

REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: GPF515 / FGE03

Product name(s): Boscalid / Prothioconazole 350 / 200 WG

Chemical active substance:

Boscalid, 350 g/kg

Prothioconazole 200 g/kg

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(Article 33 application for a new product registration)

Applicant: UPL Holdings Coöperatief U.A.

Submission date: November 2024, update March 2025, April 2025,
May 2025

MS Finalisation date: June 2025 (initial Core Assessment)

August 2025 (final Core Assessmnt)

Version history

When	What
November 2024	Applicant version
March 2025	Updated version of dRR with the additional calculations of PEC_{sw} and the risk assessment for aquatic organism for use in sunflower performed by the Applicant.
April 2025	Updated version of dRR following the submission of the final report of fish study.
May 2025	Updated version of dRR considering the risk assessment for aquatic organism with consideration the results of the new study for fish.
March 2025/ June 2025	Initial zRMS assessment The report in the dRR format has been prepared by the Applicant, therefore all comments, additional evaluations and conclusions of the zRMS are presented in grey commenting boxes. Minor changes are introduced directly in the text and highlighted in grey. Not agreed or not relevant information are ~~struck through~~ and ~~shaded~~ for transparency. Following the evaluation and before sending the document for commenting, all coloured highlighting was removed, from the parts updated by the Applicant, for better legibility.
August 2025	Final report (Core Assessment updated following the commenting period) Additional information/assessments included by the zRMS in the report in response to comments received from the CMS and the Applicant are highlighted in yellow. Not agreed or not relevant information are ~~struck through~~ and ~~shaded~~ for transparency.

Table of Contents

9	Ecotoxicology (KCP 10)	5
9.1	Critical GAP and overall conclusions.....	6
9.1.1	Overall conclusions	9
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).....	9
9.1.1.2	Effects on aquatic organisms (KCP 10.2)	9
9.1.1.3	Effects on bees (KCP 10.3.1)	11
9.1.1.4	Effects on arthropods other than bees (KCP 10.3.2) Błąd! Nie zdefiniowano zakładek.	
9.1.1.5	Effects on non-target soil meso- and macrofauna (KCP 10.4), Effects on soil microbial activity (KCP 10.5)	12
9.1.1.6	Effects on non-target terrestrial plants (KCP 10.6)	12
9.1.1.7	Effects on other terrestrial organisms (flora and fauna) (KCP 10.7).....	12
9.1.2	Grouping of intended uses for risk assessment.....	12
9.1.3	Consideration of metabolites	14
9.2	Effects on birds (KCP 10.1.1)	16
9.2.1	Toxicity data	16
9.2.1.1	Justification for new endpoints.....	18
9.2.2	Risk assessment for spray applications.....	18
9.2.2.1	First-tier assessment (screening/generic focal species)	18
9.2.2.2	Higher-tier risk assessment.....	24
9.2.2.3	Drinking water exposure	24
9.2.2.4	Effects of secondary poisoning.....	25
9.2.2.5	Biomagnification in terrestrial food chains	29
9.2.3	Risk assessment for baits, pellets, granules, prills or treated seed.....	29
9.2.4	Overall conclusions	29
9.3	Effects on terrestrial vertebrates other than birds (KCP 10.1.2).....	30
9.3.1	Toxicity data	30
9.3.1.1	Justification for new endpoints.....	31
9.3.2	Risk assessment for spray applications.....	31
9.3.2.1	First-tier assessment (screening/generic focal species)	31
9.3.2.2	Higher-tier risk assessment.....	37
9.3.2.3	Drinking water exposure	38
9.3.2.4	Effects of secondary poisoning.....	39
9.3.2.5	Biomagnification in terrestrial food chains	43
9.3.3	Risk assessment for baits, pellets, granules, prills or treated seed.....	43
9.3.4	Overall conclusions	43
9.4	Effects on other terrestrial vertebrate wildlife (reptiles and amphibians) (KCP 10.1.3).....	44
9.5	Effects on aquatic organisms (KCP 10.2)	45
9.5.1	Toxicity data	45
9.5.1.1	Justification for new endpoints.....	48
9.5.2	Risk assessment	48
9.5.3	Overall conclusions	88
9.6	Effects on bees (KCP 10.3.1)	90
9.6.1	Toxicity data	90
9.6.1.1	Justification for new endpoints.....	91
9.6.2	Risk assessment	92
9.6.2.1	Hazard quotients for bees	94
9.6.2.2	Higher-tier risk assessment for bees (tunnel test, field studies)	99
9.6.3	Effects on bumble bees.....	99
9.6.4	Effects on solitary bees.....	99

9.6.5	Overall conclusions	99
9.7	Effects on arthropods other than bees (KCP 10.3.2)	101
9.7.1	Toxicity data	101
9.7.1.1	Justification for new endpoints	101
9.7.2	Risk assessment	101
9.7.2.1	Risk assessment for in-field exposure	102
9.7.2.2	Risk assessment for off-field exposure.....	102
9.7.2.3	Additional higher-tier risk assessment.....	103
9.7.2.4	Risk mitigation measures.....	104
9.7.3	Overall conclusions	104
9.8	Effects on non-target soil meso- and macrofauna (KCP 10.4)	105
9.8.1	Toxicity data	105
9.8.1.1	Justification for new endpoints.....	107
9.8.2	Risk assessment	107
9.8.2.1	First-tier risk assessment	108
9.8.2.2	Higher-tier risk assessment.....	110
9.8.3	Overall conclusions	110
9.9	Effects on soil microbial activity (KCP 10.5)	111
9.9.1	Toxicity data	111
9.9.1.1	Justification for new endpoints.....	112
9.9.2	Risk assessment	112
9.9.3	Overall conclusions	113
9.10	Effects on non-target terrestrial plants (KCP 10.6)	114
9.10.1	Toxicity data	114
9.10.1.1	Justification for new endpoints.....	114
9.10.2	Risk assessment	114
9.10.2.1	Tier-1 risk assessment (based on screening data).....	114
9.10.2.2	Tier-2 risk assessment (based on dose-response data)	115
9.10.2.3	Higher-tier risk assessment.....	115
9.10.2.4	Risk mitigation measures.....	115
9.10.3	Risk assessment	115
9.10.3.1	Tier-1 risk assessment (based on screening data).....	115
9.10.3.2	Tier-2 risk assessment (based on dose-response data)	115
9.10.3.3	Higher-tier risk assessment.....	115
9.10.3.4	Risk mitigation measures.....	115
9.10.4	Overall conclusions	115
9.11	Effects on other terrestrial organisms (flora and fauna) (KCP 10.7).....	116
9.12	Monitoring data (KCP 10.8).....	116
9.13	Classification and Labelling	116
Appendix 1	Lists of data considered in support of the evaluation.....	117
Appendix 2	Detailed evaluation of the new studies	121

9 Ecotoxicology (KCP 10)

This document reviews the Ecotoxicology studies and risk assessment for the first authorisation of the product Boscalid / Prothioconazole 350 / 200 WG (GPF515 / FGE03) in the central zone member states Czech Republic, Hungary and Poland according to Regulation (EC) No 1107/2009.

The product is a water dispersible granule (WG) formulated fungicide containing 350 g/kg of the active substance boscalid and 200 g/kg active substance prothioconazole. It is intended to be used as fungicide to control Sclerotinia in oilseed rape.

The review reports for boscalid (SANCO/3919/2007 – rev. 5, dated 21 January 2008) and prothioconazole (SANCO/3923 /07 – final, dated 10 December 2007) are considered to provide the relevant review information or a reference to where such information can be found.

The Annex I Inclusion Directive for boscalid and prothioconazole (2008/44/EC) provides specific provisions under Part B which need to be considered by the applicant in the preparation of their submission and by the MS prior to granting an authorisation.

For the implementation of the uniform principles of Annex VI, the conclusions of the review report on boscalid, and in particular Appendices I and II thereof, shall be taken into account. In this overall assessment Member States must pay particular attention to:

- the long term risk to birds and soil organisms,
- the risk of accumulation in soil if the substance is used in perennial crops or in succeeding crops in crop rotation.

For the implementation of the uniform principles of Annex VI, the conclusions of the review report on prothioconazole, and in particular Appendices I and II thereof, shall be taken into account. In this overall assessment Member States must pay particular attention to:

- the protection of aquatic organisms. Risk mitigation measures such as buffer zones shall be applied, where appropriate,

Conditions of use shall include adequate risk mitigation measures, where appropriate.

These concerns have been addressed within the current submission.

Appendix 1 of this document contains the list of references included in this document for support of the evaluation.

Appendix 2 of this document contains the detailed information of the new studies.

Information on the detailed composition of the formulation can be found in the confidential dossier of this submission (Registration Report - Part C)

9.1 Critical GAP and overall conclusions

Table 9.1-1: Table of critical GAPs

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Use-No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g safener/ synergist per ha	Conclusion						
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthro-	Soil organisms	Non-target plants
Zonal uses (field or outdoor uses, certain types of protected crops)																				
1	CZ	Oilseed rape (winter and spring) (<i>Brassica napus</i>) BRSNN	F	Sclerotinia (<i>Sclerotinia sclerotiorum</i>) SCLESC	Spraying overall	BBCH 61-69	a) 1 b) 1	-	a) 0.7 b) 0.7	a) boscalid: 245 prothioconazole: 140 b) boscalid: 245 prothioconazole: 140	100-400	35		A	A	R	C	A	A	A
	HU	Oilseed rape (winter and spring) (<i>Brassica napus</i>) BRSNN	F	Sclerotinia (<i>Sclerotinia sclerotiorum</i>) SCLESC	Spraying overall	BBCH 61-69	a) 1 b) 1	-	a) 0.7 b) 0.7	a) boscalid: 245 prothioconazole: 140 b) boscalid: 245 prothioconazole: 140	100-400	35		A	A	R	C	A	A	A
2	CZ	Oilseed rape (winter and spring) (<i>Brassica napus</i>) BRSNN	F	Sclerotinia (<i>Sclerotinia sclerotiorum</i>) SCLESC	Spraying overall	BBCH 61-73	a) 2 b) 2	14	a) 0.7 b) 1.4	a) boscalid: 245 prothioconazole: 140 b) boscalid: 490 prothioconazole: 280	100-400	35		A	A	R	C	A	A	A
	HU	Oilseed rape (winter and spring) (<i>Brassica napus</i>) BRSNN	F	Sclerotinia (<i>Sclerotinia sclerotiorum</i>) SCLESC	Spraying overall	BBCH 61-73	a) 2 b) 2	14	a) 0.7 b) 1.4	a) boscalid: 245 prothioconazole: 140 b) boscalid: 490 prothioconazole: 280	100-400	35		A	A	R	C	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
	PL	Oilseed rape (winter) (<i>Brassica napus</i>) BRSNN	F	Sclerotinia (<i>Sclerotinia sclerotiorum</i>) SCLESC	Spraying overall	BBCH 61-73	a) 2 b) 2	14	a) 0.7 b) 1.4	a) boscalid: 245 prothioconazole: 140 b) boscalid: 490 prothioconazole: 280	100-400	35		A	A	A	A	A	A	A
Minor uses according to Article 51 (zonal uses)																				
3	PL	Spring rape gold of pleasure, winter turnip rape, mustard, sunflower, poppy linseed, flax, hemp, borage	F	Scelrotinia (<i>Sclerotinia sclerotiorum</i>) SCLESC	Spraying overall	BBCH 61-73	a) 2 per use b) 2 per crop / season	14	a) 0.7 kg/ha b) 1.4 kg/ha	a) 245 g/ha boscalid 140 g/ha prothioconazole b) 490 g/ha boscalid 280 g/ha prothioconazole	100-400	35	Use covered by the worst case use n. 2.	A	A	A	A	A	A	A

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

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

Explanation for column 15 – 21 “Conclusion”

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

**Remarks
table:**

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

9.1.1 Overall conclusions

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

Birds

Acute and long-term/reproductive TER values for the two active substances and the relevant metabolite Prothioconazole-desthio (M04) as well as combi-TER calculations exceed the relevant trigger values at the screening step and/or Tier 1 following the intended use in oilseed rape and sunflower at 2×0.7 kg product/ha covering all intended uses; thus, risk for birds can be excluded for all intended uses. Therefore, a higher-Tier risk assessment is not required.

The risk for birds due to uptake of contaminated drinking water from puddles is acceptable for all intended uses of FGE03.

The log P_{ow} of Boscalid does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required. However, the log P_{ow} of Prothioconazole and metabolites M01 and M04 exceed the trigger value of 3. The risk assessment indicates no risk due to secondary poisoning for all intended uses.

Terrestrial vertebrates other than birds

Acute and long-term/reproductive TER values for the two active substances and the relevant metabolite Prothioconazole-desthio as well as combi-TER calculations considering Prothioconazole TER values exceed the relevant trigger values at the screening step and/or Tier 1 following the intended use in oilseed rape and sunflower at 2×0.7 kg product/ha covering all intended uses.

However, when the long-term/reproductive TER values for the metabolite Prothioconazole-desthio are used for combi-TER calculations, combi-TER_{it} for the generic focal species vole at BBCH ≥ 40 is slightly below the relevant trigger value. It should be noted that the assumption of full conversion of the parent to the metabolite is worst case. Nevertheless, in a conservative approach, a higher-Tier risk assessment based on a refined deposition factor is presented.

Higher Tier TER value for vole exceeds the relevant trigger value of 5. Therefore, further assessment is not required.

No risk is indicated for the intended single application already at Tier 1.

The risk for mammals due to uptake of contaminated drinking water from puddles is acceptable for all intended uses of FGE03.

The log P_{ow} of Boscalid does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required. However, the log P_{ow} of Prothioconazole and metabolites M01 and M04 exceed the trigger value of 3. The risk assessment indicates no risk due to secondary poisoning for all intended uses.

Reptiles and amphibians

Appropriate test guidelines and guidance documents are currently not available to address the risk to reptiles and amphibians. However, due to the acceptable risk of Boscalid, Prothioconazole and metabolites to terrestrial vertebrates and fish, adverse effects following the intended uses are considered unlikely.

9.1.1.2 Effects on aquatic organisms (KCP 10.2)

For the intended uses in oilseed rape and sunflower, calculated PEC/RAC ratios at Tier 1 for the individual active substances Boscalid and Prothioconazole as well as the relevant metabolites Prothiocona-

zole-S-methyl (M01) and 1,2,4-Triazole (M13) indicate acceptable risk for all groups of aquatic organisms up to FOCUS Step 3.

However, for the relevant metabolite Prothioconazole-desthio (M04), calculated PEC/RAC ratios at Tier 1 based on the RAC of 0.334 µg/L for the most sensitive organism group fish prolonged exceed the trigger of 1 for some scenarios indicating that risk cannot be excluded. Therefore, step 4 calculations considering reduction of run-off were performed for the relevant scenarios.

For the intended uses in winter/spring oilseed rape (two applications) and sunflowers (one or two applications), calculated PEC/RAC ratios for metabolite Prothioconazole-desthio (M04) indicate an acceptable risk for the most sensitive group of aquatic organism group fish prolonged (based on a RAC of 0.334 µg/L) in all relevant FOCUS Step 4 scenarios considering 10 m vegetated buffer. Therefore, no further assessment for the individual active substances/metabolites is necessary.

For the assessment of the combined toxicity of Boscalid, Prothioconazole and metabolites, the step-wise approach according to the aquatic guidance document as well as the decision tree for metabolites according to document FAQ Aquatic MixTox Tool_v2 is considered. The assessment is performed separately for each organism group.

In summary, mixture toxicity assessment should be based on the combined toxicity of the two parents as well as on the combined toxicity of Boscalid and metabolite Prothioconazole-desthio (M04). Combined toxicity from Boscalid and Prothioconazole is relevant for all organism groups (for fish long-term and invertebrates long-term only for certain scenarios). Combined toxicity from Boscalid and metabolite M04 is relevant fish long-term and invertebrates long-term (remaining scenarios).

Regarding the combined toxicity of the two parents, a simplified approach according to the aquatic guidance document was chosen for all organism groups and scenarios where the decision tree indicates that the mixture tox assessment should be based on the two actives, since no synergistic effects are indicated and all ETR values of the individual active substances contained in the formulation are below the relevant trigger value (corresponding to a PEC/RAC ratio < 1) up to FOCUS Step 3 calculations. The simplified approach indicates no risk from the combined toxicity of Boscalid and Prothioconazole.

Regarding the combined toxicity of Boscalid and metabolite Prothioconazole-desthio (M04), a mixture toxicity risk assessment for the intended uses of FGE03 in oilseed rape according to step 8 of the AGD based on ETRmix-CA calculations for Boscalid and metabolite Prothioconazole-desthio (M04) was presented. The assessment was performed for fish chronic only. This is deemed appropriate since PEC/RAC ratios for both Boscalid and metabolite M04 are clearly worst case for this organism group covering all other organism groups.

For most FOCUS Step 3 scenarios, the mixture toxicity assessment for Boscalid and Prothioconazole-desthio (M04) indicates no risk.

However, calculated ETRmix-CA for the most sensitive organism group fish prolonged exceeds the trigger of 0.1 for some scenarios indicating that risk cannot be excluded. Therefore, step 4 calculations considering reduction of run-off were performed for the relevant scenarios.

Risk from combined exposure to Boscalid and metabolite Prothioconazole-desthio (M04) for the most sensitive organism group fish prolonged is acceptable when the following risk mitigation measures for the relevant scenarios are considered:

- two applications of 0.7 kg FGE03/ha in winter oilseed rape – 10 m vegetated buffer (scenarios R1 stream, R3 stream)
- two applications of 0.7 kg FGE03/ha in spring oilseed rape - 10 m vegetated buffer (scenario R1)
- two applications of 0.7 kg FGE03/ha in sunflower - 10 m vegetated buffer (scenarios R1 stream, R3 stream) ~~15 m vegetated buffer (scenario R4 stream).~~

It should be noted that according to the comment given by zRMS e-fate experts in e -Fate Section for **R4 scenario** - 15-meter vegetated buffer is not accepted for two applications of 0.7 kg FGE03/ha in sunflower. For this reason, further calculation of PEC_{sw} considering 20 -meter vegetated buffer zone should be calculated by the Applicant for MSs that needs this scenario for their national level.

- one application of 0.7 kg FGE03/ha in sunflower - 10 m vegetated buffer (scenario R4)

No risk mitigation measures are required for single application in winter/spring oilseed rape.

