI 100 FS pose no unacceptable risk to non-target plants according to the label.

No mitigation measures are required.

9.1.1.7 Effects on other terrestial organisms (flora and founa) (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 CHR/ZF/PROTI grouped according to crop, application rate, number of applications, timing criterion

Group	Intended uses	relevant use parameters for grouping	relevant parameter or value

[1] Candolfi MP, Barrett KL, Campbell PJ, Forster R, Grandy N, Huet M-C, Lewis G, Oomen PA, Schmuck R, Vogt H (2000) 'Guidance Document on regulatory testing procedures for plant protection products with non-target arthropods' from the workshop, European Standard Characteristics of Non-Target Arthropod Regulatory Testing (ESCORT 2) 21-23 March 2000.

Terrestrial vertebrates (Birds and Mammals; 9.2 and 9.3)	According to GAP	Scenarios according to EFSA Birds and Mammals Guidance (2009) The use in wheat, triticale and rye covered by winter cereals.	Cereals - 'Small seeds scenario' - Wheat- 0.018-0.025 kg a.s/ha Triticale: 0.015-0.025 kg a.s/ha Rye: 0.0095-0.025 kg a.s/ha Crop, application rate, timing criterion
Aquatic organisms (9.5)	According to GAP	Crops according to FOCUS surface water guidance (2015)[2]	FOCUS modelling**,** for detalis see Part B 8
Bees (9.6)	Generic risk envelope covering all product uses	Risk assessments are based on the maximum application rate	Maximum single application rate Wheat: 0.025 kg a.s/ha
Terrestrial nontarget arthropods other than bees (9.7)	According to GAP In-field	In-field ~~and off-field~~ risk assessments are based on the maximum application rate for each type of crops	Application rate and number of uses
	~~According to GAP Off-field~~		~~Crop type (height), application rate and number of uses~~
Soil meso- and macrofauna / soil microorganisms (9.8 and **9.9)**	Generic risk envelope covering all product uses	Risk assessments are based on the maximum application rate	Worst case PECsoil value taken from Section 8 (Environmental Fate)
Non-target terrestrial plants (9.10)	According to COMMISSION REGULATION (EU) No 284/2013: ,,Data for non-target terrestrial plants are: ,,where exposure is negligible, for example in the case of rodenticides, active substances used for wound protection or seed treatment, or in the case of active substances used on stored products or in glasshouses where exposure is precluded''		
~~**Non-target terrestrial plants (9.10)**~~	~~According to GAP~~	~~Risk assessments are based on the maximum application rate for winter cereals~~	~~Maximum application rate and worst case drift rate~~

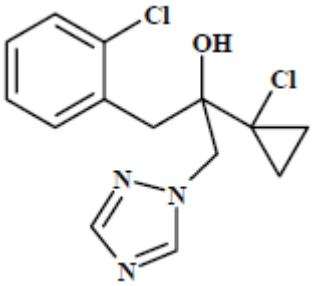
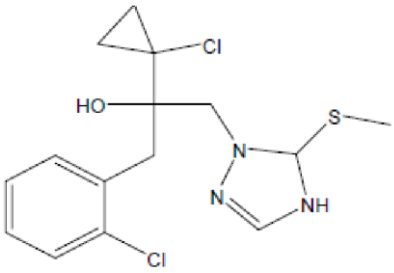
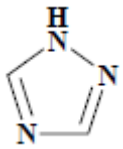
9.1.3 Consideration of metabolites

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 CHR/ZF/PROTI is indicated in the table.

Table 9.1-3 Metabolites of prothioconazole

Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?

[2] FOCUS (2015): Generic guidance for FOCUS surface water Scenarios. Version 1.4.

Prothioconazoledesthio		312.2	Soil: 49.4% Water: 56% Sediment: 26.9%	Yes, for water and soil.
ProthioconazoleS-methyl		358.8	Soil: 49.0%	Yes, for soil
1,2,4- triazole		69.065	Water/Sedminet: 32.7%	Yes, for water

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 metabolite Prothioconazole-desthio. Full details of these studies are provided in the respective EU DAR and related documents.
Effects on birds of CHR/ZF/PROTI were not evaluated as part of the EU assessment of prothioconazole.

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

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

Species	Substance	Exposure System	Results	Reference
Bobwhite quial	Prothioconazole	Acute	**LD50 > 2000 mg a.s./kg bw**	EFSA Scientific Report (2007) 106, 1-98
Bobwhite quail	Prothioconazole	5 d dietary	LC50 > 5000 mg a.s./kg diet calc. LD50 > 1413 mg a.s./kd bw/day	EFSA Scientific Report (2007) 106, 1-98

Species	Substance	Exposure System	Results	Reference
Mallard duck	Prothioconazole	5d dietary	LC50 > 5000 mg a.s./kg diet calc. LD50 > 2547 mg a.s./kg bw/day	EFSA Scientific Report (2007) 106, 1-98
Bobwhite quial	Prothioconazole	Reproduction 22 w dietary	NOEC ≥ 1000 mg a.s./kg diet calc. NOEL ≥ 86 mg a.s./kg bw/day	EFSA Scientific Report (2007) 106, 1-98
Mallard duck	Prothioconazole	Reproduction 21 w dietary	NOEC= 700 mg a.s./kg diet **calc. NOEL = 78 mg a.s./kg bw/day**	EFSA Scientific Report (2007) 106, 1-98
Bobwhite quial	Prothiconazoledesthio	Acute	**LD50 > 2000 mg p.m./kg b.w**	EFSA Scientific Report (2007) 106, 1-98
Bobwhite quial	Prothioconazoledesthio	5 d dietary	LC50 = 4090 mg p.m./kg diet calc. LD_{50} > 297 mg p.m./kg bw/d	EFSA Scientific Report (2007) 106, 1-98
Bobwhite quial	Prothioconazoledesthio	Reproduction 20 w dietary	NOEC = 173 mg p.m./kg diet **calc. NOEL= 14.8 mg p.m./kg bw/day**	EFSA Scientific Report (2007) 106, 1-98
Mallard duck	Prothioconazoledesthio	Reproduction 20 w dietary NOEC ≥ 500 mg	p.m./kg diet calc. NOEL = 63 mg p.m./kg bw/day	EFSA Scientific Report (2007) 106, 1-98

9.2.2 Risk assessment for seed treatments

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 (wheat, rye and triticale as winter cereals).

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

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

Birds feeding on treated seeds. Selection of generic focal species according to EFSA B&M guidance

Type of seeds, corresponding generic focal species and their food intake rate per body weight

Type of seeds	Generic focal species	FIR/bw
'Large seeds' (maize, beans or peas)	Large granivorous bird	0.1
'Small seeds' (not maize, beans or peas)	Small granivorous bird	0.3

According to EFSA „Prothioconazole belongs to the class of fungicides which are commonly referred to as the triazoles. This class of fungicides includes compounds such as epoxiconazole and flusilazole. It is a systemic fungicide with protective curative and eradicative activity. Its mode of action is steroid demethylation (ergosterol biosynthesis)''. Thus, Tier-1 acute and reproductive risk assessments for birds feeding on crop seedlings emerging from treated seeds have to be addressed.

Generic focal species and corresponding shortcut values for assessment of residues present in newly emerged crop shoots Generic focal species Short-cut value (SV) for acute risk**

Small omnivorous bird	0.5 x NAR*/5
*NAR-nominal loading/application rate of a.s in mg/kg seed	

**For the reproductive assessment, these shortcut values should be combined with appropriate time windows and default degradation/dissipation rates for residues (see equation below).

Calculation of the daily dry soil dose for the reproductive risk assessment for small omnivorous bird
DDSDrepro for birds= 0.025 x dosage in kg a.s/ha

For treated seeds

DDDA [mg/kg bw] = Nominal Application Rate (NAR) [mg/kg] x FIR/bw
DDDLT [mg/kg bw] = Nominal Application Rate (NAR) [mg/kg] x FIR/bw x ftwa

For seedlings

DDDA [mg/kg bw] = Residue concentration (initial) [mg/kg] x FIR/bw
DDDLT [mg/kg bw] = Residue concentration (initial) [mg/kg] x FIR/bw x ftwa

Where:

DDD daily dietary dose
FIR/bw food intake rate related to body weight
ftwa time weighted average factor (long-term considerations; default value: 0.53)

Table 9.2-2: First-tier assessment of the acute risk for birds due to the use of CHR/ZF/PROTI in cereals- Prothioconazole

Scenario	Species	LD_{50} (mg a.s./kg bw)	FIR/bw [a]	NAR (mg a.s./kg seeds)[b]	DDD ~~ETE~~ [mg a.s. /kg b.w./d]	TER [d]	Trigger value
Treated seed	Small granivorous bird	>2000	0.3	100	30.0	66.7	10
Newly emergent crop shoots	Small omnivorous bird	>2000	0.5	100/5	10.0	200.0	10

a) FIR: Food intake rate
b) NAR: Nominal loading/application rate of active substance in mg/kg seed.
c) ETE: Estimated theoretical exposure
d) TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Table 9.2-3: First-tier assessment of the acute risk for birds due to the use of CHR/ZF/PROTI in cereals- Prothioconazole-desthio

Scenario	Species	LD_{50} (mg a.s./kg bw)	FIR/bw	NAR (mg a.s./kg seeds)[b]	DDD ~~ETE~~ [mg a.s. /kg b.w./d]	TER	Trigger value
Treated seed	Small granivorous bird	>2000	0.3	104.1	31.23	64.04	10
Newly emergent crop shoots	Small omnivorous bird	>2000	0.5	104.1/5	10.41	192.12	10

a) FIR: Food intake rate
b) NAR: Nominal loading/application rate of active substance in mg/kg seed.
c) ETE: Estimated theoretical exposure
d) TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

NAR value was calculated with assumption that 100% of Prothioconazole transform into Prothioconazole-desthio. Difference between molar masses were included.

Acute risk due to the use of CHR/ZF/PROTI in cereals is acceptable for both granivorous and omnivorous birds, therefore no further risk refinement is necessary.

Table 9.2-4: First-tier assessment of the chronic/reproductive risk for birds due to the use of CHR/ZF/PROTI in cereals- Prothioconazole

Scenario	Species	LD_{50} (mg a.s./kg bw)	FIR/bw [a]	NAR (mg a.s./kg seeds)[b]	DDD ~~ETE~~ [mg a.s. /kg b.w./d]	TER [d]	Trigger value
Treated seed	Small granivorous bird	78	0.3	100	30.0	**1.17**	5 ~~10~~
Newly emergent crop shoots	Small omnivorous bird	78	0.5	100/5	10.0	7.8	5 ~~10~~

a) FIR: Food intake rate
b) NAR: Nominal loading/application rate of active substance in mg/kg seed.
c) ETE: Estimated theoretical exposure
d) TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Table 9.2-5: First-tier assessment of the chronic/reproductive risk for birds due to the use of CHR/ZF/PROTI in cereals- Prothioconazole-desthio (M4)

Scenario	Species	LD_{50} (mg a.s./kg bw)	FIR/bw	NAR (mg a.s./kg seeds)[b]	DDD ~~ETE~~ [mg a.s. /kg b.w./d]	TER	Trigger value
Treated seed	Small granivorous bird	14.8	0.3	104.1	31.23	**0.474**	5
Newly emergent crop shoots	Small omnivorous bird	14.8	0.5	104.1/5	10.41	**1.422**	5

a) FIR: Food intake rate
b) NAR: Nominal loading/application rate of active substance in mg/kg seed.
c) ETE: Estimated theoretical exposure
d) TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

NAR value was calculated with assumption that 100% of Prothioconazole transform into Prothioconazole-desthio. Difference between molar masses were included.

Chronic/reproductive risk due to the use of CHR/ZF/PROTI in cereals is not acceptable for both granivorous and omnivorous birds, therefore further risk refinement is necessary.

9.2.2.2 Higher-tier risk assessment

~~Not required~~

New study were performed to redefine risk for birds while using CHR/ZF/PROTI 100 FS: *Determination of Residues of Prothioconazole and PTZ-Desthio in Winter Wheat Seeds and Seedlings after Drilling of Coated Seeds at 4 Sites in Central Europe in 2021*, Appeltauer, A., 2022.
The objective of this study was to determine the residues of prothioconazole and PTZ-desthio in winter wheat seedlings and seeds after drilling of untreated and coated seeds treated with CHR/ZF/PROTI 100 FS under representative growing conditions in Central Europe in the field. The residues and degradation kinetics of Prothioconazole and PTZ-desthio were investigated.
Samples of seeds were taken before application in the control and the test item treatment and up to 10 days after application in the test item treatment. Samples of seedlings were taken 0 days after emergence

(0DAE) in the control and the test item treatment and up to 8DAE in the test item treatment.
For the two specimen types, the intended sampling schedule was as follows: for seeds the first sampling was before the application 0DBA (control and test item treatment) and 1, 2, 3, 5, 7, and 10 days after application (test item treatment). For seedlings the first sampling was performed 0 days after emergence (control and test item treatment), 2, 3, 4 and 8 days after emergence (test item treatment).
In all trials no residues of prothioconazole or PTZ-desthio at or above the LOD were detected in control samples of untreated seeds

Residues in seeds

Highest residues of prothioconazole in treated seeds were determined in samples taken directly before application (0DBA) with 73.2 mg/kg (trial -01), 60.4 mg/kg (trial -02), 71.0 mg/kg (trial -03) and 72.2 mg/kg (trial -04), respectively. In trials -01 and -02 residues of prothioconazole in seeds decreased subsequently to the last sampling (10DAA). In trial -03 an outlier was identified at sampling S4. Here an unexplainable high value with unknown source occurred (60.5 mg/kg). This was most likely due to a mistake during the field phase of the study. Excluding this outlier, residues of prothioconazole decreased from the first to the last sampling (10DAA) in trial -03. In trial -04 residues decreased to 1.85 mg/kg at sampling S4 (3DAA) and increased slightly to 2.30 mg/kg until sampling S6 (7DAA). At the last sampling lowest residues were observed in all trials between 1.21 mg/kg (trial -03) and 1.78 mg/kg (trial 01).

Highest residues of PTZ -desthio in treated seeds were observed at sampling S2 (1DAA) in all trials with 18.6 mg/kg (trial -01), 18.1 mg/kg (trial -02), 24.1 mg/kg (trial -03) and 11.7 mg/kg (trial -04). In trial -01 residues decreased to 12.7 mg/kg at sampling S4 (3DAA) and increased again to the last sampling to 16.4 mg/kg. In trial -02 residues decreased to 8.48 mg/kg at sampling S4 (3DAA) and increased again to 10.3 mg/kg at sampling S5 (5DAA). Subsequently residues decreased to 5.78 mg/kg at the last sampling (9.8 mg/kg). In trial -03 an outlier was identified at sampling S4. Excluding this outlier, residues of PTZdesthio decreased to 3.68 mg/kg at sampling S6 (7DAA) and increased to 5.06 mg/kg at the last sampling (10DAA). In trial -04 residues decreased to 5.94 mg/kg at sampling S3 (2DAA), increased again to

7.54 mg/kg at sampling S5 (7DAA) and decrease to 5.26 mg/kg at the last sampling (10DAA).

An overview of all residue values of prothioconazole and PTZ -desthio in seeds of trials S21-06525-01 to -04 is given in the following table:

Residues of prothioconazole and PTZ-desthio in seeds

Sampling	Timing (DAA)[1)] -01 / -02 / -03 / -04	Trial S21-06525			
		-01	-02	-03	-04
		Prothioconazole [mg/kg]			
S1	0.1 / 0.2 / 0.2 / 0.0DBA*	n.d.	n.d.	n.d.	n.d.
S1	0.1 / 0.2 / 0.2 / 0.0DBA*	73.2	60.4	71.0	72.2
S2	0.9 / 1.0 / 1.3 / 1.0	25.2	28.6	16.4	51.8
S3	2.0 / 1.9 / 2.1 / 2.0	4.58	8.06	9.86	3.72
S4	3.1 / 2.8 / 3.0 / 3.0	2.08	3.62	60.5[a]	1.85
S5	4.9 / 4.7 / 4.9 / 5.0	2.04	2.64	2.40	1.99
S6	7.1 / 6.7 / 7.0 / 7.0	1.78	1.77	1.39	2.30
S7	10.0 / 9.6 / 10.1 / 10.0	1.78	1.48	1.21	1.26
		PTZ-desthio [mg/kg]			
S1	0.1 / 0.2 / 0.2 / 0.0DBA*	n.d.	n.d.	n.d.	n.d.
S1	0.1 / 0.2 / 0.2 / 0.0DBA*	1.70	1.31	1.81	1.86
S2	0.9 / 1.0 / 1.3 / 1.0	18.6	18.1	24.1	11.7
S3	2.0 / 1.9 / 2.1 / 2.0	16.1	13.9	16.5	5.94
S4	3.1 / 2.8 / 3.0 / 3.0	12.7	8.48	2.17[a]	6.06
S5	4.9 / 4.7 / 4.9 / 5.0	12.9	10.3	5.14	7.54
S6	7.1 / 6.7 / 7.0 / 7.0	15.0	6.64	3.68	7.16
S7	10.0 / 9.6 / 10.1 / 10.0	16.4	5.78	5.06	5.26

DBA: days before application, DAA: days after application
LOQ: Level of quantification (0.01 mg/kg); LOD: level of detection (0.003 mg/kg)
* Control sample C
[a] value was identified as outlier according to expert judgement and was not used for calculation of mean value and for kinetic calculation; value represents the mean of three analyses (PTZ: first analysis: 61.2 mg/kg, second analysis: 57.2 mg/kg, third analysis: 63.0 mg/kg, PTZ-desthio: first analysis: 2.06 mg/kg, second analysis: 2.20 mg/kg, third analysis: 2.24 mg/kg)
[1)] calculated from end of application to time on dry ice or time in freezer

Prothioconazole in seeds

In trial -01 for seed samples all of the models achieved the critical values < 15 for χ^2-error and for the determination coefficient of $r^2 > 0.85$. The DFOP and HS models showed either negative confidence intervals or were not statistically significant. For the SFO model the calculated χ^2-error was 7.05 and the calculated r^2 was 0.9978. The results of the calculation showed a DT_{50} value of 0.57 days and a DT_{90} value of 1.88 days, respectively.

In trial -02 for seed samples all of the models achieved the critical values < 15 for χ^2-error and for the determination coefficient of $r^2 > 0.85$. The DFOP and HS models showed either negative confidence intervals or were not statistically significant. For the SFO model the calculated χ^2-error was 11.30 and the calculated r^2 was 0.9893. The results of the calculation showed a DT_{50} value of 0.78 days and a DT_{90} value of 2.61 days, respectively.

In trial -03 for seed samples all of the models achieved the critical values < 15 for χ^2-error and for the determination coefficient of $r^2 > 0.85$. The HS model showed negative confidence intervals and was not statistically significant. For the SFO model the calculated χ^2-error was 7.20 and the calculated r^2 was 0.9978. The results of the calculation showed a DT_{50} value of 0.66 days and a DT_{90} value of 2.19 days, respectively.

In trial -04 for seed samples none of the models achieved the critical value < 15 for χ^2-error. All models achieved the critical value of $r^2 > 0.85$ for the determination coefficient. The FOMC, DFOP and HS model showed negative confidence intervals or were not statistically significant. For the SFO model the calculated χ^2-error was 34.31 and the calculated r^2 was 0.9093. The results of the calculation showed a

DT_{50} value of 0.92 days and a DT_{90} value of 3.04 days, respectively.

$f_{twa} = (1 – e^{-kt})/ kt$, where:

f_{twa} – time weighted average factor
$k = ln(2)/DT_{50}$
t – averaging time (21 days)
DT_{50} – average DT_{50} value of 0.7325 d

$f_{twa} = 0.0503$

Table 9.2-6: Higher-tier assessment of the chronic/reproductive risk for birds due to the use of CHR/ZF/PROTI in cereals- Prothioconazole

Scenario	Species	NAR (mg a.s./kg seeds)[b]	f_{twa}	DDD (mg a.s./kg bw)	TER [d] Trigger [a]	ETE [mg a.s./kg b.w./d] [c]	NOEL FIR/bw value
Treated granivorous seed bird	Small	78	0.3	100	0.0503	1.509	51.69 5

a) FIR: Food intake rate
b) NAR: Nominal loading/application rate of active substance in mg/kg seed.
c) ETE: Estimated theoretical exposure
d) TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Prothioconazole-desthio in seeds.
In trial -01 for seed samples all of the models achieved the critical values < 15 for χ^2-error. None of the models achieved the critical value for the determination coefficient of $r^2 > 0.85$. The SFO, DFOP and HS models showed either negative confidence intervals and were not statistically significant. Therefore, none of the models can be taken for the calculation of degradation kinetics.
In trial -02 for seed samples the SFO and DFOP models achieved the critical values < 15 for χ^2-error. The DFOP model achieved the critical value for the determination coefficient of $r^2 > 0.85$. The DFOP and HS models showed either negative confidence intervals or were not statistically significant. For the SFO model the calculated χ^2-error was 14.30 and the calculated r^2 was 0.8083. The results of the calculation showed a DT_{50} value of 4.59 days and a DT_{90} value of 15.24 days, respectively.
In trial -03 for seed samples the SFO and DFOP models achieved the critical values < 15 for χ^2-error. All models achieved the critical value for the determination coefficient of $r^2 > 0.85$. The DFOP and HS models showed either negative confidence intervals or were not statistically significant. For the SFO model the calculated χ^2-error was 14.75 and the calculated r^2 was 0.952. The results of the calculation showed a DT_{50} value of 1.97 days and a DT_{90} value of 6.54 days, respectively.
In trial -04 for seed samples the DFOP and HS models achieved the critical values < 15 for χ^2-error and the critical value for the determination coefficient of $r^2 > 0.85$. The SFO, DFOP and HS models showed either negative confidence intervals or were not statistically significant. Therefore, none of the models can be taken for the calculation of degradation kinetics.

$f_{twa} = (1 – e^{-kt})/ kt$, where:

f_{twa} – time weighted average factor
$k = ln(2)/DT_{50}$
t – averaging time (21 days)

f_{twa} = 0.22267

According to maximum Prothioconazole and Prothioconazole-desthio residue values adjustment factor was estimated for all locations based on formation fraction of metabolite.

Table 9.2-7: Estimation of Adjustment factor for Prothioconazole-Desthio.

Trial	Prothioconazole residues [mg/kg]	Prothioconazole-desthio residues [mg/kg]	ff	Adjustment factor: 21-d f_{TWA} × ff
-01	73.2	18.6	0.2645	0.0589
-02	60.4	18.1	0.3120	0.0695
-03	71.0	24.1	0.3534	0.0787
-04	72.2	11.7	0.1687	0.0376
			Average adjustment factor value	**0.06118**

Table 9.2-8: Higher-tier assessment of the chronic/reproductive risk for birds due to the use of CHR/ZF/PROTI in cereals- Prothioconazole-desthio

Scenario	Species	NOEL (mg a.s./kg bw)	FIR/bw [a]	NAR (mg a.s./kg seeds) [b]	Adjustment factor (ff ×f_{twa})	ETE [mg a.s. /kg b.w./d] [c]	TER [d]	Trigger value
Treated seed	Small granivorous bird	14.8	0.3	104.1	0.06118	1.91	7.75	5

a) FIR: Food intake rate
b) NAR: Nominal loading/application rate of active substance in mg/kg seed.
c) ETE: Estimated theoretical exposure
d) TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Three relevant focal species should be considered for higher-tier risk assessment for birds: Skylark, Yellowhammer and Linnet.

Table 9.2-9: Higher-tier assessment of the chronic/reproductive risk for birds due to the use of CHR/ZF/PROTI in cereals- Prothioconazole-desthio

Species	Skylark	Yellowhammer	Linnet
Treatment	104.1 mg of desthio/kg seed		
Body weight	28.5	23	15.3
ftwa	1	1	1
FIR	0.29	0.31	0.28
PT	1	1	1
DDD	30.19	32.27	29.15
NOEL	14.8		
TER	**0.49**	**0.46**	**0.51**

Skylark (*Alauda arvensis*)

The diet of adult skylarks varies strongly with the seasons according to the availability of the particular components. Seeds are a seasonal part of this diet and are not dehusked. Green (1978) conducted a detailed study on the diet composition of skylarks in agricultural areas in the UK. The consumption data for spring-sown cereal seeds have been estimated fromthis study. The percentage of cereal grain in the diet of skylarks is estimated as 27 % in March and 29 % in April. Accordingly the fraction of cereal grain in the diet (PD) of skylarks is set at 0.29. The available literature and recent telemetry data indicate that the mean active time spent in arable crops would be clearly below the default value of 1. In a radiotelemetry study on the habitat use of skylarks in agricultural land in the UK (Crocker et al. 2001) the average active tracking time for skylarks (n = 27) in cereal fields during summer and winter was found 12 % and 25 %. The winter season data correlates with the sowing period of spring cereals, hence the relevant active tracking time is 25 % (PT = 0.25). The refined exposure assessment for the skylark consuming wheat grain treated with triticonazole is provided in table 9.1.4.-6. Such an approach was evaluated and accepted in dRR of CHR/ZF/TTC 050 FS.

Table 9.2-10: Higher-tier assessment of the chronic/reproductive risk for Skylark due to the use of CHR/ZF/PROTI in cereals- Prothioconazole-desthio

FIR/bw	NAR (mg a.s./kg seeds)	PD	PT	ETE	NOEL (mg/kg bw)	TER
0.29	104.1	0.29	0.25	2.189	14.8	6.76

Alternatively adjustment factor can be used to redefine risk for Skylark.