9.1.1.3 Effects on bees (KCP 10.3.1)

Based on the acute Tier-1 risk assessment (honeybees and bumble bees) according to SANCO/10329/2002 rev.2 (final), October 17, 2002, ~~and the chronic Tier-1 risk assessment (honeybees) according to the modified EPPQ (2010)/ECPA (2017) scheme, respectively,~~ acceptable risk to bees following the intended uses of FGE03 is indicated. ~~Risk is also acceptable considering screening/Tier-1 risk assessment for honeybees according to EFSA/2013/3295.~~

Based on the acute (honeybees and bumble bees) and chronic adult (honeybees) risk assessment according to EFSA/2013/3295, no risk is indicated following all intended uses. The chronic larval Tier-1 ETR values for honeybees are also below the relevant trigger value for the use in oilseed rape for most scenarios. However, for “treated crop” scenario (BBCH 61-69), chronic larval Tier-1 ETR exceeds the trigger when the very conservative lower limit of the ED₁₀ is used for risk assessment. It should be noted that risk is acceptable already at the screening step if the 3-fold higher median ED₁₀ or NOED, respectively, from the larval study are used. ~~In addition, considering that EFSA/2013/3295 is not taken note of yet, further risk assessment is not considered necessary here.~~

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

There is currently no validated methodology for the assessment of toxicity to bumble bees (chronic) and solitary bees (both acute and chronic). Consequently, such studies are not considered necessary for the time being. Further risk assessment is not considered necessary here.

There is currently no validated methodology for the assessment of toxicity to bumble bees (chronic) and solitary bees (both acute and chronic). Consequently, such studies are not considered necessary for the time being. Further risk assessment is not considered necessary here.

9.1.1.4 Effects on arthropods other than bees (KCP 10.3.2)

For all intended uses, Tier-1 in-field HQ values are below the relevant trigger value for the indicator species *Aphidius rhopalosiphi*. For *Typhlodromus pyri*, Tier-1 in-field HQ values based on the ER₅₀ are below the relevant trigger value. However, extended lab studies with *Typhlodromus pyri* and *Poecilus cupreus* indicate no in-field risk; thus, additional testing and/or risk mitigation measures are not required to exclude risk to the in-field.

For all intended uses, Tier-1 and higher-Tier off-field HQ values are below the relevant trigger value for both indicator species. Thus, higher-Tier risk assessment and/or risk mitigation measures are not required to protect non-target arthropods in the off-field.

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

Non-target soil meso- and macrofauna

All Tier-1 TER values for earthworms and other non-target soil organisms (meso- and macrofauna) exceed the relevant trigger values indicating no risk for all intended uses except for the Boscalid endpoint from the Annex-I inclusion for the intended multiple application when the additional approach for PEC calculation is considered (no risk for single application and/or considering FOCUS approach). However, endpoints from Annex I inclusion for the two actives and corresponding risk assessments are only presented for sake of completeness since studies were performed on the respective representative formulations and are not considered relevant for the assessment of FGE03. The risk assessment with the FGE03 study endpoint supersedes the assessment for the Boscalid endpoint. Risk is also acceptable when the two additional Boscalid endpoints (a.s. endpoint from the RAR and endpoint from study with FGG01, respectively) are used. These endpoints are potentially more appropriate for the risk assessment for earthworms from exposure to FGE03 according to the intended GAP. Therefore, higher-Tier risk assessment is not required.

Effects on soil microbial activity

The maximum concentrations with effects on micro-organisms $\leq 25\%$ exceed the respective maximum PEC_{soil} values indicating no risk for all intended uses.

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

Risk is considered acceptable since data on seedling emergence and vegetative vigour from six species do not indicate phytotoxic effects > 50% at the maximum intended application rate. Therefore, a Tier-2 risk assessment based on dose-response data is not required.

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

Not available and not required.

9.1.2 Grouping of intended uses for risk assessment

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

Table 9.1-2: Critical use pattern of FGE03 grouped according to relevant criteria

Critical use pattern of PCEs grouped according to relevant criteria			
Group	Intended uses	relevant use parameters for grouping	relevant parameter or value for sorting
Birds and mammals			
Oilseed rape (covering the minor crop uses: Spring rape gold of pleasure, winter turnip rape, mustard, sunflower, poppy linseed, flax, hemp, borage)	1, 2	Crop scenario	Number of applications: 2 × 700 g/ha
Aquatic organisms (refer to efate)			
Winter OSR, multiple application (covering gold of pleasure, winter turnip rape, mustard, poppy, linseed, flax, hemp, borage)	2	PEC _{sw} (refer to efate)	Number of applications, 2 × 700 g/ha, winter OSR
Winter OSR, single application	1		Number of applications, 1 × 700 g/ha, winter OSR
Spring OSR, multiple application	2		Number of applications, 2 × 700 g/ha, spring OSR
Spring OSR, single application	1		Number of applications, 1 × 700 g/ha, spring OSR
Sunflower	2	PEC _{sw} (refer to efate)	Number of applications, 2 × 700 g/ha, sunflower
Sunflower	2		Number of applications, 1 × 700 g/ha, sunflower
Bees			
Oilseed rape (covering minro crop uses: Spring rape gold of pleasure, winter turnip rape, mustard, sunflower, poppy linseed, flax, hemp, borage)	All	Single applicaction rate	Maximum single application rate: 700 g/ha
Non-target arthropods, in-field			
Oilseed rape (covering minor crop uses: Spring rape gold of pleasure, winter turnip rape, mustard, sunflower, poppy linseed, flax, hemp)	2	PER _{in-field}	PER _{in-field} : 1190 g/ha

Group	Intended uses	relevant use parameters for grouping	relevant parameter or value for sorting
borage)			
Oilseed rape	1		PER _{in-field} : 700 g/ha
Non-target arthropods, off-field			
Oilseed rape (covering minor crop uses: Spring rape gold of pleasure, winter turnip rape, mustard, sunflower, poppy linseed, flax, hemp, borage)	All	PER _{off-field}	PER _{off-field} : 2.832 g/ha
Soil organisms			
Oilseed rape (covering minor crop uses: Spring rape gold of 2 pleasure, winter turnip rape, mustard, poppy linseed, flax, hemp, borage)	All	PEC _{soil} (refer to efate)	Maximum PEC _{soil} : 0.039 mg/kg (Prothioconazole), 0.158 / 0.287 mg/kg (Boscalid)
Sunflower?	2	PEC _{soil} (refer to efate)	Maximum PEC _{soil} :0.048 mg/kg (prothioconazole), 0.198/0.316 Boscalid

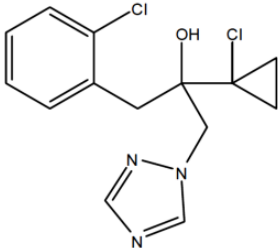
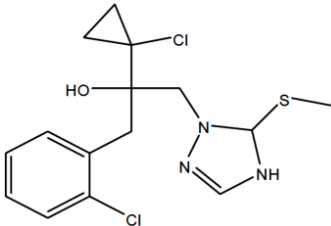
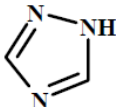
9.1.3 Consideration of metabolites

Active substance boscalid

Boscalid metabolite M510F64 occurred at amounts > 5% in 2 sequential measurements in the higher-tier outdoor water/sediment study presented for the original approval of boscalid. Metabolite M510F64 reached maximum 9.4% TAR after 30 days in the water phase. Additionally, this compound is of no toxicological and ecotoxicological relevance. Following the approaches taken for the currently authorised products containing boscalid, no exposure assessment was presented for metabolite M510F64. No other metabolites of boscalid potentially relevant for exposure assessment have been observed.

Active substance prothioconazole

Table 9.1-3: Metabolites of active substance prothioconazole potentially relevant for exposure assessment

Metabolite	Molar mass	Chemical structure	Maximum-observed occurrence in compartments	Risk assessment required?
Prothioconazole-desthio (M04) (JAU-desthio)	312.2 g/mol		Soil: 57.1 % Water: 32.3 % Sediment: 26.9 % Whole system: 54.6 %	Soil Surface water Sediment (according to residue definition for risk assessment in EFSA Scientific Report (2007) 106, 1-98)
Prothioconazole-S-methyl (M01) (JAU-S-methyl)	358.3 g/mol		Soil: 14.6 % Water/sediment: 77 % (anaerobic)	Soil Surface water (according to residue definition for risk assessment in EFSA Scientific Report (2007) 106, 1-98)
1,2,4-triazole (M13)	69.065 g/mol		Water: 37.2 % Sediment: 4.6 % Whole system: 41.8 %	Surface water (according to residue definition for risk assessment in EFSA Scientific Report (2007) 106, 1-98)

zRMS comments:

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

The maximum occurrence is relevant for exposure evaluation, for information agreed in this area please refer to the Core Assessment, Part B, Section 8, where all respective data are provided and used in calculation of PEC_{soil} and $PEC_{sw/sed}$ values, considered further in the risk assessment.

As the information on the maximum occurrence was not checked in detail, it was struck through in Table 9.1-3.

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

Avian toxicity studies have been carried out with Boscalid, 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 FGE03 were not evaluated as part of the EU assessment of Boscalid and Prothioconazole, respectively. However, the provision of further data on the formulation FGE03 is not considered essential, because the risk can be addressed based on active substance/metabolite data considering the combi-TER approach assuming dose addition. FGE03 does not contain any toxicologically classified co-formulants (refer to Part C of the present dossier); thus, the formulation is not expected to be more toxic than the active substances/metabolites.

The selection of studies and endpoints for the risk assessment of the individual active substances and the metabolite Prothioconazole-desthio is in line with the results of the EU review process but considers extrapolation factors according to current guidance. Justifications are provided below.

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

Species	Substance	Exposure System	Results	Reference
<i>Colinus virginianus</i>	Boscalid	Oral 1 d Acute	LD ₅₀ > 2000 mg/kg bw Extrapolated LD₅₀ = 3776 mg/kg bw^a	Review report, SANCO/3919 /2007-rev. 5, 21 January 2008 and DAR (2002)
<i>Colinus virginianus</i>	Boscalid	Dietary 5 d Short-term	LC ₅₀ > 5000 mg/kg feed	Review report, SANCO/3919 /2007-rev. 5, 21 January 2008 and DAR (2002)
<i>Anas platyrhynchos</i>	Boscalid	Dietary 5 d Short-term	LC ₅₀ > 5000 mg/kg feed	Review report, SANCO/3919 /2007-rev. 5, 21 January 2008 and DAR (2002)
<i>Colinus virginianus</i>	Boscalid	Dietary Reproductive toxicity	NOEC = 300 mg/kg feed NOAED = 24.1 mg/kg bw/d	Review report, SANCO/3919 /2007-rev. 5, 21 January 2008 and DAR (2002)
<i>Anas platyrhynchos</i>	Boscalid	Dietary Reproductive toxicity	NOEC = 1000 mg/kg feed	Review report, SANCO/3919 /2007-rev. 5, 21 January 2008 and DAR (2002)

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

^a An extrapolation factor of 1.888 is applied according to EFSA/2009/1438 since 10 animals were tested at each dose level and no effects were observed.

Table 9.2-2: Endpoints and effect values relevant for the risk assessment for birds - Prothioconazole

Species	Substance	Exposure System	Results	Reference
<i>Colinus virginianus</i>	Prothioconazole	Oral 1 d Acute	LD ₅₀ > 2000 mg/kg bw Extrapolated LD₅₀ = 3776 mg/kg bw^a	EFSA Scientific Report (2007) 106, 1-98, and DAR (2005)
<i>Colinus virginianus</i>	Prothioconazole	Dietary 5 d Short-term	LC ₅₀ > 5000 mg/kg bw/d LD ₅₀ > 1413 mg/kg bw/d	EFSA Scientific Report (2007) 106, 1-98, and DAR (2005)
<i>Anas platyrhynchos</i>	Prothioconazole	Dietary 5 d Short-term	LC ₅₀ > 5000 mg/kg feed LD ₅₀ > 2457 mg/kg bw/d	EFSA Scientific Report (2007) 106, 1-98, and DAR (2005)
<i>Colinus virginianus</i>	Prothioconazole	Dietary Reproductive toxicity	NOEC = 1000 mg/kg feed NOEL = 86 mg/kg bw/d	EFSA Scientific Report (2007) 106, 1-98, and DAR (2005)
<i>Anas platyrhynchos</i>	Prothioconazole	Dietary Reproductive toxicity	NOEC = 700 mg/kg feed NOEL = 78 mg/kg bw/d	EFSA Scientific Report (2007) 106, 1-98, and DAR (2005)
<i>Colinus virginianus</i>	Prothioconazole-desthio	Oral 1 d Acute	LD₅₀ > 2000 mg/kg bw^b	EFSA Scientific Report (2007) 106, 1-98, and DAR (2005)
<i>Colinus virginianus</i>	Prothioconazole-desthio	Dietary 5 d Short-term	LC ₅₀ > 4090 mg/kg feed LD₅₀ > 297 mg/kg bw/d^c LD₅₀ = 614 mg/kg bw/d^d	EFSA Scientific Report (2007) 106, 1-98, and DAR (2005)
<i>Colinus virginianus</i>	Prothioconazole-desthio	Dietary Reproductive toxicity	NOEC = 173 mg/kg feed NOEL = 14.8 mg/kg bw/d	EFSA Scientific Report (2007) 106, 1-98, and DAR (2005)
<i>Anas platyrhynchos</i>	Prothioconazole-desthio	Dietary Reproductive toxicity	NOEC = 500 mg/kg feed NOEL = 63 mg/kg bw/d	EFSA Scientific Report (2007) 106, 1-98, and DAR (2005)

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

a An extrapolation factor of 1.888 is applied according to EFSA/2009/1438 since 10 animals were tested at each dose level and no effects were observed.

The following endpoints are used in the acute risk assessment for Prothioconazole-desthio:

b acute LD₅₀ > 2000 mg/kg bw (EU-agreed endpoint, EFSA Conclusion 2007)

c short-term dietary LD₅₀ > 297 mg/kg bw/d (EFSA Conclusion 2007), used for acute risk assessment in line with EFSA (2009) together with the acute endpoint since the endpoint from the 5-d study is lower

d short-term dietary LD₅₀ = 614 mg/kg bw/d (re-calculated in RAR 2018), used for acute risk assessment in line with EFSA (2009) together with the acute endpoint since the endpoint from the 5-d study is lower

Since Prothioconazole-desthio is major plant metabolite of Prothioconazole with higher occurrence in plant material and higher toxicity compared to the parent, it is also considered in the risk assessment for both the individual active substances and the combination toxicity of the product.

zRMS comments:

Avian toxicity data for boscalid, prothioconazole and prothioconazole metabolite JAU 6476-desthio provided in Tables 9.2-1 and 9.2-2 above were verified by zRMS and then confirmed that they are in line with EU agreed endpoints reported in Review report, SANCO/3919 /2007-rev. 5, 21 January 2008 and DAR (2002) and EFSA Scientific Report (2007) 106, respectively.

9.2.1.1 Justification for new endpoints

The selection of studies and endpoints for the risk assessment of the individual active substances and the metabolite Prothioconazole-desthio is in line with the results of the EU review process. Acute active substance endpoints were extrapolated according to EFSA (2009) since 10 animals were tested at each dose level and no effects were observed.

For Prothioconazole-desthio, LD₅₀ values from the 5-d dietary toxicity study are used in the acute risk assessment in addition to the EU-agreed endpoint from the acute study. This is in line with the current guidance document for birds and mammals (EFSA 2009) since the short-term dietary endpoint is worst case. The guidance was not yet into force at the time of the Annex-1 inclusion of Prothioconazole. Two endpoints are available from the 5-d study and considered for risk assessment, a “higher than” endpoint from the EFSA Conclusion and a recalculated definite endpoint from the RAR (2018).

zRMS comments:

zRMS agrees that for metabolite Prothioconazole-desthio the lowest value of LD₅₀ >297 mg metabolite/kg bw from 5-d dietary toxicity study should be used in the risk assessment.

For both active substances prothioconazole and boscalid extrapolated values of LD₅₀ of 3776 mg/kg bw used in the risk assessment has been validated by zRMS. For MSs that they prefer lower value of LD₅₀ of >2000 mg /kg the additional calculations have been added.

9.2.2 Risk assessment for spray applications

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

Here, the assessment for the use group oilseed rape (2×0.7 kg product/ha; use 2) covers the risk for birds from all intended uses (see 9.1.2).

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

The results of the acute and reproductive screening/Tier-1 risk assessments are summarised in the following tables.

Table 9.2-3: Screening/First-Tier assessment of the acute and long-term/reproductive risk for birds due to exposure to Boscalid following the use of FGE03 in oilseed rape (use 2) and sunflower

Intended use	Oilseed rape and sunflower				
Active substance	Boscalid				
Application rate (kg/ha)	2 × 0.245 (14 days interval)				
Acute toxicity (mg/kg bw)	3776 (extrapolated value), 2000*				
TER criterion	10				
Crop scenario Growth stage	Indicator species	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a
Bulbs and onion like crops, cereals, fruiting vegetables, leafy vegetables, legume forage, maize, oilseed rape, potatoes, pulses, root and stem vegetables, strawberries, sugar beet, and sunflower	Small omnivorous bird	158.8	1.2	46.7	80.9 42.8*
Reprod. toxicity (mg/kg bw/d)	24.1				
TER criterion	5				
Crop scenario Growth stage	Generic focal species	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{lt}
Oilseed rape BBCH ≥ 40	medium herbivorous/ granivorous bird "pigeon"	0.9	1.4 × 0.53	0.164	147
Oilseed rape BBCH ≥ 40	Small omnivorous bird "lark"	2.7		0.491	49.1
~~Oilseed rape BBCH 30–39~~	~~medium herbivorous/ granivorous bird "pigeon"~~	~~1.1~~		~~0.200~~	~~121~~
~~Oilseed rape BBCH 30–39~~	~~Small omnivorous bird "lark"~~	~~3.3~~		~~0.600~~	~~40.2~~
Oilseed rape late – late (with seeds) (BBCH 30-99)	Small insectivorous bird "dunnock"	2.7		0.491	49.1
Sunflower BBCH 61 - 92	Small granivorous/insectivorous bird "bunting"	10.0		1.818	13.3

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

*based on the lower value

Table 9.2-4: Screening/First-Tier assessment of the acute and long-term/reproductive risk for birds due to exposure to Prothioconazole following the use of FGE03 in oilseed rape (use 2) and sunflower

Intended use	Oilseed rape and sunflower				
Active substance	Prothioconazole				
Application rate (kg/ha)	2 × 0.14 (14 days interval)				
Acute toxicity (mg/kg bw)	3776 (extrapolated value), 2000 *				
TER criterion	10				
Crop scenario Growth stage	Indicator species	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a
Bulbs and onion like crops, cereals, fruiting vegetables, leafy vegetables, legume forage, maize, oilseed rape, potatoes, pulses, root and stem vegetables, strawberries, sugar beet, and sunflower	Small omnivorous bird	158.8	1.2	26.7	142 74.90*
Reprod. toxicity (mg/kg bw/d)	78				
TER criterion	5				
Crop scenario Growth stage	Generic focal species ^a	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{it}
Oilseed rape BBCH ≥ 40	medium herbivorous/ granivorous bird "pigeon"	0.9	1.4 × 0.53	0.093	834
Oilseed rape BBCH ≥ 40	Small omnivorous bird "lark"	2.7		0.280	278
Oilseed rape BBCH 30–39	medium herbivorous/ granivorous bird "pigeon"	1.1		0.114	683
Oilseed rape BBCH 30–39	Small omnivorous bird "lark"	3.3		0.343	228
Oilseed rape late – late (with seeds) (BBCH 30-99)	Small insectivorous bird "dunnock"	2.7		0.280	278
Sunflower BBCH 61 - 92	Small granivorous/insectivorous bird "bunting"	10.0		1.039	75.1

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

a Risk for the individual active substance would already be acceptable at the screening step based on indicator species. Tier-1 calculations are required for combination toxicity assessment only.

*Based on lower value

Table 9.2-5: Screening/First-Tier assessment of the acute and long-term/reproductive risk for birds due to exposure to Prothioconazole-desthio following the use of FGE03 in oilseed rape (use 2) and sunflower

Intended use	Oilseed rape and sunflower				
Active substance	Prothioconazole-desthio				
Application rate (kg/ha)	2 × 0.127 (14 days interval) ^a				
Acute toxicity (mg/kg bw)	> 2000 ^b / > 297 ^c / 614 ^d				
TER criterion	10				
Crop scenario Growth stage	Indicator species	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a
Bulbs and onion like crops, cereals, fruiting vegetables, leafy vegetables, legume forage, maize, oilseed rape, potatoes, pulses, root and stem vegetables, strawberries, sugar beet, and sunflower	Small omnivorous bird	158.8	1.2	24.2	> 82.6 / > 12.3 / 25.4
Reprod. toxicity (mg/kg bw/d)	14.8				
TER criterion	5				
Crop scenario Growth stage	Generic focal species	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{lt}
Oilseed rape BBCH ≥ 40	medium herbivorous/ granivorous bird "pigeon"	0.9	1.4 × 0.53	0.085	175
Oilseed rape BBCH ≥ 40	Small omnivorous bird "lark"	2.7		0.254	58.2
Oilseed rape BBCH 30–39	medium herbivorous/ granivorous bird "pigeon"	1.1		0.104	143
Oilseed rape BBCH 30–39	Small omnivorous bird "lark"	3.3		0.311	47.6
Oilseed rape late – late (with seeds) (BBCH 30-99)	Small insectivorous bird "dunnock"	2.7		0.254	58.2
Sunflower BBCH 61 - 92	Small granivorous/insectivorous bird "bunting"	10.0		0.942	15.7

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

- a assuming full conversion of Prothioconazole to Prothioconazole-desthio as worst case, considering a factor 0.907 based on the molecular weight ratio of 312.20 (metabolite) : 344.26 (parent)
b acute LD₅₀ > 2000 mg/kg bw (EU-agreed endpoint, EFSA Conclusion 2007)
c short-term dietary LD₅₀ > 297 mg/kg bw/d (EFSA Conclusion 2007)
d short-term dietary LD₅₀ = 614 mg/kg bw/d (re-calculated in RAR 2018)

zRMS comments:

TER_A and TER_{LT} values for the exposure to boscalid, prothioconazole, its metabolite prothioconazole-desthio are above the trigger of 10 and 5 for acute and long-term exposure, indicating acceptable risk for birds. It should be noted that the acute risk for both active substances was performed by the Applicant with consideration of extrapolated LD₅₀ of 3776 kg bw value. This approach is validated by zRMS. However, for MSs that prefers lower value, the evaluations presented in Tables 9.2-3 and 9.2-4 above were amended accordingly with consideration of the LD₅₀ of 2000 mg pm/kg bw/d value for both active substances.

Overall, acceptable acute and long-term risk may be concluded for birds exposed to boscalid, prothioconazole, metabolite JAU 6476-desthio in FGE03 for all intended uses in GE03.

Mixture toxicity

FGE03 contains two active substances, and a toxic metabolite is formed in plant material. Therefore, mixture toxicity needs to be assessed.

In a first step, the surrogate LD₅₀ values for acute effects are calculated and potential single substance drivers of the risk are identified according to Appendix B of EFSA/2009/1438 (for equations please refer to the guidance document).

To consider two extreme scenarios, it is assumed that exposure takes place to

- A. Boscalid + Prothioconazole
- B. Boscalid + Prothioconazole-desthio (assuming full conversion of the parent to the metabolite)

Table 9.2-6: Calculation of the surrogate LD₅₀ value for acute effects to birds and assessment of single substance driver of the risk – Boscalid and Prothioconazole

Substance	Concentration in the product (g/kg)	Fraction of a.s. in the product	LD ₅₀ (mg/kg bw)	LD ₅₀ mix (mg/kg bw) and tox per fraction mix	Tox per fraction a.s.	Deviation from tox per fraction mix (%)
Boscalid	350	0.64	3776	3776	5900 ~~5934~~	64 ~~57~~
Prothioconazole	200	0.36	3776		10488.9 ~~10384~~	36 ~~175~~

Table 9.2-7: Calculation of the surrogate LD₅₀ value for acute effects to birds and assessment of single substance driver of the risk – Boscalid and Prothioconazole-desthio (M04)

Substance	Concentration in the product (g/kg)	Fraction of a.s./met. in the product	LD ₅₀ (mg/kg bw)	LD ₅₀ mix (mg/kg bw) and tox per fraction mix	Tox per fraction a.s.	Deviation from tox per fraction mix (%)
Boscalid	350	0.66	3776	2898	5721.2 ~~5723~~	51 ~~98~~
M04	181.4 ^a	0.34	> 2000 ^b		5882.35 ~~5859~~	49 ~~102~~

a assuming full conversion of Prothioconazole to Prothioconazole-desthio as worst case, considering a factor 0.907 based on the molecular weight ratio of 312.20 (metabolite) : 344.26 (parent)

b in case lower endpoints from 5-d dietary study are considered, deviation from tox per fraction is still > 10% indicating that no toxic driver can be identified

None of the active substances/metabolites can be identified where the two quotients “tox per fraction (a.s.)” and “tox per fraction (mix)” deviate by ≤ 10 %, i.e. contributes to ≥ 90 % to mixture toxicity. Thus, the predicted LD₅₀(mix) should be used in the risk assessment together with appropriate exposure estimates.

Potential risk from combined exposure to active substances and/or relevant metabolites is assessed based on the combi-TER approach assuming dose additivity. The combined TER value is calculated according to the equation $TER_{combi} = 1 / (1/TER_{substance\ 1} + 1/TER_{substance\ 2})$. An acceptable risk is expected when $TER_{combi} > trigger$.

Please note that the combi-TER approach will obtain the same results as the risk assessment based on the calculated mixture endpoint.

For sub-lethal effects and the effects on reproduction, the EFSA Guidance recommends only performing a risk assessment for combined toxicity on a case-by case basis. However, in the central zone it has been agreed that potential combined toxicity should be taken into account for the sub-lethal effects, as well as acute/lethal effects. This is in line with the aquatic guidance document (EFSA, 2013) and the EFSA guidance on mixture toxicity (2019). Therefore, in the current risk assessment, combined toxicity is also considered for reproductive/long-term effects.

Table 9.2-8: TER values for individual active substances/metabolites and combi-TER calculations for birds following the use of FGE03 in oilseed rape (use 2) and sunflower

Crop scenario Growth stage	Indicator species	TER _a Boscalid	TER _a Prothiocona- zole	TER _a Prothiocona- zole-desthio	TER _a , combi Boscalid + Prothiocona- zole ^a	TER _a , combi Boscalid + Prothiocona- zole-desthio ^b
Bulbs and onion like crops, cereals, fruiting vegetables, leafy vegetables, legume forage, maize, oilseed rape, potatoes, pulses, root and stem vegetables, strawberries, sugar beet, and sunflower	Small omnivorous bird	80.9 42.8*	142 74.90*	> 82.6 ^c / > 12.3 ^d / 25.4 ^e	51.5 27.32*	40.9 ^c / 10.7 ^d / 19.3 ^e
Crop scenario Growth stage	Generic focal species	TER _{lt} Boscalid	TER _{lt} Prothiocona- zole	TER _{lt} Prothiocona- zole-desthio	TER _{lt} , combi Boscalid + Prothiocona- zole ^a	TER _{lt} , combi Boscalid + Prothiocona- zole-desthio ^b
Oilseed rape BBCH ≥ 40	medium herbivorous/ granivorous bird "pigeon"	147	834	175	125	79.7
Oilseed rape BBCH ≥ 40	Small omnivorous bird "lark"	49.1	278	58.2	41.7	26.6
Oilseed rape BBCH 30– 39	medium herbivorous/ granivorous bird "pigeon"	121	683	143	102	65.2
Oilseed rape BBCH 30– 39	Small omnivorous bird "lark"	40.2	228	47.6	34.1	21.7
Oilseed rape late – late (with seeds) (BBCH 30– 99)	Small insectivorous bird "dunnock"	49.1	278	58.2	41.7	26.6
Sunflower BBCH 61 - 92	Small granivorous/insectivoro us bird "bunting"	13.3	75.1	15.7	11.3	7.2

TER: toxicity to exposure ratio. TER values shown **in bold** fall below the relevant trigger (10 for acute toxicity, 5 for longterm/reproductive toxicity)

a scenario A: exposure to Boscalid + Prothioconazole

b. scenario B: exposure to Boscalid + Prothioconazole-desthio (assuming full conversion of the parent to the metabolite as worst case)

c based on acute LD₅₀ > 2000 mg/kg bw (EU-agreed endpoint, EFSA Conclusion 2007)

d based on short-term dietary LD₅₀ > 297 mg/kg bw/d (EFSA Conclusion 2007)

e based on short-term dietary LD₅₀ = 614 mg/kg bw/d (re-calculated in RAR 2018)

* With consideration of LD₅₀ >2000 mg a.s./kg bw

Acute and long-term/reproductive TER values for the two active substances and the relevant metabolite Prothioconazole-desthio as well as combi-TER calculations exceed the relevant trigger values at the screening step and/or Tier 1 following the intended use in oilseed rape and sunflower at 2 × 0.7 kg product/ha covering all intended uses; thus, risk for birds can be excluded for all intended uses. Therefore, a higher-Tier risk assessment is not required.

zRMS comments:

The acute and long-term combined risk assessment provided in the Table 9.2-8 have been validated by zRMS. In addition, zRMS added in the Table above the TER_A combi taking into account LD₅₀ >2000 mg a.s./kg bw value.

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

9.2.2.2 Higher-tier risk assessment

Not required.

9.2.2.3 Drinking water exposure

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

Leaf scenario

Since FGE03 is not intended to be applied on leafy vegetables forming heads or crop plants with comparable water collecting structures at principal growth stage 4 or later, the leaf scenario does not have to be considered.

Puddle scenario

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

With a $K(f)_{oc}$ of 743 L/kg (geometric mean), Boscalid belongs to the group of more sorptive substances. With a $K(f)_{oc}$ of 1765 L/kg (based on aged soil column leaching study), Prothioconazole belongs to the group of more sorptive substances.

With a $K(f)_{oc}$ of 573.5 L/kg (geometric mean), Prothioconazole-desthio belongs to the group of more sorptive substances.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use oilseed rape at 2×0.7 kg product/ha also covers the risk for birds from all other intended uses in terms of maximum effective application rate (see 9.1.2).

Boscalid

Effective application rate (g/ha) =	490 ^a			
Acute toxicity (mg/kg bw) =	3776	quotient	=	0.130
	2000*			0.245*
Reprod. toxicity (mg/kg bw/d) =	24.1	quotient	=	20.3

a assuming no degradation between applications

Prothioconazole

Effective application rate (g/ha) =	280 ^a			
Acute toxicity (mg/kg bw) =	3776	quotient	=	0.074
	2000*			0.14*
Reprod. toxicity (mg/kg bw/d) =	78	quotient	=	3.59

a assuming no degradation between applications

Prothioconazole-desthio (M04)

Effective application rate (g/ha) =	254 ^a			
Acute toxicity (mg/kg bw) >	2000	quotient	<	0.127
	>297			0.85*
Reprod. toxicity (mg/kg bw/d) =	14.8	quotient	=	17.2

a assuming no degradation between applications and full conversion of Prothioconazole to Prothioconazole-desthio as worst case, considering a factor 0.907 based on the molecular weight ratio of 312.20 (metabolite) : 344.26 (parent)

*Lower LD50 value used

The ratios of effective application rate (in g/ha) to relevant endpoints (in mg/kg bw/d) do not exceed the relevant trigger value of 3000 with high margin of safety, even if risk quotients are summed up to account

for combination toxicity. Thus, risk to birds from exposure to drinking water following the intended uses of FGE03 can be excluded.

zRMS comments:

Ratios between the application rates and toxicity endpoints are below the respective triggers (<50) for active substances and metabolite Prothioconazole-desthio (M04) demonstrating acceptable risk resulting from exposure of birds to a.s. and this metabolite M 04 via drinking water in puddles.

9.2.2.4 Effects of secondary poisoning

The log P_{ow} of Boscalid amounts to 2.96 (Review report, SANCO/3919 /2007-rev. 5, 21 January 2008) and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

However, the log P_{ow} values of Prothioconazole, Prothioconazole-desthio and Prothioconazole-S-methyl amount to 3.82 (pH 7; EFSA Scientific Report (2007) 106, 1-98), 3.04 and 4.19 (EFSA Scientific Report (2007) 106, 1-98), respectively, and thus exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is therefore 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 predicted concentrations in soil.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the screening assessment for the use group oilseed rape covers the risk for birds from all intended uses (see 9.1.2).

Table 9.2-9: Assessment of the risk for earthworm-eating birds due to exposure to Prothioconazole via bioaccumulation in earthworms (secondary poisoning) for the intended use in oilseed rape (covers all intended uses)

Parameter	Prothioconazole	Comments
PEC _{soil} (mg kg ⁻¹)	0.014	Long-term PEC _{soil} (dry weight) after last application, TWA, 21 days, in OSR (worst case); please refer to dRR Part B8, environmental fate
log P_{ow} / P_{ow}	3.82 / 6607	Partition coefficient octanol-water (EFSA Scientific Report (2007) 106, 1-98)
K _{oc} (L kg ⁻¹)	1765	Partition coefficient soil organic carbon-water (EFSA Scientific Report (2007) 106, 1-98)
f _{oc}	0.02	Organic carbon content of soil; default (EFSA/2009/1438)
BCF _{earthworm}	2.27	Bioconcentration factor for earthworms related to dry soil; BCF _{earthworm} = PEC _{earthworm fwt} / PEC _{soil dwt} = (0.84 + 0.012 × P_{ow}) / (f _{oc} × K _{oc})
PEC _{earthworm}	0.0318	PEC _{earthworm} = PEC _{soil} × BCF _{earthworm}
DDD (mg kg bw d ⁻¹)	0.0334	Daily dietary dose; DDD = PEC _{worm} × 1.05
NOAEL (mg kg bw d ⁻¹)	78	No-observed-adverse-effect level (EFSA Scientific Report (2007) 106, 1-98)
TER _{it}	2338	Toxicity exposure ratio (TER criterion: 5)

TER values shown **in bold** fall below the relevant trigger.

Table 9.2-10: Assessment of the risk for earthworm-eating birds due to exposure to Prothioconazole-S-methyl (M01) via bioaccumulation in earthworms (secondary poisoning) for the intended use in oilseed rape (covers all intended uses)

Parameter	Prothioconazole-S-methyl	Comments
PEC _{soil} (mg kg ⁻¹)	0.009	Long-term PEC _{soil} (dry weight) after last application, TWA, 21 days, in OSR (worst case); please refer to dRR Part B8, environmental fate
log P _{ow} / P _{ow}	4.19 / 15488	Partition coefficient octanol-water (EFSA Scientific Report (2007) 106, 1-98)
K _{oc} (L kg ⁻¹)	2526	Partition coefficient soil organic carbon-water (geomean, refer to environmental fate section)
f _{oc}	0.02	Organic carbon content of soil; default (EFSA/2009/1438)
BCF _{earthworm}	3.70	Bioconcentration factor for earthworms related to dry soil; $BCF_{earthworm} = PEC_{earthworm\ fwt} / PEC_{soil\ dwt} = (0.84 + 0.012 \times P_{ow}) / (f_{oc} \times K_{oc})$
PEC _{earthworm}	0.0333	$PEC_{earthworm} = PEC_{soil} \times BCF_{earthworm}$
DDD (mg kg bw d ⁻¹)	0.0349	Daily dietary dose; $DDD = PEC_{worm} \times 1.05$
NOAEL (mg kg bw d ⁻¹)	7.8	No-observed-adverse-effect level, assuming 10-fold higher toxicity compared to the parent (EFSA Scientific Report (2007) 106, 1-98)
TER _{it}	223	Toxicity exposure ratio (TER criterion: 5)

TER values shown **in bold** fall below the relevant trigger.

Table 9.2-11: Assessment of the risk for earthworm-eating birds due to exposure to Prothioconazole-desthio (M04) via bioaccumulation in earthworms (secondary poisoning) for the intended use in oilseed rape (covers all intended uses)

Parameter	Prothioconazole-desthio	Comments
PEC _{soil} (mg kg ⁻¹)	0.0332	Long-term PEC _{soil} (dry weight) after last application, TWA, 21 days, in Sunflower OSR (worst case); please refer to dRR Part B8, environmental fate
log P _{ow} / P _{ow}	3.04 / 1096	Partition coefficient octanol-water (EFSA Scientific Report (2007) 106, 1-98)
K _{oc} (L kg ⁻¹)	574	Partition coefficient soil organic carbon-water (geomean, refer to environmental fate section)
f _{oc}	0.02	Organic carbon content of soil; default (EFSA/2009/1438)
BCF _{earthworm}	1.22	Bioconcentration factor for earthworms related to dry soil; $BCF_{earthworm} = PEC_{earthworm\ fwt} / PEC_{soil\ dwt} = (0.84 + 0.012 \times P_{ow}) / (f_{oc} \times K_{oc})$
PEC _{earthworm}	0.0391	$PEC_{earthworm} = PEC_{soil} \times BCF_{earthworm}$
DDD (mg kg bw d ⁻¹)	0.0410	Daily dietary dose; $DDD = PEC_{worm} \times 1.05$
NOAEL (mg kg bw d ⁻¹)	14.8	No-observed-adverse-effect level (EFSA Scientific Report (2007) 106, 1-98)
TER _{it}	361	Toxicity exposure ratio (TER criterion: 5)

TER values shown **in bold** fall below the relevant trigger.

zRMS comments:

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

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

In conclusion, an acceptable risk of secondary exposure from all relevant compounds could be concluded for birds.