Table 9.2-11: Higher-tier assessment of the chronic/reproductive risk for Skylark due to the use of CHR/ZF/PROTI in cereals- Prothioconazole-desthio

FIR/bw	NAR (mg a.s./kg seeds)	PD	PT	Adjustment factor (ff × f_{twa})	ETE	NOEL (mg/kg bw)	TER
0.29	104.1	1	1	0.06118	1.85	14.8	8.0

Yellowhammer (*Emberiza citrinella*)

The elements of cracking the husk and dehusking itself are strongly fixed components of the foraging behaviour (Ziswiler, 1965). In the Prosser (2001) study, seeds were placed on bait stations on mixed farmland, and video cameras used to record bird activity (See also chapter 5.5 in SANCO/4145/2000, which refers to Prosser). According to Prosser, normally granivorous species that are capable of dehusking seeds do so and, in case of pesticide-treated seeds, this would reduce their pesticide intake for an estimated amount of 85 %. Hence, birds of the Emberizidae (example: yellowhammer), the Fringilidae family (examples: chaffinch, greenfinch), tree sparrows and house sparrows are exposed to smaller amounts of seed treatments than assumed in Tier 1 assessment. It is thus seen as a conclusive approach when employing an exposure factor of 0.15 for the dehusked proportion of cereal seeds for a refined exposure assessment. The seeds evaluated by Prosser (2001) represent both, cereal species with naked seeds (wheat, rye) and those with glumed seeds (barley, oat, rice). The results of this study thus allow to estimate the influence of dehusking on the exposure of granivorous birds to seed treatments for all species of cereal seeds. Dehusking is probably not quite the right term to use for what birds do with cereal seeds. When birds "dehusk" these

seeds, they crack the caryopse in their beaks, and then manoeuvre it around while they scrape out the floury centre with the tongue. Such an approach was evaluated and accepted in dRR of CHR/ZF/TTC 050 FS. Beside that according to Prosser (2010) PT 0.85 can be used for Yellowhammer feeding with cereals seeds.

Table 9.2-12: Higher-tier assessment of the chronic/reproductive risk for Yellowhammer due to the use of CHR/ZF/PROTI in cereals- Prothioconazole-desthio

FIR/bw	NAR (mg a.s./kg seeds)	DF	PT	ETE	NOEL (mg/kg bw)	TER
0.31	104.1	0.15	0.85	4.11	14.8	**3.60**

Adjustment factor can be used to redefine risk for Skylark.

Table 9.2-13: Higher-tier assessment of the chronic/reproductive risk for Yellowhammer due to the use of CHR/ZF/PROTI in cereals- Prothioconazole-desthio

FIR/bw	NAR (mg a.s./kg seeds)	DF	PT	Adjustment factor (ff × f_{twa})	ETE	NOEL (mg/kg bw)	TER
0.31	104.1	1	1	0.06118	1.97	14.8	7.51

Linnet (*Linaria cannabina*)

Evans et al (1997)[3] carried out winter surveys of birds on 20 arable farms in Devon and 20 in East Anglia. They report only on red-list species and found that grey partridges, skylarks, song thrushes, linnets, cirl buntings and yellowhammers all avoided winter cereals, and all except song thrushes preferred set-aside and stubbles. Song thrushes and linnets were also significantly more likely to be found among brassicas.

Considering the linnet's feeding behaviour and habitat selection, it becomes obvious that linnets rely very much on small weed seeds throughout the year. To some extent, also crop seeds may be consumed during their milk ripe stages in summer or on stubble fields in autumn and winter. Linnets are not considered to be the focal species for freshly drilled cereal fields, as cereal seeds tend to be slightly too big in size to fully match the linnets preference for smaller seeds (refer to Eybert & Constant (1998)[4]). Moreover, the feeding activity of linnets is reported to be low on freshly drilled fields in cases where the number of seeds remaining on the soil surface is not very high.

Alternatively adjustment factor can be used to redefine risk for Linnet.

[3] Evans, A. D., Henderson, I. G. (1997): *Responses of farmland birds to set-aside and its management*; Ecology and Conservation of Lowland Farmland Birds, 69

[4] Eybert, M.; Constant, P. (1998): Diet of nestling linnets (*Acanthis cannabina* L.); Journal of Ornithology 139, 277 286. M- 266848-01-1.

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Table 9.2-14: Higher-tier assessment of the chronic/reproductive risk for Linnet due to the use of CHR/ZF/PROTI in cereals- Prothioconazole-desthio

FIR/bw	NAR (mg a.s./kg seeds)	DF	PT	Adjustment factor (ff × f_{twa})	ETE	NOEL (mg/kg bw)	TER
0.28	104.1	1	1	0.06118	1.78	14.8	8.31

S8	7.2 / 9.6 / 10.0 / 20.9*	n.d.	n.d.	n.d.	n.d.
S8	7.2 / 9.6 / 10.0 / 20.9	n.d.	n.d.	n.d.	n.d.
S9	9.0 / 11.6 / 12.0 / 23.0	n.d.	n.d.	n.d.	n.d.
S10	10.0 / 12.8 / 13.0 / 24.0	n.d.	n.d.	n.d.	n.d.
S11	11.0 / 13.7 / 13.9 / 25.0	n.d.	n.d.	n.d.	n.d.
S12	15.0 / 17.7 / 17.9 / 28.9	n.d.	n.d.	n.d.	n.d.
			PTZ-desthio [mg/kg]		
S8	7.2 / 9.6 / 10.0 / 20.9*	n.d.	n.d.	n.d.	n.d.
S8	7.2 / 9.6 / 10.0 / 20.9	0.0187	0.0131	0.0146	< LOQ (0.00512)
S9	9.0 / 11.6 / 12.0 / 23.0	0.0187	0.0115	0.0117	0.0132
S10	10.0 / 12.8 / 13.0 / 24.0	0.0140	< LOQ (0.00768)	< LOQ (0.00548)	< LOQ (0.00656)
S11	11.0 / 13.7 / 13.9 / 25.0	n.d.[1]	n.d.	n.d.	< LOQ (0.00552)

DAA: days after application
LOQ: Level of quantification (0.01 mg/kg); LOD: level of detection (0.003 mg/kg)
* Control sample C
[1] calculated from end of application to time on dry ice or time in freezer

Residues in seedlings

In all trials no residues of prothioconazole or PTZ-desthio at or above the LOD were detected in control samples of seedlings. In all trials no residues of prothioconazole at or above the LOD were detected in seedlings samples of test item treatment T. Highest residues of PTZ-desthio in seedlings of the test item treatment were observed at sampling S9 in trial -01 with 0.0187 mg/kg (9.0DAA) and in trial -04 with 0.0132 mg/kg (23.0DAA). In trial -02 and -03 highest residues were observed at sampling S8 with 0.0131 mg/kg (9.6DAA) and 0.0146 mg/kg (10.0 DAA), respectively. In trial -01 residues decreased subsequently to <LOQ at the last sampling (15.0DAA). In trial -02 residues decreased to 0.0115 mg/kg in sampling S9 (11.6DAA). At sampling S10 and S11 (12.8 and 13.0DAA) residues were <LOQ and at the last sampling (17.7DAA) <LOD. In trial -03 residues decreased to 0.0117 mg/kg in sampling S9 (12.0DAA). At sampling S10 and S11 (13.0 and 13.9DAA) residues were <LOQ and at the last sampling (17.9DAA) <LOD. In trial -04 residues were <LOQ in the following sampling until the last sampling (28.9DAA).

An overview of all residue values of prothioconazole and PTZ-desthio in seedlings of trials S21-06525 -01 to -04 is given in the following table:

Residues in seedlings

The calculation of the degradation kinetics of prothioconazole in seedlings was not possible as no residues were detected in all trials.

Residues in seedlings

In trial -01 for seedlins none of the models achieved the critical value < 15 for χ^2-error. The SFO, DFOP and HS model achieved the critical value for the determination coefficient of $r^2 > 0.85$. The DFOP and HS models showed either negative confidence intervals or where not statistically significant. For the SFO model the calculated χ^2-error was 15.62 and the calculated r^2 was 0.9882. The results of the calculation showed a DT_{50} value of 4.85 days and a DT_{90} value of 16.11 days, respectively

In trial -02 for seedlins none of the models achieved the critical value < 15 for χ^2-error. The SFO, DFOP and HS model achieved the critical value for the determination coefficient of $r^2 > 0.85$. The DFOP and HS models showed either negative confidence intervals or where not statistically significant. For the SFO model the calculated χ^2-error was 16.95 and the calculated r^2 was 0.931. The results of the calculation showed a DT_{50} value of 4.61 days and a DT_{90} value of 15.32 days, respectively.

In trial -03 for seedlins none of the models achieved the critical value < 15 for χ^2-error. The SFO and DFOP model achieved the critical value for the determination coefficient of $r^2 > 0.85$. The DFOP and HS models showed either negative confidence intervals or where not statistically significant. For the SFO model the calculated χ^2-error was 25.75 and the calculated r^2 was 0.9025. The results of the calculation showed a DT_{50} value of 4.57 days and a DT_{90} value of 15.17 days, respectively.
In trial -04 for seedlins none of the models achieved the critical values of < 15 for χ^2-errorand for the determination coefficient of $r^2 > 0.85$. All models showed either negative confidence intervals or where not statistically significant. Therefore, none of the models can be taken for the calculation of degradation kinetics.

Maximum prothioconazole concentrations in seeds over all trials ranged between 60.4 mg/kg (trial -02 sampling S1) and 73.2 mg/kg (trial -01 sampling S1).
Maximum PTZ-desthio concentrations in seeds over all trials ranged between 18.1 mg/kg (trial -02 sampling S2) and 24.1 mg/kg (trial -03 sampling S2).
No prothioconazole residues were detected in seedlings in all trials at all samplings.
Maximum PTZ-desthio concentrations in seedlings over all trials ranged between 0.0131 mg/kg (trial -02 sampling S8) and 0.0187 mg/kg (trial -01 sampling S9).
For the degradation of prothioconazole in seeds, the single first order (SFO) degradation model was used – as recommended in Appendix H of the EFSA Birds and Mammals Guidance Document (2009). The DT_{50} values over trials was calculated between 0.57 days and 0.92 days. The DT_{90} values over all trials was calculated between 1.88 days and 3.04 days.
For the degradation of PTZ-desthio in seeds, only the results of trials -02 and -03 can be concidered reliable. In these trials the single first order (SFO) degradation model was used – as recommended in Appendix H of the EFSA Birds and Mammals Guidance Document (2009). The DT_{50} values over these trials was calculated between 1.97 days and 4.59 days. The DT_{90} values over these trials was calculated between 6.54 days and 15.24 days.
The degradation of prothioconazole in seeds could not be calculated as no residues were detected in any of the samplings over all trials.
For the degradation of PTZ-desthio in seedlings, only the results of trials -01, -02 and -03 can be concidered reliable. In these trials the single first order (SFO) degradation model was used – as recommended in Appendix H of the EFSA Birds and Mammals Guidance Document (2009). The DT_{50} values over these trials was calculated between 4.57 days and 4.85 days. The DT_{90} values over these trials was calculated between 15.17 days and 16.11 days.

$f_{twa} = (1 – e^{-kt})/ kt$, where:

f_{twa} – time weighted average factor
$k = ln(2)/DT_{50}$
t – averaging time (21 days)
f_{twa}
= 0.3072

Table 9.2-15: Higher-tier assessment of the chronic/reproductive risk for birds due to the use of CHR/ZF/PROTI in cereals- Prothioconazole-desthio

	Scenario	Species	LD_{50} (mg a.s./kg bw)	FIR/bw [a] (mg a.s./kg bw)	NAR (mg seeds)[b]	f_{twa}	ETE [mg a.s. b.w./d][c]	TER [d]	Trigger value
Newly emergent	Small omnivorous crop bird	shoots	14.8	0.5	104.1/5	0.3072	3.20	**4.625**	5

a) FIR: Food intake rate

b) NAR: Nominal loading/application rate of active substance in mg/kg seed.
c) ETE: Estimated theoretical exposure
d) TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Adjustment factor can be used to redefine risk for omnivorous bird based on formation fraction of Prothioconazole-desthio in seeds, as the amount of Prothioconazole-Desthio in seedlings depends of the amount in seeds.

Trial	Prothioconazole residues [mg/kg]	Prothioconazoledesthio residues [mg/kg]	ff	Adjustment factor: 21d fTWA × ff
-01	73.2	18.6	0.2645	0.0813
-02	60.4	18.1	0.3120	0.0958
-03	71.0	24.1	0.3534	0.1086
-04	72.2	11.7	0.1687	0.0518
			Average adjustment factor value	**0.0844**

Table 9.2-16: Higher-tier assessment of the chronic/reproductive risk for birds due to the use of CHR/ZF/PROTI in cereals- Prothioconazole-desthio

Scenario	Species	LD_{50} (mg a.s./kg b.w.)	FIR/bw [a] (f_{twa})	NAR (mg a.s. /kg seeds)[b]	Adjustment factor (ff × bw)	ETE [mg a.s./kg b.w./d][c]	TER [d]	Trigger value
Newly emergent crop shoots	Small omnivorous bird	14.8	0.5	104.1/5	0.0844	0.879	16.84	5

a) FIR: Food intake rate
b) NAR: Nominal loading/application rate of active substance in mg/kg seed.
c) ETE: Estimated theoretical exposure
d) TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Chronic risk due to the use of CHR/ZF/PROTI in cereals is acceptable for both granivorous and omnivorous birds, therefore no further risk refinement is necessary.

Review comments

As a Refinement the Applicant presented:

Residue field study, Prothioconazole and for Prothioconazole-Desthio in seeds

Residue study was to determine the residues of prothioconazole and PTZ-desthio in winter wheat seedlings and seeds after drilling of untreated and coated seeds treated with CHR/ZF/PROTI 100 FS under representative growing conditions in Central Europe in the field. The residues and degradation kinetics of

Prothioconazole and PTZ-desthio were investigated. The agricultural practices and cereals varieties were in accordance with the local farming practice.
The study was conducted in compliance with the EFSA Guidance document on Risk Assessment for Birds and Mammals (2009), SANCO/4145/2000 and SANTE/2020/12830 rev.
Since sufficient information was presented in the study izRMS accepted use of this study for the refinement on the risk assessment.

9.2.2.3 Drinking water exposure

According to Birds and Mammals Guidance (Chapter 5.2. Risk assessment for treated seed) is stated that: *„Significant contamination of drinking water after the use of pesticide as seed treatment seem equally unlikely to be a critical rout or to lead to TER greater than direct dietary consumption''*. Therefore, the risk assessment focuses on the dietary route of exposure. Drinking water exposure is not required.

~~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 CHR/ZF/PROTI is not a product for spray applications and it's not intended to be applied on leafy vegetables forming heads or crop plants with comparable water collecting structures at principal growth stage 4 or later, the leaf scenario does not have to be considered.~~

~~**Puddle scenario**~~

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

~~With a K(f)oc of 1765, prothioconazole belongs to the group of less/more sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied. (see 9.1.2).~~

~~**Table 9.2-6: Puddle scenario for birds due to the use of CHR/ZF/PROTI in cereals - Prothioconazole and Prothioconazole-desthio**~~

~~Compound~~	~~Koc [L/kg]~~	~~Application rate [g/ha]~~	~~Toxicity[mg ai/kg bw]~~	~~Ratio Application rate: toxicity~~	~~Trigger~~
~~Prothioconazole~~	~~1765~~	~~25~~	~~>2000~~	~~0.0125~~	~~3000~~
~~JAU 6476-desthio~~	~~575.4~~	~~25~~	~~>2000~~	~~0.0125~~	~~3000~~

9.2.2.4 Effects of secondary poisoning

The log P_{ow} of Prothioconazole amounts to 3.82 and thus exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is required.

The log P_{ow} of Prothioconazole-desthio amounts to 3.04 and thus exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is required.

The log P_{ow} of Prothioconazole-S-methyl amounts to 4.19 and thus exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is required.

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.

Table 9.2-7: Assessment of the risk for earthworm-eating birds due to exposure to Prothioconazole and its metabolites via bioaccumulation in earthworms (secondary poisoning) for the intended use in cereals (worse case use from GAP table in soil)

Parameter	Prothioconazole	Prothioconazole-desthio	Prothioconazole-Smethyl	comments
PEC_{soil} (mg/kg soil)	0.0333	0.0078	0.0019	
log P_{ow} / P_{ow}	3.82/6607	3.04/1096	4.19/15488	
Koc	1765	575.4	2556.3	Mean
foc	0.02	0.02	0.02	Default
BCF_{worm}	2.270	1.216	3.652	$BCF_{worm/soil}$ = ($PEC_{worm,ww}/PEC_{soil,dw}$) = (0.84 + 0.012 × P_{ow}) / foc × Koc
PEC_{worm}	0.0756	0.0095	0.00694	PEC_{worm} = PEC_{soil} × $BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.0794	0.00998	0.00729	DDD = PEC_{worm} × 1.05
NOEL (mg/kg bw/d)	78	14.8	78*****	LoEP
TER_{lt}	982.37	1482.97	10699.59	
Trigger value	5	5	5	EC1107/2009
Refinement required	No	No	No	

TER values shown in bold fall below the relevant trigger.
***** NOEL of the parent compound Prothioconazole

All TER values are above the trigger of 5. Accordingly the risk to earthworm-eating birds from the use of the product on cereals is acceptable.

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 prothioconazole in water.

Table 9.2-8: Assessment of the risk for fish-eating birds due to exposure to Prothioconazole and its metabolite via bioaccumulation in fish (secondary poisoning) for the intended use in forestry tree (worse case use from GAP table in surface water)

Parameter	Prothioconazole	Prothioconazole-desthio	comments
$PEC_{sw;}$ (mg/L)	0.46 (Step 2)	0.1265 (D6 ditch)	
BCF_{fish}	18.8	45.0	LoEP
BMF	-	-	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	8.648	5.6925	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Parameter	**Prothioconazole**	**Prothioconazole-desthio**	**comments**
Daily dietary dose (mg/kg bw/d)	1.375	0.905	$DDD = PEC_{fish} \times 0.159$
NOEL (mg/kg bw/d)	78	14.8	LoEP
TER_{lt}	56.73	16.35	
Trigger value	5	5	EC1107/2009
Refinement required	No	No	

TER values shown in bold fall below the relevant trigger.

All TER values are above the trigger of 5. Accordingly the risk to fish-eating birds from the use of the product on cereals is acceptable.

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

All TER values exceed the relevant triggers indicating that CHR/ZF/PROTI does not pose an unacceptable risk to birds following applications according to recommended use pattern.

Evaluation of exposing to birds through the drinking water demonstrated the acceptable risk. The risk to earthworm- and fish-eating brids from secondary poisoning is low.

Review comments:

Acute risk due to the use of CHR/ZF/PROTI in cereals is acceptable.

Long-term risk for use of the product in cereals showed unacceptable risk at TIER 1. Higher risk assessment was performed.

For use in cereals the refinement based on DT_{50} for prothioconazole and its relevant metabolite Prothioconazole-desthio (M4) was provided and accepted.

The acceptability of the refinement should be considered at the MS level.

CHR/ZF/PROTI 100 FS is a seed treatment used in cereals it is recommended to minimise the exposure of birds by inclusion of following safety phrases on the label:

SPe 5: To protect birds/wild mammals the treated seeds should be entirely incorporated in the soil; ensure that the treated seeds are also fully incorporated at the end of rows. SPe 6: To protect birds/wild mammals remove spillages.

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 and its relevant metabolite Prothioconazole-desthio. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on mammals of CHR/ZF/PROTI were not evaluated as part of the EU assessment of Prothioconazole.

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	Acute Oral	LD50 > **6200 mg a.s./kg bw/d**	EFSA Scientific Report (2007) 106, 1-98,
Rat	Prothioconazole	Long-term (2generation), gavage	$NOEL_{parental}$= 9.7 mg a.s./kg bw/d NOELreproduction= **95.6 mg a.s./kg bw/d**	EFSA Scientific Report (2007) 106, 1-98,
Rat	Prothioconazoledesthio	Acute Oral	$LD50_{(female)}$= 2506 mg p.m./kg bw/d $LD50_{(male)}$= 2806 mg p.m./kg bw/d	EFSA Scientific Report (2007) 106, 1-98,
Mouse	Prothioconazoledesthio	Acute Oral	$LD50_{(female)}$= 3459 mg p.m./kg bw/d $LD50_{(male)}$= **2235 mg p.m./kg bw/d**	EFSA Scientific Report (2007) 106, 1-98,

Rat	Prothioconazoledesthio	long-term (2generation) oral	$NOEL_{(parental)}$= 2.5 mg p.m./kg bw/d $NOEL_{(male)}$= **10 mg p.m./kg bw/d**	EFSA Scientific Report (2007) 106, 1-98,

9.3.2 Risk assessment for seed treatments

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.

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

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

Table 9.3-2: First-tier assessment of the acute risk for mammals due to the use of CHR/ZF/PROTI in cereals- Prothioconazole

Scenario	Species	NAR [mg a.s. /kg bw)	DDD seeds)[b]	TER	Trgger (mg b.w./d]	(mg	LD_{50} ~~ETE~~	FIR/bw value a.s./kg a.s./kg
Treated seed	Small granivorous mammals	>6200	0.24	100	24		258	10
Newly crop shoots	Small mammal	>6200	0.24	100/5	4.8	1292	10 emergent	omnivorous

a) FIR: Food intake rate
b) NAR: Nominal loading/application rate of active substance in mg/kg seed.
c) ETE: Estimated theoretical exposure
d) TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Table 9.3-3: First-tier assessment of the acute risk for mammals due to the use of CHR/ZF/PROTI in cereals- Prothioconazole-desthio

Scenario	Species	NAR [mg a.s./kg bw)	DDD seeds)[b]	TER	Trgger (mg b.w./d]	(mg a.s./kg	~~ETE~~	LD_{50} value a.s./kg	FIR/bw [mg a.s. /kg
Treated seed	Small granivorous mammals	2235	0.24	104.1		24.98		89.47	10
Newly crop shoots	Small mammal	2235	0.24	104.1/5		5.00	447	10 emergent	omnivorous

a) FIR: Food intake rate
b) NAR: Nominal loading/application rate of active substance in mg/kg seed.
c) ETE: Estimated theoretical exposure
d) TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

NAR value was calculated with assumption that 100% of Prothioconazole Prothioconazole-transform into desthio. Difference between molar masses were included.

Acute risk due to the use of CHR/ZF/PROTI in cereals is acceptable for mammals. ~~not acceptable for both granivorous and omnivorous birds, therefore further risk refinement is necessary.~~

Table 9.3-4: First-tier assessment of the chronic/reproductive risk for mammals due to the use of CHR/ZF/PROTI in cereals- Prothioconazole

Scenario	Species	LD_{50} (mg a.s./kg bw)	FIR/bw [a]	NAR (mg a.s./kg seeds)[b]	f_{TWA}	DDD ~~ETE~~ [ng a.s./kg b.w./d]	TER	Trigger value
Treated seed	Small granivorous mammals	95.6	0.24	100	1	24	**3.98**	5
Newly emergent crop shoots	Small omnivorous mammal	95.6	0.24	100/5	1	4.8	19.9	5

a) FIR: Food intake rate
b) NAR: Nominal loading/application rate of active substance in mg/kg seed.
c) ETE: Estimated theoretical exposure
d) TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Table 9.3-5: First-tier assessment of the chronic/reproductive risk for mammals due to the use of CHR/ZF/PROTI in cereals- Prothioconazole-desthio

Scenario	Species	LD_{50} (mg a.s./kg bw)	FIR/bw [a]	NAR (mg a.s./kg seeds)[b]	f_{TWA}	DDD ~~ETE~~ [mg a.s./kg b.w./d]	TER	Trigger value
Treated seed	Small granivorous mammals	10	0.24	104.1	1	24.98	**0.40**	5
Newly emergent crop shoots	Small omnivorous mammal	10	0.24	104.1/5	1	5.00	**2.0**	5

a) FIR: Food intake rate
b) NAR: Nominal loading/application rate of active substance in mg/kg seed.
c) ETE: Estimated theoretical exposure
d) TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

NAR value was calculated with assumption that 100% of Prothioconazole transform into Prothioconazole-desthio. Difference between molar masses were included.

Chronic/reproductive risk due to the use of CHR/ZF/PROTI in cereals is not acceptable for both granivorous and omnivorous birds, therefore further risk refinement is necessary.

9.3.2.2 Higher-tier risk assessment

~~Not required.~~

New study were performed to redefine risk for birds while using CHR/ZF/PROTI 100 FS: *Determination of Residues of Prothioconazole and PTZ-Desthio in Winter Wheat Seeds and Seedlings after Drilling of Coated Seeds at 4 Sites in Central Europe in 2021*, Appeltauer, A., 2022. Study were briefly described in birds higher-tier risk assessment.

Prothioconazole in seeds

$f_{twa} = (1 - e^{-kt})/ kt$, where:

f_{twa} – time weighted average factor
$k = \ln(2)/DT_{50}$
t – averaging time (21 days)
DT_{50} – average DT_{50} value of 0.7325 d

$f_{twa} = 0.0503$

Table 9.3-6: Higher-tier assessment of the chronic/reproductive risk for mammals due to the use of CHR/ZF/PROTI in cereals- Prothioconazole

Scenario	Species	LD_{50} (mg a.s./kg	FIR/bw [a] a.s./kg [mg	NAR (mg a.s. bw)	f_{TWA} seeds)[b] /kg	DDD ~~ETE~~ b.w./d] [c]	TER [d]	Trigger value
	Small	95.6	0.24	100	0.0503	1.21	79.01	5
Treated seed	granivorous mammals							

a) FIR: Food intake rate
b) NAR: Nominal loading/application rate of active substance in mg/kg seed.
c) ETE: Estimated theoretical exposure
d) TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Prothioconazole-dethio in seeds

$f_{twa} = (1 - e^{-kt})/ kt$, where:

f_{twa} – time weighted average factor
$k = \ln(2)/DT_{50}$
t – averaging time (21 days)

f_{twa} = 0.22267
According to maximum Prothioconazole and Prothioconazole-desthio residue values adjustment factor was estimated for all locations based on formation fraction of metabolite.

Table 9.3-7: Estimation of Adjustment factor for Prothioconazole-Desthio.

Trial	Prothioconazole residues [mg/kg]	Prothioconazoledesthio residues [mg/kg]	ff	Adjustment factor: 21-d f_{TWA} × ff
-01	73.2	18.6	0.2645	0.0589
-02	60.4	18.1	0.3120	0.0695
-03	71.0	24.1	0.3534	0.0787
-04	72.2	11.7	0.1687	0.0376
			Average adjustment factor value	**0.06118**

Table 9.3-8: Higher-tier assessment of the chronic/reproductive risk for mammals due to the use of CHR/ZF/PROTI in cereals- Prothioconazole-desthio Scenario

		Species	DDD (mg a.s./kg	TER[a] a.s./kg ×f_{twa})	LD_{50} Trigger (mg	FIR/bw factor (ff [mg bw) seeds)[b]	NAR a.s.	Adjustment ETE /kg b.w./d] [c]	f_{TWA} [d]	value
Treated granivorous seed	Small mammals	10	0.24	104.1	0.06118	0.0612	1.53	6.53	5	

a) FIR: Food intake rate
b) NAR: Nominal loading/application rate of active substance in mg/kg seed.
c) ETE: Estimated theoretical exposure
d) TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

One relevant focal species should be considered for higher-tier risk assessment for mammals: Wood mouse.

The wood mouse (*Apodemus sylvaticus*)

According to Prosser (2010) publication PT value of 0.70 can be used for wood mouse feeding with cereals seeds between October to February.
The wood mouse (*Apodemus sylvaticus*), which is considered as relevant species, would not feed exclusively on freshly sown cereal. Thus the Tier 1 assumption of a PD = 1 for treated seeds leads to an overestimation of exposure under field conditions. Based on a study on seasonal diet composition (Abt & Bock 1998[3]), the representative summer diet of wood mice consists of approx. 60 % grains/seeds, 20 % green plant material and 20 % invertebrates.
Adjustment factor based on amount of Prothioconazole-Desthio in comparison to parent compound can be used.

Table 9.3-9: Higher-tier assessment of the chronic/reproductive risk for wood mouse due to the use of CHR/ZF/PROTI in cereals- Prothioconazole-desthio FIR/bw NAR PD PT

	Adjustment (mg/kg a.s./kg	ETE ×f_{twa})	NOEL /kg	TER (mg bw) seeds)	factor (ff b.w./d]	[mg a.s.	
0.23	104.1	0.60	0.70	0.06118	0.615	10.0	16.26
0.23	104.1	1	0.70	0.06118	1.025	10.0	9.75
Prothioconazole-dethio in seedlings							

$f_{twa} = (1 – e^{-kt})/ kt$, where:

f_{twa} – time weighted average factor
$k = ln(2)/DT_{50}$
t – averaging time (21 days)

– ion in farmland field mice *Apodemus spp. and bank voles Clethrionomys glareolus*, Acta Theriologica 43, 379-389

f_{twa} = 0.3072

Table 9.3-10: Higher-tier assessment of the chronic/reproductive risk for mammals due to the use of CHR/ZF/PROTI in cereals- Prothioconazole-desthio Scenario

	Species	LD_{50}	FIR/bw	NAR	f_{TWA}	ETE	TER [d]
	Trigger	(mg seeds)[b]	(mg b.w./d]	value [mg a.s./kg bw] [c]		a.s./kg	a.s. /kg bw)
Newly emergent crop shoots	Small omnivorous mammal	10	0.24	104.1/5	0.3072	1.54	6.49

a) FIR: Food intake rate
b) NAR: Nominal loading/application rate of active substance in mg/kg seed.
c) ETE: Estimated theoretical exposure
d) TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Chronic risk due to the use of CHR/ZF/PROTI in cereals is acceptable for both granivorous and omnivorous mammals, therefore no further risk refinement is necessary.

9.3.2.3 Drinking water exposure

According to Birds and Mammals Guidance (Chapter 5.2. Risk assessment for treated seed) is stated that: ,,Significant contamination of drinking water after the use of pesticide as seed treatment seem equally unlikely to be a critical rout or to lead to TER greater than direct dietary consumption''. Therefore, the risk assessment focuses on the dietary route of exposure. Drinking water exposure is not required.

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

~~**Puddle scenario**~~

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

~~With a K(f)oc of 1765, Prothioconazole belongs to the group of less sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied. (see 9.1.2).~~

~~**Table 9.3-6: Puddle scenario for mammals due to the use of CHR/ZF/PROTI in cereals- Prothioconazole and Prothioconazole-desthio**~~

~~Compound~~	~~Koc [L/kg]~~	~~Application rate [g/ha]~~	~~Toxicity[mg ai/kg bw]~~	~~Ratio Application rate: toxicity~~	~~Trigger~~
~~Prothioconazole~~	~~1765~~	~~25~~	~~>6200~~	~~0.00403~~	~~3000~~
~~JAU 6476-desthio~~	~~575.4~~	~~25~~	~~>2235~~	~~0.0112~~	~~3000~~

9.3.2.4 Effects of secondary poisoning

The log P_{ow} of Prothioconazole amounts to 3.82 and thus exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is required.

The log P_{ow} of Prothioconazole-desthio amounts to 3.04 and thus exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is required.

The log P_{ow} of Prothioconazole-S-methyl amounts to 4.19 and thus exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is required.

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. (see 9.1.2).

Table 9.3-7: Assessment of the risk for earthworm-eating mammals due to exposure to Prothioconazole and its metabolites via bioaccumulation in earthworms (secondary poisoning) for the intended use in winter cereals (worse case use from GAP table in soil)

Parameter	Prothioconazole	Prothioconazole-desthio	Prothioconazole-Smethyl	comments
PEC_{soil} (mg/kg soil)	0.0333	0.0078	0.0019	
log P_{ow} / P_{ow}	3.82/6607	3.04/1096	4.19/15488	
Koc	1765	575.4	2556.3	Mean
foc	0.02	0.02	0.02	Default
BCF_{worm}	2.270	1.216	3.652	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC_{worm}	0.0756	0.0095	0.00694	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.0794	0.00998	0.00729	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	95.6	10	**95.6***	LoEP
TER_{lt}	1264.6	1002.0	13113.9	
Trigger value	5	5	5	EC1107/2009
Refinement required	No	No	No	

TER values shown in bold fall below the relevant trigger.
***** NOEL of the parent compound Prothioconazole

All TER values are above the trigger of 5. Accordingly the risk to earthworm-eating mammals from the use of the product on cereals is acceptable.

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 of Prothioconazole in water.

Table 9.3-8: Assessment of the risk for fish-eating mammals due to exposure to Prothioconazole and Prothioconazole-desthio via bioaccumulation in fish (secondary poisoning) for the intended use in forestry tree (worse case use from GAP table in surface water)

Parameter	Prothioconazole	Prothioconazole-desthio	comments
$PEC_{sw;}$ (mg/L)	0.46 (Step 2)	0.1265 (D6 ditch)	
BCF_{fish}	18.8	45.0	LoEP
BMF	-	-	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	8.648	5.6925	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	1.375	0.905	$DDD = PEC_{fish} \times 0.159$
NOEL (mg/kg bw/d)	95.6	10	LoEP
TER_{lt}	69.53	11.05	
Trigger value	5	5	EC1107/2009
Refinement required	No	No	

TER values shown in bold fall below the relevant trigger.

All TER values are above the trigger of 5. Accordingly the risk to fish-eating mammals from the use of the product on cereals is acceptable.

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

All TER values exceed the relevant triggers indicating that CHR/ZF/PROTI does not pose an unacceptable risk to mammals 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 mammals from secondary poisoning is low.

Review comments:

Acute risk due to the use of CHR/ZF/PROTI in cereals is acceptable.
Chronic/reproductive risk due to the use of CHR/ZF/PROTI in cereals was not acceptable for both granivorous and omnivorous birds at Tier 1, therefore further risk refinement is necessary.
For use in cereals the refinement based on DT_{50} for prothioconazole and its relevant metabolite Prothioconazole-desthio (M4) was provided and accepted. Wood mouse was chosen as a focal species. And refinement of PT was also accepted, taking to consideration BBCH phases and type of crop. Based on literature on seasonal diet composition (Abt & Bock 1998) The Applicant presented PD refinement, however since no details were presented worst case scenario of 100% diet for wood mouse was taken to consideration. The acceptability of the refinement should be considered at the MS level.

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

Not required

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

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

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

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
Oncorhynchus mykiss	Prothioconazole	acute	LC_{50} = 1.83 mg a.s/L	EFSA Scientific Report (2007) 106,
Lepomis macrochirus	Prothioconazole	acute	LC_{50}= 4.59 mg a.s/L	EFSA Scientific Report (2007) 106,
Cyprinus carpio	Prothioconazole	acute	LC50= 6.91 mg a.s./L	EFSA Scientific Report (2007) 106,
Oncorhynchus mykriss	Prothioconazole	chronic	NOEC= 0.308 mg a.s./L	EFSA Scientific Report (2007) 106,

Daphnia magna	Prothioconazole	Acute	EC_{50}= 1.3 mg a.s/L	EFSA Scientific Report (2007) 106,
Daphnia Magna	Prothioconazole	Chronic	NOEC= 0.56 mg a.s./L	EFSA Scientific Report (2007) 106,
Species	**Substance**	**Exposure System**	**Results**	**Reference**
Pseudokirchneriella subcapitata	Prothioconazole	Sub-chronic	72h E_rC_{50} = 2.18 mg a.s./L 72h E_bC_{50} = 1.10 mg a.s./L	EFSA Scientific Report (2007) 106,
Chironomus riparius	Prothioconazole	Chronic	NOEC= 9.14 mg a.s/L	EFSA Scientific Report (2007) 106,
Oncorhynchus mykiss	Prothioconazoledesthio	Acute	LC_{50}= 6.63 mg p.m/L	EFSA Scientific Report (2007) 106,
Leuciscus idus melanotus	Prothioconazoledesthio	Acute	LC_{50}= 13.2 mg p.m/L	EFSA Scientific Report (2007) 106,
Oncorhychus mykiss	Prothioconazoledesthio	Chronic	NOEC= 3.34 µg p.m./L	EFSA Scientific Report (2007) 106,
Daphnia magna	Prothioconazoledesthio	Acute	EC_{50} > 10 mg p.m./L	EFSA Scientific Report (2007) 106,
Daphnia magna	Prothioconazoledesthio	Chronic	NOEC= 0.10 mg p.m./L	EFSA Scientific Report (2007) 106,
Scenedesmus subspicatus	Prothioconazoledesthio	Sub-chronic	E_bC_{50}= 0.073 mg p.m./L E_rC_{50}= 0.55 mg p.m./L	EFSA Scientific Report (2007) 106,
Chironomus riparius	Prothioconazoledesthio	Chronic	NOEC= 2.0 mg p.m./L	EFSA Scientific Report (2007) 106,
Oncorhychus mykiss	1,2,4-triazole	Acute	LC_{50}= 498 mg p.m/L	EFSA Scientific Report (2007) 106,
Oncorhychus mykiss	1,2,4-triazole	Chronic	NOErC= 3.2 mg a.s./L	EFSA Scientific Report (2007) 106,
Dpahnia magna	1,2,4-triazole	Acute	EC_{50}= 900 mg p.m./L	EFSA Scientific Report (2007) 106,
Daphnia magna	1, 2, 4-triazole	48h	EC_{50} > 100 mg pm/L	EU agreed endpoints derived from PRAPeR 13, 2007 (on triazole metabolites)
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	EFSA Scientific Report (2007) 106,
Higher-tier studies (micro- or mesocosm studies)				

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

Table 9.5-2: Endpoints and effect values relevant for the risk assessment for aquatic organisms – CHR/ZF/PROTI

Species	Substance	Exposure System	Results	Reference
Daphnia magna	CHR/ZF/PROTI 100	48 h, s	EC_{50} = 19.76 mg/L	T. Turek-Lipka,
Species	**Substance**	**Exposure System**	**Results**	**Reference**
	FS		NOEC = 12.5 mg/L	2021, Study code: W-54-20
Raphidocelis subcapitata	CHR/ZF/PROTI 100 FS	72 h, s	E_rC_{50} = 14.69 mg/L (m) E_yC_{50} = 5.53 mg/L (m) ~~E_rC_{50} = 28.37 mg/L~~ ~~E_yC_{50} = 10.69 mg/L~~	T. Turek-Lipka, 2021, Study code: W-55-20
Higher-tier studies (micro- or mesocosm studies)				

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

Review comments:

In the study W-55-20 by T. Turek-Lipka, 2021, the analytical measurements demonstrated that the test item concentrations throughout the test was outside 80-120% of nominal and for this reason endpoints should be expressed as measured concentrations.
Following endpoints based on measured test item concentrations would be used for risk assessment purposes:
ErC_{50}= 14.69 mg formulation/L
EyC_{50}= 5.53 mg formulation/L

9.5.2 Risk assessment

The evaluation of the risk for aquatic and sediment-dwelling organisms was performed in accordance with the recommendations of the "Guidance document on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters in the context of Regulation (EC) No 1107/2009", as provided by the Commission Services (SANTE-2015-00080, 15 January 2015).
The relevant global maximum FOCUS Step 1, 2 and 3 PEC_{SW} for risk assessments covering the proposed use pattern and the resulting PEC/RAC ratios are presented in the table below. To achieve a concise risk assessment, the risk envelope approach is applied.

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, ~~2~~ calculations for the use of CHR/ZF/PROTI in winter cereals

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		LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}/E_yC_{50}	NOEC
(µg/L)		1830	308	1300	560	1100	9410
AF		100	10	100	10	10	10
RAC (µg/L)		18.3	30.8	13	56	110	941
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**						
Step 1							
N-Europe	2.49	0.136	0.081	0.192	0.044	0.023	0.003

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

Table 9.5-4: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Prothioconazole-desthio for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of CHR/ZF/PROTI in winter cereals

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		LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}/E_yC_{50}	NOEC
(µg/L)		6630	3.34	10000	100	73	2000
AF		100	10	100	10	10	10
RAC (µg/L)		66.3	0.334	100	10	7.3	200
FOCUS Scenario PEC gl-max (µg/L)							
Step 1							
N-Europe	**3.82**	0.058	**11.437**	0.038	0.382	0.523	0.019
Step 2							
N-Europe	**1.43**	-	**4.281**	-	-	-	-
Step 3							
D3/ditch	0.0037	-	0.011	-	-	-	-
D4/pond	0.0036	-	0.011	-	-	-	-
D4/stream	0.0152	-	0.046	-	-	-	-
D5/pond	0.0013	-	0.004	-	-	-	-
D5/stream	0.0082	-	0.025	-	-	-	-
D6/ditch	0.1265	-	0.379	-	-	-	-
R1/pond	0.0057	-	0.017	-	-	-	-

R1/stream	0.0329	-	0.099	-	-	-	-
R3/stream	0.0427	-	0.128	-	-	-	-
Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Sed. dwell. prolonged**
R4/stream	0.0939	-	0.281	-	-	-	-

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

Table 9.5-5: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for 1,2,4-triazole for each organism group based on FOCUS Steps 1~~, 2~~ calculations for the use of CHR/ZF/PROTI in winter cereals

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Algae**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Pseudokirchn. subcapitata***
Endpoint		LC_{50}	NOEC	EC_{50}	E_rC_{50}/E_yC_{50}
(µg/L)		498000	3200	100000	8200
AF		100	10	100	10
RAC (µg/L)		4980	320	1000	820
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**				
Step 1					
N-Europe	0.56	0.0001	0.0018	0.0006	0.0007

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

Table 9.5-6: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for CHR/ZF/PROTI for each organism group based on Drift Calculator SWASH MODEL ver 5.3 calculations for the use in winter cereals

Intended use	Winter cereals
Formulation	CHR/ZF/PROTI 100 FS
Application rate (g[prod]/ha)	1 x 272 g
Entry into surface water via spray drift (Drift calculator from SWASH)	
Buffer zone (m)	**PECsw µg prod/L**
1	1.7475 (focus ditch)
Entry into surface water via spray drift (Drift alculator from SWASH)	
Buffer zone (m)	**RAC/PEC ratio** **Daphnia magna =EC_{50} 19 760 µg/L** **RAC=197.6 (AF=100)**
1	0.00884
Buffer zone (m)	**RAC/PEC ratio** **Pseudokirchmeirella subcapitata =E_rC_{50}** 14690 ~~28 370~~ **µg/L** **RAC=1469** ~~2 837~~ **(AF=10)**
1	~~0.00062~~

9.5.3 Overall conclusions

Based on the predicted rates of CHR/ZF/PROTI 100 FS, the TER values describing the risk for aquatic species following exposure to CHR/ZF/PROTI 100 FS according to the GAP of the formulation CHR/ZF/PROTI 100 FS achieve the acceptability criteria without applying mitigation measures.

Review comments:

The evaluation of the risk for aquatic was performed in accordance with Guidance document on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters in the context of Regulation (EC) No 1107/2009 (EFSA Journal 2013;11(7):3290).

Calculated PEC/RAC values for prothioconazole and its relevant metabolites are below the trigger value of 1 at steps 1 (prothioconazole, 1,2,4-triazole) and 3 (Prothioconazole-desthio), indicating low risk to aquatic organisms. No mitigation measures are required.

Acceptable Risk may be concluded for aquatic organisms exposed to the formulation at application rate 1 x 272 g (g[prod]/ha)

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

Effects on bees of CHR/ZF/PROTI were not evaluated as part of the EU assessment of Prothioconazole. New data submitted with this application are listed in Table 9.6-1 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. Justifications are provided below.

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

Species	Substance	Exposure System	Results	Reference
Apis mellifera	Prothioconazole	Oral	LD_{50} > 71 µg/bee	EFSA Scientific Report (2007) 106
Apis mellifera	Prothioconazole	Contact	LD_{50} > 200 µg/bee	EFSA Scientific Report (2007) 106
Apis mellifera	CHR/ZF/PROTI 100 FS	Oral	LD_{50} > 200 µg prod./bee LD_{50} > 20 µg a.s./bee	Kulec-Płoszczyca, E., 2021, B-24-21
Apis mellifera	CHR/ZF/PROTI 100 FS	Contact	LD_{50} > 200 µg prod./bee LD_{50} > 20 µg a.s./bee	Kulec-Płoszczyca, E., 2021, B-25-21
Higher-tier studies (tunnel test, field studies)				

9.6.2 Risk assessment

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

9.6.2.1 Hazard quotients for bees

Table 9.6-2: First-tier assessment of the risk for bees due to the use of CHR/ZF/PROTI

Intended use	Cereals		
Active substance	conazole		
Application rate (g a.s/ha)	1 × 25		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**

Oral toxicity	71	25	0.352
Contact toxicity	200		0.125
Oral toxicity- CHR/ZF/PROTI 100 FS	20		1.25
Contact toxicity- CHR/ZF/PROTI 100 FS	20		1.25

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

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

Not relevant.

Review Comments:
Since acceptable acute risk have been concluded for bees exposed to prothioconazole from the use of CHR/ZF/PROTI 100 FS – Gamelan 100 FS at the Tier 1, a higher-tier risk assessment is not required for the proposed uses of CHR/ZF/PROTI 100 FS – Gamelan 100 FS/ Doraltes 100 FS.

9.6.3 Effects on bumble bees

Not required.

Review Comments:
According to SANCO/10329/2002 rev 2 final, the risk assessment for bumblebees is not required.

9.6.4 Effects on solitary bees

Not required.

Review Comments:
According to SANCO/10329/2002 rev 2 final, the risk assessment for bumblebees is not required.

9.6.5 Overall conclusions

All hazard quotients (HQ) are considerably less than 50, indicating that CHR/ZF/PROTI 100 FS applied at the maximum use rate poses low risk to bees.

Review comments

The evaluation has been performed in line with SANCO/10329/2002 rev 2 final based on the endpoints derived from the studies for active substance and formulation.
All hazard quotients calculated are lower than 50, indicating that the acute oral and contact risk to bees is acceptable following the use according to the proposed use pattern of CHR/ZF/PROTI 100 FS – Gamelan 100 FS/ Doraltes 100 FS.

9.7 Effects on arthropods other than bees (KCP 10.3.2)

9.7.1 Toxicity data

CHR/ZF/PROTI 100 FS – Gamelan 100 FS/ Doraltes 100 FS is a seed treatment product used on cereals. According to ESCORT 2. ("Guidance document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods", 2000) and the "Guidance Document on Terrestrial Ecotoxicology" (SANCO/10329/2002 rev 2 final, 17 October 2002) in the case of a seed treatment, a risk assessment for parasitoids and for foliage dwelling predators is not deemed appropriate.

~~Studies on the toxicity to non-target arthropods 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 non-target arthropods of CHR/ZF/PROTI were not evaluated as part of the EU assessment of Prothioconazole. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.
~~The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process. Justifications are provided below.~~

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

Species	Substance	Exposure System	Results	Reference
Ground beetle (*Poecilus cupreus)*	CHR/ZF/PROTI 100 FS	Laboratory test (2D)	LR_{50} > 0.018 kg a.s./ha NOER* > 0.018 kg a.s./ha	D. Stonham, 2021, Study code: CHR-2101
Rove beetle (*Aleochara bilineata)*	CHR/ZF/PROTI 100 FS	Laboratory test (2D)	ER_{50} > 0.018 kg a.s./ha	Tew, G., 2021, Study code: CHR-21-02
Field or semi-field tests				
* NOER with respect to both beetle survival and feeding				

9.7.2 Risk assessment

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

9.7.2.1 Risk assessment for in-field exposure

To achieve a concise risk assessment, the risk envelope approach is applied.

Table 9.7-2: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of CHR/ZF/PROTI in cereals (worse case use from GAP table)

Intended use	Winter cereals

Active substance/product	CHR/ZF/PROTI
Application rate (g a.s./ha)	1 x 25
MAF	1

Test species Tier I	**LR_{50} (lab.) (g a.s./ha) NOER**	**$PER_{in\text{-}field}$ (g a.s./ha)**	**$HQ_{in\text{-}field}$ criterion: HQ ≤ 2**
Poecilus cupreus	> 18 g	25	1.39
Aleochara bilineata	> 18 g	25	1.39

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.

* If an LR_{50} or ER_{50} from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

9.7.2.2 Risk assessment for off-field exposure

Review comments:
Since formulation CHR/ZF/PROTI 100 FS is a seed treatment no spray drift is expected. The risk for offfield population of non-target arthropods other than bees is acceptable.

~~To achieve a concise risk assessment, the risk envelope approach is applied.~~

~~**Table 9.7-3: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of CHR/ZF/PROTI in cereals**~~

~~**Intended use**~~	~~Winter cereals~~
~~**Active substance/product**~~	~~CHR/ZF/PROTI~~
~~**Application rate (g a.s/ha)**~~	~~1 × 25~~
~~**MAF**~~	~~1~~
~~**vdf**~~	~~10 (Tier 1)~~

~~**Test species Tier I**~~	~~**LR_{50} (lab.) (g a.s /ha)**~~	~~**Drift rate**~~	~~**$PER_{off\text{-}field}$ (g/ha)**~~	~~**CF**~~	~~**$HQ_{off\text{-}field}$ criterion: HQ ≤ 2**~~
~~*Poecilus cupreus*~~	~~> 18 g~~	~~0.0277~~	~~0.0693~~	~~10~~	~~0.0385~~
~~*Aleochara bilineata*~~	~~> 18 g~~	~~0.0277~~	~~0.0693~~		~~0.0385~~

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

All hazard quotients (HQ) are considerably less than 2, indicating that CHR/ZF/PROTI 100 FS applied at the maximum use rate poses no risk to non-target arthropods. No risk mitigation needed.

Review comments:

Tests for Ground beetle (*Poecilus cupreus*) and Rove beetle (Aleochara bilineata) are considered adequate for CHR/ZF/PROTI 100 FS used as seed treatment.
Studies with *Hypoaspis aculeifer* and *Folsomia candida were also conducted with CHR/ZF/PROTI 100 FS. Acceptable* Risk to collembolan and soil mites could be concluded for non-target soil organisms (meso- and macrofauna).
The risk assessment performed by the Applicant for arthropods other than bees is acceptable.

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 and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents. Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of CHR/ZF/PROTI were not evaluated as part of the EU assessment of prothioconazole. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.
The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process. Justifications are provided below.