Risk assessment for fish-eating birds via secondary poisoning

According to EFSA/2009/1438, the risk for piscivorous birds is assessed for a bird of 1000 g body weight with a daily food consumption of 159 g. Bioaccumulation in fish is estimated based on predicted concentrations in surface water / is based on the regulatory acceptable concentration for aquatic organisms as a limit value for admissible concentrations of Prothioconazole in water.

Table 9.2-12: Assessment of the risk for fish-eating birds due to exposure to Prothioconazole via bioaccumulation in fish (secondary poisoning) for the intended use in oilseed rape (covers all intended uses)

Parameter	Prothioconazole	Comments
PEC _{sw} (mg L ⁻¹)	0.0125	Tier-1 RAC-aqua is used instead of PEC at screening step
log P _{ow} / P _{ow}	3.82 / 6607	Partition coefficient octanol-water (EFSA Scientific Report (2007) 106, 1-98)
BCF _{fish}	19.7 ~~20~~	Whole body bioconcentration factor fish (EFSA Scientific Report (2007) 106, 1-98)
BMF (L kg _{wwt} ⁻¹)	1	Biomagnification factor (relevant for BCF ≥ 2000 and log P _{ow} > 3); default values according to the Aquatic Guidance Document (SANTE-2015-00080)
PEC _{fish}	0.2463	Concentration in fish; PEC _{fish} = PEC _{sw} × BCF _{fish}
DDD (mg kg bw d ⁻¹)	0.03915	Daily dietary dose; DDD = PEC _{fish} × 0.159
NOAEL (mg kg bw d ⁻¹)	78	No-observed-adverse-effect level (EFSA Scientific Report (2007) 106, 1-98)
TER _{it}	1992	Toxicity exposure ratio (TER criterion: 5)

TER values shown **in bold** fall below the relevant trigger.

Table 9.2-13: Assessment of the risk for fish-eating birds due to exposure to Prothioconazole-S-methyl (M01) via bioaccumulation in fish (secondary poisoning) for the intended use in oilseed rape and sunflower (covers all intended uses)

Parameter	Prothioconazole-S-methyl	Comments
PEC _{sw} (mg L ⁻¹)	0.00641 ~~0.00172091~~	Initial STEP 1 PEC _{sw} in OSR and Sunflower, multiple application (worst case); please refer to dRR Part B8., ~~environmental fate; multiplied with a twa of 0.53 according to the reproductive RA~~ Initial PEC _{surface water} in OSR (worst case); please refer to dRR Part B8, environmental fate; multiplied with a twa of 0.53 according to the reproductive RA
log P _{ow} / P _{ow}	4.19 / 15488	Partition coefficient octanol-water (EFSA Scientific Report (2007) 106, 1-98)
BCF _{fish}	727	Whole body bioconcentration factor fish (estimated from the equation log BCF fish = 0.85 × log Kow – 0.70, according to TGD, part II, 2003)
BMF (L kg _{wwt} ⁻¹)	1	Biomagnification factor (relevant for BCF ≥ 2000 and log P _{ow} > 3); default values according to the Aquatic Guidance Document (SANTE-2015-00080)
PEC _{fish}	4.66 ~~1.2510~~	Concentration in fish; PEC _{fish} = PEC _{sw} × BCF _{fish}
DDD (mg kg bw d ⁻¹)	0.74 ~~0.19891~~	Daily dietary dose; DDD = PEC _{fish} × 0.159
NOAEL (mg kg bw d ⁻¹)	7.8	No-observed-adverse-effect level, assuming 10-fold higher toxicity compared to the parent (EFSA Scientific Report (2007) 106, 1-98)
TER _{it}	10.54 ~~39.2~~	Toxicity exposure ratio (TER criterion: 5)

TER values shown **in bold** fall below the relevant trigger.

Table 9.2-14: Assessment of the risk for fish-eating birds due to exposure to Prothioconazole-desthio (M04) via bioaccumulation in fish (secondary poisoning) for the intended use in oilseed rape (covers all intended uses)

Parameter	Prothioconazole-desthio	Comments
PEC _{sw} (mg L ⁻¹)	0.000334	Tier-1 RAC-aqua is used instead of PEC at screening step
log P _{ow} / P _{ow}	3.04 / 1096	Partition coefficient octanol-water (EFSA Scientific Report (2007) 106, 1-98)
BCF _{fish}	65	Whole body bioconcentration factor fish (EFSA Scientific Report (2007) 106, 1-98)
BMF (L kg _{wwt} ⁻¹)	1	Biomagnification factor (relevant for BCF ≥ 2000 and log P _{ow} > 3); default values according to the Aquatic Guidance Document (SANTE-2015-00080)
PEC _{fish}	0.0217	Concentration in fish; PEC _{fish} = PEC _{sw} × BCF _{fish}
DDD (mg kg bw d ⁻¹)	0.00345	Daily dietary dose; DDD = PEC _{fish} × 0.159
NOAEL (mg kg bw d ⁻¹)	14.8	No-observed-adverse-effect level (EFSA Scientific Report (2007) 106, 1-98)
TER _{it}	4288	Toxicity exposure ratio (TER criterion: 5)

TER values shown **in bold** fall below the relevant trigger.

The risk assessment indicates no risk due to secondary poisoning for all intended uses. ~~in oilseed rape. Considering the high margin of safety of the risk assessment, uses in sunflower are deemed covered.~~

zRMS comments:

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

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

9.2.2.5 Biomagnification in terrestrial food chains

According to information from the toxicology section on the ADME studies (DAR 2005), Prothioconazole shows no potential for accumulation due to the extensive metabolism and rapid and effective excretion. Thus, further assessment is not required.

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

Not relevant.

9.2.4 Overall conclusions

Acute and long-term/reproductive TER values for the two active substances and the relevant metabolite Prothioconazole-desthio (M04) as well as combi-TER calculations exceed the relevant trigger values at the screening step and/or Tier 1 following the intended use in oilseed rape and sunflower at 2×0.7 kg product/ha covering all intended uses; thus, risk for birds can be excluded for all intended uses. Therefore, a higher-Tier risk assessment is not required.

The risk for birds due to uptake of contaminated drinking water from puddles is acceptable for all intended uses of FGE03.

The log P_{ow} of Boscalid does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required. However, the log P_{ow} of Prothioconazole and metabolites M01 and M04 exceed the trigger value of 3. The risk assessment indicates no risk due to secondary poisoning for all intended uses.

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 Boscalid, 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 FGE03 were not evaluated as part of the EU assessment of Boscalid and Prothioconazole, respectively. However, the provision of further data on the formulation FGE03 is not considered essential, because the risk can be addressed based on active substance/metabolite data considering the combi-TER approach assuming dose addition. FGE03 does not contain any toxicologically classified co-formulants (refer to Part C of the present dossier); thus, the formulation is not expected to be more toxic than the active substances/metabolites.

The selection of studies and endpoints for the risk assessment of the individual active substances and the metabolite Prothioconazole-desthio 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 - Boscalid

Species	Substance	Exposure System	Results	Reference
Rat	Boscalid	Oral 1 d Acute	LD₅₀ > 5000 mg/kg bw	Review report, SANCO/3919 /2007-rev. 5, 21 January 2008 and DAR (2002)
Rat	Boscalid	Oral Reproductive toxicity	NOAED= 67 mg/kg bw/d	Review report, SANCO/3919 /2007-rev. 5, 21 January 2008 and DAR (2002)

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

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

Species	Substance	Exposure System	Results	Reference
Rat	Prothioconazole	Oral 1 d Acute	LD₅₀ > 6200 mg/kg bw	EFSA Scientific Report (2007) 106, 1-98, and DAR (2005)
Rat	Prothioconazole	Oral Reproductive toxicity	NOEL = 9.7 mg/kg bw/d (parental) NOEL = 95.6 mg/kg bw/d (reproduction)^b	EFSA Scientific Report (2007) 106, 1-98, and DAR (2005)
Rat	Prothioconazole-desthio	Oral 1 d Acute	LD ₅₀ = 2506 mg/kg bw (female) LD ₅₀ = 2806 mg/kg bw (male) LD ₅₀ = 2652 mg/kg bw (geomean ^a)	EFSA Scientific Report (2007) 106, 1-98, and DAR (2005)
Mouse	Prothioconazole-desthio	Oral 1 d Acute	LD ₅₀ = 3459 mg/kg bw (female) LD₅₀ = 2235 mg/kg bw (male)	EFSA Scientific Report (2007) 106, 1-98, and DAR (2005)
Rat	Prothioconazole-desthio	Oral Reproductive toxicity	NOEL = 2.5 mg/kg bw/d (parental) NOEL = 10 mg/kg bw/d (reproduction)^b	EFSA Scientific Report (2007) 106, 1-98, and DAR (2005)

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

- a according to EFSA (2009)
- b reproductive endpoints were used for risk assessment according to the EFSA Conclusion (2007) and were also considered appropriate following re-assessment in the RAR (2018)

Since Prothioconazole-desthio is major plant metabolite of Prothioconazole with higher occurrence in plant material and higher toxicity compared to the parent, it is also considered in the risk assessment for both the individual active substances and the combination toxicity of the product.

zRMS comments:

Mammalian toxicity data for boscalid, prothioconazole and prothioconazole metabolite JAU 6476-desthio provided in Tables 9.3-1 and 9.3-2 have been confirmed that they are in line with EU agreed endpoints reported in Review report, SANCO/3919 /2007-rev. 5, 21 January 2008 and DAR (2002) and EFSA Scientific Report (2007) 106, respectively.

zRMS agrees that further data on the formulation FGE03 is not considered essential, because the risk can be addressed based on active substance/metabolite data considering the combi-TER approach assuming dose addition. FGE03 does not contain any toxicologically classified co-formulants (refer to Part C of the present dossier); thus, the formulation is not expected to be more toxic than the active substances/metabolites.

9.3.1.1 Justification for new endpoints

The selection of studies and endpoints for the risk assessment of the individual active substances and the metabolite desthio-prothioconazole is in line with the results of the EU review process.

9.3.2 Risk assessment for spray applications

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

Here, the assessment for the use group oilseed rape (2×0.7 kg product/ha; use 2) covers the risk for birds from all intended uses (see 9.1.2).

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

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

Table 9.3-3: Screening/First-Tier assessment of the acute and long-term/reproductive risk for mammals due to exposure to Boscalid following the use of FGE03 in oilseed rape (use 2) and sunflower

Intended use	Oilseed rape and sunflower				
Active substance	Boscalid				
Application rate (kg/ha)	2 × 0.245 (14 days interval)				
Acute toxicity (mg/kg bw)	> 5000				
TER criterion	10				
Crop scenario Growth stage	Indicator species	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a
Bulbs and onion like crops, cereals, oilseed rape, potatoes, root and stem vegetables, strawberries, sugar beet, and sunflower	Small herbivorous mammal	118.4	1.2	34.8	> 144
Reprod. toxicity (mg/kg bw/d)	67				
TER criterion	5				
Crop scenario Growth stage	Generic focal species ^a	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{it}
Oilseed rape All season	Large herbivorous mammal “lagomorph”	14.3	1.4 × 0.53	2.60	25.8
Oilseed rape BBCH ≥ 20	Small insectivorous mammal "shrew"	1.9		0.345	194
Oilseed rape BBCH ≥ 40	Small herbivorous mammal "vole"	18.1		3.29	20.4
Oilseed rape BBCH ≥ 40	Small omnivorous mammal “mouse”	1.9		0.345	194
Oilseed rape BBCH 30–39	Small omnivorous mammal “mouse”	2.3		0.418	160
Sunflower BBCH ≥ 20	Small insectivorous mammal "shrew"	1.9		0.345	194
Sunflower BBCH ≥ 40	Large herbivorous mammal “lagomorph	14.3		2.60	25.8
Sunflower BBCH ≥ 40	Small herbivorous mammal "vole"	18.1		3.29	20.4
Sunflower BBCH ≥ 40	Small omnivorous mammal “mouse”	1.9		0.345	194

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

a Risk for the individual active substance would already be acceptable at the screening step based on indicator species. Tier-1 calculations are required for combination toxicity assessment only.

Table 9.3-4: Screening/First-Tier assessment of the acute and long-term/reproductive risk for mammals due to exposure to Prothioconazole following the use of FGE03 in oilseed rape (use 2) and sunflower

Intended use	Oilseed rape and sunflower				
Active substance	Prothioconazole				
Application rate (kg/ha)	2 × 0.14 (14 days interval)				
Acute toxicity (mg/kg bw)	> 6200				
TER criterion	10				
Crop scenario Growth stage	Indicator species	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a
Bulbs and onion like crops, cereals, oilseed rape, potatoes, root and stem vegetables, strawberries, sugar beet, and sunflower	Small herbivorous mammal	118.4	1.2	19.9	> 312
Reprod. toxicity (mg/kg bw/d)	95.6				
TER criterion	5				
Crop scenario Growth stage	Generic focal species ^a	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{it}
Oilseed rape All season	Large herbivorous mammal “lagomorph”	14.3	1.4 × 0.53	1.49	64.4
Oilseed rape BBCH ≥ 20	Small insectivorous mammal "shrew"	1.9		0.197	484
Oilseed rape BBCH ≥ 40	Small herbivorous mammal "vole"	18.1		1.88	50.8
Oilseed rape BBCH ≥ 40	Small omnivorous mammal “mouse”	1.9		0.197	484
Oilseed rape BBCH 30–39	Small omnivorous mammal “mouse”	2.3		0.239	400
Sunflower BBCH ≥ 20	Small insectivorous mammal "shrew"	1.9		0.197	484
Sunflower BBCH ≥ 40	Large herbivorous mammal “lagomorph	14.3		1.49	64.4
Sunflower BBCH ≥ 40	Small herbivorous mammal "vole"	18.1		1.88	50.8
Sunflower BBCH ≥ 40	Small omnivorous mammal “mouse”	1.9		0.197	484

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

a Risk for the individual active substance would already be acceptable at the screening step based on indicator species. Tier-1 calculations are required for combination toxicity assessment only.

Table 9.3-5: Screening/First-Tier assessment of the acute and long-term/reproductive risk for mammals due to exposure to Prothioconazole-desthio following the use of FGE03 in oilseed rape (use 2) and sunflower

Intended use	Oilseed rape and sunflower				
Active substance	Prothioconazole-desthio				
Application rate (kg/ha)	2 × 0.127 (14 days interval) ^a				
Acute toxicity (mg/kg bw)	2235				
TER criterion	10				
Crop scenario Growth stage	Indicator species	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a
Bulbs and onion like crops, cereals, oilseed rape, potatoes, root and stem vegetables, strawberries, sugar beet, and sunflower	Small herbivorous mammal	118.4	1.2	18.0	124
Reprod. toxicity (mg/kg bw/d)	10				
TER criterion	5				
Crop scenario Growth stage	Generic focal species	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{it}
Oilseed rape All season	Large herbivorous mammal “lagomorph”	14.3	1.4 × 0.53	1.35	7.42
Oilseed rape BBCH ≥ 20	Small insectivorous mammal "shrew"	1.9		0.179	55.9
Oilseed rape BBCH ≥ 40	Small herbivorous mammal "vole"	18.1		1.71	5.86
Oilseed rape BBCH ≥ 40	Small omnivorous mammal “mouse”	1.9		0.179	55.9
Oilseed rape BBCH 30–39	Small omnivorous mammal “mouse”	2.3		0.217	46.2
Sunflower BBCH ≥ 20	Small insectivorous mammal "shrew"	1.9		0.179	55.9
Sunflower BBCH ≥ 40	Large herbivorous mammal “lagomorph	14.3		1.35	7.42
Sunflower BBCH ≥ 40	Small herbivorous mammal "vole"	18.1		1.71	5.86
Sunflower BBCH ≥ 40	Small omnivorous mammal “mouse”	1.9		0.179	55.9

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

a assuming full conversion of Prothioconazole to Prothioconazole-desthio as worst case, considering a factor 0.907 based on the molecular weight ratio of 312.20 (metabolite) : 344.26 (parent)

zRMS comments:

The screening step and Tier 1 risk assessment has been validated by the zRMS.

Based on performed calculations acceptable acute and chronic risk may be concluded for mammals exposed to the both active substances and the metabolite prothioconazole-desthio following the use of FGE03.

Mixture toxicity

FGE03 contains two active substances, and a toxic metabolite is formed in plant material. Therefore, mixture toxicity needs to be assessed.

In a first step, the surrogate LD₅₀ values for acute effects are calculated and potential single substance drivers of the risk are identified according to Appendix B of EFSA/2009/1438 (for equations please refer to the guidance document).

To consider two extreme scenarios, it is assumed that exposure takes place to

- A. Boscalid + Prothioconazole
- B. Boscalid + Prothioconazole-desthio (assuming full conversion of the parent to the metabolite)

Table 9.3-6: Calculation of the surrogate LD₅₀ value for acute effects to mammals and assessment of single substance driver of the risk – Boscalid and Prothioconazole

Substance	Concentration in the product (g/kg)	Fraction of a.s. in the product	LD ₅₀ (mg/kg bw)	LD ₅₀ mix (mg/kg bw) and tox per fraction mix	Tox per fraction a.s.	Deviation from tox per fraction mix (%)
Boscalid	350	0.64	> 5000	> 5379	7812.5 5934	69.0 46
Prothioconazole	200	0.36	> 6200		17222.2 10384	31.0 217

Table 9.3-7: Calculation of the surrogate LD₅₀ value for acute effects to mammals and assessment of single substance driver of the risk – Boscalid and Prothioconazole-desthio (M04)

Substance	Concentration in the product (g/kg)	Fraction of a.s./met. in the product	LD ₅₀ (mg/kg bw)	LD ₅₀ mix (mg/kg bw) and tox per fraction mix	Tox per fraction a.s.	Deviation from tox per fraction mix (%)
Boscalid	350	0.66	> 5000	3515	7575.75 7591	46.0 116
M04	181.4 ^a	0.34	2235		6573.5 6547	54.0 86

a assuming full conversion of Prothioconazole to Prothioconazole-desthio as worst case, considering a factor 0.907 based on the molecular weight ratio of 312.20 (metabolite) : 344.26 (parent)

None of the active substances/metabolites can be identified where the two quotients “tox per fraction (a.s.)” and “tox per fraction (mix)” deviate by ≤ 10 %, i.e. contributes to ≥ 90 % to mixture toxicity. Thus, the predicted LD₅₀(mix) should be used in the risk assessment together with appropriate exposure estimates.

Potential risk from combined exposure to active substances and/or relevant metabolites is assessed based on the combi-TER approach assuming dose additivity. The combined TER value is calculated according to the equation $TER_{combi} = 1 / (1/TER_{substance\ 1} + 1/TER_{substance\ 2})$. An acceptable risk is expected when $TER_{combi} > trigger$.

Please note that the combi-TER approach will obtain the same results as the risk assessment based on the calculated mixture endpoint.

For sub-lethal effects and the effects on reproduction, the EFSA Guidance recommends only performing a risk assessment for combined toxicity on a case-by case basis. However, in the central zone it has been agreed that potential combined toxicity should be taken into account for the sub-lethal effects, as well as acute/lethal effects. This is in line with the aquatic guidance document (EFSA, 2013) and the EFSA guidance on mixture toxicity (2019). Therefore, in the current risk assessment, combined toxicity is also considered for reproductive/long-term effects.

Table 9.3-8: TER values for individual active substances/metabolites and combi-TER calculations for mammals following the use of FGE03 in oilseed rape (use 2) and sunflower

Crop scenario Growth stage	Indicator species	TER _a Boscalid	TER _a Prothiocona- zole	TER _a Prothiocona- zole-desthio	TER _a , combi Boscalid + Prothiocona- zole ^a	TER _a , combi Boscalid + Prothiocona- zole-desthio ^b
Bulbs and onion like crops, cereals, oilseed rape, potatoes, root and stem vegetables, strawberries, sugar beet, and sunflower	Small herbivorous mammal	> 144	> 312	82.7	> 98.3	66.5
Crop scenario Growth stage	Generic focal species	TER _{lt} Boscalid	TER _{lt} Prothiocona- zole	TER _{lt} Prothiocona- zole-desthio	TER _{lt} , combi Boscalid + Prothiocona- zole ^a	TER _{lt} , combi Boscalid + Prothiocona- zole-desthio ^b
Oilseed rape All season	Large herbivorous mammal "lagomorph"	25.8	64.4	7.42	18.4	5.75
Oilseed rape BBCH ≥ 20	Small insectivorous mammal "shrew"	194	484	55.9	138.5	43.3
Oilseed rape BBCH ≥ 40	Small herbivorous mammal "vole"	20.4	50.8	5.86	14.5	4.55
Oilseed rape BBCH ≥ 40	Small omnivorous mammal "mouse"	194	484	55.9	138.5	43.3
Oilseed rape BBCH 30– 39	Small omnivorous mammal "mouse"	160	400	46.2	114.5	35.8
Sunflower BBCH ≥ 20	Small insectivorous mammal "shrew"	194	484	55.9	138.5	43.3
Sunflower BBCH ≥ 40	Large herbivorous mammal "lagomorph"	25.8	64.4	7.42	18.4	5.75
Sunflower BBCH ≥ 40	Small herbivorous mammal "vole"	20.4	50.8	5.86	14.5	4.55
Sunflower BBCH ≥ 40	Small omnivorous mammal "mouse"	194	484	55.9	138.5	43.3

TER: toxicity to exposure ratio. TER values shown **in bold** fall below the relevant trigger (10 for acute toxicity, 5 for longterm/reproductive toxicity).

a. scenario A: exposure to Boscalid + Prothioconazole

b. scenario B: exposure to Boscalid + Prothioconazole-desthio (assuming full conversion of the parent to the metabolite as worst case)

Acute and long-term/reproductive TER values for the two active substances and the relevant metabolite Prothioconazole-desthio as well as combi-TER calculations considering Prothioconazole TER values exceed the relevant trigger values at the screening step and/or Tier 1 following the intended use in oilseed rape and sunflower at 2×0.7 kg product/ha covering all intended uses.

However, when the long-term/reproductive TER values for the metabolite Prothioconazole-desthio are used for combi-TER calculations, combi-TER_{lt} for the generic focal species vole at BBCH ≥ 40 is slightly below the relevant trigger value. It should be noted that the assumption of full conversion of the parent to the metabolite is worst case. Nevertheless, in a conservative approach, a higher-Tier risk assessment is presented below.

For use 1 (1×0.7 kg product/ha), all TER values are 1.4 times higher since no MAF is to be considered; thus, risk is acceptable for uses 1 and 2.

zRMS comments:

Based on calculations performed in the Table 9.3-8 acute and long-term/reproductive TER values for the two active substances and the relevant metabolite Prothioconazole-desthio as well as combi-TER calculations considering Prothioconazole TER values exceed the relevant trigger values at the screening step and/or Tier 1 following the intended use in oilseed rape and sunflower at 2×0.7 kg product/ha covering all intended uses.

However, when the long-term/reproductive TER values for the metabolite Prothioconazole-desthio are used for combi-TER calculations, combi-TER_{LT} for the generic focal species vole at BBCH ≥ 40 is slightly below the relevant trigger value of 5 (being **4.55**). It should be noted that the assumption of full conversion of the parent to the metabolite is worst case. Higher Tier risk assessment is presented in Point 9.3.2.2 below.

9.3.2.2 Higher-tier risk assessment

At Tier-1, combi-TER_{lt} for the generic focal species vole at BBCH ≥ 40 is slightly below the relevant trigger value when the longterm/reproductive TER values for the metabolite Prothioconazole-desthio is used for combi-TER calculations. Therefore, in the following a higher-Tier risk assessment is presented for the concerned scenario.

Deposition factor (DF)

A deposition factor of 0.25 is used in the Tier-1 risk assessment for application in oilseed rape at BBCH ≥ 40 according to EFSA/2009/1438. However, this does not reflect the current scientific knowledge and is not consistent with other guidance documents published since then, e.g., in the Environmental Fate Section. According to EFSA (2014)¹ and FOCUS (2023)², a lower **DF of 0.2** is deemed appropriate for oilseed rape application at BBCH 20-89 (relevant here for small herbivorous “vole”, BBCH 39-69). This is also acknowledged in the new guidance document for birds and mammals³ (not yet taken note of) and in the Central Zone Evaluation Manual⁴, respectively. It is also in line with the interception of 80% used in exposure calculations in the efate section and considered for risk assessment for other organism groups. Considering a deposition factor of 0.2 instead of 0.25, TER_{lt, combi} Boscalid + Prothioconazole-desthio would be 1.25 times higher (~~5.69~~ **5.43** instead of 4.55), thus exceeding the relevant trigger value of 5. Therefore, further assessment is not required.

zRMS comments:

zRMS agrees with DF of 0.2 proposed by the Applicant to refine the risk assessment for vole at BBCH for oilseed rape application and sunflower at BBCH 20-89 (relevant here for small herbivorous “vole”, BBCH >40).

Considering a deposition factor of 0.2 instead of 0.25 for Prothioconazole - desthio TER_{LT} has been calculated and further calculations for TER_{lt, combi} Boscalid + Prothioconazole-desthio have been conducted.

Screening/First-Tier assessment of the acute and long-term/reproductive risk for mammals due to exposure to Prothioconazole-desthio following the use of FGE03 in oilseed rape (use 2) with DF=2*

Intended use	Oilseed rape and sunflower
Active substance	Prothioconazole-desthio
Application rate (kg/ha)	2×0.127 (14 days interval) ^a

¹ EFSA (European Food Safety Authority), 2014. EFSA Guidance Document for evaluating laboratory and field dissipation studies to obtain DegT50 values of active substances of plant protection products and transformation products of these active substances in soil. EFSA Journal 2014;12(5):3662, 37 pp., doi:10.2903/j.efsa.2014.3662.

² FOCUS Generic Guidance, 2023: Generic Guidance for Tier 1 FOCUS Ground Water Assessments, version 2.4, March 2023

³ EFSA (European Food Safety Authority), Aagaard A, Berny P, Chaton PF, Antia AL, McVey E, Arena M, Fait G, Ippolito A, Linguadoca A, Sharp R, Theobald A and Brock T, 2023. Guidance on the risk assessment for Birds and Mammals. EFSA Journal 2023;21(2):7790, 300 pp. <https://doi.org/10.2903/j.efsa.2023.7790>.

⁴ Working document on Risk Assessment of Plant Protection Products in the Central Zone Ecotoxicology. Version 2.0, August 2023

Reprod. toxicity (mg/kg bw/d)	10				
TER criterion	5				
Crop scenario Growth stage	Generic focal species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{lt}
Oilseed rape BBCH ≥ 40	Small herbivorous mammal "vole"	14.4	1.4 x 0.53	1.35	7.4*
Sunflower BBCH ≥ 40	Small herbivorous mammal "vole"	14.4		1.35	7.4*

TER values for individual active substances/metabolites and combi-TER calculations for mammals following the use of FGE03 in oilseed rape (use 2) and sunflower

Crop scenario Growth stage	Generic focal species	TER _{lt} Boscalid	TER _{lt} Prothioconazole	TER _{lt} Prothioconazole- desthio	TER _{lt} , combi Boscalid + Prothioconazole	TER _{lt} , combi Boscalid + Prothioconazole- desthio
Oilseed rape BBCH ≥ 40	Small herbivorous mammal "vole"	20.4	50.8	7.4*	14.5	5.43*
Sunflower BBCH ≥ 40	Small herbivorous mammal "vole"	20.4	50.8	7.4*	14.5	5.43*

*DF=0.2 for metabolite Prothioconazole-desthio

The TER_{LT} values are above trigger of 5, indicating an acceptable long-term risk for mammals.

Overall, acceptable risk to mammals may be concluded from the intended Central Zone uses of FH03.

9.3.2.3 Drinking water exposure

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

Puddle scenario

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

With a $K(f)_{oc}$ of 743 L/kg (geometric mean), Boscalid belongs to the group of more sorptive substances. With a $K(f)_{oc}$ of 1765 L/kg (based on aged soil column leaching study), Prothioconazole belongs to the group of more sorptive substances.

With a $K(f)_{oc}$ of 573.5 L/kg (geometric mean), Prothioconazole-desthio belongs to the group of more sorptive substances.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use oilseed rape at 2×0.7 kg product/ha also covers the risk for mammals from all other intended uses in terms of maximum effective application rate (see 9.1.2).

Boscalid

Effective application rate (g/ha) =	490 ^a			
Acute toxicity (mg/kg bw) >	5000	quotient	<	0.098
Reprod. toxicity (mg/kg bw/d) =	67	quotient	=	7.31

^a assuming no degradation between applications

Prothioconazole

Effective application rate (g/ha) =	280 ^a			
Acute toxicity (mg/kg bw) >	6200	quotient	<	0.045
Reprod. toxicity (mg/kg bw/d) =	95.6	quotient	=	2.93

a assuming no degradation between applications

Prothioconazole-desthio

Effective application rate (g/ha) =	254 ^a			
Acute toxicity (mg/kg bw) =	2235	quotient	=	0.114
Reprod. toxicity (mg/kg bw/d) =	10	quotient	=	25.4

a assuming no degradation between applications and full conversion of Prothioconazole to Prothioconazole-desthio as worst case, considering a factor 0.907 based on the molecular weight ratio of 312.20 (metabolite) : 344.26 (parent)

The ratios of effective application rate (in g/ha) to relevant endpoints (in mg/kg bw/d) do not exceed the relevant trigger values with high margin of safety, even if risk quotients are summed up to account for combination toxicity. Thus, risk to mammals from exposure to drinking water following the intended uses of FGE03 can be excluded.

zRMS comments:

Ratios between the application rates and toxicity endpoints are below the respective triggers for active substances and metabolite Prothioconazole-desthio (M04) demonstrating acceptable risk resulting from exposure of mammals to a.s. and this metabolite via drinking water in puddles.

9.3.2.4 Effects of secondary poisoning

The log P_{ow} of Boscalid amounts to 2.96 (Review report, SANCO/3919 /2007-rev. 5, 21 January 2008) and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

However, the log P_{ow} values of Prothioconazole, Prothioconazole-desthio and Prothioconazole-S-methyl amount to 3.82 (pH 7; EFSA Scientific Report (2007) 106, 1-98), 3.04 and 4.19 (EFSA Scientific Report (2007) 106, 1-98), respectively, and thus exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is therefore 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 predicted concentrations in soil.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the screening assessment for the use group oilseed rape covers the risk for mammals from all intended uses (see 9.1.2).

Table 9.3-9: Assessment of the risk for earthworm-eating mammals due to exposure to Prothioconazole via bioaccumulation in earthworms (secondary poisoning) for the intended use in oilseed rape (covers all intended uses)

Parameter	Prothioconazole	Comments
PEC _{soil} (mg kg ⁻¹)	0.014	Long-term PEC _{soil} (dry weight) after last application, TWA, 21 days, in OSR (worst case); please refer to dRR Part B8, environmental fate
log P _{ow} / P _{ow}	3.82 / 6607	Partition coefficient octanol-water (EFSA Scientific Report (2007) 106, 1-98)
K _{oc} (L kg ⁻¹)	1765	Partition coefficient soil organic carbon-water (EFSA Scientific Report (2007) 106, 1-98)
f _{oc}	0.02	Organic carbon content of soil; default (EFSA/2009/1438)
BCF _{earthworm}	2.27	Bioconcentration factor for earthworms related to dry soil; $BCF_{earthworm} = PEC_{earthworm\ fwt} / PEC_{soil\ dwt} = (0.84 + 0.012 \times P_{ow}) / (f_{oc} \times K_{oc})$
PEC _{earthworm}	0.0318	$PEC_{earthworm} = PEC_{soil} \times BCF_{earthworm}$
DDD (mg kg bw d ⁻¹)	0.0407	Daily dietary dose; $DDD = PEC_{worm} \times 1.28$
NOAEL (mg kg bw d ⁻¹)	95.6	No-observed-adverse-effect level (EFSA Scientific Report (2007) 106, 1-98)
TER _{it}	2350	Toxicity exposure ratio (TER criterion: 5)

TER values shown **in bold** fall below the relevant trigger.

Table 9.3-10: Assessment of the risk for earthworm-eating mammals due to exposure to Prothioconazole-S-methyl (M01) via bioaccumulation in earthworms (secondary poisoning) for the intended use in oilseed rape (covers all intended uses)

Parameter	Prothioconazole-S-methyl	Comments
PEC _{soil} (mg kg ⁻¹)	0.009	Long-term PEC _{soil} (dry weight) after last application, TWA, 21 days, in OSR (worst case); please refer to dRR Part B8, environmental fate
log P _{ow} / P _{ow}	4.19 / 15488	Partition coefficient octanol-water (EFSA Scientific Report (2007) 106, 1-98)
K _{oc} (L kg ⁻¹)	2526	Partition coefficient soil organic carbon-water (geomean, refer to environmental fate section)
f _{oc}	0.02	Organic carbon content of soil; default (EFSA/2009/1438)
BCF _{earthworm}	3.70	Bioconcentration factor for earthworms related to dry soil; $BCF_{earthworm} = PEC_{earthworm\ fwt} / PEC_{soil\ dwt} = (0.84 + 0.012 \times P_{ow}) / (f_{oc} \times K_{oc})$
PEC _{earthworm}	0.0333	$PEC_{earthworm} = PEC_{soil} \times BCF_{earthworm}$
DDD (mg kg bw d ⁻¹)	0.0426	Daily dietary dose; $DDD = PEC_{worm} \times 1.28$
NOAEL (mg kg bw d ⁻¹)	9.56	No-observed-adverse-effect level, assuming 10-fold higher toxicity compared to the parent (EFSA Scientific Report (2007) 106, 1-98)
TER _{it}	225	Toxicity exposure ratio (TER criterion: 5)

TER values shown **in bold** fall below the relevant trigger.

Table 9.3-11: Assessment of the risk for earthworm-eating mammals due to exposure to Prothioconazole-desthio (M04) via bioaccumulation in earthworms (secondary poisoning) for the intended use in oilseed rape (covers all intended uses)

Parameter	Prothioconazole-desthio	Comments
PEC _{soil} (mg kg ⁻¹)	0.0332	Long-term PEC _{soil} (dry weight) after last application, TWA, 21 days, in Sunflower OSR (worst case); please refer to dRR Part B8, environmental fate
log P _{ow} / P _{ow}	3.04 / 1096	Partition coefficient octanol-water (EFSA Scientific Report (2007) 106, 1-98)
K _{oc} (L kg ⁻¹)	574	Partition coefficient soil organic carbon-water (geomean, refer to environmental fate section)
f _{oc}	0.02	Organic carbon content of soil; default (EFSA/2009/1438)
BCF _{earthworm}	1.22	Bioconcentration factor for earthworms related to dry soil; $BCF_{earthworm} = PEC_{earthworm\ fwt} / PEC_{soil\ dw} = (0.84 + 0.012 \times P_{ow}) / (f_{oc} \times K_{oc})$
PEC _{earthworm}	0.0391	$PEC_{earthworm} = PEC_{soil} \times BCF_{earthworm}$
DDD (mg kg bw d ⁻¹)	0.0500	Daily dietary dose; $DDD = PEC_{worm} \times 1.28$
NOAEL (mg kg bw d ⁻¹)	10	No-observed-adverse-effect level (EFSA Scientific Report (2007) 106, 1-98)
TER _{it}	200	Toxicity exposure ratio (TER criterion: 5)

TER values shown **in bold** fall below the relevant trigger.

zRMS comments:

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

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

Risk assessment for fish-eating mammals via secondary poisoning

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

Table 9.3-12: Assessment of the risk for fish-eating mammals due to exposure to Prothioconazole via bioaccumulation in fish (secondary poisoning) for the intended use in oilseed rape (covers all intended uses)

Parameter	Prothioconazole	Comments
PEC _{sw} (mg L ⁻¹)	0.0125	Tier-1 RAC-aqua is used instead of PEC at screening step
log P _{ow} / P _{ow}	3.82 / 6607	Partition coefficient octanol-water (EFSA Scientific Report (2007) 106, 1-98)
BCF _{fish}	20	Whole body bioconcentration factor fish (EFSA Scientific Report (2007) 106, 1-98)
BMF (L kg _{wwt} ⁻¹)	1	Biomagnification factor (relevant for BCF ≥ 2000 and log P _{ow} > 3); default values according to the Aquatic Guidance Document (SANTE-2015-00080)
PEC _{fish}	0.2463	Concentration in fish; PEC _{fish} = PEC _{sw} × BCF _{fish}
DDD (mg kg bw d ⁻¹)	0.03497	Daily dietary dose; DDD = PEC _{fish} × 0.142
NOAEL (mg kg bw d ⁻¹)	95.6	No-observed-adverse-effect level (EFSA Scientific Report (2007) 106, 1-98)
TER _{it}	2734	Toxicity exposure ratio (TER criterion: 5)

TER values shown in **bold** fall below the relevant trigger.