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

Species	Substance	Exposure System	Results	Reference
Eisenia fetida	Prothioconazoledesthio	long term	NOEC 1 mg p.m./ :g dw NOECcorr=0.5 mg p.m./kg dw	EFSA Scientific Report (2007) 106
Eisenia fetida	Protioconazole-Smethyl	long-term	NOEC= 100 mg p.m./kg dw NOECcorr= 50 mg p.m./kg dw	EFSA Scientific Report (2007) 106
Eisenia andrei	CHR/ZF/PROTI	long term	EC_{10}=313.1 mg	A. Wróbel, 2021,

Species	Substance	Exposure System	Results	Reference

			formulation/kg dw soil corresponding to: EC_{10}=30.2 mg of a.s/kg dw soil EC_{10corr} =156.5 mg formulation/kg dw soil corresponding to: EC_{10corr}=15.1 mg of a.s/kg dw soil NOEC ≥ 560 mg test item/kg dw $NOEC_{corr}$ ≥ 280 mg test item/kg dw NOEC ≥ 53.9 mg a.s/kg dw $NOEC_{corr}$ ≥ 26.9 mg a.s/kg dw	Study code: G-67-20
Folsomia candida	Prothioconazole	long term	NOEC= 64 mg a.s/kg dw NOECcorr= 32 mg a.s/kg dw	EFSA Scientific Report (2007) 106
Folsomia candida	Prothioconazoledethio	long term	NOEC= 62.5 mg p.m./kg dw NOECcorr=31.25 mg p.m./kg dw	EFSA Scientific Report (2007) 106
Folsomia candida	Prothioconazole-Smethyl	long term	NOEC= 31.6 mg p.m./kg bw NOECcorr= 15.8 mg p.m./kg bw	EFSA Scientific Report (2007) 106
Folsomia candida	CHR/ZF/PROTI	long term	Reproduction EC_{10}= 236.8 mg formulation/kg dw soil EC_{corr}=118.4 formulation/kg dry weight of the artificial soil NOEC=180 mg formulation/kg dw soil NOECcorr=90 mg formulation/kg dw soil Surviv al	A. Wróbel, 2021, Study code: G-68-20

			LC_{10}= 407.2 mg test item/kg dw NOEC ≥ 1000 mg	
Species	**Substance**	**Exposure System**	**Results**	**Reference**
			test item/kg dw NOECcorr≥ 500 mg test item/kg dw NOEC ≥ 96.3 mg a.s./kg dw NOECcorr ≥ 48.15 mg a.s./kg dw	
Hypoaspis aculeifer	Prothioconazole	long term	NOEC= 100 mg a.s./kg dw NOECcorr= 50 mg a.s./kg dw	EFSA Scientific Report (2007) 106
Hypoaspis aculeifer	CHR/ZF/PROTI	long term	$EC_{10reproduction}$=524.2 mg test item/kg dw ($EC_{10reproductio}$ corr: 262.1 mg test item/kg dw) $NOEC_{surv.}$ ≥ 1000 mg test item/kg dw $NOEC_{surv.corr}$ ≥ 500 mg test item/kg dw $NOEC_{rep.}$ ≥ 560 mg test item/kg dw $NOEC_{rep.corr}$ ≥ 280 mg test item/kg dw $NOEC_{rep.}$ ≥ 53.9 mg a.s/kg dw $NOEC_{rep.corr}$ ≥ 26.95 mg a.s/kg dw	A. Wróbel, 2021, Study code: G-69-20
Field studies				
Litter bag test				

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

Review comments:

Currently endpoints for soil organisms must be corrected by a factor of 2 for substances with log Pow > 3 regardless of the peat content in the study, log Pow of Prothioconazole and its metabolites exceeds the trigger value of 3 so endpoints would be divided by 2.
Where EC_{10} was more relevant than NOEC the risk assessment has been updated.

9.8.2 Risk assessment

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

9.8.2.1 First-tier risk assessment

The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2, Table 8.7-3. According to the assessment of environmental-fate data, multi-annual accumulation in soil is need to be considered for prothioconazole. To achieve a concise risk assessment, the risk envelope approach is applied.

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 CHR/ZF/PROTI in cereals (worst case use from GAP table in soil)

Intended use			
Acute effects on earthworms			
Product/active substance	**LC_{50} (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_a (criterion TER ≥ 10)**
Not required			
Chronic effects on earthworms			
Product/active substance	**NOEC or EC_{10} (mg/kg dw)***	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
Prothioconazole	-	-	-
Prothioconazole-desthio	0.5	0.0152 ~~0.0078~~	32.89 ~~64.10~~
Prothioconazole-S-methyl	50	0.0043 ~~0.0019~~	11627.91 ~~26315.79~~
CHR/ZF/PROTI	156.5 ~~280~~	0.3627	431.48 ~~771.9~~
Chronic effects on other soil macro- and mesofauna Folsomia candida			
Product/active substance	**NOEC* (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
Prothioconazole	32	0.0333	960.96
Prothioconazole-desthio (M04)	31.25	0.0152 ~~0.0078~~	2055.92 ~~4006.41~~
Prothioconazole-S-methyl (M01)	15.8	0.0043 ~~0.0019~~	3674.42 ~~8315.79~~
CHR/ZF/PROTI	90	0.3627	248.14

	~~500~~		~~1378.~~ ~~5~~ ~~5~~
Chronic effects on other soil macro- and mesofauna Hypoaspis aculeifer			
Product/active substance	**NOEC* (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
Prothioconazole	50	0.0333	1501.50
CHR/ZF/PROTI	262.1 ~~280~~	0.3627	722.6 3 ~~771.9~~ ~~9~~

TER values shown in bold fall below the relevant trigger. *
NOEC or EC_{10} correct by factor 2 (log Pow > 2)

9.8.2.2 Higher-tier risk assessment

Not relevant.

Review comments:

A higher tier assessment is not required based on the low risk indicated in the First-tier chronic assessment for earthworms, collembolan, and soil mite.

9.8.3 Overall conclusions

The long term risk to earthworms and other non-target soil organisms (meso- and macrofauna) was assessed as low for CHR/ZF/PROTI 100 FS in a first-tier risk assessment.

Review comments:

The risk assessment for earthworms exposed to prothioconazole and its relevant metabolites following application of CHR/ZF/PROTI 100 FS – Gamelan 100 FS/ Doraltes 100 FS in cereals was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology" (SANCO/10329/2002) and was accepted by the izRMS

Risk to collembolan and soil mites was estimated by calculating TERs based on the appropriate PECsoil NOECs/EC_{10} from the studies. For assessment of risk from CHR/ZF/PROTI 100 FS, the calculated PECsoil value for formulated product was used. PEC_{soil} has been updated accordingly in table 9.8-2. For details please see Section 8.

TER values calculated for all considered compounds and CHR/ZF/PROTI 100 FS – Gamelan 100 FS/ Doraltes 100 FS were above the triggers indicating acceptable long-term risk to earthworms and other soil macro-organisms. No further evaluation is deemed necessary.

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

Effects on soil microorganisms of CHR/ZF/PROTI were not evaluated as part of the EU assessment of prothioconazole. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.
The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process. Justifications are provided below.

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, aerobic soil type	2.0 kg a.s./ha	EFSA Scientific Report (2007) 106,
N-mineralisation	Prothioconazole-desthio	28 d, aerobic soil type	0.2 kg p.m./ha	EFSA Scientific Report (2007) 106,
N-mineralisation	Prothioconazoledesthio	28 d, aerobic soil type	1.0 kg p.m./ha	EFSA Scientific Report (2007) 106,
N-mineralisation	Prothioconazole-Smethyl	28 d, aerobic type soil	2.0 kg p.m/ha	EFSA Scientific Report (2007) 106,
N-mineralisation	CHR/ZF/PROTI	28 d, aerobic type soil	1.31 mg test item/kg dry weight of soil (i.e. 0.13 mg of prothioconazole/kg dry weight of soil) and 5 x PEC: 6.55 mg of the test item / kg dry weight of soil (i.e. 0.65 mg of prothioconazole/kg dry weight of soil) did not have any long-term adverse effects on the process of nitrogen transformation in aerobic surface soils.	A. Wróbel, 2021, Study code: G-70-20

9.9.2 Risk assessment

The evaluation of the risk for soil microorganisms was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev 2 (final), October 17, 2002).
The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2, Table 8.7-3 and were already used in the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) (see 9.8). To achieve a concise risk assessment, the risk envelope approach is applied.

Table 9.9-2: Assessment of the risk for effects on soil micro-organisms due to the use of CHR/ZF/PROTI in cereals (worse case use from GAP table in soil)

Intended use	Cereals		
N-mineralisation			
Product/active substance	**Max. conc. with effects ≤ 25 % (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**Risk acceptable?**
Prothioconazole	2.71	0.0333	yes
Prothioconazole-desthio	1.37	0.0152 ~~0.0078~~	yes
Prothioconazole-S-methyl	2.69	0.0043 ~~0.0019~~	yes
CHR/ZF/PROTI	1.31 mg test item/kg dry weight of soil (i.e. 0.13 mg of prothioconazole/kg dry weight of soil) and 5 x PEC: 6.55 mg of the test item / kg dry weight of soil (i.e. 0.65 mg of prothioconazole/kg dry weight of soil) did not have any long-term adverse effects on the process of nitrogen transformation in aerobic surface soils.	0.3627	yes
C-mineralisation			
Product/active substance	**Max. conc. with effects ≤ 25 % (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**Risk acceptable?**
Not required			

9.9.3 Overall conclusions

The Predicted Environmental Concentrations of the formulation CHR/ZF/PROTI 100 FS and its active substance prothioconazole in soil are below the concentrations at which no unacceptable effects (< 25%) regarding the soil microbial activity were observed after 42 days or more of exposure, indicating that the proposed use of CHR/ZF/PROTI 100 FS poses an acceptable risk to soil microorganisms.

Review comments:

The risk assessment for soil micro-organisms exposed to CHR/ZF/PROTI 100 FS, following the proposed uses of the formulation, was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology" (SANCO/10329/2002).
The risk assessment presented in Table 9.9-2 is agreed by the izRMS. The relevant PECsoil for risk assessments is taken from Section 8 (Environmental Fate), for details please, refer to Section 8. Based on the obtained results, soil nitrate formation rates were below the 25% trigger value. Thus, it is concluded that CHR/ZF/PROTI 100 FS had no significant impact on soil microorganisms when applied at test item concentrations up 6.55 mg formulation/kg soil dry weight.

On the basis of results it was assumed that CHR/ZF/PROTI 100 FS, does not pose unacceptable risk to soil microorganisms. The risk to soil micro-organisms is considered to be low for all representative uses.

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

9.10.1 Risk assessment

The formulation CHR/ZF/PROTI is a fungicide seed treatment and as such an evaluation of the effects on non-target terrestrial plants in the field is not required according SANCO/10329/2002 rev 2 final 17.10.2002.
As was written in COMMISSION REGULATION (EU) No 284/2013 of 1 March 2013: Data are not required, where exposure is negligible, for example in the case of rodenticides, active substances used for wound protection or seed treatment, or in the case of active substances used on stored products or in glasshouses where exposure is precluded.

Review comments:

Since CHR/ZF/PROTI 100 FS – Gamelan 100 FS/ Doraltes 100 FS is a fungicide seed treatment studies of the effects on non-target terrestrial plants are not required.

9.10.1.1 Tier-1 risk assessment (based screening data)

Not relevant.

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

Not relevant.

9.10.1.3 Higher-tier risk assessment

Not relevant.

9.10.1.4 Risk mitigation measures

No risk mitigation is required.

9.10.2 Overall conclusions

No new studies were necessary. As was written in COMMISSION REGULATION (EU) No 284/2013 of 1 March 2013: Data are not required, where exposure is negligible, for example in the case of rodenticides, active substances used for wound protection or seed treatment, or in the case of active substances used on stored products or in glasshouses where exposure is precluded.

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

Not relevant

9.12 Monitoring data (KCP 10.8)

Not available

9.13 Classification and Labelling

According to calculation method of ecotoxicological classification and data obtained from acute aquatic toxicity tests, product CHR/ZF/PROTIO 100 FS is classified as Aquatic Chronic 2, H411.

Review comments:

The Applicant present proposition of the classification. However, izRMS added detailed information for for the formulation CHR/ZF/PROTI 100 FS – Gamelan 100 FS/ Doraltes 100 FS.

CHR/ZF/PROTI 100 FS was classified and labeled according to REGULATION (EC) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006.

Prothioconazole: 100 g/L (10%). Substance is not ready biodegradable.

CLASSIFICATION	
Hazard classes, categories:	No acute classification, Aquatic Chronic 2, H411
LABELLING	
Hazard pictograms:	 GHS09
Signal word:	Warning
Hazard statements:	H411 – Toxic to aquatic life with long lasting effects
Precautionary statements:	P391 - Collect spillage. P501 - Dispose of contents/container to an approved waste disposal plant.
EUH401	To avoid risks to man and the environment, comply with the instructions for use.

Standard phrases under Regulation (EU) No 547/2011

SP 1	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).
SPe5	To protect birds/wild mammals the treated seeds should be entirely incorporated in the soil; ensure that the treated seeds are also fully incorporated at the end of rows.
SPe6	To protect birds/wild mammals remove spillages.

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.1.1	Adamczak, A.	2021	*CHR/ZF/PROTI - TER Calculations for Terrestial Verterbrates* no GLP Unpublished	N	Chemirol
KCP 10.1.1/02	Appeltauer, A.	2021	*Determination of Residues of Prothioconazole and PTZ-Desthio in Winter Wheat Seeds and Seedlings after Drilling of Coated Seeds at 4 Sites in Central Europe in 2021* Eurofins Agroscience Services Ecotox GmbH, Niefern-Öschelbronn, Germany Study code: S21-06525 GLP Unpublished	N	Chemirol
KCP 10.1.2	Adamczak, A.	2021	*CHR/ZF/PROTI - TER Calculations for Terrestial Verterbrates* no GLP Unpublished	N	Chemirol
KCP 10.1.2/02	Appeltauer, A.	2021	*Determination of Residues of Prothioconazole and PTZ-Desthio in Winter Wheat Seeds and Seedlings after Drilling of Coated Seeds at 4 Sites in Central Europe in 2021* Eurofins Agroscience Services Ecotox GmbH, Niefern-Öschelbronn, Germany Study code: S21-06525 GLP Unpublished	N	Chemirol
KCP 10.2/01	Turek-Lipka, T.	2021	*CHR/ZF/PROTI 100 FS – Daphnia Magna, Acute Immobilisation Test* Łukasiewicz Research Network - Institute of Industrial Organic Chemistry, Branch Pszczyna, Poland Study code: W-54-20 GLP Unpublished	N	Chemirol

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 10.2/02	Turek-Lipka, T.	2021	*CHR/ZF/PROTI 100 FS – Daphnia Magna, Acute Immobilisation Test* Łukasiewicz Research Network - Institute of Industrial Organic Chemistry, Branch Pszczyna, Poland Study code: W-54-20 GLP Unpublished	N	Chemirol
10.3.1/01	Kulec-Płoszczyca, E.	2021	*CHR/ZF/PROTI 100 FS Honeybees (Apis mellifera L.), Acute Oral Toxicity Test* Łukasiewicz Research Network - Institute of Industrial Organic Chemistry, Branch Pszczyna, Poland Study code: B-24-21 GLP Unpublished	N	Chemirol
10.3.1/02	Kulec-Płoszczyca, E.	2021	*CHR/ZF/PROTI 100 FS Honeybees (Apis mellifera L.), Acute Contact Toxicity Test* Łukasiewicz Research Network - Institute of Industrial Organic Chemistry, Branch Pszczyna, Poland Study code: B-25-21 GLP Unpublished	N	Chemirol
10.3.2/01	Stonham, D.	2021	*CHR/ZF/PROTI 100 FS – An Extended Laboratory Test to Evaluate the Effects of Treated Wheat Seed on the Ground Beetle, Poecilus cupreus (Coleoptera: Carabidae)* Mambo- Tox A Division of Cawood Scientific Ltd., Southampton, UK Study code: CHR-21-01 GLP Unpublished	N	Chemirol
10.3.2/02	Tew, G.	2021	*CHR/ZF/PROTI 100 FS – An extended laboratory test to evaluate the effects of treated wheat seed on the rove beetle Aleochara bilineata (Coleoptera; Staphylinidae)* Mambo- Tox A Division of Cawood Scientific Ltd., Southampton, UK Study code: CHR-21-02 GLP Unpublished	N	Chemirol

10.4/01	Wróbel, A.	2021	*CHR/ZF/PROTI 100 FS – Earthworm reproduction test (Eisenia andrei)* Łukasiewicz Research Network - Institute of Industrial Organic Chemistry, Branch Pszczyna, Poland Study code: G-67-20	N	Chemirol
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**
			GLP Unpublished		
10.4/02	Wróbel, A.	2021	*CHR/ZF/PROTI 100 FS – Collembolan (Folsomia candida) Reproduction Test* Łukasiewicz Research Network - Institute of Industrial Organic Chemistry, Branch Pszczyna, Poland Study code: G-68-20 GLP Unpublished	N	Chemirol
10.4/03	Wróbel, A.	2021	*CHR/ZF/PROTI 100 FS – Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil* Łukasiewicz Research Network - Institute of Industrial Organic Chemistry, Branch Pszczyna, Poland Study code: G-69-20 GLP Unpublished	N	Chemirol
10.5	Wróbel, A.	2021	*CHR/ZF/PROTI 100 FS – Soil Microorganisms: Nitrogen Transformation Test* Łukasiewicz Research Network - Institute of Industrial Organic Chemistry, Branch Pszczyna, Poland Study code: G-70-20 GLP Unpublished	N	Chemirol

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 10.1/01	XXX	1999	JAU 6476 techn.ai: Acute oral toxicity for bobwhite quail (Colinus virginianus) XXX	Y	BAY

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**
KCP 10.1/02	XXX	1990	SXX 0665 (Technical Grade) acute oral LD50 to bobwhite quail XXX	Y	BAY
KCP 10.1/03	XXX	2001	JAU 6476 techn.: 5 day-dietary LC50 for bobwhite quail (Colinus virginianus) XXX	Y	BAY
KCP 10.1/04	XXX	2001	JAU 6476 techn.: 5-day-dietary LC50 to mallard duck (Anas platyrhynchos) XXX	Y	BAY
KCP 10.1/05	XXX	2001	JAU 6476-desthio.: 5-day-dietary LC50 for bobwhite quail (Colinus virginianus) XXX	Y	BAY
KCP 10.1/06	XXX.	2000	Reproduction study in bobwhite quail with JAU 6476 (by dietary admixture) XXX	Y	BAY
KCP 10.1/07	XXX	2000	Reproduction study in mallard duck with JAU 6476 (by dietary admixture) XXX	Y	BAY
KCP 10.1/08	XXX	2002	JAU6476-desthio techn. Ai.: Effects of a subcronic dietary exposure to the northern bobwhite quial including effects on reproduction and behaviour XXX	Y	BAY

KCP 10.1/09	XXX	2001	Desthio JAU-6476: A reproduction study with the mallard (Anas platyrhynchos) XXX	Y	BAY
KCP 10.2/01	XXX	1999	JAU 6476 – Acute toxicity (96 haours) to Rainbow trout (Oncorhynchus mykiss) in a static test XXX	Y	BAY
KCP 10.2/02	XXX	1999	JAU 6476 – Acute toxicity (96 hours) to bluegill (Lepomis macrochirus) in a static test XXX	Y	BAY
KCP	XXX	2000	JAU 6476 – Acute toxicity (96 hours) to common carp (cyprinus carpio) in a static test	Y	BAY

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**
10.2/03			XXX		
KCP 10.2/04	XXX	1990	SXX 0665 techn. Acute toxicity to rainbow trout in a static test XXX	Y	BAY
KCP 10.2/05	XXX	1991	SXX 0665: Acute toxicity to golden orfe in a static test XXX	Y	BAY
KCP 10.2/06	XXX	2001	JAU 6476-S-methyl – Acute toxicity (96 hours) to raumbow trout (Oncorhynchus mykiss) in a semi-static test XXX	Y	BAY
KCP 10.2/07	XXX.	1983	Report on the test for acute toxicity of CGA 98032 to rainbow trout XXX	Y	Bay

KCP 10.2/08	XXX	2002	1,2,4-Triazole-Juvenile growth test, fish (Oncorhynchus mykiss) XXX	Y	BAY
KCP 10.2/09	XXX	2001	JAU 6476 – Early life-stage toxicity test with rainbow trout (Oncorhynchus mykiss) under flow-through conditions XXXgr	Y	BAY
KCP 10.2/10	XXX	2002	JAU 6476-desthio: Early life-stage toxicity test with rainbow trout (Oncorhynchus mykiss) under flowthrough conditions XXX	Y	BAY
KCP 10.2/11	XXX.	2001	(14C)-JAU 6476 – Bioconcentration and biotransformation in bluegill (Lepomis macrochirus) under flowthrough conditions XXX	Y	BAY
KCP 10.2/12	XXX	2001	[14]-JAU 6476-desthio- Bioconcentration and biotransformation in bluegill (Lepomis macrochirus) under flow-through conditions XXX	Y	BAY
KCP 10.2/13	Schneider, J.	2002	Estimation of Partition Coefficient in Octanol-Water of JAU 6476-S-methyl Report No.: MO-02-002532 Bayer AG	N	BAY

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**
			non GLP Unpublished		
KCP 10.2/14	Heimbach, F.	1999	Acute toxicity of JAU 6476 (tech.) to water fleas (Dpahnia magna) Report No.: HBF/DM 212 Bayer AG GLP Unpublished	N	BAY

KCP 10.2/15	Heimbach, F.	1990	Acute toxicity of SXX 0665 (techn.) to waterfleas (Daphnia magna) Report No.: HBF/DM 95 Bayer AG GLP Unpublished	N	BAY
KCP 10.2/16	Dorgerloh, M. Sommer, H.	2001	Acute toxicity of JAU 6476-S-methyl to waterfleas (Daphnia magna) Report No.: DOM 21055 Bayer AG GLP Unpublished	N	BAY
KCP 10.2/17	Rufli, H.	1983	Report on the test for acute toxicity of CGA 98032 to Daphnia magna Report No.: 821416 Ciba-Gergy Limited, Basel, Switzerland Bayer AG non GLP Unpublished	N	BAY
KCP 10.2/18	Hendel, B. Sommer, H.	2001	Influence of JAU 6476 (tech) on the reproduction rate of water fleas Report No.: HDB/RDM 67 Bayer AG GLP Unpublished	N	BAY
KCP	Dorgerloh, M.	2001	Influence of JAU 6476-desthio on the reproduction rate of water fleas in a static renewal laboratory test	N	BAY

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**

10.2/19	Sommer, H.		system Report No.: DOM 21036 Bayer AG GLP Unpublished		
KCP 10.2/20	Dorgerloh, M.	2000	JAU 6476 – Influence on the growth of the green alga, Selenastrum capricornutum Report No.: DOM 99107 Bayer AG GLP Unpublished	N	BAY
KCP 10.2/21	Heimbach, F.	1990	Growth inhibition of green algae (Scendedesmus subspicatus) by SXX 0665 (tech.) Report No.: HBF/AL 78 Bayer AG GLP Unpublished	N	BAY
KCP 10.2/22	Dorgerloh, M. Sommer, H.	2001	JAU 6476-S-methyl – Influence on the growth of the green alga, Selenastrum capricornutum Report No.: DOM 21028 Bayer AG GLP Unpublished	N	BAY
KCP 10.2/23	Palmer, S.J. Kendall, T.Z. Krueger, H.O.	2001	1,2,4-Triazole: A 96-hour toxicity test with the freshwater alga (Selenastrum capricornutum) Report No.: 528A-101 Wildlife International Ltd., Easton, MD, USA Bayer AG GLP Unpublished	N	BAY
KCP 10.2/24	Hendel, B.	2000	Influence of JAU 6476 (tech.) on development and emergence of larvae of Chironomus riparius in a water-sedmient system Raport No.: HDB/CH 42 Bayer AG	N	BAY

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**
			GLP Unpublished		
KCP 10.2/25	Hendel, B.	2000	Influence of SXX 0665 (tech.) on development and emergence of larvae of Chironomus riparius in a water-sedmient system Report No.: HDB/CH 43 Bayer AG GLP Unpublished	N	BAY
KCP 10.3	Wilhelmy, H.	1999	JAU 6476 a.i. – Acute effects on the honeybee Apis mellifera Report No.: IBA64051 Noack Laboratorium, Sarstedt, Germany Bayer AG GLP Unpublished	N	BAY
KCP 10.4/01	Meisner, P.	2000	Influence of JAU 6476 EC 250 on the reproduction of earthworms (Eisenia fetida) Report No.: MPE/RG 235 Bayer AG GLP Unpublished	N	BAY
KCP 10.4/02	Meisner, P.	2000	Influence of JAU 6476-desthio on the reproduction of earthworms (Eisenia fetida) Report No.: MPE/RG 332/00 Bayer AG GLP Unpublished	N	BAY

KCP 10.4/03	Heimbach, F.	2000	Influence of JAU 6476-S-Methyl on the reproduction of earthworms (Eisenia fetida) Report No.: HBF/RG 317 Bayer AG GLP Unpublished	N	BAY

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 10.5/01	Anderson, J. P. E.	1999	Influence of JAU 6476 technical ingredient on glucose simulated respiration in soils Report No.: AJO/203099 Bayer AG GLP Unpublished	N	BAY
KCP 10.5/02	Anderson, J. P. E.	1999	Influence of JAU 6476 technical ingredient on the microbial mineralization of nitrogen in soils Report No.: AJO/203199 Bayer AG GLP Unpublished	N	BAY
KCP 10.5/03	Anderson, J. P. E.	2000	Influence of the metabolite JAU-6476-desthio on the icrobial mineralization of nitrogen in soils Report No.: AJO/209400 Bayer AG GLP Unpublished	N	BAY
KCP 10.5/04	Anderson, J. P. E.	2001	Influence of the metabolite JAU 6476-desthio on the microbial mineralization of nitrogen in soils Report No.: AJO/219101 Bayer Ag GLP Unpublished	N	BAY

KCP 10.5/05	Anderson, J. P. E.	1999	Influence of the metabolite JAU 6476-S-methyl on glucose stimulated respiration in soils Reoirt No.: AJO/203399 Bayer AG GLP Unpublished	N	BAY
KCP 10.5/06	Anderson, J. P. E.	1999	Influence of the metabolite Jau 6476-S-methyl on the microbial mineralization of nitrogen in soils Report No.: AJO/203399 Bayer AG GLP Unpublished	N	BAY

Data point	**Author(s)**	**Year**	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
KCP 10.4/04	Nienstedt, K. M.	2002	Reproduction toxicity test exposing Folsomia candida (collembola) to JAU 6476 technical Report No.: 1022.028.641 Springborn Laboratories AG, Horn, Switzerland Bayer AG GLP Unpublished	N	BAY
KCP 10.4/05	Hoogendoom, G. M.	2000	An extended laboratory study to evaluate the effects of JAU 6476 on the predaceous mite Hypoaspis aculeifer canestrini (acari: Laelapidae) Report No.: B060HAE MITOX Stichting Bevordering Duurzame Plaagbestrijding, Amsterdam, Netherlands Bayer AG GLP Unpublished	N	BAY

KCP 10.4/06	Moser, T. Roembke, J.	2001	Acute and reproduction toxicity of JAU 6476-Desthio to the collembolan species Folsomia candida according to the ISO Guideline 11267 Report No.: P1CR ECT GmbH, Floersheim, Germany Bayer AG GLP Unpublished	N	BAY
KCP 10.4/07	Nienstedt, K. M. Novent, O.	2001	Reproduction toxicity test exposing Folsomia candida (Collembola) to JAU 6476-desthio Report No.: 1022.020.641 Springborn Laboratories AG, Horn, Switzerland Bayer AG GLP Unpublished	N	BAY
KCP 10.4/08	Moser, T. Scheffczyk, A.	2001	Acute and reproduction toxicity of JAU 6476-S-methyl to the collembolan species Folsomia candida Report No.: P35CR ECT GmbH, Floersheim, Germany Bayer AG	N	BAY

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**
			GLP Unpublished		
KCP 10.5/07	Mueller, G.	1999	Investigation of the cological properties of JAU 6476 Report No.: 839 N/99 Bayer AG GLP Unpublished	N	BAY

Appendix 2 Detailed evaluation of the new studies

Review Comment:
In order to provide sufficient detail, where appropriate, the following studies summaries have been adapted by the izRMS. Details were taken directly from the full studies reports provided in the dossier. zRMS text is highlighted in grey. The comments on individual studies are provided in grey comment boxes.