Table 9.3-13: Assessment of the risk for fish-eating mammals due to exposure to Prothioconazole-S-methyl (M01) via bioaccumulation in fish (secondary poisoning) for the intended use in oilseed rape and sunflower (covers all intended uses)

Parameter	Prothioconazole-S-methyl	Comments
PEC _{sw} (mg L ⁻¹)	0.00641 0.00172091	Initial STEP 1 PEC _{sw} in OSR and Sunflower, multiple application please refer to dRR Part B8, environmental fate; multiplied with a two of 0.53 according to the reproductive RA
log P _{ow} / P _{ow}	4.19 / 15488	Partition coefficient octanol-water (EFSA Scientific Report (2007) 106, 1-98)
BCF _{fish}	727	Whole body bioconcentration factor fish (estimated from the equation log BCF fish = 0.85 × log Kow - 0.70, according to TGD, part II, 2003)
BMF (L kg _{wwt} ⁻¹)	1	Biomagnification factor (relevant for BCF ≥ 2000 and log P _{ow} > 3); default values according to the Aquatic Guidance Document (SANTE-2015-00080)
PEC _{fish}	4.66 1.2510	Concentration in fish; PEC _{fish} = PEC _{sw} × BCF _{fish}
DDD (mg kg bw d ⁻¹)	0.66 0.17764	Daily dietary dose; DDD = PEC _{fish} × 0.142
NOAEL (mg kg bw d ⁻¹)	9.56	No-observed-adverse-effect level, assuming 10-fold higher toxicity compared to the parent (EFSA Scientific Report (2007) 106, 1-98)
TER _{it}	14.5 53.8	Toxicity exposure ratio (TER criterion: 5)

TER values shown in **bold** fall below the relevant trigger.

Table 9.3-14: Assessment of the risk for fish-eating mammals due to exposure to Prothioconazole-desthio (M04) via bioaccumulation in fish (secondary poisoning) for the intended use in oilseed rape (covers all intended uses)

Parameter	Prothioconazole-desthio	Comments
PEC _{sw} (mg L ⁻¹)	0.000334	Tier-1 RAC-aqua is used instead of PEC at screening step
log P _{ow} / P _{ow}	3.04 / 1096	Partition coefficient octanol-water (EFSA Scientific Report (2007) 106, 1-98)
BCF _{fish}	65	Whole body bioconcentration factor fish (EFSA Scientific Report (2007) 106, 1-98)
BMF (L kg _{wwt} ⁻¹)	1	Biomagnification factor (relevant for BCF ≥ 2000 and log P _{ow} > 3); default values according to the Aquatic Guidance Document (SANTE-2015-00080)
PEC _{fish}	0.0217	Concentration in fish; PEC _{fish} = PEC _{sw} × BCF _{fish}
DDD (mg kg bw d ⁻¹)	0.00308	Daily dietary dose; DDD = PEC _{fish} × 0.142
NOAEL (mg kg bw d ⁻¹)	10	No-observed-adverse-effect level (EFSA Scientific Report (2007) 106, 1-98)
TER _{it}	3244	Toxicity exposure ratio (TER criterion: 5)

TER values shown **in bold** fall below the relevant trigger.

The risk assessment indicates no risk due to secondary poisoning for all intended uses. ~~in oilseed rape. Considering the high margin of safety of the risk assessment, uses in sunflower are deemed covered.~~

zRMS comments:

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

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

In conclusion, an acceptable risk of secondary exposure from all relevant compounds could be concluded for mammals.

9.3.2.5 Biomagnification in terrestrial food chains

According to information from the toxicology section on the ADME studies (DAR 2005), Prothioconazole shows no potential for accumulation due to the extensive metabolism and rapid and effective excretion. Thus, further assessment is not required.

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

Not relevant.

9.3.4 Overall conclusions

Acute and long-term/reproductive TER values for the two active substances and the relevant metabolite Prothioconazole-desthio as well as combi-TER calculations considering Prothioconazole TER values exceed the relevant trigger values at the screening step and/or Tier 1 following the intended use in oilseed rape and sunflower at 2 × 0.7 kg product/ha covering all intended uses.

However, when the long-term/reproductive TER values for the metabolite Prothioconazole-desthio are used for combi-TER calculations, combi-TER_{it} for the generic focal species vole at BBCH ≥ 40 is slightly

below the relevant trigger value. It should be noted that the assumption of full conversion of the parent to the metabolite is worst case. Nevertheless, in a conservative approach, a higher-Tier risk assessment based on a refined deposition factor is presented.

Higher Tier TER value for vole exceeds the relevant trigger value of 5. Therefore, further assessment is not required.

No risk is indicated for the intended single application already at Tier 1.

The risk for mammals due to uptake of contaminated drinking water from puddles is acceptable for all intended uses of FGE03.

The log P_{ow} of Boscalid does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required. However, the log P_{ow} of Prothioconazole and metabolites M01 and M04 exceed the trigger value of 3. The risk assessment indicates no risk due to secondary poisoning for all intended uses.

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

Appropriate test guidelines and guidance documents are currently not available to address the risk to reptiles and amphibians. However, due to the acceptable risk of Boscalid and Prothioconazole to terrestrial vertebrates and fish, adverse effects following the intended uses are considered unlikely.

zRMS comments:

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

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

Effects on aquatic organisms of FGE03 were not evaluated as part of the EU assessment of Boscalid and Prothioconazole, respectively. 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 of the active substances and metabolites is in line with the results of the EU review process.

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

Species	Substance	Exposure System	Results	Reference
Rainbow trout	Boscalid	96 h, s	LC₅₀ = 2.7 mg a.s./L_{mm}	Review report, SANCO/3919 /2007-rev. 5, 21 January 2008 and DAR (2002)
Rainbow trout	Boscalid	97 d (ELS), f	NOEC = 0.125 mg a.s./L_{nom}	Review report, SANCO/3919 /2007-rev. 5, 21 January 2008 and DAR (2002)
<i>Daphnia magna</i>	Boscalid	48 h, s	EC₅₀ = 5.33 mg a.s./L_{mm}	Review report, SANCO/3919 /2007-rev. 5, 21 January 2008 and DAR (2002)
<i>Daphnia magna</i>	Boscalid	21 d, ss	NOEC = 1.31 mg a.s./L_{mm}	Review report, SANCO/3919 /2007-rev. 5, 21 January 2008 and DAR (2002)
<i>Chironomus riparius</i>	Boscalid	28 d, spiked water, s	NOEC = 1.0 mg a.s./L_{nom}	Review report, SANCO/3919 /2007-rev. 5, 21 January 2008 and DAR (2002)
<i>Chironomus riparius</i>	Boscalid	28 d, spiked sediment, s	NOEC = 23.26 mg a.s./kg sed._{im}	Review report, SANCO/3919 /2007-rev. 5, 21 January 2008 and DAR (2002)
<i>Pseudokirchneriella subcapitata</i>	Boscalid	96 h, s	E_rC₅₀ = 3.75 mg a.s./L_{mm} E_yC₅₀ = 1.34 mg a.s./L_{mm}	Review report, SANCO/3919 /2007-rev. 5, 21 January 2008 and DAR (2002)
Higher-tier studies				
Not relevant.				

s: static; ss: semi-static; f: flow-through; nom: based on nominal concentrations; mm: based on mean measured concentrations; im: based on initial measured concentrations
Endpoints used for risk assessment are highlighted in **bold**.

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

Species	Substance	Exposure System	Results	Reference
Rainbow trout	Prothioconazole	96 h, s	LC₅₀ = 1.83 mg a.s./L_{mm}	EFSA Scientific Report (2007) 106, 1-98, and DAR (2005)
<i>Lepomis macrochirus</i>	Prothioconazole	96 h, s	LC ₅₀ = 4.59 mg a.s./L _{mm}	EFSA Scientific Report (2007) 106, 1-98, and DAR (2005)
<i>Cyprinus carpio</i>	Prothioconazole	96 h, s	LC ₅₀ = 6.91 mg a.s./L _{nom}	EFSA Scientific Report (2007) 106, 1-98, and DAR (2005)
Rainbow trout	Prothioconazole	97 d (ELS), f	NOEC = 0.308 mg a.s./L_{mm}	EFSA Scientific Report (2007) 106, 1-98, and DAR (2005)
<i>Daphnia magna</i>	Prothioconazole	48 h, s	EC₅₀ = 1.3 mg a.s./L_{nom}	EFSA Scientific Report (2007) 106, 1-98, and DAR (2005)
<i>Daphnia magna</i>	Prothioconazole	21 d, ss	NOEC = 0.56 mg a.s./L_{nom}	EFSA Scientific Report (2007) 106, 1-98, and DAR (2005)
<i>Chironomus riparius</i>	Prothioconazole	28 d, spiked water, s	NOEC = 9.14 mg a.s./L_{nom}	EFSA Scientific Report (2007) 106, 1-98, and DAR (2005)
<i>Pseudokirchneriella subcapitata</i>	Prothioconazole	96 h, s	ErC₅₀ = 2.18 mg a.s./L_{im} EbC ₅₀ = 1.10 mg a.s./L _{im}	EFSA Scientific Report (2007) 106, 1-98, and DAR (2005)
Rainbow trout	Prothioconazole-desthio (M04)	96 h, s	LC₅₀ = 6.63 mg/L_{nom}	EFSA Scientific Report (2007) 106, 1-98, and DAR (2005)
<i>Leuciscus idus melanotus</i>	Prothioconazole-desthio (M04)	96 h, s	LC ₅₀ = 13.2 mg/L _{nom}	EFSA Scientific Report (2007) 106, 1-98, and DAR (2005)
Rainbow trout	Prothioconazole-desthio (M04)	97 d (ELS), f	NOEC = 0.00334 mg/L_{nom}	EFSA Scientific Report (2007) 106, 1-98, and DAR (2005)
<i>Daphnia magna</i>	Prothioconazole-desthio (M04)	48 h, s	EC₅₀ > 10 mg/L_{nom}	EFSA Scientific Report (2007) 106, 1-98, and DAR (2005)
<i>Daphnia magna</i>	Prothioconazole-desthio (M04)	21 d, ss	NOEC = 0.1 mg/L_{nom}	EFSA Scientific Report (2007) 106, 1-98, and DAR (2005)
<i>Chironomus riparius</i>	Prothioconazole-desthio (M04)	28 d, spiked water, s	NOEC = 2 mg/L_{nom}	EFSA Scientific Report (2007) 106, 1-98, and DAR (2005)
<i>Scenedesmus subspicatus</i>	Prothioconazole-desthio (M04)	96 h, s	ErC₅₀ = 0.55 mg a.s./L_{nom} EbC ₅₀ = 0.073 mg a.s./L _{nom}	EFSA Scientific Report (2007) 106, 1-98, and DAR (2005)
Rainbow trout	Prothioconazole-S-methyl (M01)	96 h, ss	LC₅₀ = 1.8 mg/L_{mm}	EFSA Scientific Report (2007) 106, 1-98, and DAR (2005)
<i>Daphnia magna</i>	Prothioconazole-S-methyl (M01)	48 h, s	EC₅₀ = 2.8 mg/L_{nom}	EFSA Scientific Report (2007) 106, 1-98, and DAR (2005)
<i>Pseudokirchneriella</i>	Prothioconazole-S-	72 h, s	ErC₅₀ = 47.4 mg a.s./L_{im}	EFSA Scientific Report

Species	Substance	Exposure System	Results	Reference
<i>subcapitata</i>	methyl (M01)		E _b C ₅₀ = 3.77 mg a.s./L _{im}	(2007) 106, 1-98, and DAR (2005)
Rainbow trout	1,2,4-Triazole (M13)	96 h, s	LC₅₀ = 498 mg/L_{mm}	EFSA Scientific Report (2007) 106, 1-98, and DAR (2005)
Rainbow trout	1,2,4-Triazole (M13)	97 d (ELS), f	NOEC = 3.2 mg/L_{nom}	EFSA Scientific Report (2007) 106, 1-98, and DAR (2005)
<i>Daphnia magna</i>	1,2,4-Triazole (M13)	24 h, s	EC₅₀ = 900 mg/L_{nom}	EFSA Scientific Report (2007) 106, 1-98, and DAR (2005)
<i>Pseudokirchneriella subcapitata</i>	1,2,4-Triazole (M13)	96 h, s	E_rC₅₀ = 22.5 mg a.s./L_{mm} E _b C ₅₀ = 8.2 mg a.s./L _{mm}	EFSA Scientific Report (2007) 106, 1-98, and DAR (2005)
Higher-tier studies				
Not relevant.				

s: static; ss: semi-static; f: flow-through; nom: based on nominal concentrations; mm: based on mean measured concentrations; im: based on initial measured concentrations
Endpoints used for risk assessment are highlighted **in bold**.

zRMS comments:

Aquatics toxicity data for boscalid, prothioconazole and prothioconazole's metabolites provided in Tables 9.5-1 and 9.5-2 have been confirmed that they are in line with EU agreed endpoints reported in Review report, SANCO/3919 /2007-rev. 5, 21 January 2008 and DAR (2002) and EFSA Scientific Report (2007) 106, respectively.

Table 9.5-3: Endpoints and effect values relevant for the risk assessment for aquatic organisms – FGE03

Species	Substance	Exposure System	Results	Reference
<i>Oncorhynchus mykiss</i>	Boscalid 350 g/kg + Prothioconazole 200 g/kg WG (343.2 g/kg Boscalid, 344.3 g/kg Prothioconazole)	96 h, ss	LC ₅₀ = 8.329 mg/L _{nom} corresponding to 4.638 mg a.s./L _{nom} ^b	New endpoint Report No. 503-3-07-38626, UPL/2024/1451 KCP 10.2.1/01
<i>Daphnia magna</i>	Boscalid 350 g/kg + Prothioconazole 200 g/kg WG (35.88% Boscalid w/w, 19.99% Prothioconazole w/w)	48 h, s	EC ₅₀ = 5.012 mg/L _{nom} corresponding to 2.800 mg a.s./L _{nom} ^a	New endpoint Kamle (2023a) Report No. 502-3-07-33000, UPL/2022/2968 KCP 10.2.1/01
<i>Pseudokirchneriella subcapitata</i>	Boscalid 350 g/kg + Prothioconazole 200 g/kg WG (35.88% Boscalid w/w, 19.99% Prothioconazole w/w)	96 h, s	E _r C ₅₀ = 6.353 mg/L _{nom} corresponding to 3.549 mg a.s./L _{nom} ^a	New endpoint Kamle (2023b) Report No. 502-3-07-33000, UPL/2022/2968 KCP 10.2.1/02
Higher-tier studies (micro- or mesocosm studies)				
Not relevant.				

s: static, ss: semi static; nom: based on nominal concentrations; twa: time-weighted average
a sum of active substances

For fish, no product study with FGE03 was initially conducted to account for animal welfare and as it is not deemed required for the following reasons: Based on active substance data, toxicity endpoints for fish, *Daphnia* and algae are in the same range (factor 1.5 to 2). In addition, FGE03 does not contain any (eco-) toxicologically classified co-formulants (refer to Part C of the present dossier); thus, the formulation is not expected to be more toxic than the active substance. Data on invertebrates and algae indicate no synergistic effects, i.e. the product is not expected to be more toxic than the active substances. Therefore, it is reasonable and sufficiently worst case to extrapolate from a.s. data considering mixture toxicity. In addition, it is obvious that the aquatic risk assessment is driven by the fish long-term toxicity of the metabolite Prothioconazole-desthio (M04) together with Boscalid covering the assessment for the formulation. An acute fish study with the formulation would not provide additional value to the risk assessment. Nevertheless, since explicitly requested by the zRMS for the Southern zone and after their approval for running this vertebrate study, an acute fish study with the formulation will be is being has been submitted to zRMS-PL. when final report becomes available (please refer to the study plan with code UPL/2024/1451 attached to this section and in the reference list).

zRMS comments:

Studies on toxicity of FGE03 to aquatic organisms were evaluated and agreed by the zRMS.

Summary of the performed studies together with zRMS evaluation may be found in Appendix 2.

For details of the evaluation please refer to Appendix 2.

9.5.1.1 Justification for new endpoints

For algae, the lowest ErC_{50} from the product study is used for risk assessment since this is the regulatory relevant endpoint.

9.5.2 Risk assessment

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

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

- **Table 9.5-4:** one or two applications of 0.7 kg FGE03/ha in winter oilseed rape - Boscalid
- **Table 9.5-5:** one or two applications of 0.7 kg FGE03/ha in spring oilseed rape - Boscalid
- **Table 9.5-6:** one or two applications of 0.7 kg FGE03/ha in winter and spring oilseed rape - Prothioconazole
- **Table 9.5-7:** one or two applications of 0.7 kg FGE03/ha in winter and spring oilseed rape - Prothioconazole-S-methyl (M01)
- **Table 9.5-8:** two applications of 0.7 kg FGE03/ha in winter oilseed rape - Prothioconazole-desthio (M04)
- **Table 9.5-9:** one applications of 0.7 kg FGE03/ha in winter oilseed rape - Prothioconazole-desthio (M04)
- **Table 9.5-10:** two applications of 0.7 kg FGE03/ha in spring oilseed rape - Prothioconazole-desthio (M04)
- **Table 9.5-11:** one application of 0.7 kg FGE03/ha in spring oilseed rape - Prothioconazole-desthio (M04)
- **Table 9.5-12:** one or two applications of 0.7 kg FGE03/ha in winter and spring oilseed rape - 1,2,4-Triazole (M13)
- **Table 9.5-13:** one or two applications of 0.7 kg FGE03/ha in sunflower - Boscalid

- **Table 9.5-14:** one or two applications of 0.7 kg FGE03/ha in sunflower - Prothioconazole
- **Table 9.5-15:** one or two applications of 0.7 kg FGE03/ha in sunflower - Prothioconazole-S-methyl (M01)
- **Table 9.5-16:** two applications of 0.7 kg FGE03/ha in sunflower - Prothioconazole-desthio (M04)
- **Table 9.5-17:** one application of 0.7 kg FGE03/ha in sunflower - Prothioconazole-desthio (M04)
- **Table 9.5-18:** one or two applications of 0.7 kg FGE03/ha in sunflower - 1,2,4-Triazole (M13)

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

Table 9.5-4: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for each organism group based on FOCUS Steps 1, 2 and 3 calculations for one or two applications of FGE03 to winter oilseed rape at 0.7 kg/ha - Boscalid

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged		Sed. dwell. prolonged
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>P. subcapitata</i>	<i>C. riparius</i>		<i>C. riparius</i>
Endpoint (µg/L)		LC ₅₀ 2700	NOEC 125	EC ₅₀ 5330	NOEC 1310	ErC ₅₀ 3750	NOEC 1000		NOEC 23260
AF		100	10	100	10	10	10		10
RAC (µg/L)		27	12.5	53.3	131	375	100		2326
FOCUS Scenario	PEC _{gl-max} (µg/L)							PEC _{gl-max} (µg/kg)	
Step 1									
	86.556	3.21	6.92	1.62	0.661	0.231	0.866	609.628	0.262
Step 2									
Northern Europe, March-May	6.151	0.228	0.492	0.115	0.047	0.016	0.062	42.925	0.018
Northern Europe, June-September	36.151	1.34	2.89	0.678	0.276	0.096	0.362	42.925	0.018
Southern Europe, March-May	10.037	0.372	0.803	0.188	0.077	0.027	0.100	71.777	0.031
Southern Europe, June-September	8.094	0.300	0.648	0.152	0.062	0.022	0.081	57.351	0.025

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. pro- longed	Algae	Sed. dwell. pro- longed		Sed. dwell. pro- longed
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>P. subcapitata</i>	<i>C. riparius</i>		<i>C. riparius</i>
Endpoint (µg/L)		LC ₅₀ 2700	NOEC 125	EC ₅₀ 5330	NOEC 1310	ErC ₅₀ 3750	NOEC 1000		NOEC 23260
AF		100	10	100	10	10	10		10
RAC (µg/L)		27	12.5	53.3	131	375	100		2326
FOCUS Scenario	PEC _{gl-max} (µg/L)							PEC _{gl-max} (µg/kg)	
Step 3									
D3 / ditch ^a	1.555	0.058	0.124						
D4 / pond	0.533	0.020	0.043						
D4 / stream ^a	1.341	0.050	0.107						
D5 / pond	0.597	0.022	0.048						
D5 / stream ^a	1.447	0.054	0.116						
R1 / pond	0.275	0.010	0.022						
R1 / stream	3.144	0.116	0.252						
R3 / stream	2.666	0.099	0.213						

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

a PEC from single application

Table 9.5-5: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for each organism group based on FOCUS Steps 1, 2 and 3 calculations for one or two applications of FGE03 to spring oilseed rape at 0.7 kg/ha - Boscalid

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged		Sed. dwell. prolonged
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>P. subcapitata</i>	<i>C. riparius</i>		<i>C. riparius</i>
Endpoint (µg/L)		LC ₅₀ 2700	NOEC 125	EC ₅₀ 5330	NOEC 1310	ErC ₅₀ 3750	NOEC 1000		NOEC 23260
AF		100	10	100	10	10	10		10
RAC (µg/L)		27	12.5	53.3	131	375	100		2326
FOCUS Scenario	PEC _{gl-max} (µg/L)							PEC _{gl-max} (µg/kg)	
Step 1									
	86.556	3.21	6.92	1.62	0.661	0.231	0.866	610	0.262
Step 2									
Northern Europe, March-May	6.151	0.228	0.492	0.115	0.047	0.016	0.062	42.925	0.018
Northern Europe, June-September	36.151	1.34	2.89	0.678	0.276	0.096	0.362	42.925	0.018
Southern Europe, March-May	10.037	0.372	0.803	0.188	0.077	0.027	0.100	71.777	0.031
Southern Europe, June-September	8.094	0.300	0.648	0.152	0.062	0.022	0.081	57.351	0.025

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. pro- longed	Algae	Sed. dwell. pro- longed		Sed. dwell. pro- longed
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>P. subcapitata</i>	<i>C. riparius</i>		<i>C. riparius</i>
Endpoint (µg/L)		LC ₅₀ 2700	NOEC 125	EC ₅₀ 5330	NOEC 1310	ErC ₅₀ 3750	NOEC 1000		NOEC 23260
AF		100	10	100	10	10	10		10
RAC (µg/L)		27	12.5	53.3	131	375	100		2326
FOCUS Scenario	PEC _{gl-max} (µg/L)							PEC _{gl-max} (µg/kg)	
Step 3									
D1 / ditch	5.466	0.202	0.437						
D1 / stream	3.666	0.136	0.293						
D3 / ditch ^a	1.556	0.058	0.124						
D4 / pond	0.730	0.027	0.058						
D4 / stream	1.433	0.053	0.115						
D5 / pond	0.515	0.019	0.041						
D5 / stream ^a	1.446	0.054	0.116						
R1 / pond	0.675	0.025	0.054						
R1 / stream	2.483	0.092	0.199						

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

^a PEC from single application

Table 9.5-6: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for each organism group based on FOCUS Step 1 and 2 calculations for one or two applications of FGE03 to winter and spring oilseed rape at 0.7 kg/ha - Prothioconazole

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>P. subcapitata</i>	<i>C. riparius</i>
Endpoint (µg/L)		LC ₅₀ 1830	NOEC 308	EC ₅₀ 1300	NOEC 560	ErC ₅₀ 2180	NOEC 9140
AF		100	10	100	10	10	10
RAC (µg/L)		18.3	30.8	13	56	218	914
FOCUS Scenario	PEC _{gl-max} (µg/L)						
Step 1							
	30.408	1.662	0.987	2.34	0.543	0.139	0.033
Step 2							
Northern Europe, March-May	1.505	0.082	0.049	0.116	0.027	0.007	0.002
Northern Europe, June-September	1.505	0.082	0.049	0.116	0.027	0.007	0.002
Southern Europe, March-May	1.505	0.082	0.049	0.116	0.027	0.007	0.002
Southern Europe, June-September	1.505	0.082	0.049	0.116	0.027	0.007	0.002

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 9.5-7: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for each organism group based on FOCUS Step 1 and 2 calculations for one or two applications of FGE03 to winter and spring oilseed rape at 0.7 kg/ha - Prothioconazole-S-methyl (M01)

Group		Fish acute	Inverteb. acute	Algae
Test species		<i>O. mykiss</i>	<i>D. magna</i>	<i>P. subcapitata</i>
Endpoint (µg/L)		LC ₅₀ 1800	EC ₅₀ 2800	E _r C ₅₀ 47400
AF		100	100	10
RAC (µg/L)		18	28	4740
FOCUS Scenario	PEC _{gl-max} (µg/L)			
Step 1				
	6.41**/3.21* 3.247	0.36**/0.18* 0.180	0.22/0.015 0.116	0.0013/0.00067 6.9E-04
Step 2				
	-	-	-	-
Northern Europe, March-May	0.20**/0.17* 0.105	0.01**/0.0094* 5.8E-03	0.007/0.006 3.8E-03	0.0000042/0.000035 2.2E-05
Northern Europe, June-September	0.20**/0.17* 0.105	0.01**/0.0094* 5.8E-03	0.007/0.006 3.8E-03	0.0000042/0.000035 2.2E-05
Southern Europe, March-May	0.32**/0.20* 0.209	0.0172**/0.01* 1.2E-02	0.011/0.007 7.5E-03	0.000067/0.0000042 4.4E-05
Southern Europe, June-September	0.26**/0.17* 0.157	0.014**/0.0094* 8.7E-03	0.0092/0.006 5.6E-03	0.000055/0.000035 3.3E-05

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration, ** multiple application, *single application

Table 9.5-8: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for each organism group based on FOCUS Steps 1, 2 and 3 calculations for two applications of FGE03 to winter oilseed rape at 0.7 kg/ha - Prothioconazole-dethio (M04)

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>S. subspicatus</i>	<i>C. riparius</i>
Endpoint		LC ₅₀	NOEC	EC ₅₀	NOEC	ErC ₅₀	NOEC
(µg/L)		6630	3.34	> 10000	100	550	2000
AF		100	10	100	10	10	10
RAC (µg/L)		66.3	0.334	> 100	10	55	200
FOCUS Scenario	PEC _{gl-max} (µg/L)						
Step 1							
	54.845	0.827	164	0.548	5.48	1.00	0.274
Step 2							
Northern Europe, March-May	1.736	0.026	5.20	0.017	0.174	0.032	0.009
Northern Europe, June-September	1.736	0.026	5.20	0.017	0.174	0.032	0.009
Southern Europe, March-May	2.802	0.042	8.39	0.028	0.280	0.051	0.014
Southern Europe, June-September	2.269	0.034	6.79	0.023	0.227	0.041	0.011
Step 3							
D3 / ditch	0.044		0.132				
D4 / pond	0.010		0.030				
D4 / stream	0.022		0.066				
D5 / pond	0.010		0.030				
D5 / stream	0.032		0.096				
R1 / pond	0.040		0.120				
R1 / stream	0.614		1.838				
R3 / stream	0.408		1.222				

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 9.5-9: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for each organism group based on FOCUS Steps 1, 2 and 3 calculations for one application of FGE03 to winter oilseed rape at 0.7 kg/ha - Prothioconazole-desthio (M04)

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>S. subspicatus</i>	<i>C. riparius</i>
Endpoint (µg/L)		LC ₅₀ 6630	NOEC 3.34	EC ₅₀ > 10000	NOEC 100	ErC ₅₀ 550	NOEC 2000
AF		100	10	100	10	10	10
RAC (µg/L)		66.3	0.334	> 100	10	55	200
FOCUS Scenario	PEC _{gl-max} (µg/L)						
Step 1							
	27.423	0.414	82.1	0.274	2.74	0.50	0.137
Step 2							
Northern Europe, March-May	1.075	0.016	3.22	0.011	0.108	0.020	0.005
Northern Europe, June-September	1.075	0.016	3.22	0.011	0.108	0.020	0.005
Southern Europe, March-May	1.746	0.026	5.23	0.017	0.175	0.032	0.009
Southern Europe, June-September	1.411	0.021	4.22	0.014	0.141	0.026	0.007
Step 3							
D3 / ditch	0.036		0.108				
D4 / pond	0.007		0.021				
D4 / stream	0.026		0.078				
D5 / pond	0.006		0.018				
D5 / stream	0.036		0.108				
R1 / pond	0.056		0.168				
R1 / stream	0.173		0.518				
R3 / stream	0.216		0.647				

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 9.5-10: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for each organism group based on FOCUS Steps 1, 2 and 3 calculations for two applications of FGE03 to spring oilseed rape at 0.7 kg/ha - Prothioconazole-desthio (M04)

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>S. subspicatus</i>	<i>C. riparius</i>
Endpoint (µg/L)		LC ₅₀ 6630	NOEC 3.34	EC ₅₀ > 10000	NOEC 100	ErC ₅₀ 550	NOEC 2000
AF		100	10	100	10	10	10
RAC (µg/L)		66.3	0.334	> 100	10	55	200
FOCUS Scenario	PEC _{gl-max} (µg/L)						
Step 1							
	54.845	0.827	164	0.548	5.48	1.00	0.274
Step 2							
Northern Europe, March-May	1.736	0.026	5.20	0.017	0.174	0.032	0.009
Northern Europe, June-September	1.736	0.026	5.20	0.017	0.174	0.032	0.009
Southern Europe, March-May	2.802	0.042	8.39	0.028	0.280	0.051	0.014
Southern Europe, June-September	2.269	0.034	6.79	0.023	0.227	0.041	0.011
Step 3							
D3 / ditch	0.047		0.141				
D4 / pond	0.010		0.030				
D4 / stream	0.022		0.066				
D5 / pond	0.010		0.030				
D5 / stream	0.032		0.096				
R1 / pond	0.126		0.377				
R1 / stream	0.519		1.554				

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 9.5-10: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for each organism group based on FOCUS Steps 1, 2 and 3 calculations for two applications of FGE03 to spring oilseed rape at 0.7 kg/ha - Prothioconazole-dethio (M04)

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>S. subspicatus</i>	<i>C. riparius</i>
Endpoint (µg/L)		LC ₅₀ 6630	NOEC 3.34	EC ₅₀ > 10000	NOEC 100	ErC ₅₀ 550	NOEC 2000
AF		100	10	100	10	10	10
RAC (µg/L)		66.3	0.334	> 100	10	55	200
FOCUS Scenario	PEC _{gl-max} (µg/L)						
Step 1							
	27.423	0.414	82.1	0.274	2.74	0.50	0.137
Step 2							
Northern Europe, March-May	1.075	0.016	3.22	0.011	0.108	0.020	0.005
Northern Europe, June-September	1.075	0.016	3.22	0.011	0.108	0.020	0.005
Southern Europe, March-May	1.746	0.026	5.23	0.017	0.175	0.032	0.009
Southern Europe, June-September	1.411	0.021	4.22	0.014	0.141	0.026	0.007
Step 3							
D3 / ditch	0.043		0.129				
D4 / pond	0.009		0.027				
D4 / stream	0.026		0.078				
D5 / pond	0.006		0.018				
D5 / stream	0.036		0.108				
R1 / pond	0.052		0.156				
R1 / stream	0.207		0.620				

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 9.5-12: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for each organism group based on FOCUS Step 1 and 2 calculations for one or two applications of FGE03 to winter and spring oilseed rape at 0.7 kg/ha - 1,2,4-triazole (M13)

Group		Fish acute	Fish prolonged	Inverteb. acute	Algae
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>S. subspicatus</i>
Endpoint (µg/L)		LC ₅₀ 498000	NOEC 3200	EC ₅₀ 900000	ErC ₅₀ 22500
AF		100	10	100	10
RAC (µg/L)		4980	320	9000	2250
FOCUS Scenario	PEC _{gl-max} (µg/L)				
Step 1					
	7.27 6.804	0.0014 1.4E-03	0.0227 0.021	0.00080 7.6E-04	0.0032 3.0E-03
Step 2					
Northern Europe, March-May	0.189	3.8E-05	5.9E-04	2.1E-05	8.4E-05
Northern Europe, June-September	0.189	3.8E-05	5.9E-04	2.1E-05	8.4E-05
Southern Europe, March-May	0.222	4.5E-05	6.9E-04	2.5E-05	9.9E-05
Southern Europe, June-September	0.206	4.1E-05	6.4E-04	2.3E-05	9.2E-05

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

Table 9.5-13: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for each organism group based on FOCUS Steps 1, 2 and 3 calculations for one or two applications of FGE03 to sunflower at 0.7 kg/ha - Boscalid

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged		Sed. dwell. prolonged
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>P. subcapitata</i>	<i>C. riparius</i>		<i>C. riparius</i>
Endpoint (µg/L)		LC ₅₀ 2700	NOEC 125	EC ₅₀ 5330	NOEC 1310	ErC ₅₀ 3750	NOEC 1000		NOEC 23260
AF		100	10	100	10	10	10		10
RAC (µg/L)		27	12.5	53.3	131	375	100		2326
FOCUS Scenario	PEC _{gl-max} (µg/L)							PEC _{gl-max} (µg/kg)	
Step 1									
	86.556	3.21	6.92	1.62	0.661	0.231	0.866	609.628	0.262
Step 2									
Northern Europe, March-May	6.151	0.228	0.492	0.115	0.047	0.016	0.062	42.925	0.018
Northern Europe, June-September	6.151	1.34	2.89	0.678	0.276	0.096	0.362	42.925	0.018
Southern Europe, March-May	10.037	0.372	0.803	0.188	0.077	0.027	0.100	71.777	0.031
Southern Europe, June-September	8.094	0.300	0.648	0.152	0.062	0.022	0.081	57.351	0.025

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. pro- longed	Algae	Sed. dwell. pro- longed		Sed. dwell. pro- longed
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>P. subcapitata</i>	<i>C. riparius</i>		<i>C. riparius</i>
Endpoint (µg/L)		LC ₅₀ 2700	NOEC 125	EC ₅₀ 5330	NOEC 1310	ErC ₅₀ 3750	NOEC 1000		NOEC 23260
AF		100	10	100	10	10	10		10
RAC (µg/L)		27	12.5	53.3	131	375	100		2326
FOCUS Scenario	PEC ^{gl-max} (µg/L)							PEC ^{gl-max} (µg/kg)	
Step 3									
D3 / ditch ^a	1.285	0.05	0.10						
D4 / pond	0.925	0.03	0.07						
D4 / stream ^a	1.607	0.06	0.13						
D5 / pond	0.278	0.01	0.02						
D5 / stream ^a	1.256	0.05	0.10						
R1 / pond	0.535	0.02	0.04						
R1 / stream	3.090	0.11	0.25						
R3 / stream	2.599	0.10	0.21						
R4 / stream	3.917	0.15	0.31						

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

Table 9.5-14: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for each organism group based on FOCUS Step 1 and 2 calculations for one or two applications of FGE03 to sunflower at 0.7 kg/ha - Prothioconazole

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>P. subcapitata</i>	<i>C. riparius</i>
Endpoint (µg/L)		LC ₅₀ 1830	NOEC 308	EC ₅₀ 1300	NOEC 560	ErC ₅₀ 2180	NOEC 9140
AF		100	10	100	10	10	10
RAC (µg/L)		18.3	30.8	13	56	218	914
FOCUS Scenario	PEC _{gl-max} (µg/L)						
Step 1							
	30.408	1.662	0.987	2.34	0.543	0.139	0.033
Step 2							
Northern Europe, March-May	1.505	0.082	0.049	0.116	0.027	0.007	0.002
Northern Europe, June-September	1.505	0.082	0.049	0.116	0.027	0.007	0.002
Southern Europe, March-May	1.505	0.082	0.049	0.116	0.027	0.007	0.002
Southern Europe, June-September	1.505	0.082	0.049	0.116	0.027	0.007	0.002

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 9.5-15: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for each organism group based on FOCUS Step 1 and 2 calculations for one or two applications of FGE03 to sunflower rape at 0.7 kg/ha - Prothioconazole-S-methyl (M01)

Group		Fish acute	Inverteb. acute	Algae
Test species		<i>O. mykiss</i>	<i>D. magna</i>	<i>P. subcapitata</i>
Endpoint (µg/L)		LC ₅₀ 1800	EC ₅₀ 2800	ErC ₅₀ 47400
AF		100	100	10
RAC (µg/L)		18	28	4740
FOCUS Scenario	PEC _{gl-max} (µg/L)			
Step 1				
	6.41**/3.247*	0.356/0.180	0.22/0.116	0.0013/6.9E-04
Step 2				
Northern Europe, March-May	0.20** / 0.17* 0.105 / 0.068	0.01/0.0094 5.8E-03	0.007/0.006 3.8E-03	0.0000042/0.000035 2.2E-05
Northern Europe, June-September	0.20** / 0.17* 0.105 / 0.068	0.01/0.0094 5.8E-03	0.007/0.006 3.8E-03	0.0000042/0.000035 2.2E-05
Southern Europe, March-May	0.32** / 0.20* 0.209 / 0.136	0.0172/0.01 1.2E-02	0.011/0.007 7.5E-03	0.000067/0.000042 4.4E-05
Southern Europe, June-September	0.26 / 0.17* 0.157 / 0.102	0.014/0.0094 8.7E-03	0.0092/0.006 5.6E-03	0.000055/0.000035 3.3E-05

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration, * single, ** multiple application

Table 9.5-16: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for each organism group based on FOCUS Steps 1, 2 and 3 calculations for two applications of FGE03 to sunflower at 0.7 kg/ha - Prothioconazole-desthio (M04)