A 2.1 KCP 10.1 Effects on birds and other terrestrial vertebrates

A 2.1.1 KCP 10.1.1 Effects on birds

No new studies was performed.

A.2.1.1.1 KCP 10.1.1.1 Acute oral toxicity No

additional studies were performed.

A.2.1.1.2 KCP 10.1.1.2 Higher tier data on birds No

additional studies were performed.

A 2.1.2 KCP 10.1.2 Effects on terrestrial vertebrates other than birds No

additional studies were performed.

A 2.1.2.1 KCP 10.1.2.1 Acute oral toxicity to mammals

No additional studies were performed.

Summarised in Section 6 (Mammalian Toxicology)

A 2.1.2.2 KCP 10.1.2.2 Higher tier data on mammals

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

A 2.2 KCP 10.2 Effects on aquatic organisms

Comments of izRMS:	The study was conducted to OECD guideline 202 and according to the principles of GLP. In the definitive test the validity criteria were met according to OECD Guideline No. 202. No deviations from the guideline were noted during the study. The analytical measurements demonstrated that the test item concentrations throughout the test was within 80-120% of nominal and for this reason endpoints are expressed as nominal concentrations. The study is reliable and suitable for the risk assessment. All results refer to nominal concentrations. Following endpoints based on nominal test item concentrations would be used for risk assessment purposes: EC_{50}/48 h is 19.76 mg/L. LOEC/48 h value is 25.0 mg/L. NOEC/48 h value is 12.5 mg/L.

A 2.2.1.1.1 Study 1

Reference:	KCP 10.2/01
Report	CHR/ZF/PROTI 100 FS – Daphnia Magna, Acute Immobilisation Test, T. Turek-Lipka, 2021, Study code: W-54-20
Guideline(s):	the OECD Guideline No. 202 (2004)/ EU method C.2.
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

The aim of the study was to determine the test item concentration causing 50% immobilisation of Daphnia magna i.e. EC50 value after 48 h of exposure. The LOEC and NOEC values were also determined.

Materials and methods

Test item:	Protiokonazol 100 FS [CHR/ZF/PROTI 100 FS]; batch no.: 202003, the (determined) content of prothioconazole: 9.63 ± 0.20 %; density: 1.088 g/mL; production date: April 01, 2020, expiry date: April 01, 2022.
Test organism:	Daphnia magna Straus (< 24 h old at exposure initiation); not first brood progeny; neonates collected from a laboratory culture cultivated at the Łukasiewicz Research Network - Institute of Industrial Organic Chemistry, Branch Pszczyna.
Test design:	Static test (48 h of exposure); 4 replicates per test item concentration and the control; 5 Daphnia magna in each replicate.

Nominal test item concentrations:	100, 50, 25, 12.5, 6.25 mg/L plus the control.
Test conditions:	Temperature: 20.4 – 22.0°C; pH of the control: 7.46 – 7.51; dissolved oxygen concentration in the control: 8.8 – 8.9 mg/L; daily cycle 16 h light : 8 h dark; fluorescent light source; no feeding; no aeration; medium: Elendt M7.
Statistics:	Probit method calculations and analyses by: Stepdown Cochran-Armitage Test Procedure.
Chemical determinations:	The concentrations of prothioconazole were determined with the validated high performance liquid chromatographic method with DAD detection.
Endpoint values:	EC50/48 h, NOEC/48 h, and LOEC/48 h.

Preliminary test (non-GLP)
In the test, the recorded temperature was in a range of 20.2 – 20.9°C. The pH values measured in all test item concentrations and the control were in the ranges of 7.52 – 7.62 at exposure initiation and 7.46 – 7.55 at exposure termination. The dissolved oxygen concentrations measured in all test item concentrations and the control were in the ranges of 8.4 – 8.8 mg/L at exposure initiation and 8.2 – 8.4 mg/L at exposure termination.

Nominal test item concentration [mg/L]	Measured at exposure initiation #		Measured at exposure termination *	
	pH value	Dissolved oxygen concentration [mg/L]	pH value	Dissolved oxygen concentration [mg/L]
Control	7.54	8.4	7.55	8.4
0.1	7.57	8.6	7.54	8.3
1.0	7.60	8.7	7.48	8.4
10	7.62	8.8	7.46	8.3
100	7.52	8.8	7.48	8.2

#- pH values and dissolved oxygen concentrations measured in samples before split up into replicates
*- pH values and dissolved oxygen concentrations measured in samples of pooled replicates

At exposure termination, in the control and in the test item concentrations of 0.1, 1.0 and 10 mg/L no immobilisation of Daphnia magna was observed. In the test item concentration of 100 mg/L, the immobilisation of Daphnia magna was 100 %.

Nominal test item concentration [mg/L]	Number of *Daphnia magna*	Number of immobilised *Daphnia magna*								Total of immobilised *Daphnia magna* [%]	
		24 h				48 h					
		Replicates									
		A	B	C	D	A	B	C	D	24 h	48 h
Control	20	0	0	0	0	0	0	0	0	0	0
0.1	20	0	0	0	0	0	0	0	0	0	0
1.0	20	0	0	0	0	0	0	0	0	0	0
10	20	0	0	0	0	0	0	0	0	0	0
100	20	5	5	5	5	5	5	5	5	100	100

Time of exposure: 20.01.2021 – 22.01.2021

Results of chemical determinations

In the stability test, the concentrations of prothioconazole were chemically determined using the validated high performance liquid chromatographic method with DAD detection [SOP/C/407]. Samples of the test item concentrations of 10 and 100 mg/L, and the control collected at exposure initiation, after 24 and 48 hours of exposure initiation were chemically determined [SOP/W/83].

At exposure initiation, the determined concentrations of prothioconazole were 94.4 and 93.9% of the nominal concentration in the test item concentrations of 10 and 100 mg/L, respectively. The results confirm that the test item concentrations were prepared correctly.

After 24 hours of exposure, the determined concentrations of prothioconazole were 90.2 and 90.9% of the nominal concentration in the test item concentrations of 10 and 100 mg/L, respectively. At exposure termination, the determined concentration of prothioconazole was 84.9 and 90.6% of the nominal concentration in the test item concentrations of 10 and 100 mg/L, respectively. Therefore, the concentration of prothioconazole was stable under test conditions. The results are given in table below. Based on the chemical determinations results, the definitive test was planned to be performed in a static design.

Nominal test item concentration [mg/L]	Nominal concentration of prothioconazole [mg/L]	Average determined concentration of prothioconazole (n=3) in samples collected					
		at exposure initiation [mg/L]	% of nominal concentration	after 24 h of exposure [mg/L]	% of nominal concentration	after 48 h of exposure [mg/L]	% of nominal concentration
Control	–	< LoD	–	< LoD	–	< LoD	–
10	0.963	0.909	94.4	0.868	90.2	0.818	84.9
100	9.630	9.047	93.9	8.757	90.9	8.729	90.6

LOQ = 0.05 mg/L
LOD = 0.015 mg/L
– no value

Definitive test

In the definitive test, the recorded temperature during exposure was in the range of 20.4 – 22.0°C and constant within 1.6ºC. The measured pH values were in the range of 7.45 – 7.53 in the test item concentrations and the control at exposure initiation, and in the range of 7.25 – 7.51 in the test item concentrations and the control at exposure termination. The measured dissolved oxygen concentrations were in the range of 8.9 – 9.0 mg/L in the test item concentrations and the control at exposure initiation, and in the range of 8.0 – 8.8 mg/L in the test item concentrations and the control at exposure termination (table below).

Nominal test item concentration [mg/L]	Measured at exposure initiation #		Measured at exposure termination *	
	pH value	Dissolved oxygen concentration [mg/L]	pH value	Dissolved oxygen concentration [mg/L]
Control	7.46	8.9	7.51	8.8
6.25	7.51	8.9	7.45	8.7
12.5	7.53	9.0	7.39	8.8
25	7.50	8.9	7.38	8.6
50	7.48	8.9	7.28	8.3
100	7.45	8.9	7.25	8.0

#- pH values and dissolved oxygen concentrations measured in samples before split up into replicates
*- pH values and dissolved oxygen concentrations measured in samples of pooled replicates

At exposure termination in the control and in the test item concentrations of 6.25, 12.5, 25, 50 and 100 mg/L, the immobilisation of Daphnia magna was 5, 0, 5, 80, 100 and 100 %, respectively.
No abnormal behaviour of Daphnia magna was observed during exposure.
No abnormal behaviour of Daphnia magna was observed during exposure.
The immobilisation of Daphnia magna after 24 h and 48 h of exposure is given in the table below.

Nominal test item concentration [mg/L]	Number of *Daphnia magna*	Number of immobilised *Daphnia magna*								Total of immobilised *Daphnia magna* [%]	
		24 h				48 h					
		Replicates									
		A	B	C	D	A	B	C	D	24 h	48 h
Control	20	0	0	0	0	0	0	1	0	0	5
6.25	20	0	0	0	0	0	0	0	0	0	0
12.5	20	1	0	0	0	1	0	0	0	5	5
25	20	3	0	2	0	4	5	3	4	25	80
50	20	4	3	5	2	5	5	5	5	70	100
100	20	5	5	5	5	5	5	5	5	100	100

Time of exposure: 17.02.2021 – 19.02.2021

Results of chemical determinations

In the definitive test, the recorded temperature during exposure was in the range of 20.4 – 22.0°C and constant within 1.6ºC (Figure 8). The measured pH values were in the range of 7.45 – 7.53 in the test item concentrations and the control at exposure initiation, and in the range of 7.25 – 7.51 in the test item concentrations and the control at exposure termination. The measured dissolved oxygen concentrations were in the range of 8.9 – 9.0 mg/L in the test item concentrations and the control at exposure initiation, and in the range of 8.0 – 8.8 mg/L in the test item concentrations and the control at exposure termination. At exposure termination in the control and in the test item concentrations of 6.25, 12.5, 25, 50 and 100 mg/L, the immobilisation of Daphnia magna was 5, 0, 5, 80, 100 and 100 %, respectively. No abnormal behaviour of Daphnia magna was observed during exposure. No abnormal behaviour of Daphnia magna was observed during exposure. The immobilisation of Daphnia magna after 24 h and 48 h of exposure is given in the table below.

Nominal test item concentration [mg/L]	Number of *Daphnia magna*	Number of immobilised *Daphnia magna*								Total of immobilised *Daphnia magna* [%]	
		24 h				48 h					
		Replicates									
		A	B	C	D	A	B	C	D	24 h	48 h
Control	20	0	0	0	0	0	0	1	0	0	5
6.25	20	0	0	0	0	0	0	0	0	0	0
12.5	20	1	0	0	0	1	0	0	0	5	5
25	20	3	0	2	0	4	5	3	4	25	80
50	20	4	3	5	2	5	5	5	5	70	100
100	20	5	5	5	5	5	5	5	5	100	100

Time of exposure: 17.02.2021 – 19.02.2021

Endpoint values

The endpoint values were determined based on the nominal test item concentrations. The endpoint values were calculated with a probit method. The lowest observed effect concentration (LOEC) and the no observed effect concentration (NOEC) were estimated on the basis of statistical analysis. To conduct statistical analysis, the ToxRat Professional commercial software was used [SOP/W/68]. The endpoint values are presented in table below.

Endpoint values [mg/L]	Time of exposure	
	24 h	48 h
EC_{50}	34.92 (28.26 – 43.28)	19.76 (16.74 – 23.06)
EC_{20}	22.15 (15.87 – 27.47)	15.65 (11.93 – 18.20)
EC_{10}	17.46 (11.35 – 22.41)	13.85 (9.79 – 16.42)
LOEC	25	25
NOEC	12.5	12.5

Calculations were made according to [7], [SOP/W/68].
(-) - 95% confidence interval

The median concentration causing 50% immobilisation of Daphnia magna after 24 h of exposure, i.e. the EC50/24 h value is 34.92 mg/L (95% confidence interval 28.26 – 43.28). The EC20/24 h value is 22.15 mg/L (95% confidence interval 15.87 – 27.47). The EC10/24 h value is 17.46 mg/L (95% confidence interval 11.35 – 22.41). The median concentration causing 50% immobilisation of Daphnia magna after 48 h of exposure, i.e. the EC50/48 h value is 19.76 mg/L (95% confidence interval 16.74 – 23.06).
The EC20/48 h value is 15.65 mg/L (95% confidence interval 11.93 – 18.20). The EC10/48 h value is 13.85 mg/L (95% confidence interval 9.79 – 16.42). The data on immobilisation of the Daphnia magna at exposure termination were analysed using Step-down Cochran-Armitage Test Procedure, which showed significant differences between the nominal test item concentrations in the range of 25 – 100 mg/L and the control. Therefore, the LOEC/48 h value is 25 mg/L and the NOEC/48 h value is 12.5 mg/L.

Validity criteria

In the definitive test, the validity criteria were met according to the OECD Guideline No.

202 (2004):
- the percentage of immobilisation of Daphnia magna in the control was 5% (criterion: not more than 10%),
- the dissolved oxygen concentrations in the test vessels were within the range of 8.0 – 9.0 mg/L (criterion: not less than 3 mg/L).

Results
The effect of the test item on immobilisation of Daphnia magna was assessed. The test item concentrations used in the definitive test were determined on the basis of the preliminary tests results. The Daphnia magna were considered immobile if they showed no ability to swim within 15 seconds after gentle swirling of the test vessel.

A 2.2.1.1.2 Study 2

Comments of izRMS:	The study was conducted to OECD guideline 201 and according to the principles of GLP. In the definitive test the validity criteria were met according to OECD Guideline No. 201. No deviations from the guideline were noted during the study. The analytical measurements demonstrated that the test item concentrations throughout the test was outside 80-120% of nominal and for this reason endpoints should be expressed as measured concentrations. Following endpoints based on measured test item concentrations would be used for risk assessment purposes: ErC_{50}= 14.69 mg formulation/L (measured) EyC_{50}= 5.53 mg formulation/L (measured)

Reference:	KCP 10.2/02
Report	CHR/ZF/PROTI 100 FS – *Raphidocelis subcapitata* SAG 61.81 (formerly Pseudokirchneriella subcapitata), Growth inhibition test, T. Turek-Lipka, 2021, Study code: W-55-20
Guideline(s):	the OECD Guideline No. 201 (2006)/EU method C.3.
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

The aim of the study was to determine the test item concentrations causing 50% inhibition of growth rate and yield of the algae, Raphidocelis subcapitata SAG 61.81 (formerly Pseudokirchneriella subcapitata) (ErC50 and EyC50 after 72 hours of exposure, respectively). The LOEC and NOEC values were also determined.

Materials and methods

Test item:	Protiokonazol 100 FS [CHR/ZF/PROTI 100 FS]; batch no.:202003, the (determined) content of prothioconazole: 9.63 ± 0.20 %; density: 1.088 g/mL; production date: April 01, 2020, expiry date: April 01, 2022.

Test organism:	The unicellular freshwater green algae, Raphidocelis subcapitata (formerly Pseudokirchneriella subcapitata (Korshikov) Hindák, Selenastrum capricornutum Prinz) SAG 61.81 cultivated at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna, Department of Ecotoxicological Studies, Laboratory of Aquatic Organisms Toxicology. The algae were obtained from the Culture Collection of Algae at Göttingen University, Germany.
Test design:	72 hours of exposure; three replicates per each test item concentration; six replicates per control; initial algal cell density: 1 x 10^4 cells/mL.
Nominal test item concentrations:	100, 33, 11, 3.70, 1.23 mg/L plus the control.
Test conditions:	Temperature: 21.8 – 22.5°C; pH of the control: 7.54 – 7.69; mean light intensity: 7010 – 7225 lux; constant illumination and shaking; medium: AAP.
Statistics:	Probit method calculations and analyses by: Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Williams Multiple Sequential t-test Procedure, Multiple Sequentially-rejective Welsht-test After Bonferroni- Holm.
Chemical determinations:	The concentrations of prothioconazole were determined with the validated high performance liquid chromatographic method with DAD detection.
Endpoint values:	ErC50/72 h, EyC50/72 h, NOEC/72 h, LOEC/72 h.

Results and discussions
The effect of the test item on the green algal growth was assessed. The range of the test item concentrations used in the definitive test was determined on the basis of the preliminary test results. The growth inhibition was estimated on the basis of the density of the algae cells determined in the definitive test.

Preliminary test (non–GLP)
The recorded temperature was in the range of 21.6 – 22.2°C. The mean light intensity was in the range of 7498 - 7515 lux. The pH values were in the ranges of 7.56 – 7.78 at exposure initiation and 7.80 – 8.11 at exposure termination.

Nominal test item concentration [mg/L]	pH values# at exposure initiation	pH values* at exposure termination
Control	7.56	8.07
0.1	7.78	8.08
1.0	7.71	8.11
10	7.67	7.96
100	7.59	7.80

- pH measured in samples before splitting up into replicates
* - pH measured in samples of pooled replicates

The average transmittance values were between 52.7 – 100.0% at exposure initiation and 45.5 – 100.0% at exposure termination when compared with the control [SOP/W/12]. Hence, the number of cells was determined with a direct method which involves counting the number of cells in the Bürker chamber under a microscope [SOP/W/9, SOP/W/19]. In case of each replicate, the number of cells was determined after 72 h of exposure.
No growth rate inhibition was observed in the test item concentrations of 1.0 mg/L. The growth rate inhibition after 72 hours of exposure was 0.18, 17.25, and 99.89 % in the test item concentrations of 0.1, 10 and 100 mg/L, respectively. No yield inhibition was observed in the test item concentration of 1.0 mg/L. The yield inhibition after 72 hours of exposure was 1.42, 51.45, and 99.88 % in the test item concentrations of 0.1, 10 and 100 mg/L, respectively. The inhibition of growth rate and yield estimated in comparison to the control after 72 hours of exposure are given in the table below.

Nominal test item concentration [mg/L]	% inhibition after 72 h of exposure (growth rate)	% inhibition after 72 h of exposure (yield)
Control	0.00	0.00
0.1	0.18	1.42
1.0	-2.84*	-11.84*
10	17.25	51.45
100	99.89	99.88

Definitive test
The recorded temperature was in the range of 21.8 – 22.5°C and constant within 0.7ºC. The mean light intensity was in the range of 7010 – 7225 lux. The pH values measured at exposure initiation were in the range of 7.40 – 7.54 and at exposure termination were in the range of 7.38 – 7.69.

Nominal test item concentration [mg/L]	pH values# at exposure initiation	pH values* at exposure termination
Control	7.54	7.69
1.23	7.51	7.60
3.70	7.48	7.58
11	7.50	7.51
33	7.45	7.44
100	7.40	7.38

#- pH measured in samples before splitting up into replicates
*- pH measured in samples of pooled replicates

Morphology observations of the algae cells were performed at exposure termination. In all test item concentrations, no differences in shape, size and colour of algal cells were reported as compared to the algae cells in the control.

Nominal test item concentration [mg/L]	Observations
Control	Normal colour, shape and size of the algal cells
1.23	No changes
3.70	No changes
11	No changes
33	No changes
100	No changes

Algal cell density are presented in the table below.

Nominal test item concentration [mg/L]	Algal cell density [x10^6 cells/mL]		
	24 h	48 h	72 h
Control	0.029	0.208	0.704
	0.029	0.175	0.900
	0.038	0.188	0.913
	0.033	0.183	0.746
	0.042	0.175	0.738
	0.025	0.167	0.933
mean	**0.033**	**0.183**	**0.822**
standard deviation	*0.006*	*0.014*	*0.103*
1.23	0.029	0.167	0.908
	0.033	0.200	1.133
	0.029	0.192	1.021
mean	**0.030**	**0.186**	**1.021**
standard deviation	*0.002*	*0.017*	*0.113*
3.70	0.029	0.121	0.704
	0.029	0.146	0.879
	0.025	0.138	0.808
mean	**0.028**	**0.135**	**0.797**
standard deviation	*0.002*	*0.013*	*0.088*
11	0.021	0.104	0.354
	0.033	0.096	0.421
	0.021	0.092	0.408
mean	**0.025**	**0.097**	**0.394**
standard deviation	*0.007*	*0.006*	*0.036*
33	0.017	0.033	0.071
	0.025	0.025	0.079
	0.017	0.038	0.071
mean	**0.020**	**0.032**	**0.074**
standard deviation	*0.005*	*0.007*	*0.005*
100	0.013	0.013	0.013
	0.017	0.017	0.013
	0.017	0.013	0.013
mean	**0.016**	**0.014**	**0.013**
standard deviation	*0.002*	*0.002*	*0.000*

Time of exposure: 08.02.2021 – 11.02.2021

The average section-by-section, specific growth rates and yield calculated for the whole exposure are provided in.

Nominal test item concentration [mg/L]	Growth rate* [10^6 cells/mL]				Yield** [10^6 cells/mL]
	0-24 h	24-48 h	48-72 h	0-72 h	72 h
Control	1.065	1.970	1.219	1.418	0.694
	1.065	1.797	1.638	1.500	0.890
	1.335	1.599	1.580	1.505	0.903
	1.194	1.713	1.405	1.437	0.736
	1.435	1.427	1.439	1.434	0.728
	0.916	1.899	1.720	1.512	0.923
mean	**1.168**	**1.734**	**1.500**	**1.468**	**0.812**
standard deviation	*0.192*	*0.200*	*0.182*	*0.042*	*0.103*
1.23	1.065	1.751	1.693	1.503	0.898
	1.194	1.802	1.734	1.577	1.123
	1.065	1.890	1.671	1.542	1.011
mean	**1.108**	**1.814**	**1.700**	**1.541**	**1.011**
standard deviation	*0.075*	*0.071*	*0.032*	*0.037*	*0.113*
3.70	1.065	1.428	1.761	1.418	0.694
	1.065	1.616	1.795	1.492	0.869
	0.916	1.708	1.767	1.464	0.798
mean	**1.015**	**1.584**	**1.774**	**1.458**	**0.787**
standard deviation	*0.086*	*0.143*	*0.018*	*0.037*	*0.088*
11	0.742	1.600	1.225	1.189	0.344
	1.194	1.068	1.478	1.247	0.411
	0.742	1.477	1.489	1.236	0.398
mean	**0.893**	**1.382**	**1.398**	**1.224**	**0.384**
standard deviation	*0.261*	*0.279*	*0.150*	*0.031*	*0.036*
33	0.531	0.663	0.766	0.653	0.061
	0.916	0.000	1.151	0.689	0.069
	0.531	0.804	0.625	0.653	0.061
mean	**0.659**	**0.489**	**0.847**	**0.665**	**0.064**
standard deviation	*0.223*	*0.430*	*0.272*	*0.021*	*0.005*
100	0.262	0.000	0.000	0.087	0.003
	0.531	0.000	-0.268	0.087	0.003
	0.531	-0.268	0.000	0.087	0.003
mean	**0.441**	**-0.089**	**-0.089**	**0.087**	**0.003**
standard deviation	*0.155*	*0.155*	*0.155*	*0.000*	*0.000*

The relationship between the inhibition of growth rate and the nominal test item concentrations and between the inhibition of yield and the nominal test item at 72 h are provided in the table below.

Nominal test item concentration [mg/L]	% inhibition after 72 h of exposure (growth rate)	% inhibition after 72 h of exposure (yield)
Control	0.0	0.0
1.23	-5.0*	-24.4*
3.70	0.7	3.1
11	16.6	52.7
33	54.7	92.2
100	94.0	99.6

Results of the chemical determinations

The concentrations of prothioconazole were determined using a validated liquid chromatographic method with DAD detection [SOP/C/407]. Samples of each test item concentration and the control were collected at exposure initiation and at exposure termination. The results are presented in table below.

Nominal test item concentration [mg/L]	Nominal concentration of prothioconazole [mg/L]	Geometric mean of determined concentration of prothioconazole* [mg/L]	Average determined concentration of prothioconazole (n=3) in samples collected			
			at exposure initiation [mg/L]	% of nominal concentration	at exposure termination [mg/L]	% of nominal concentration
Control	–	-	< LoD	–	< LoD	–
1.23	0.118	0.087	0.113	95.8	0.067	56.8
3.70	0.356	0.235	0.324	91.0	0.171	48.0
11	1.059	0.647	0.938	88.6	0.446	42.1
33	3.178	1.943	2.788	87.7	1.354	42.6
100	9.630	7.969	8.848	91.9	6.694	69.5

LOQ = 0.05 mg/L
LOD = 0.015 mg/L
– no value
*geometric mean was calculated according to the formula [10]

At exposure initiation, the determined concentrations of prothioconazole were in the range of 87.7 – 95.8% of the nominal concentration. The results confirm that the test item concentrations were prepared correctly. At exposure termination, the determined concentrations of prothioconazole were in the range of 42.1 – 69.5% of the nominal concentration.
The results showed that concentrations of prothioconazole were lower than 80% of nominal concentrations at exposure termination. Therefore, the concentrations of prothioconazole were not stable under test conditions.

Endpoint values
The endpoint values are based on the nominal test item concentrations. The ECx values were calculated with a probit method. The lowest observed effect concentration (LOEC) and the no observed effect concentration (NOEC) were estimated on the basis of statistical analyses. To conduct statistical analyses, the ToxRat Professional commercial software was used [SOP/W/68]. The endpoint values are presented in the tables below.

Endpoint value [mg/L]	Time of exposure:		
	24 h	48 h	72 h
E_rC_{50}	49.24 (28.76 – 116.02)	24.85 (21.76 – 28.42)	28.37 (27.01 – 29.80)
E_rC_{20}	7.27 (1.89 – 13.61)	9.02 (7.12 – 10.84)	13.16 (12.12 – 14.15)
E_rC_{10}	2.67 (0.32 – 6.34)	5.31 (3.85 – 6.76)	8.80 (7.89 – 9.69)
LOEC	11	3.70	11
NOEC	3.70	1.23	3.70

(-) – 95% confidence interval
Calculations were made according to [8], [SOP/W/68]
n.d. – not determined

Endpoint value [mg/L]	Time of exposure:		
	24 h	48 h	72 h
E_yC_{50}	22.91 (12.03 – 51.29)	9.48 (8.07 – 11.13)	10.69 (9.87 – 11.58)
E_yC_{20}	3.46 (0.58 – 7.34)	3.27 (2.45 – 4.07)	5.82 (4.98 – 6.54)
E_yC_{10}	1.29 (0.09 – 3.51)	1.88 (1.27 – 2.50)	4.23 (3.42 – 4.95)
LOEC	11	3.70	11
NOEC	3.70	1.23	3.70

(-) – 95% confidence interval
Calculations were made according to [8], [SOP/W/68]
n.d. – not determined

The median test item concentration causing 50% inhibition of the average specific growth rate of Raphidocelis subcapitata, i.e. the ErC50/72 h value is 28.37 mg/L (95% confidence interval: 27.01 – 29.80). The ErC20/72 h value is 13.16 mg/L (95% confidence interval: 12.12 – 14.15) and the ErC10/72 h value is 8.80 mg/L (95% confidence interval: 7.89 – 9.69). Statistical tests based on the growth rate data were the Shapiro-Wilk's Test on Normal Distribution which confirmed normal distribution of the data, the Levene's Test on Variance Homogeneity (with Residuals) which showed that the variances were homogenous and the Williams Multiple Sequential t-test Procedure which showed significant differences between the test item concentrations in the range of 11 – 100 mg/L and the control. The lowest concentration of the test item causing an effect on growth rate, i.e. LOEC/72 h value is 11 mg/L and the highest concentration of the test item not causing any effect on growth rate, i.e. NOEC/72 h value is 3.7 mg/L. The median test item concentration causing 50% yield inhibition of Raphidocelis subcapitata, i.e. the EyC50/72 h value is 10.69 mg/L (95% confidence interval: 9.87 – 11.58). The EyC20/72 value is 5.82 mg/L (95% confidence interval: 4.98 – 6.54) and EyC10/72 h value is 4.23 mg/L (95% confidence interval: 3.42 – 4.95). Statistical tests based on the yield data were the Shapiro-Wilk's Test on Normal Distribution which confirmed normal distribution of the data, the Levene's Test on Variance Homogeneity (with Residuals) which showed that the variances were heterogenous and the Multiple Sequentially-rejective Welsh-t-test After Bonferroni-Holm which showed significant differences between the test item concentrations in the range of 11 - 100 mg/L and the control. The lowest concentration of the test item causing an effect on yield, i.e. LOEC/72 h value is 11 mg/L. The highest concentration of the test item not causing any effect on yield, i.e. NOEC/72 h value is 3.70 mg/L.

Validity criteria
In the definitive test, the following validity criteria specified in the OECD Guideline No.
201 (2006) were met:
- the biomass in the control increased by a factor of 82.2 within the 72-hour test period (criterion: at least a 16-fold growth),
- the coefficient of variation of the mean specific growth rate after the 72-hour test period (exposure initiation – exposure termination) in the control culture was 2.9% (criterion: it must not exceed 7%), - the mean coefficient of variation for the section-by-section growth rate in the control culture was 20.6% (criterion: it must not exceed 35%).

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

A 2.3.1.1.2 Study 1

Comments of izRMS:	The study was conducted to OECD guideline 213 and according to the principles of GLP. No deviations to the guideline were noted. In the definitive test the validity criteria were met according to OECD Guideline The study is reliable and suitable for the risk assessment.

Reference: KCP 10.3.1/01

Report: CHR/ZF/PROTI 100 FS Honeybees (Apis mellifera L.), Acute Oral Toxicity Test, Kulec-Płoszczyca, E., 2021, Study code: B-24-21

Guideline(s): OECD Guideline for the Testing of Chemicals No. 213 (1998) and the EU Method C.16. (2008)

Deviations: No

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study): No

Materials and methods

Test item: CHR/ZF/PROTI 100 FS content: 9.63 ± 0.20% (104.8 g/L) of Prothioconazole batch no.: 202003 production date: 01.04.2020 expiry date: 01.04.2022

the honeybee, *Apis mellifera* L., strain: carnica

Biological test system:

– age: approximately 3 weeks

– source: an apiary at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna [SOP/B/14],

Test design: – the test item: exposure duration: 48 hours number of doses: 5 doses and a control number of replicates: 3 replicates number of bees: 10 bees/replicate – the reference item: exposure duration: 24 hours number of doses: 3 doses number of replicates: 3 replicates number of bees: 10 bees/replicate

Test item doses: 12.5, 25.0, 50.0, 100.0 and 200.0 µg test item/bee and a control (0.0 µg/bee)

Reference item doses: 0.1, 0.2 and 0.4 µg a.i./bee

Test conditions: 24.0-26.0°C, 63-66% humidity

Preliminary test

Mortality of the control group after 48 hours of exposure was 0.0%. After 48 hours the percentages of mortality of the bees treated with the test item at the doses of 8.0, 40.0 and 200.0 µg/bee, were 0.0, 10.0 and 20.0%. No abnormal behavioural effects were observed during the test.

Definitive test

Mortality of the treated insects is presented in the tables below.
After 4 hours of exposure, mortality of the control group and the groups treated with the test item at the doses of 12.5, 25.0, 50.0, 100.0 and 200.0 µg/bee was 0.0%.
After 24 hours of exposure, mortality of the control group was 0.0% and for the treated groups' mortality percentages at the doses of 12.5, 25.0, 50.0, 100.0 and 200.0 µg/bee, were 0.0, 0.0, 3.3, 3.3 and 13.3%, respectively.
After 48 hours of exposure, mortality of the control group was 3.3%. For the treated groups' mortality percentages (at the doses of 12.5, 25.0, 50.0, 100.0 and 200.0 µg/bee, were 0.0, 0.0, 0.0, 0.0 and 10.3%, respectively.
The median lethal doses LD50/24 h and LD50/48 h are higher than the highest test item dose used in the test, i.e. 200.0 µg/honeybee.

Table 4. Honeybee mortality after 4 hours of exposure – definitive test

Dose [µg/bee]	Number of tested bees [no.]	Mortality				
		Number of dead bees [no.] replicates			Total	
		I	II	III	[no.]	[%]
0.0 (control)	30	0	0	0	0	0.0
12.5	30	0	0	0	0	0.0
25.0	30	0	0	0	0	0.0
50.0	30	0	0	0	0	0.0
100.0	30	0	0	0	0	0.0
200.0	30	0	0	0	0	0.0

Table 5. Honeybee mortality and the LD_{50} after 24 hours of exposure – definitive test

Dose [µg/bee]	Number of tested bees [no.]	Mortality					LD_{50} [µg/bee]
		Number of dead bees [no.] replicates			Total		
		I	II	III	[no.]	[%]	
0.0 (control)	30	0	0	0	0	0.0	> 200.0
12.5	30	0	0	0	0	0.0	
25.0	30	0	0	0	0	0.0	
50.0	30	1	0	0	1	3.3	
100.0	30	1	0	0	1	3.3	
200.0	30	1	1	2	4	13.3	

Table 6. Honeybee mortality and the LD_{50} after 48 hours of exposure – definitive test

Dose [µg/bee]	Number of tested bees [no.]	Mortality						LD_{50} [µg/bee]
		Number of dead bees [no.] replicates			Total			
		I	II	III	[no.]	[%]	[%][a]	
0.0 (control)	30	0	1	0	1	3.3	-	> 200.0
12.5	30	0	0	1	1	3.3	0.0	
25.0	30	0	1	0	1	3.3	0.0	
50.0	30	1	0	0	1	3.3	0.0	
100.0	30	1	0	0	1	3.3	0.0	
200.0	30	1	1	2	4	13.3	10.3	

[a]: mortality corrected according formula of Abbott's [6]

Definition of the endpoints

The LD50 (median lethal dose) oral is a statistically derived single dose of a test or reference item that can cause death in 50 per cent of biological test systems when administered by the oral route. The LD50 is expressed in µg of the test item per bee or in µg of the active ingredient contained in the reference item per bee. It was calculated with the log-probit method using ToxRat Proffesional software, version 3.3.0 [9, SPO/B/67]
Mortality: a honeybee is considered dead if it is completely immobile.

Test validity criteria

The following validity criteria were met during the test:

- the mortality for the control was 3.3% at the end of the experiment (criterion: it must not exceed 10%).
- the LD50/24 h of the reference item (dimethoate) was 0.15 µg a.i./bee (criterion: 0.10 – 0.35 µg a.i./bee).

A 2.3.1.1.3 KCP 10.3.1.1.2 Acute contact toxicity to bees

A 2.3.1.1.4 Study 1

Comments of izRMS:	The study was conducted to OECD guideline 214 and according to the principles of GLP. According to the Guideline No. 214/ EU Method C.17., the honeybees may be anesthetized with carbon dioxide for application of the test item. Anesthesia was replaced with mechanical immobilization. The mentioned deviation had not effect on the results of the study In the definitive test the validity criteria were met according to OECD Guideline The study is reliable and suitable for the risk assessment.

Reference:	KCP 10.3.1/02
Report Guideline(s):	CHR/ZF/PROTI 100 FS Honeybees (Apis mellifera L.), Acute Contact Toxicity Test, Kulec-Płoszczyca, E., 2021, Study code: B-25-21 OECD Guideline for the Testing of Chemicals No. 214 (1998) and the EU Method C.17. (2008)

Deviations:	Yes, minor ~~No~~	
	GLP:	Yes
	Acceptability:	Yes
Duplication (if vertebrate study)	No	

Materials and methods

Test item:		~~CHR/ZF/PROTI 100 FS~~ content: 202003 production date: 01.04.2020 expiry date: 01.04.2022
Biological test system:		The honeybee, *Apis*
- age:	*mellifera* L., strain: carnica	approximately 3 weeks
-source:	an apiary at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna,	
Test design:		– the test item: exposure duration: 48 hours number of doses: 5 doses and one control number of replicates: 3 replicates number of bees: 10 bees/replicate – the reference item: exposure duration: 24 hours number of doses: 3 doses number of replicates: 3 replicates number of bees: 10 bees/replicate
Test item doses:		12.5, 25.0, 50.0, 100.0 and 200.0 µg test item/bee and a control (0.0 µg/bee)
Reference item doses:		0.1, 0.2 and 0.4 µg a.i./bee
Test conditions: -temperature: -relative air humidity:	24.0 – 25.5°C 67 – 69%	
Place:	Dark room	
Statistical analysis:		regression analysis using the log-probit method

Endpoints: – honeybee mortality after 24 and 48 hours of the exposure,
– the contact LD_{50}/24 h of the reference item (dimethoate).

Preliminary test

Mortality results obtained in the preliminary experiment are presented in Tables 1 and 2. Mortality of the control groups after 48 hours of exposure was 0.0%. After 24 and 48 hours the percentages of mortality of the bees treated with the test item at the doses of 8.0, 40.0 and 200.0 µg/honeybee were 0.0%. No abnormal behavioural effects were observed during the test

Definitive test

Mortality of the treated insects is presented in the tables below.
Mortality of the control group after 4 hours of the test was 0.0%. After 24 hours the percentage of mortality of the bees in control group was 3.3%. After 48 hours the percentages of mortality of the bees in control group was 10.0%.
After 4 hours of exposure, the percentages of mortality of the bees treated with the test item at the doses of 12.5, 25.0, 50.0, 100.0 and 200.0 µg/honeybee were 0.0%.
After 24 hours of exposure, the percentages of mortality, corrected according to the formula of Abbott, of the bees treated with the test item at the doses of 12.5, 25.0, 50.0, 100.0 and 200.0 µg/honeybee were -3.5, -3.5, 0.0 -3.5 and 3.5%, respectively. The negative values indicate that the mortality in the group treated with the test item was lower than in the control group.
After 48 hours of exposure, the percentages of mortality, corrected according to the formula of Abbott, of the bees treated with the test item at the doses of 12.5, 25.0, 50.0, 100.0 and 200.0 µg/honeybee were 11.1, -11.1, -7.4, -11.1 and -3.7%, respectively. The negative values indicate that the mortality in the group treated with the test item was lower than in the control group.
The median lethal doses (LD50/24 h and LD50/48 h contact) are higher than 200.0 µg/honeybee.

Table 4. Honeybee mortality after 4 hours of exposure – definitive test

Dose [µg/bee]	Number of tested bees [no.]	Mortality				
		Number of dead bees [no.] replicates			Total	
		I	II	III	[no.]	[%]
0.0 (control)	30	0	0	0	0	0.0
12.5	30	0	0	0	0	0.0
25.0	30	0	0	0	0	0.0
50.0	30	0	0	0	0	0.0
100.0	30	0	0	0	0	0.0
200.0	30	0	0	0	0	0.0

Table 5. Honeybee mortality and the LD_{50} after 24 hours of exposure – definitive test

Dose [µg/bee]	Number of tested bees [no.]	Mortality						LD_{50} [µg/bee]
		Number of dead bees [no.] replicates			Total			
		I	II	III	[no.]	[%]	[%] Corr[a]	
0.0 (control)	30	0	1	0	1	3.3	–	> 200.0
12.5	30	0	0	0	0	0.0	-3.5*	
25.0	30	0	0	0	0	0.0	-3.5*	
50.0	30	1	0	0	1	3.3	0.0	
100.0	30	0	0	0	0	0.0	-3.5*	
200.0	30	0	0	2	2	6.7	3.5	

[a]: mortality corrected according to the Abbott formula

*: the negative value indicates that the mortality in the group treated with the test item was lower than in the control group

Table 6. Honeybee mortality and the LD_{50} after 48 hours of exposure – definitive test

Dose [µg/bee]	Number of tested bees [no.]	Mortality						LD_{50} [µg/bee]
		Number of dead bees [no.] replicates			Total			
		I	II	III	[no.]	[%]	[%] Corr[a]	
0.0 (control)	30	1	2	0	3	10.0	–	> 200.0
12.5	30	0	0	0	0	0.0	-11.1*	
25.0	30	0	0	0	0	0.0	-11.1*	
50.0	30	1	0	0	1	3.3	-7.4*	
100.0	30	0	0	0	0	0.0	-11.1*	
200.0	30	0	0	2	2	6.7	-3.7	

[a]: mortality corrected according to the Abbott formula

*: the negative value indicates that the mortality in the group treated with the test item was lower than in the control group

The preliminary non-GLP test was conducted between 16-18.06.2021.

Definition of the endpoints

The LD50 (median lethal dose) contact, is a statistically derived single dose of a substance tha t can cause death in 50 per cent of biological test system when administered by contact rout e. The LD50 is expressed in µg test item per bee or in µg of the active ingredient contained in the reference item per bee. It was calculated with the log-probit method.

Mortality: a honeybee is dead if it is completely immobile.

Test validity criteria

The following validity criteria were met during the test:

- the mortality for the control was 10.0% after 48 h (criterion: it must not exceed 10.0%),

- the LD50/24 h of the reference item (dimethoate) was 0.28 µg a.i./bee (criterion: 0.10 – 0.30 µg a.i./bee).

A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

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

A 2.3.1.4 KCP 10.3.1.4 Sub-lethal effects

A 2.3.1.4.1 Study 1

Comments of izRMS:	The study was conducted to Heimbach et al. (2000). Method. All the validity criteria were met. The study is acceptable. LR_{50} value was > 0.018 kg a.s./ha CHR/ZF/PROTI 100 FS

Reference:	KCP 10.3
Report	CHR/ZF/PROTI 100 FS – An Extended Laboratory Test to Evaluate the Effects of Treated Wheat Seed on the Ground Beetle, Poecilus cupreus (Coleoptera: Carabidae), D. Stonham, 2021, Study code: CHR-21-01
Guideline(s):	Heimbach et al. (2000). A method for testing effects of plant protection products on the carabid beetle Poecilus cupreus (Coleoptera, Carabidae) under laboratory and semi-field conditions
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

The test item for this study, CHR/ZF/PROTI 100 FS containing the active substance Prothioconazole (nominally 100 g/L), was supplied as a dressing treatment to wheat seed. The aim of this study was to determine the effects of this test item on the ground beetle Poecilus cupreus (Coleoptera, Carabidae), under extended laboratory test conditions.

Materials and methods

Test item:	Protiokonazol 100 FS [CHR/ZF/PROTI 100 FS]; batch no.: 202003, the (determined) content of prothioconazole: 9.63 ± 0.20 %; density: 1.088 g/mL; production date: April 01, 2020, expiry date: April 01, 2022.
Test organism:	The test species was Poecilus cupreus and adult beetles were obtained from a commercial supplier (Bio-Test Labor Gmbh, Sagerheide, Germany).

Test design:	The test method was adapted from the guideline of Heimbach et al. (2000) and the test design was in line with current European guidance documents (Barrett et al., 1994; Candolfi et al., 2001), which themselves meet the relevant requirements of Commission Regulations (EU) No. 283/2013 and 284/2013 (EU, 2013).
Nominal test item concentrations:	The test item for this study, CHR/ZF/PROTI 100 FS containing the active substance Prothioconazole (nominally 100 g/L), was supplied as a dressing treatment to wheat seed.
Test conditions:	The test arenas were set up in a controlledenvironment room operating at 19.8-20.0°C and 72-77% RH, with light levels of 475 lux provided for a 16 h photoperiod.
Statistics:	Statistical analyses were performed following the recommendations given in OECD Guidance Document 54 (2006), using validated computer software ToxRatPro® (ToxRat Solutions GmbH, 2018).
Endpoint values:	The endpoints for the bioassay were assessments of mortality and the feeding activity of the beetles over 14 days.

RESULTS

Mortality of Ground Beetle

There were no beetle deaths (0% mortality) in the control treatment, the 0.018, or the 0.015 kg a.s./ha test item rates of CHR/ZF/PROTI 100 FS. The corrected mortality for all of the test item treatment rates was 0%. As neither of the test item treatments resulted in >50% corrected mortality, the 14-day LR50 value for the test item was > 0.018 kg a.s./ha, the highest rate tested. As both test item treatments showed no significant statistical difference from the control treatment (Fisher's exact binomial test, one-sided, > control, $\alpha = 0.05$) the NOER with respect to beetle survival was 0.018 kg a.s./ha. There was 93.3% mortality (93.3% corrected mortality) in the toxic reference treatment.

Treatment	Test item rate (kg a.s./ha)	% mortality at 14 days [a]	% corrected mortality at 14 days [b]
Control	-	0	-
CHR/ZF/PROTI 100 FS	0.018	0	0
	0.015	0	0
Toxic reference	-	93.3 *	93.3

a) Treatments were compared to the control using Fisher's exact binomial test (one-sided, > control, α = 0.05). Any statistically significant difference is indicated by an asterisk.

b) Corrected using Abbott's formula.

Feeding assessments

In the control treatment, an average of 0.37 of the pupae supplied were consumed per beetle per feeding occasion, during the 14 days of assessments. This compared with 0.52 and 0.54 in the 0.018 and 0.015 kg a.s./ha treatment rates of CHR/ZF/PROTI 100 FS, respectively. When compared statistically, none of the feeding activity of the beetles in the CHR/ZF/PROTI 100 FS treatments were significantly lower than in the control treatment (Williams' multiple sequential t-test or Dunnet's multiple t-test, one-sided, > control, $\alpha = 0.05$). Individuals in the toxic reference treatment consumed 0% of the pupae supplied.

Treatment	Test item rate (kg a.s./ha)	Mean number of fly pupae eaten per surviving beetle per feeding occasion [a)]	Overall % change in feeding relative to the control [b)]
Control	-	0.37	~
CHR/ZF/PROTI 100 FS	0.018	0.52	42
	0.015	0.54	47
Toxic reference	-	0.00 *	-100

a) The portion of food items taken per beetle in each treatment were compared by Williams' multiple sequential t-test (one-sided, < control, $\alpha = 0.05$), Dunnett's multiple t-test (one-sided, < control, $\alpha = 0.05$) or Welch's t-test (one-sided, < control, $\alpha = 0.05$). Treatments that resulted in a significant reduction in feeding when compared to the control are indicated with and asterisk.

b) A positive value indicates an increase, a negative value a decrease, relative to the control.

Validity criteria

For this test to be deemed valid, the study plan and the guideline of Heimbach et al. (2000), indicated that:
- Mortality in the control treatment should not exceed 6.7% over the initial 14 days of the test.
- Mortality in the toxic reference treatment should be 65 ± 35% after 14 days. These criteria were met.

Conclusions

In an extended laboratory test to determine the effects of fresh residues of CHR/ZF/PROTI 100 FS and the ground beetle *Poecilus cupreus*, the 14-day LR_{50} value was > 0.018 kg a.s./ha CHR/ZF/PROTI 100 FS, the highest rate tested. Based on statistical comparison with the control, the NOER with respect to both beetle survival and feeding was 0.018 kg a.s./ha.

A 2.3.1.4.2 Study 2

Comments of izRMS:	The study was conducted to Grimm et al. (2000) and according to the principles of GLP. No deviations occurred during the study. Validity criteria For the bioassay to be considered valid, the guideline of Grimm et al. (2000) indicates that the mean number of beetles emerging from parasitized fly pupae in the control treatment should be > 400 per replicate (nominally 26.7% of those provided). Also, the mean number of beetles emerging in the toxic reference treatment should be reduced by > 50%, relative to the control. Both criteria were met in this study The study is reliable and suitable for the risk assessment.

Reference: KCP 10.3.2/02

Report	CHR/ZF/PROTI 100 FS – An extended laboratory test to evaluate the effects of treated wheat seed on the rove beetle Aleochara bilineata (Coleoptera; Staphylinidae), G., Tew, 2021, Study code: CHR-21-02
Guideline(s):	Grimm et al. (2000). A test for evaluating the chronic effects of plant protection products on the rove beetle Aleochara bilineata Gyll. (Coleoptera: Staphylinidae) under laboratory and extended laboratory test conditions.
Deviations: No	
GLP: Yes	
Acceptability:	Yes
Duplication (if vertebrate study) No	

Materials and methods

Test item:	Protiokonazol 100 FS [CHR/ZF/PROTI 100 FS]; batch no.: 202003, the (determined) content of prothioconazole: 9.63 ± 0.20 %; density: 1.088 g/mL; production date: April 01, 2020, expiry date: April 01, 2022.
Test organism:	The test insects (A. bilineata) were obtained as parasitised pupae of the onion fly, Delia antiqua Meig. (Diptera: Anthomyiidae), from a commercial supplier (De Groene Vlieg, Nieuwe Tonge, the Netherlands).
Test design:	Two application rates of CHR/ZF/PROTI 100 FS were evaluated alongside an untreated control and a toxic reference treatment. Test item treatments were applied to replicated boxes of sandy soil, in which the test insects (adult rove beetles) were then confined. The test item treatment (dressed seed) and control (undressed seed) were drilled into furrows made in the substrate. Fly pupae for the beetles to parasitise were provided on 3 occasions and any surviving beetles were removed at 28 DAT. The pupae were later separated from the soil and the number of offspring that subsequently hatched were recorded.
Nominal test item concentrations:	The test item for this study, CHR/ZF/PROTI 100 FS containing the active substance Prothioconazole (nominally 100 g/L), was supplied as a dressing treatment to wheat seed.
Test conditions:	The intended ambient conditions for all stages of the bioassay were 18-22°C and 60-90% relative humidity (RH), with a 16 h photoperiod of 2001000 lux, although minor fluctuations outside of these ranges for periods of less than 2 h were not to be considered as Study Plan deviations in which case

the threshold value has been reported. During the time that the adult beetles were in the test arenas, the conditions recorded in the room were 18.4-21.3°C and 74-90% RH). During the emergence of the F1 progeny, the conditions recorded were 18.8-21.1°C and 76-89% RH. Lighting of 800-900 lux was provided for a 16 h photoperiod throughout the bioassay.

Endpoint values:

The endpoint of the bioassay was an assessment of the numbers of progeny (F1 beetles) that successfully emerged from host fly pupae provided for parasitism during the first phase of the experiment. The intention was to determine values for both the *median effect concentration* (EC50) and the *no-observed effect concentration* (NOEC), with respect to reproduction. **Results**

Mortality assessment

The results of assessments of the condition of the beetles over the first 28 days of the test are given in Appendix II and summarised in Table 1. Mortality amongst the adult beetles originally introduced is not considered to be a key assessment criterion in the guideline of Grimm et al. (2000) due to the relatively short lifespan of the beetles and the consequent potential for high natural mortality over the bioassay period. However, the results can sometimes provide an early indication of acute harmful treatment effects. At 28 DAT, there was 18.8% mortality in the control treatment. This compared with -16.3% and 18.8% mortality or -3.1% and 0.0% corrected mortality in the 0.018 and 0.015 kg a.s./ha treatment rates of CHR/ZF/PROTI 100 FS, respectively.
The toxic reference treatment resulted in 93.8% mortality (92.3% corrected) by 28 DAT.

Table 1. The percentage mortality of beetles in each treatment at 28 days.

Treatment	Test item rate (kg a.s./ha)	% mortality	Corrected % mortality [a)]
Control	-	18.8	-
CHR/ZF/PROTI 100 FS	0.018	16.3	-3.1
	0.015	18.8	0
Toxic reference	-	93.8	92.3

a) Corrected percentage mortality calculated using Abbott's formula (Abbott, 1925). Negative values indicate a decrease and positive values an increase in mortality relative to the control.

Reproduction assessment

The results of the assessments of beetle reproduction (numbers of F1 progeny) are given in Appendix III and summarised in Table 2 and Figure 1. The mean number of progeny produced per replicate was 552.3 in the control treatment, compared with values of, 604.8 and 492.3 in the, 0.018 and 0.015 kg a.s./ha treatment rates of CHR/ZF/PROTI 100 FS respectively. Based on the nominal 1500 fly pupae provided per replicate for parasitisation, this equated to parasitism success of 36.8% in the control treatment. The changes in reproduction compared to the control treatment were -9.5% and 10.9% for the respective test item treatments. As both test item treatment rates resulted in <50% reduction in reproduction, the ER50 value was > 0.018 kg a.s./ha. None of the test item treatments differed significantly from the control treatment (multiple sequentially-rejective U-test after Bonferroni-Holm, one-sided, < control, $\alpha = 0.05$) and so the NOER value was 0.018 kg a.s./ha.

The mean number of progeny in the toxic reference treatment was 10.5, indicating a parasitism success of only 0.7%. The toxic reference differed significantly from the control (student-t test for homogeneous variances, $\alpha = 0.05$, one-sided, < control).

Table 2. The reproductive success of the beetles. The mean (n = 4) number of F_1 progeny that developed from the nominal 1500 onion fly pupae provided per replicate are indicated.

Treatment	Test item rate (kg a.s./ha)	Mean number of F_1 progeny [a)]	% change in reproduction [b)]
Control	-	552.3	-
CHR/ZF/PROTI 100 FS	0.018	604.8	-9.5
	0.015	492.3	10.9
Toxic reference	-	10.5 *	98.1

a) Individual test-item treatments were compared to the control by multiple sequentially-rejective U-test after Bonferroni-Holm (one-sided, > control, $\alpha = 0.05$). The toxic reference was compared to the control by Student-t test for homogeneous variances (one-sided, < control, $\alpha = 0.05$). An asterisk (*) indicates where there was a significant reduction in numbers of progeny.

b) The percentage change in numbers of F1 progeny, relative to the control. A positive value indicates a decrease and a negative value an increase, relative to the control.

Conclusions

In an extended laboratory test where adults of the rove beetle *Aleochara bilineata* were exposed to fresh residues of CHR/ZF/PROTI 100 FS on a natural soil substrate, the NOER value with respect to the reproductive success of the beetles was 0.018 kg a.s./ha, the maximum rate tested, and the ER50 value was > 0.018 kg a.s./ha.

A 2.3.1.5 KCP 10.3.1.5 Cage and tunnel tests

A 2.3.1.6 KCP 10.3.1.6 Field tests with honeybees

A 2.4 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

A 2.4.1.1.1 Study 1

Comments of izRMS:	The study was conducted to OECD guideline 222 and according to the principles of GLP. No deviations occurred during the study. In the definitive test all the validity criteria were met according to OECD Guideline No. 222. No deviations from OECD Guideline occurred. Overall, the study is considered acceptable with following endpoints: EC_{10}=313.1 mg formulation/kg dw soil corresponding to: EC_{10}=30.2 mg of a.s/kg dw soil; NOEC ≥ 560 mg test item/kg dw soil corresponding to: NOEC ≥ 53.9 mg a.s/kg dw soil

Reference:	KCP 10.4/01
Report	CHR/ZF/PROTI 100 FS – Earthworm reproduction test (*Eisenia andrei*), A. Wróbel, 2021, Study code: G-67-20
Guideline(s):	According to the OECD Guideline No. 222 (2016)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

The aims of the study were to assess the impact of the test item on reproduction of the earthworm, Eisenia andrei and to determine the EC10, EC20, EC50, and NOEC.

Materials and methods

Test item:	Protiokonazol 100 FS [CHR/ZF/PROTI 100 FS] batch no.: 202003
Active substances:	prothioconazole – 9.63 ± 0.20%
Artificial soil:	10% sphagnum peat, 20% kaolin clay, 70% airdried quartz sand
Test organism:	the earthworm, *Eisenia andrei* obtained from a standard laboratory culture cultivated at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna, Department of Ecotoxicological Studies, Laboratory of Soil Organisms Toxicology
Test design:	test duration: 8 weeks; number of replicates: 4
Concentrations of the test item:	replicates/concentration + 8 replicates/control; number of earthworms: 10 earthworms/replicate control, 5.6, 10.0, 18.0, 32.0, 56.0, 100.0, 180.0, 320.0, 560.0 and 1000.0 mg/kg dry weight of the artificial soil

Test conditions:	temperature: 19.8 – 22.0°C; pH at the beginning of the experiment: 5.60 – 5.72; pH at the end of the experiment: 5.51 – 5.98; soil moisture content at the beginning of the experiment: 23.1 – 27.8% (44.3 – 53.3% of the maximum water holding capacity); soil moisture content at the end of the experiment: 22.8 – 27.0% (43.7 – 51.8% of the maximum water holding capacity); light-dark cycle: 16h : 8h; light intensity at the beginning of the experiment: 449.0 – 651.5 lux light intensity at the end of the experiment: 453.1 – 621.3 lux
Statystical analysis:	EC10, EC20, EC50, LC50 – probit analysis using linear max. likelihood regression, NOEC (reproduction) – Shapiro-Wilk's Test on Normal Distribution, Bartlett's Test Procedure on Variance Homogeneity (with Residuals), Williams Multiple Sequential t-test Procedure, NOEC (survival) – Fisher's Exact Binomial Test with Bonferroni Correction LOEC: a values suggested by the ToxRat Professional 2.10 statistical computer software
Endpoint:	EC10, EC20, EC50, NOEC, LOEC (reproduction) LC50, NOEC, LOEC (survival)

RESULTS

Mortality of the adult earthworms

The impact of the test item on mortality of the earthworms is presented in the table below.

Concentration of the test item [mg/kg dry weight of the artificial soil]	Replicate	Number of tested earthworms [no.]	Number of alive earthworms [no.]	Total mortality	
				[no.]	[%]
0.0 (control)	1	10	8	5	6.3
	2	10	8		
	3	10	10		
	4	10	10		
	5	10	10		
	6	10	9		
	7	10	10		
	8	10	10		
5.6	1	10	9	5	12.5
	2	10	10		
	3	10	8		
	4	10	8		
10.0	1	10	10	2	5.0
	2	10	9		
	3	10	9		
	4	10	10		
18.0	1	10	10	2	5.0
	2	10	9		
	3	10	9		
	4	10	10		
32.0	1	10	8	5	12.5
	2	10	9		
	3	10	8		
	4	10	10		
56.0	1	10	9	4	10.0
	2	10	10		
	3	10	7		
	4	10	10		
100.0	1	10	9	3	7.5
	2	10	8		
	3	10	10		
	4	10	10		
180.0	1	10	10	0	0.0
	2	10	10		
	3	10	10		
	4	10	10		
320.0	1	10	10	0	0.0
	2	10	10		
	3	10	10		
	4	10	10		
560.0	1	10	9	4	10.0
	2	10	9		
	3	10	9		
	4	10	9		
1000.0	1	10	10	1	2.5
	2	10	9		
	3	10	10		
	4	10	10		

No statistically significant difference (Fisher's Exact Binomial Test with Bonferroni Correction, alpha = 0.05, one-sided greater)

After 4 weeks of the experiment, at the control group mortality of adult earthworms was equal to 6.3%. At concentrations ranging from 5.6 to 1000.0 mg of the test item/kg dry weight of artificial soil, after 4 weeks of exposure to the test item, mortality of the adult earthworms was between 0.0% and 12.5%. The concentration of the test item causing 50% mortality of the adult earthworms (LC50) is above 1000.0 mg/kg dry weight of the artificial soil (above 96.3 mg of prothioconazole/kg dry weight of the artificial soil).

Parameter	Value [mg test item/kg dry weight of artificial soil]	Value [mg of prothioconazole/kg dry weight of artificial soil]
EC_{10}	313.1 (1.8 – 518.0)	30.2 (0.2 – 49.9)
EC_{20}	590.7 (152.5 – >1000.0)	56.9 (14.7 – >96.3)
EC_{50}	>1000.0	>96.3
NOEC (reproduction)	560.0	53.9
LOEC (reproduction)	1000.0	96.3
LC_{50}	>1000.0	>96.3
NOEC (survival)	≥1000.0	≥96.3
LOEC (survival)	>1000.0	>96.3

Observations of the earthworms

After 4 weeks of the experiment, at the concentrations between 5.6 and 1000.0 mg of the test item/kg dry weight of the artificial soil, the changes in appearance and behaviour of the adult earthworms were not observed.
The results of the observations of the earthworms for changes in behaviour and in morphology are presented in the table below

Concentration of the test item [mg/kg dry weight of the artificial soil]	Replicate	Number of tested earthworms [no.]	Changes in behaviour and in morphology
0.0 (control)	1	10	8 nc 2 d
	2	10	8 nc 2 d
	3	10	10 nc
	4	10	10 nc
	5	10	10 nc
	6	10	9 nc 1 d
	7	10	10 nc
	8	10	10 nc
5.6	1	10	9 nc 1 d
	2	10	10 nc
	3	10	8 nc 2 d
	4	10	8 nc 2 d
10.0	1	10	10 nc
	2	10	9 nc 1 d
	3	10	9 nc 1 d
	4	10	10 nc
18.0	1	10	10 nc
	2	10	9 nc 1 d
	3	10	9 nc 1 d
	4	10	10 nc
32.0	1	10	8 nc 2 d
	2	10	9 nc 1 d
	3	10	8 nc 2 d
	4	10	10 nc
56.0	1	10	9 nc 1 d
	2	10	10 nc
	3	10	7 nc 3 d
	4	10	10 nc
100.0	1	10	9 nc 1 d
	2	10	8 nc 2 d
	3	10	10 nc
	4	10	10 nc
180.	1	10	10 nc
	2	10	10 nc
	3	10	10 nc
	4	10	10 nc
320.0	1	10	10 nc
	2	10	10 nc
	3	10	10 nc
	4	10	10 nc
560.0	1	10	9 nc 1 d
	2	10	9 nc 1 d
	3	10	9 nc 1 d
	4	10	9 nc 1 d
1000.0	1	10	10 nc
	2	10	9 nc 1 d
	3	10	10 nc
	4	10	10 nc

Body weights of the living adult earthworms

The table below illustrates body weights of the living adult earthworms, whereas second table below presents the average body weight and changes in body weight recorded after 4 weeks of the experiment. After 4 weeks of the exposure period of the test item at the concentrations ranging from 5.6 to 1000.0 mg/kg dry weight of artificial soil, the body weight increase was between 2.7 and 9.6%. As for the control group, the body weight increase was equal to 11.4%.

Concentration of the test item [mg/kg dry weight of the artificial soil]	Replicate	Weight of living earthworms at the beginning of the experiment [mg]	Weight of living earthworms after 4 weeks of the experiment [mg]
0.0 (control)	1	3590	4150
	2	3780	3140
	3	3650	4140
	4	3630	3840
	5	3870	4130
	6	4010	3740
	7	3790	4010
	8	4350	4660
5.6	1	4730	4390
	2	4230	4280
	3	3570	3110
	4	3730	3340
10.0	1	3750	3970
	2	4220	3850
	3	3820	3580
	4	3690	3870
18.0	1	3710	4200
	2	4130	4040
	3	3700	3730
	4	3810	3980
32.0	1	4370	3550
	2	4030	3740
	3	3820	3210
	4	3450	3610
56.0	1	4090	3870
	2	3920	3980
	3	3900	2900
	4	3510	3940
100.0	1	3790	3550
	2	4280	3650
	3	3510	3630
	4	3840	4040
180.0	1	4240	4350
	2	4030	4050
	3	3350	3550
	4	3500	3860
320.0	1	4340	4470
	2	4560	4670
	3	3860	3950
	4	3470	3570

Concentration of the test item [mg/kg dry weight of the artificial soil]	Replicate	Weight of living earthworms at the beginning of the experiment [mg]	Weight of living earthworms after 4 weeks of the experiment [mg]
560.0	1	4200	3880
	2	4040	3820
	3	3650	3450
	4	3330	3330
1000.0	1	3980	4130
	2	3940	3760
	3	4250	4470
	4	3490	3570

Concentration of the test item [mg/kg dry weight of the artificial soil]	Replicate	Mean weight of 1 earthworm at the beginning of the experiment [mg]	Mean weight of 1 earthworm after 4 weeks of the experiment [mg]	Body weight increase		Mean body weight increase	
				[mg]	[%]	[mg]	[%]
0.0 (control)	1	359	519	160	44.5	42.2	11.4
	2	378	393	15	3.8		
	3	365	414	49	13.4		
	4	363	384	21	5.8		
	5	387	413	26	6.7		
	6	401	416	15	3.6		
	7	379	401	22	5.8		
	8	435	466	31	7.1		
5.6	1	473	488	15	3.1	24.0	6.3
	2	423	428	5	1.2		
	3	357	389	32	8.9		
	4	373	418	45	11.9		
10.0	1	375	397	22	5.9	15.4	4.1
	2	422	428	6	1.4		
	3	382	398	16	4.1		
	4	369	387	18	4.9		
18.0	1	371	420	49	13.2	36.6	9.6
	2	413	449	36	8.7		
	3	370	414	44	12.0		
	4	381	398	17	4.5		
32.0	1	437	444	7	1.5	13.6	3.6
	2	403	416	13	3.1		
	3	382	401	19	5.0		
	4	345	361	16	4.6		
56.0	1	409	430	21	5.1	23.6	6.3
	2	392	398	6	1.5		
	3	390	414	24	6.2		
	4	351	394	43	12.3		
100.0	1	379	394	15	4.1	18.9	4.8
	2	428	456	28	6.6		
	3	351	363	12	3.4		
	4	384	404	20	5.2		
180.0	1	424	435	11	2.6	17.3	4.8
	2	403	405	2	0.5		
	3	335	355	20	6.0		
	4	350	386	36	10.3		
320.0	1	434	447	13	3.0	10.8	2.7
	2	456	467	11	2.4		
	3	386	395	9	2.3		
	4	347	357	10	2.9		

Concentration of the test item [mg/kg dry weight of the artificial soil]	Replicate	Mean weight of 1 earthworm at the beginning of the experiment [mg]	Mean weight of 1 earthworm after 4 weeks of the experiment [mg]	Body weight increase		Mean body weight increase	
				[mg]	[%]	[mg]	[%]
560.0	1	420	431	11	2.6	21.7	6.0
	2	404	424	20	5.1		
	3	365	383	18	5.0		
	4	333	370	37	11.1		
1000.0	1	398	413	15	3.8	17.2	4.3
	2	394	418	24	6.0		
	3	425	447	22	5.2		
	4	349	357	8	2.3		

Impact of the test item on reproduction of the earthworms

After the application of the test item at the concentrations ranging from 5.6 to 1000.0 mg/kg dry weight of the artificial soil, the mean number of juveniles was between 83.5 and 140.8 per replicate. The mean number of juveniles in the control group was equal to 131.4 per replicate.
After 8 weeks of the experiment, it was concluded that Protiokonazol 100 FS [CHR/ZF/PROTI 100 FS] had a statistically significant impact on reproduction of the earthworms at the concentration equal to 1000.0 mg/kg dry weight of artificial soil.
The concentration of the test item causing a 10% reduction in the number of juveniles produced within the exposure period (EC10) is equal to 313.1 mg/kg dry weight of the artificial soil (equal to 30.2 mg of prothioconazole/kg dry weight of the artificial soil).
The concentration of the test item causing a 20% reduction in the number of juveniles produced within the exposure period (EC20) is equal to 590.7 mg/kg dry weight of the artificial soil (equal to 56.9 mg of prothioconazole/kg dry weight of the artificial soil).
The concentration of the test item causing a 50% reduction in the number of juveniles produced within the exposure period (EC50) is above 1000 mg/kg dry weight of the artificial soil (above 96.3 mg of prothioconazole/kg dry weight of the artificial soil).
The highest concentration at which the test item is observed to have no statistically significant effects on reproduction (NOEC) is equal to 560.0 mg/kg dry weight of the artificial soil (equal to 53.9 mg of prothioconazole/kg dry weight of the artificial soil).
The lowest concentration at which the test item is observed to have a statistically significant effect on reproduction (LOEC) is equal to 1000.0 mg/kg dry weight of the artificial soil (equal to 96.3 mg of prothioconazole/kg dry weight of the artificial soil).

Observations of the juveniles of earthworms
After 8 weeks of the experiment, the juveniles of earthworms did not exhibit any changes in appearance and behaviour.

Results of the reference test

According to the OECD Guideline No. 222, the LOEC should be between 1 – 5 mg/kg dry weight of the artificial soil; hence, it may be concluded that the sensitivity of the test organisms was proper.

Concentration [mg/kg dry soil]	Replicate	Number of juveniles [no.]	Mean ± SD	Comparison to the control [%]	CV* [%]
0.0 (control with acetone)	1 2 3 4 5 6 7 8	147 143 127 136 134 149 174 123	141.6 ± 16.0	-	11.3
0.0 (control)	1 2 3 4 5 6 7 8	138 166 160 154 163 172 144 127	153.0 ± 15.4	108.0	10.1
1.0	1 2 3 4	148 151 136 121	139.0 ± 13.6	98.1	9.8
1.5	1 2 3 4	135 120 146 139	135.0 ± 11.0	95.3	8.1
2.25	1 2 3 4	102 109 123 132	116.5† ± 13.5	82.3	11.6
3.37	1 2 3 4	88 130 91 60	92.3† ± 28.8	65.1	31.2
5.0	1 2 3 4	39 31 18 27	28.8† ± 8.7	20.3	30.4
NOEC (mg/kg dry weight of the artificial soil)		1.50			
LOEC (mg/kg dry weight of the artificial soil)		2.25			

Validity criteria

The results are considered valid because the following criteria were satisfied in the controls:
- each replicate produced from 109 to 164 juveniles (131.4 mean) at the end of the experiment (criterion: ≥ 30 juveniles by the end of the experiment),
- the coefficient of variation of reproduction was 14.6% (criterion: ≤ 30%),
- adult mortality over the initial 4 weeks of the experiment was 6.3% (criterion: ≤ 10%).

A 2.4.1.2 KCP 10.4.1.2 Earthworms - field studies

A 2.4.2 KCP 10.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

A 2.4.2.1.1 Study 1

Comments of izRMS:	The study was conducted to OECD guideline 232 and according to the principles of GLP. Following deviations to the guideline were noted: - culturing of collembolans takes place in plastic containers containing an artificial substrate consisting of plaster and charcoal in ratio 9:1 and not 10:1 or 8:1 as is mentioned in OECD Guideline No. 232 (2016) - at the end of the test the soil moisture content was determined by drying small sample of the artificial soil in 105°C instead of weighing the test vessels as it is mentioned in OECD Guideline No. 232 (2016) Since In the definitive test all the validity criteria were met according to OECD Guideline No. 232: the deviations did not affect the results of the study. The study is reliable and suitable for the risk assessment.

Reference:	KCP 10.4/02
Report	CHR/ZF/PROTI 100 FS – Collembolan (*Folsomia candida*) Reproduction Test, A. Wróbel, 2021, Study code: G-68-20
Guideline(s):	OECD Guideline No. 232 (2016)
Deviations:	Yes
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

The aims of the study were to assess the impact of the test item on reproduction of the collembolan, Folsomia candida and to determine the EC10, EC20, EC50, and NOEC.

Materials and methods

Test item:	Protiokonazol 100 FS [CHR/ZF/PROTI 100 FS] batch no.: 202003
Test organism:	The collembolan, Folsomia candida obtained from a standard laboratory culture at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna, Laboratory of Soil Organisms Toxicology. The collembolans used in the study were between 9 to 12 days old.
Test design:	test duration: 28 days number of replicates: 4 replicates / concentration + 8 replicates / control; number of collembolans: 10 / replicate
Nominal test item concentrations:	a control, 5.6, 10.0, 18.0, 32.0, 56.0, 100.0, 180.0, 320.0, 560.0 and 1000.0 mg of the test item/kg of dry weight of the artificial soil
Test conditions:	temperature: 21.1 – 22.0°C; pH at the beginning of the test: 5.57 – 5.64;

	pH at the end of the test: 5.52 – 5.57; soil moisture content at the beginning of the test: 14.8 – 15.9% (45.6 – 48.9% of the maximum water holding capacity); soil moisture content at the end of the test: 14.5 – 16.4% (44.5 – 50.4% of the maximum water holding capacity); lighting: 16 h light and 8h dark; light intensity at the beginning of the experiment: 586.0 – 668.0 lux; light intensity at the end of the experiment: 578.5 – 675.1 lux
Statistics:	EC10, EC20, EC50 – probit analysis using linear max. likelihood regression LC10, LC20 and LC50 – logit analysis using linear max. likelihood regression NOEC (number of juveniles): - Shapiro-Wilk's Test on Normal Distribution, - Bartlett's Test Procedure on Variance Homogeneity, - Williams Multiple Sequential t-test Procedure. NOEC (survival): - Fisher's Exact Binomial Test with Bonferroni Correction.
Endpoint values:	EC10, EC20, EC50, NOEC LC10, LC20, LC50, NOEC

RESULTS Mortality

Mortality of the adults after 28 days of the experiment is presented in the table below, whereas the results showing the impact of the test item on mortality are shown in the second table below.

At the concentrations ranging from 5.6 to 1000.0 mg/kg dry weight of the artificial soil, the mortality of adults ranged from 0.0 to 15.0%. As for the control group, it was equal to 3.8%.

The concentration of the test item causing a 50% mortality of adults within the exposure period (LC50) is above 1000.0 mg/kg dry weight of the artificial soil (above 96.3 mg of prothioconazole/kg dry weight of the artificial soil).

Concentration [mg/kg dry weight of the artificial soil]	Replicate	Number of tested collembolans	Number of living collembolans after 28 days [no.]	Total mortality	
				No.	%
control	1 2 3 4 5 6 7 8	10 10 10 10 10 10 10 10	10 9 10 9 10 9 10 10	3	3.8
5.6	1 2 3 4	10 10 10 10	10 10 10 10	0	0.0
10.0	1 2 3 4	10 10 10 10	9 10 10 8	3	7.5
18.0	1 2 3 4	10 10 10 10	10 10 7 10	3	7.5
32.0	1 2 3 4	10 10 10 10	9 9 10 10	2	5.0
56.0	1 2 3 4	10 10 10 10	10 10 9 10	1	2.5
100.0	1 2 3 4	10 10 10 10	9 9 10 9	3	7.5
180.0	1 2 3 4	10 10 10 10	9 9 10 10	2	5.0
320.0	1 2 3 4	10 10 10 10	8 8 10 10	4	10.0
560.0	1 2 3 4	10 10 10 10	8 8 10 8	6	15.0
1000.0	1 2 3 4	10 10 10 10	7 8 9 10	6	15.0

No statistically significant differences between the control and the treatment groups were noticed (Fisher's Exact Binomial Test with Bonferroni Correction, significance level = 0.05, one-sided greater).

Endpoint	Value [mg test item/kg dry weight of the artificial soil]	Value [mg of prothioconazole/kg dry weight of the artificial soil]
LC_{10}	407.2 (132.3 – >1000.0)	39.2 (12.7 – >96.3)
LC_{20}	> 1000.0	> 96.3
LC_{50}	> 1000.0	> 96.3
NOEC	≥ 1000.0	≥ 96.3

Impact on reproduction

The number of juveniles at the end of the test is presented in first table below, whereas the results showing the impact of the test item on reproduction are shown in the second table below.

After the application of the test item at the concentrations ranging from 5.6 to 1000.0 mg/kg dry weight of the artificial soil, the mean number of juveniles was between 862.0 and 1152.8 per replicate. As for the control group, the number of juveniles was equal to 1132.1 per replicate.

The obtained results led to the following conclusions:

- The concentration of the test item causing a 10% reduction in the number of juveniles produced within the exposure period (EC10) is equal to 236.8 mg/kg dry weight of the artificial soil (i.e. 22.8 mg of prothioconazole/kg dry weight of the artificial soil).
- The concentration of the test item causing a 20% reduction in the number of juveniles produced within the exposure period (EC20) is equal to 643.6 mg/kg dry weight of the artificial soil (i.e. 62.0 mg of prothioconazole/kg dry weight of the artificial soil). - The concentration of the test item causing a 50% reduction in the number of juveniles produced within the exposure period (EC50) is above 1000.0 mg/kg dry weight of the artificial soil (i.e. above 96.3 mg of prothioconazole/kg dry weight of the artificial soil).
- The highest concentration at which the test item is observed to have no statistically significant effects on collembolan reproduction (NOEC) is equal to 180.0 mg/kg dry weight of the artificial soil (i.e. 17.3 mg of

prothioconazole/kg dry weight of the artificial

Concentration [mg/kg dry weight of the artificial soil]	Replicate	Number of juveniles	Mean ±SD	Comparison to the control [%]	Coefficient of variation [%]
control	1 2 3 4 5 6 7 8	1173 1007 1211 1282 1112 1021 1038 1213	1132.1 ± 102.9	-	9.1
5.6	1 2 3 4	1215 946 1102 1348	1152.8 ± 170.6	101.8	14.8
10.0	1 2 3 4	1183 1121 909 1282	1123.8 ± 157.8	99.3	14.0
18.0	1 2 3 4	996 1155 903 1174	1057.0 ± 130.0	93.4	12.3
32.0	1 2 3 4	1018 1145 1023 1298	1121.0 ± 131.8	99.0	11.8
56.0	1 2 3 4	1017 1023 1092 1169	1075.3 ± 71.2	95.0	6.6
100.0	1 2 3 4	1007 902 1102 1340	1087.8 ± 187.0	96.1	17.2
180.0	1 2 3 4	1209 1161 1202 993	1141.3 ± 101.1	100.8	8.9
320.0	1 2 3 4	903 814 1112 963	948.0* ± 125.3	83.7	13.2
560.0	1 2 3 4	966 790 854 932	885.5* ± 79.1	78.2	8.9
1000.0	1 2 3 4	686 797 781 1184	862.0* ± 220.2	76.1	25.5

"*" - statistically significant differences between the control and the treatment group (Williams Multiple Sequential t-test Procedure, significance level = 0.05, one-sided smaller).

soil).

Endpoint	Value [mg test item/kg dry weight of the artificial soil]	Value [mg of prothioconazole/kg dry weight of the artificial soil]
EC_{10}	236.8 (73.0 – 375.5)	22.8 (7.0 – 36.2)
EC_{20}	643.6 (413.2 – >1000.0)	62.0 (39.8 – >96.3)
EC_{50}	> 1000.0	> 96.3
NOEC	180.0	17.3

Observations of the collembolans
After 4 weeks of the experiment, at the concentrations between 5.6 and 1000.0 mg of the test item/kg dry weight of the artificial soil, the changes in appearance and behaviour of the collembolans were not observed.

Results of the reference test concentration of boric acid causing a 50% reduction in the number of juveniles produced within the exposure period (EC50) is 101.7 mg/kg dry weight of the artificial soil.
According to the OECD Guideline No. 232, the EC50 should be about 100 mg/kg dry weight of the artificial soil; hence, it may be concluded that the sensitivity of the test organisms was proper. The test was conducted 27.08.2020 – 28.09.2020.

Validity criteria
The results are considered valid because the following criteria were satisfied in the controls:
- mean adult mortality: 3.8% (criterion: ≤ 20%),
- the mean number of juveniles per vessel at the end of the test: 1132.1 (criterion: ≥100 juveniles at the end of the test),
- the coefficient of variation calculated for the number of juveniles: 9.1% (criterion: ≤ 30%).

Deviations from the study plan
- culturing of collembolans takes place in plastic containers containing an artificial substrate consisting of plaster and charcoal in ratio 9:1 and not 10:1 or 8:1 as is mentioned in OECD Guideline No. 232 (2016), - at the end of the test the soil moisture content was determined by drying small sample of the artificial soil in 105°C instead of weighing the test vessels as it is mentioned in OECD Guideline No. 232 (2016). The deviations did not affect the results of the study.

A 2.4.2.1.2 Study 2

Comments of izRMS:	The study was conducted to OECD guideline 226 and according to the principles of GLP. Following deviations to the guideline were noted: 1. According to the OECD Guideline No. 226 (2016) the water content of the soil substrate should be maintained throughout the test by weighing and if needed rewatering the vessels periodically. In the study to maintain proper moisture content, a small sample of soil was drying at 105°C and re-weighing at the beginning, after 7 days of the test and at the end of the test. 2. Due to the use of the temperature extraction method there was no need for euthanasia of the extracted organisms since the mites are fixed in a 70% ethanol solution 3. Due to the use of the temperature extraction method, it was not possible to record the symptoms with behavioral and morphology changes of the extracted predatory mites These deviations did not affect the results, since all validity criteria were met. The study is reliable and suitable for the risk assessment

Reference:	KCP 10.4/03
Report	CHR/ZF/PROTI 100 FS – Predatory mite (*Hypoaspis (Geolaelaps) aculeifer*) reproduction test in soil, A. Wróbel, 2021, Study code: G-69-20
Guideline(s):	OECD Guideline No. 226 (2016)
Deviations:	Yes

GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

The aims of the study were to assess the effects of the test item on the reproductive output of the predatory mite, Hypoaspis aculeifer and to determine the EC10, EC20, EC50, and NOEC.

Materials and methods

Test item:	Protiokonazol 100 FS [CHR/ZF/PROTI 100 FS] batch no.: 202003
Test organism:	The predatory mites, Hypoaspis (Geolaelaps) aculeifer (adult female mites from a synchronized culture) obtained from a standard laboratory culture at the Łukasiewicz Research Network - Institute of Industrial Organic Chemistry Branch Pszczyna, Ecotoxicology Research Group, Laboratory of Soil Organisms Toxicology. The mites were introduced 7 – 14 days after becoming adult (28-35 days after the start of egg lying in the synchronization).
Test design:	test duration: 14 days number of replicates: 4 replicates / concentration + 8 replicates / control; number of mites: 10 mites / replicate
Nominal test item concentrations:	a control, 5.6, 10.0, 18.0, 32.0, 56.0, 100.0, 180.0, 320.0, 560.0 and 1000.0 mg test item/kg dry weight of the artificial soil.
Test conditions:	temperature: 20.1 – 22.0°C pH at the beginning of the test: 5.55 – 5.62 pH at the end of the test: 5.62 – 5.67 soil moisture content at the beginning of the test: 14.5 – 15.5% (44.5 – 47.8% of the maximum water holding capacity) soil moisture content in the middle of the test: 14.3 – 16.0% (44.1 – 49.4% of the maximum water holding capacity) soil moisture content at the end of the test: 13.9 – 15.2% (42.9 – 46.7% of the maximum water holding capacity) light-dark cycle: 16 h light and 8 h dark light intensity at the beginning of the test: 576 – 634 lux light intensity at end of the test: 612 – 671 lux

Statistics:	EC10, EC20, EC50 – a logit analysis using linear max. likelihood regression LC10, LC20, LC50 – a probit analysis using linear max. likelihood regression NOEC: - offspring number – Shapiro-Wilk's Test on Normal Distribution, Bartlett's Test Procedure on Variance Homogeneity, Williams Multiple Sequential t-test Procedure - survival – Fisher's Exact Binomial Test with Bonferroni Correction
Endpoint values:	EC10, EC20, EC50, NOEC LC10, LC20, LC50, NOEC

RESULTS Mortality of adult females

Mortality of adult mites after 14 days of the experiment is presented in first table below. The endpoint values are given in the second table below.

Mortality of the predatory mites exposed to the test item at the concentrations ranging from 5.6 to 1000.0 mg/kg dry weight of the artificial soil was between 5.0% and 17.5%. Mortality of the control group was equal to 7.5%.

The concentration of the test item causing a 50% mortality of adults within the exposure period (LC50) is above 1000.0 mg/kg dry weight of the artificial soil (above 96.3 mg of prothioconazole/kg dry weight of the artificial soil).

Concentration [mg/kg dry weight of the artificial soil]	Replicate	Number of tested mites	Number of alive mites after 14 days [no.]	Mortality	
				no.	%
0 (control)	1 2 3 4 5 6 7 8	10 10 10 10 10 10 10 10	8 10 10 9 10 10 9 8	6	7.5
5.6	1 2 3 4	10 10 10 10	9 7 8 10	6	15.0
10.0	1 2 3 4	10 10 10 10	10 10 8 9	3	7.5
18.0	1 2 3 4	10 10 10 10	10 8 7 9	6	15.0
32.0	1 2 3 4	10 10 10 10	10 9 9 10	2	5.0
56.0	1 2 3 4	10 10 10 10	8 8 9 8	7	17.5
100.0	1 2 3 4	10 10 10 10	7 8 10 9	6	15.0
180.0	1 2 3 4	10 10 10 10	10 8 10 9	3	7.5
320.0	1 2 3 4	10 10 10 10	7 10 10 10	3	7.5
560.0	1 2 3 4	10 10 10 10	8 10 10 10	2	5.0
1000.0	1 2 3 4	10 10 10 10	10 10 10 8	2	5.0

Endpoint	Value [mg/kg dry weight of the artificial soil]	Value [mg of prothioconazole/kg dry weight of the artificial soil]
LC_{10}	> 1000.0	> 96.3
LC_{20}	> 1000.0	> 96.3
LC_{50}	> 1000.0	> 96.3
NOEC	≥ 1000.0	≥ 96.3

Impact on reproduction

The number of juveniles at the end of the test is presented in the first table below, whereas the endpoints showing the impact of the test item on reproduction are given in the second table below.

After the application of the test item at the concentrations ranging from 5.6 to 1000.0 mg/kg dry weight of the artificial soil, the mean number of juveniles was between 109.3 and 219.0 per replicate. The mean number of juveniles in the control group was equal to 196.1 per replicate.

The obtained results led to the following conclusions:

- The concentration of the test item causing a 10% reduction in the number of mites produced within the exposure period (EC10) is equal to 524.2 mg/kg dry weight of the artificial soil (equal to 50.5 mg of prothioconazole/kg dry weight of the artificial soil).
- The concentration of the test item causing a 20% reduction in the number of mites produced within the exposure period (EC20) is equal to 687.4 mg/kg dry weight of the artificial soil (equal to 66.2 mg of prothioconazole/kg dry weight of the artificial soil).
- The concentration of the test item causing a 50% reduction in the number of mites produced within the exposure period (EC50) is above 1000.0 mg/kg dry weight of the artificial soil (above 96.3 mg of prothioconazole/kg dry weight of the artificial soil).
- The highest concentration at which the test item is observed to have no statistically significant effects on mite reproduction (NOEC) is equal to 560.0 mg/kg dry weight of the artificial soil (equal to 53.9 mg of prothioconazole/kg dry weight of the artificial soil).

Concentration [mg/kg dry weight of soil]	Replicate	Number of juvenile mites	Mean ±SD	Comparison to the control [%]	CV* [%]
0 (control)	1 2 3 4 5 6 7 8	220 235 212 188 187 195 181 151	196.1 ± 26.0	-	13.3
5.6	1 2 3 4	195 178 226 184	195.8 ± 21.4	99.8	10.9
10.0	1 2 3 4	191 239 199 206	208.8 ± 21.1	106.4	10.1
18.0	1 2 3 4	200 195 253 189	209.3 ± 29.5	106.7	14.1
32.0	1 2 3 4	209 204 198 225	209.0 ± 11.6	106.6	5.5
56.0	1 2 3 4	180 218 237 195	207.5 ± 25.1	105.8	12.1
100.0	1 2 3 4	186 249 244 197	219.0 ± 32.1	111.7	14.7
180.0	1 2 3 4	144 172 190 199	176.3 ± 24.3	89.9	13.8
320.0	1 2 3 4	154 186 182 200	180.5 ± 19.3	92.0	10.7
560.0	1 2 3 4	165 142 199 214	180.0 ± 32.6	91.8	18.1
1000.0	1 2 3 4	114 100 106 117	109.3⁺ ± 7.7	55.7	7.1

Endpoint	Value [mg/kg dry weight of the artificial soil]	Value [mg of protioconazole/ kg dry weight of the artificial soil]
EC_{10}	**524.2** (284.1 – 647.5)	**50.5** (27.4 – 62.4)
EC_{20}	**687.4** (488.4 – 789.9)	**66.2** (47.0 – 76.1)
EC_{50}	**> 1000.0**	**> 96.3**
NOEC	**560.0**	**53.9**

Results of the reference test
The concentration of boric acid causing a 50% reduction in the number of juveniles produced within the exposure period (EC50) is 241.250 mg/kg dry weight of the artificial soil.
According to the OECD Guideline No. 226, the EC50 should be between 100 and 500 mg/kg dry weight of the artificial soil; hence, it may be concluded that the sensitivity of the test organisms was proper.

Validity criteria
The results are considered valid because the following criteria were satisfied in the control:
- mean adult mortality: 7.5% (criterion: ≤ 20%),
- the mean number of juveniles per vessel at the end of the test: 196.1 (criterion: ≥ 50 juveniles at the end of the test),
- the coefficient of variation for the number of juveniles: 13.3 % (criterion: ≤ 30%).

Deviations from the study plan
There are three deviations from the OECD Guideline No. 226 (2016), however they did not affect the results:
1. According to the OECD Guideline No. 226 (2016) the water content of the soil substrate should be maintained throughout the test by weighing and if needed re-watering the vessels periodically. In the study to maintain proper moisture content, a small sample of soil was drying at 105°C and re-weighing at the beginning, after 7 days of the test and at the end of the test (Chapter 3.5.7).
2. Due to the use of the temperature extraction method, there was no need for euthanasia of the extracted organisms since the mites are fixed in a 70% ethanol solution (Chapter 3.5.8).
3. Due to the use of the temperature extraction method, it was not possible to record the symptoms with behavioral and morphology changes of the extracted predatory mites (Chapter 3.4.8).

Deviation from the study plan:
Department of Ecotoxicological Studies was changed into Ecotoxicology Research Group. This deviation did not affect the results of the study. Deviations from SOP/G/94
No deviations from the SOP/G/94 were noticed.

A 2.5 KCP 10.5 Effects on soil nitrogen transformation

A 2.5.1.1.1 Study 1

Comments of izRMS:	The study was conducted to OECD guideline 216 and according to the principles of GLP. Following deviations occurred in the study: - According the Guideline, the soil extraction should be conducted at 150 rpm for 60 min. However, in this study, the extraction was performed at 90 rpm for 24 hours. The modification resulted from the optimization of the nitrate extraction which showed that the extraction was more effective when the shaking rate was lower, and the extraction lasted longer - The predicted environmental concentration (PEC) was calculated assuming 1 cm of the soil depth according to the German conditions for the substances with the mobility in soil KFoc > 500 mL/g and correspondence with the Sponsor. Thus, the applied soil depth is a deviation from OECD Guideline No. 216 (2000), and EU Method C.21, where the PEC is calculated by using 5 cm of the soil depth. Since all validity criteria were fulfilled these deviations did not affect the results of the study. In the definitive test all the validity criteria were met according to OECD Guideline No. 216: It was concluded that CHR/ZF/PROTI 100 FS at the concentrations corresponding to the PEC: 1.31 mg test item/kg dry weight of soil (i.e. 0.13 mg of prothioconazole/kg dry weight of soil) and 5 x PEC: 6.55 mg of the test item / kg dry weight of soil (i.e. 0.65 mg of prothioconazole/kg dry weight of soil) did not have any long-term adverse effects on the process of nitrogen transformation in aerobic surface soils.

Reference:	KCP 10.5
Report	CHR/ZF/PROTI 100 FS – Soil Microorganisms: Nitrogen Transformation Test, A. Wróbel, 2021, Study code: G-70-20,
Guideline(s):	OECD Guideline No. 216 (2000)/EU Method C.21.
Deviations:	Yes
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item:	Protiokonazol 100 FS [CHR/ZF/PROTI 100 FS] batch no.: 202003
Soil:	Agricultural soil collected from a place belonging to the Łukasiewicz Research Network - Institute of Industrial Organic Chemistry Branch Pszczyna.

Test design:	Three portions of soil (3 x 1500 g), i.e. one control group and two treated groups. Every portion was divided into three replicates (3 x 500 g). The soil was enriched with the organic substrate, i.e. lucerne at dose of 5 g/kg dry weight of soil. Test duration: 42 days.
Nominal test item concentrations:	control; PEC: 1.31 mg test item/kg dry weight of soil (i.e. 0.13 mg of prothioconazole/kg dry weight of soil); 5 x PEC: 6.55 mg of the test item/kg dry weight of soil (i.e. 0.65 mg of prothioconazole/kg dry weight of soil)
Test conditions:	temperature: 19.6 – 21.8°C, soil moisture: 45.8 – 53.5% of the maximum water holding capacity, incubation in darkness
Statistics:	- Shapiro-Wilk's test on Normal Distribution - Levene's Test on Variance Homogeneity (with Residuals) - Williams Multiple Sequential t-test Procedure
Endpoint values:	The concentration of nitrate [mg/kg dry soil] after 0, 7, 14, 28 and 42 days of incubation. The nitrate formation rate [mg/kg dry weight of soil/day] for selected time intervals of soil incubation, i.e. 0 – 7, 0 – 14, 0 – 28, 0 - 42 days. Percent deviation from the control in nitrate formation rate calculated for selected time intervals i.e. 0 – 7, 0 – 14, 0 – 28, 0 - 42 days.

Results

The nitrate ions concentrations on 0, 7, 14, 28 and 42 day of incubation are shown in Tables 5 – 9. Nitrate formation rates [mg nitrate/kg dry weight soil/day] for selected time intervals, i.e. 0 – 7, 0 – 14, 0 – 28, 0 - 42 days are given in Table 10. Deviations from the control based on nitrogen ions formation rates are shown in the table below.

Time interval [d]	PEC	5 x PEC
0 – 7	-79.4	-68.0
0 – 14	-98.2	-87.1
0 – 28	47.6	-4.4
0 – 42	17.7	-17.5

Values obtained using ToxRat 2.10. computer software.

After 0, 7 and 14 days of incubation, there were statistically significant differences in nitrate ions concentration between the control and the groups treated with the test item at both concentrations i.e. PEC and 5xPEC.

Concentration	Control			PEC			5 x PEC		
Replicate	I	II	III	I	II	III	I	II	III
Reading* [mg/L]	7.823	9.463	8.513	15.013	15.903	15.663	15.693	15.043	16.263
Nitrate ion concentration [mg/kg of dry soil]	39.12	47.32	42.57	75.07	79.52	78.32	78.47	75.22	81.32
Mean nitrate ion concentration [mg/kg of dry soil] ± SD	43.00 ± 4.12			77.63⁺ ± 2.30			78.33⁺ ± 3.05		
CV	9.6			3.0			3.9		

* - values adjusted for the value of the blank sample

⁺ statistically significant difference between the control and the treatment group (Williams Multiple Sequential t-test Procedure, significance level = 0.05, two sided)

Concentration	Control			PEC			5 x PEC		
Replicate	I	II	III	I	II	III	I	II	III
Reading* [mg/L]	0.628	0.600	0.536	1.139	1.106	1.204	2.181	2.223	2.214
Nitrate ion concentration [mg/kg of dry soil]	3.14	3.00	2.68	5.70	5.53	6.02	10.91	11.12	11.07
Mean nitrate ion concentration [mg/kg of dry soil] ± SD	2.94 ± 0.24			5.75⁺ ± 0.25			11.03⁺ ± 0.11		
CV	8.0			4.3			1.0		

* - values adjusted for the value of the blank sample

⁺ - statistically significant difference between the control and the treatment group (Williams Multiple Sequential t-test Procedure, significance level = 0.05, two sided)

Concentration	Control			PEC			5 x PEC		
Replicate	I	II	III	I	II	III	I	II	III
Reading* [mg/L]	46.515	44.735	45.415	46.555	44.985	46.235	58.595	59.395	59.305
Nitrate ion concentration [mg/kg of dry soil]	232.58	223.68	227.08	232.78	224.93	231.18	292.98	296.98	296.53
Mean nitrate ion concentration [mg/kg of dry soil] ± SD	227.78 ± 4.49			229.63 ± 4.15			295.49⁺ ± 2.19		
CV	2.0			1.8			0.7		

* - values adjusted for the value of the blank sample

⁺ - statistically significant difference between the control and the treatment group (Williams Multiple Sequential t-test Procedure, significance level = 0.05, two sided)

After 28 and 42 days of incubation the statistically significant differences in nitrate ions concentration between the control and the group treated with the test item, i.e. 5xPEC were noticed. No statistically significant differences in nitrate ions concentration between the control and the group treated with the test item, i.e. PEC were noticed.

Concentration	Control			PEC			5 x PEC		
Replicate	I	II	III	I	II	III	I	II	III
Reading* [mg/L]	1.650	1.921	1.945	2.070	2.153	2.162	3.062	2.970	3.022
Nitrate ion concentration [mg/kg of dry soil]	8.25	9.61	9.73	10.35	10.77	10.81	15.31	14.85	15.11
Mean nitrate ion concentration [mg/kg of dry soil] ± SD	9.19 ± 0.82			10.64⁺ ± 0.25			15.09⁺ ± 0.23		
CV	8.9			2.4			1.5		

* - values adjusted for the value of the blank sample

⁺ - statistically significant difference between the control and the treatment group (Williams Multiple Sequential t-test Procedure, significance level = 0.05, two sided)

Concentration	Control			PEC			5 x PEC		
Replicate	I	II	III	I	II	III	I	II	III
Reading* [mg/L]	22.812	25.192	24.262	22.792	22.982	25.162	30.422	32.212	32.862
Nitrate ion concentration [mg/kg of dry soil]	114.06	125.96	121.31	113.96	114.91	125.81	152.11	161.06	164.31
Mean nitrate ion concentration [mg/kg of dry soil] ± SD	120.44 ± 6.00			118.23 ± 6.58			159.16⁺ ± 6.32		
CV	5.0			5.6			4.0		

* - values adjusted for the value of the blank sample

⁺ - statistically significant difference between the control and the treatment group (Williams Multiple Sequential t-test Procedure, significance level = 0.05, two sided)

At the time intervals: 0 – 7, 0 – 14 and 0 – 42 there were statistically significant differences in nitrate formation rate between the control and the both groups treated with test item, i.e. PEC and 5xPEC. At the time interval 0 – 28 the statistically significant difference in nitrate formation rate between the control and the lowest concentration of the test item, i.e. PEC was noticed. No statistically significant difference in nitrate formation rate between the control and the highest concentration of the test item, i.e. 5xPEC was noticed.

Time interval [d]	Control						PEC						5 x PEC					
	Replicate			Mean	±	SD	Replicate			Mean	±	SD	Replicate			Mean	±	SD
	I	II	III				I	II	III				I	II	III			
0 – 7	-5.694	-5.714	-5.760	-5.723	±	0.03	-10.277	-10.300	-10.230	-10.269⁺	±	0.04	-9.632	-9.602	-9.609	-9.615⁺	±	0.02
0 – 14	-2.482	-2.385	-2.377	-2.415	±	0.06	-4.806	-4.776	-4.773	-4.785⁺	±	0.02	-4.502	-4.534	-4.516	-4.517⁺	±	0.02
0 – 28	2.538	2.963	2.797	2.766	±	0.21	1.297	1.331	1.721	1.450⁺	±	0.24	2.635	2.955	3.071	2.887	±	0.23
0 – 42	4.514	4.302	4.383	4.399	±	0.11	3.694	3.507	3.656	3.619⁺	±	0.10	5.111	5.206	5.195	5.170⁺	±	0.05

* - Rate of nitrate ions formation per a day = [(mg nitrate / kg of soil dry weight on sampling day 'a') - (mg nitrate / kg of soil dry weight on day 0)]/ 'a' day; 'a' = 7, 14, 28 and 42 day

⁺ - statistically significant difference between the control and the treatment group (Williams Multiple Sequential t-test Procedure, significance level = 0.05, two sided)

On 28 day of analysis the percent deviation from the control calculated on the basis of the nitrate formation rate of the soil treated with the test item at the concentration corresponding to the PEC exceeded 25%, therefore, according to the OECD No. 216, EU Method C.21 and the study plan, the experiment was continued.

Time interval [d]	PEC	5 x PEC
0 – 7	-79.4	-68.0
0 – 14	-98.2	-87.1
0 – 28	47.6	-4.4
0 – 42	17.7	-17.5

Values obtained using ToxRat 2.10. computer software.

The difference in the nitrate formation rate between the control soil and the ones treated with the test item at the concentrations corresponding to the PEC: 1.31 mg test item/kg dry weight of soil (i.e. 0.13 mg of prothioconazole/kg dry weight of soil) and 5 x PEC: 6.55 mg of the test item / kg dry weight of soil (i.e. 0.65 mg of prothioconazole/kg dry weight of soil) did not exceed 25% on 42 day of analysis.
When the difference in the nitrates formation rate between the lower treatment (PEC) and a control is equal to or less than 25% at any sampling day after day 28, the product can be evaluated as having no long-term influence on nitrogen transformation in soil.

Conclusions
On the basis of the results, it was concluded that Protiokonazol 100 FS [CHR/ZF/PROTI 100 FS] at the concentrations corresponding to the PEC: 1.31 mg test item/kg dry weight of soil (i.e. 0.13 mg of prothioconazole/kg dry weight of soil) and 5 x PEC: 6.55 mg of the test item / kg dry weight of soil (i.e. 0.65 mg of prothioconazole/kg dry weight of soil) did not have any long-term adverse effects on the process of nitrogen transformation in aerobic surface soils.

Validity criterion
The coefficients of variation (CV) in the control group were 9.6, 8.0, 8.9, 5.0 and 2.0%, after 0, 7, 14, 28 and 42 days of incubation. The Validity criterion was met, because the variation between replicate control samples is less than 15%.

Deviations from the study plan
Deviation from the OECD Guideline No. 216 (2000), the EU Method C.21:
According the Guideline, the soil extraction should be conducted at 150 rpm for 60 min. However, in this study, the extraction was performed at 90 rpm for 24 hours. The modification resulted from the optimization of the nitrate extraction which showed that the extraction was more effective when the shaking rate was lower and the extraction lasted longer (point 3.4.4.4.).
The predicted environmental concentration (PEC) was calculated assuming 1 cm of the soil depth according to the German conditions for the active substances with the mobility in soil KFoc > 500 mL/g. Thus, the applied soil depth is a deviation from the OECD Guideline No. 216 (2000) and EU Method C.21 where the PEC is calculated by using 5 cm of the soil depth (point 3.3.). These deviations did not affect the results of the study.

A 2.6 KCP 10.6 Effects on terrestrial non-target higher plants

N/A

A 2.6.1 KCP 10.6.1 Summary of screening data

N/A

izRMS version

A 2.6.2 KCP 10.6.2 Testing on non-target plants

N/A

A 2.6.3 KCP 10.6.3 Extended laboratory studies on non-target plants

N/A

A 2.7 KCP 10.7 Effects on other terrestrial organisms (flora and fauna)

N/A

A 2.8 KCP 10.8 Monitoring data

N/A