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>S. subspicatus</i>	<i>C. riparius</i>
Endpoint (µg/L)		LC ₅₀ 6630	NOEC 3.34	EC ₅₀ > 10000	NOEC 100	ErC ₅₀ 550	NOEC 2000
AF		100	10	100	10	10	10
RAC (µg/L)		66.3	0.334	> 100	10	55	200
FOCUS Scenario	PEC _{gl-max} (µg/L)						
Step 1							
	54.845	0.827	164	0.548	5.48	1.00	0.274
Step 2							
Northern Europe, March-May	1.736	0.026	5.20	0.017	0.174	0.032	0.009
Northern Europe, June-September	1.736	0.026	5.20	0.017	0.174	0.032	0.009
Southern Europe, March-May	2.802	0.042	8.39	0.028	0.280	0.051	0.014
Southern Europe, June-September	2.269	0.034	6.79	0.023	0.227	0.041	0.011

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>S. subspicatus</i>	<i>C. riparius</i>
Endpoint (µg/L)		LC ₅₀ 6630	NOEC 3.34	EC ₅₀ > 10000	NOEC 100	ErC ₅₀ 550	NOEC 2000
AF		100	10	100	10	10	10
RAC (µg/L)		66.3	0.334	> 100	10	55	200
FOCUS Scenario	PEC _{gl-max} (µg/L)						
Step 3							
D3 / ditch	0.029		0.087				
D4 / pond	0.009		0.027				
D4 / stream	0.027		0.081				
D5 / pond	0.015		0.045				
D5 / stream	0.029		0.087				
R1 / pond	0.095		0.284				
R1 / stream	0.544		1.629				
R3 / stream	0.421		1.260				
R4 / stream	0.677		2.027				

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 9.5-11: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for each organism group based on FOCUS Steps 1, 2 and 3 calculations for one application of FGE03 to sunflower at 0.7 kg/ha - Prothioconazole-desthio (M04)

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>S. subspicatus</i>	<i>C. riparius</i>
Endpoint (µg/L)		LC ₅₀ 6630	NOEC 3.34	EC ₅₀ > 10000	NOEC 100	ErC ₅₀ 550	NOEC 2000
AF		100	10	100	10	10	10
RAC (µg/L)		66.3	0.334	> 100	10	55	200
FOCUS Scenario	PEC _{gl-max} (µg/L)						
Step 1							
	27.423	0.414	82.1	0.274	2.74	0.50	0.137
Step 2							
Northern Europe, March-May	1.075	0.016	3.22	0.011	0.108	0.020	0.005
Northern Europe, June-September	1.075	0.016	3.22	0.011	0.108	0.020	0.005
Southern Europe, March-May	1.746	0.026	5.23	0.017	0.175	0.032	0.009
Southern Europe, June-September	1.411	0.021	4.22	0.014	0.141	0.026	0.007

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>S. subspicatus</i>	<i>C. riparius</i>
Endpoint (µg/L)		LC ₅₀ 6630	NOEC 3.34	EC ₅₀ > 10000	NOEC 100	ErC ₅₀ 550	NOEC 2000
AF		100	10	100	10	10	10
RAC (µg/L)		66.3	0.334	> 100	10	55	200
FOCUS Scenario	PEC _{gl-max} (µg/L)						
Step 3							
D3 / ditch	0.033		0.099				
D4 / pond	0.030		0.090				
D4 / stream	0.015		0.045				
D5 / pond	0.009		0.027				
D5 / stream	0.034		0.102				
R1 / pond	0.014		0.042				
R1 / stream	0.164		0.491				
R3 / stream	0.222		0.665				
R4 / stream	0.462		1.383				

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 9.5-18: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for each organism group based on FOCUS Step 1 and 2 calculations for one or two applications of FGE03 to sunflower at 0.7 kg/ha - 1,2,4-triazole (M13)

Group		Fish acute	Fish prolonged	Inverteb. acute	Algae
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>S. subspicatus</i>
Endpoint (µg/L)		LC ₅₀ 498000	NOEC 3200	EC ₅₀ 900000	ErC ₅₀ 22500
AF		100	10	100	10
RAC (µg/L)		4980	320	9000	2250
FOCUS Scenario	PEC _{gl-max} (µg/L)				
Step 1					
	7.27 6.804	0.0014 1.4E-03	0.023 0.021	0.00080 7.6E-04	0.0032 3.0E-03
Step 2					
Northern Europe, March-May	0.189	3.8E-05	5.9E-04	2.1E-05	8.4E-05
Northern Europe, June-September	0.189	3.8E-05	5.9E-04	2.1E-05	8.4E-05
Southern Europe, March-May	0.222	4.5E-05	6.9E-04	2.5E-05	9.9E-05
Southern Europe, June-September	0.206	4.1E-05	6.4E-04	2.3E-05	9.2E-05

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

For the intended uses in oilseed rape and sunflower, calculated PEC/RAC ratios at Tier 1 for the individual active substances Boscalid and Prothioconazole as well as the relevant metabolites Prothioconazole-S-methyl (M01) and 1,2,4-Triazole (M13) indicate acceptable risk for all groups of aquatic organisms up to FOCUS Step 3.

However, for the relevant metabolite Prothioconazole-desthio (M04), calculated PEC/RAC ratios at Tier 1 based on the RAC of 0.334 µg/L for the most sensitive organism group fish prolonged exceed the trigger of 1 for some scenarios indicating that risk cannot be excluded. Therefore, further PEC/RAC ratios were calculated based on FOCUS Step 4 PEC_{SW} considering reduced exposure of surface water bodies:

- **Table 9.5-19:** two applications of 0.7 kg FGE03/ha in winter oilseed rape - Prothioconazole-desthio (M04)
- **Table 9.5-20:** two applications of 0.7 kg FGE03/ha in spring oilseed rape - Prothioconazole-desthio (M04)
- **Table 9.5-21:** two applications of 0.7 kg FGE03/ha in sunflower - Prothioconazole-desthio (M04)

Table 9.5-22: one application of 0.7 kg

Table 9.5-12: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for Prothioconazole-desthio (M04) based on FOCUS Step 4 calculations and toxicity data for fish prolonged (based on RAC = 0.334 µg/L) with mitigation of run-off for two applications of 0.7 kg FGE03/ha in winter oilseed rape

Intended use	Winter oilseed rape, two applications	
Metabolite	Prothioconazole-desthio (M04)	
Application rate (g/ha)	2 × 245	
Vegetated filter strip (m)	0	10
R1 / stream	0.614	0.278
R3 / stream	0.408	0.182
RAC (µg/L)	PEC/RAC ratio	
0.334		
R1 / stream	1.838	0.832
R3 / stream	1.222	0.545

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

Table 9.5-13: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for Prothioconazole-desthio (M04) based on FOCUS Step 4 calculations and toxicity data for fish prolonged (based on RAC = 0.334 µg/L) with mitigation of run-off for two applications of 0.7 kg FGE03/ha in spring oilseed rape

Intended use	Spring oilseed rape, two applications	
Metabolite	Prothioconazole-desthio (M04)	
Application rate (g/ha)	2 × 245	
Vegetated filter strip (m)	0	10
R1 / stream	0.519	0.237
RAC (µg/L)	PEC/RAC ratio	
0.334		
R1 / stream	1.554	0.710

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

Table 9.5-14: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for Prothioconazole-desthio (M04) based on FOCUS Step 4 calculations and toxicity data for fish prolonged (based on RAC = 0.334 µg/L) with mitigation of run-off for two applications of 0.7 kg FGE03/ha in sunflower

Intended use	Sunflower, two applications	
Metabolite	Prothioconazole-desthio (M04)	
Application rate (g/ha)	2 × 245	
Vegetated filter strip (m)	0	10
R1 / stream	0.544	0.247
R3 / stream	0.421	0.192
R4 / stream	0.677	0.307
RAC (µg/L) 0.334	PEC/RAC ratio	
R1 / stream	1.629	0.740
R3 / stream	1.260	0.575
R4 / stream	2.027	0.919

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

Table 9.5-15: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for Prothioconazole-desthio (M04) based on FOCUS Step 4 calculations and toxicity data for fish prolonged (based on RAC = 0.334 µg/L) with mitigation of run-off for one application of 0.7 kg FGE03/ha in sunflower

Intended use	Sunflower, one application	
Metabolite	Prothioconazole-desthio (M04)	
Application rate (g/ha)	1 × 245	
Vegetated filter strip (m)	0	10
R4 / stream	0.462	0.210
RAC (µg/L) 0.334	PEC/RAC ratio	
R4 / stream	1.383	0.629

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

For the intended uses in winter/spring oilseed rape (two applications) and sunflowers (one or two applications), calculated PEC/RAC ratios for metabolite Prothioconazole-desthio (M04) indicate an acceptable risk for the most sensitive aquatic organism group fish prolonged (based on a RAC of 0.334 µg/L) in all relevant FOCUS Step 4 scenarios. Therefore, no further assessment for the individual active substances/metabolites is necessary.

zRMS comments:

For the intended uses in oilseed rape and sunflower, calculated PEC/RAC ratios at Tier 1 for the individual active substances Boscalid and Prothioconazole as well as the relevant metabolites Prothioconazole-S-methyl (M01) and 1,2,4-Triazole (M13) indicate acceptable risk for all groups of aquatic organisms up to FOCUS Step 3.

However, for the relevant metabolite **Prothioconazole-desthio (M04)**, calculated PEC/RAC ratios at Tier 1 based on the **RAC of 0.334 µg/L** for the most sensitive organism group fish prolonged exceed the trigger of 1 for the following scenarios indicating that risk cannot be excluded.

- R1 (stream) and R3 (stream) scenarios – two applications in winter oilseed rape
- R1 (stream) scenario – two applications in spring oilseed rape
- R1-, R3 - and R4 – (stream) scenarios - two applications in sunflower
- R4 (stream) scenario - one application in sunflower

Therefore, STEP 4 calculations have been provided and acceptable risk for these scenarios can be concluded when **10-meter vegetated buffer zone to surface water bodies is applied.**

Mixture toxicity

For the assessment of the combined toxicity of Boscalid, Prothioconazole and metabolites, the step-wise approach according to the aquatic guidance document as well as the document FAQ Aquatic MixTox Tool_v2⁵ is considered (decision tree for metabolites; see below). The decision tree also follows the aquatic guidance document and provides a basis for the decision on the compounds to be considered in the mixture toxicity assessment for each organism group. The assessment is performed separately for each organism group.

5 Frequently Asked Questions in the context of the aquatic Mixture Toxicity and in relation with the use of the MixTox Tool. Version 2: December 2022.

- 1- Is the metabolite toxic of equal toxicity (endpoint value within a factor 3 of parent endpoint) or of higher toxicity compared to parent A? Please note that the comparison should be done for the same species.

Yes: Go to 2

No: No further consideration of metabolite(s) needed in the mixture toxicity risk assessment. Conduct a mixture toxicity risk assessment based simply on *A* and *B* using the MixTox Tool.

- 2- Is the metabolite contributing to the risk with more than 90% compared to the a.s. (i.e. > 90% of risk due to $PEC_{metab}/RAC_{metab} + PEC_A/RAC_A$ attributed to PEC_{metab}/RAC_{metab} at the most critical/ worst-case FOCUS Step and scenario(s))?

Yes: Conduct a mixture toxicity risk assessment according to step 8 based on the *metab.* and *B* using the MixTox Tool.

No: Go to 3

- 3- Is the metabolite of parent A only formed in water¹ (e.g. based on fate studies such as hydrolysis and/or phytolysis degradation studies)?

Yes: Go to 4

**No or
unknown:** Go to 6

- 4- Is the maximum formation rate of the metabolite occurring within the duration² of the test performed with the parent (e.g. based on information available from water-sediment degradation tests)?

Yes: Go to 5

No: Go to 6

- 5- Conduct a mixture toxicity risk assessment based on *A*, *B* and *metab.* according to step 8 in the AGD. If relevant, FOCUS Step 4 PEC_{sw}-values considering risk mitigation measures should be used. Please use information provided below under section 1.2, for the expression of the test endpoints. Does the assessment lead to an unacceptable risk?

Yes: Consider if mixture toxicity can be refined using additional data e.g. geomean or SSD.

No: No unacceptable risk identified, no further steps are needed.

- 6- Conduct a mixture toxicity risk assessment solely based on *A* and *B* according to step 8 in the AGD (i.e. no consideration of *metab.*). If relevant, FOCUS Step 4 PEC_{sw}-values considering risk mitigation measures should be used. Please use information provided below under section 1.2, for the expression of the test endpoints. Does the assessment lead to an unacceptable risk?

Yes: Consider if mixture toxicity can be refined using additional data e.g. geomean or SSD.

No: No unacceptable risk identified, no further steps are needed.

An overview on the parent and metabolite endpoints and assessment is presented in **Table 9.5-23**.

Table 9.5-23: Endpoints and assessment for Boscalid, Prothioconazole and their relevant metabolites in surface water

Organism group	Species	End-point	Endpoint in mg/L ^a					Decision tree for metabolites ^c				
			Boscalid	Prothioconazole	M04	M01	M13	Metabolite	Step 1	Step 2	Step 3	Conclusion
Fish acute	<i>O. mykiss</i>	LC ₅₀	2.7	1.83	6.63	1.8	498	M04	No	-	-	Step 8 AGD MixTox parents
								M01	Yes → step 2	No → step 3	No → step 6	
								M13	No	-	-	
Fish long-term	<i>O. mykiss</i>	NOEC	0.125	0.308	0.00334		3.2	M04	Yes → step 2	Yes/no → step 3 (some scenarios)	No → step 6	Step 8 AGD MixTox parents / Boscalid + M04
								M01	-	-	-	
								M13	No	-	-	
Invert. acute	<i>D. magna</i>	EC ₅₀	5.33	1.3	> 10	2.8	900	M04	No	-	-	Step 8 AGD MixTox parents
								M01	Yes → step 2	No → step 3	No → step 6	
								M13	No	-	-	
Invert long-term	<i>D. magna</i>	NOEC	1.3	0.56	0.1			M04	Yes → step 2	Yes/no → step 3 (some scenarios)	No → step 6	Step 8 AGD MixTox parents / Boscalid + M04
								M01	-	-	-	
								M13	-	-	-	
Algae	<i>P. subcapitata</i>	E _r C ₅₀	3.75	2.18	0.55 ^b	47.4	22.5	M04	Yes → step 2	No → step 3	No → step 6	Step 8 AGD MixTox parents
								M01	No	-	-	
								M13	No	-	-	
Sediment-dwellers	<i>C. riparius</i>	NOEC	1.0	9.14	2			M04	Yes → step 2	No → step 3	No → step 6	Step 8 AGD MixTox parents
								M01	-	-	-	
								M13	-	-	-	

^a Metabolite endpoints in the same range (factor 3) or of higher toxicity compared to the parent are highlighted **in bold** (step 1 of decision tree).

^b *Scenedesmus subspicatus* (green algae of the same order as *Pseudokirchneriella subcapitata*)

^c according to FAQ Aquatic MixTox Tool_v2 (see above)

Fish acute: Endpoints are available for the two actives and the three Prothioconazole metabolites. Only metabolite **M01** is of higher or comparable toxicity as the parent (step 1); thus, the remaining metabolites do not need to be considered further in the mixture toxicity assessment. Based on FOCUS Step 1/2 PEC_{sw} values, M01 is contributing to the risk with less than 90% compared to the parent (step 2, **Tables 9.5-23, 25**). Since it is not formed only in water (step 3), mixture toxicity risk assessment should be based only on the two actives according to step 8 of the AGD.

Table 9.5-24: Contribution of metabolite M01 to risk for fish acute compared to the parent (winter and spring OSR and sunflower, multiple application)

FOCUS Scenario	PEC/RAC Prothioconazole	PEC/RAC M01	Contribution metabolite (%)
Step 1	1.662	0.36 0.180	17 9.8
Step 2			
Northern Europe, March-May	0.082	0.01 0.006	10.87 6.6
Northern Europe, June-September	0.082	0.01 0.006	10.87 6.6
Southern Europe, March-May	0.082	0.0172 0.012	17.33 12.4
Southern Europe, June-September	0.082	0.014 0.009	14.6 9.6

Table 9.5-25: Contribution of metabolite M01 to risk for fish acute compared to the parent (winter and spring OSR and sunflower, single application)

FOCUS Scenario	PEC/RAC Prothioconazole	PEC/RAC M01	Contribution metabolite (%)
Step 1	0.831	0.18 0.090	17.80 9.8
Step 2			
Northern Europe, March-May	0.070	0.0094 0.004	11.83 5.1
Northern Europe, June-September	0.070	0.0094 0.004	11.83 5.1
Southern Europe, March-May	0.070	0.01 0.008	12.5 9.7
Southern Europe, June-September	0.070	0.094 0.006	11.83 7.5

Since for fish acute there is also a product endpoint available, MDR calculation according to step 2 of the AGD is done to check if CA (concentration addition) holds or if antagonism or synergism is indicated (Table 9.5-26).

Table 9.5-16: MDR calculation for fish acute

Species	Substance	Concentration (C _i) in formulation (g a.s./L)	Fraction in product (P _i)	EC _{xi} (mg a.s./L)	EC _{xmix-CA} (mg sum a.s./L)	EC _{xPPP} (mg sum a.s./L)	MDR
<i>O. mykiss</i>	Boscalid	350	0.64	2.7	2.3	4.64 ^a	0.49
	Prothioconazole	200	0.36	1.83			

a based on nominal contents of active substances

The MDR is between 0.2 and 5. No synergism or antagonism is indicated. Thus, the “concentration addition” concept holds and step 8 of the AGD is deemed appropriate. A risk assessment based on the measured formulation endpoint is not required.

Fish long-term: Endpoints are available for the two actives and Prothioconazole metabolites M04 and M13. Only metabolite **M04** is of higher or comparable toxicity as the parent (step 1); thus, the remaining metabolites do not need to be considered further in the mixture toxicity assessment. Based on FOCUS Step 1 to 3 PEC_{sw} values, M04 is contributing to the risk with more than 90% compared to the parent for several scenarios (step 2, Tables 9.5-14 - 17). Thus, for these scenarios, mixture toxicity risk assessment would be based only on Boscalid and metabolite M04 according to step 8 of the AGD. For the remaining scenarios, the decision tree is continued. Since the metabolite is not formed only in water (step 3), mixture toxicity risk assessment would be based only on the two actives according to step 8 of the AGD for the corresponding scenarios.

Table 9.5-27: Contribution of metabolite M04 to risk for fish long-term compared to the parent (winter OSR, multiple application)

FOCUS Scenario	PEC/RAC Prothioconazole	PEC/RAC M04	Contribution metabolite (%) ^a
Step 1	0.987	164	99.4
Step 2			
Northern Europe, March-May	0.049	5.20	99.1
Northern Europe, June-September	0.049	5.20	99.1
Southern Europe, March-May	0.049	8.39	99.4
Southern Europe, June-September	0.049	6.79	99.3
Step 3			
D3 / ditch	0.025	0.132	83.9
D4 / pond	0.001	0.030	95.4
D4 / stream	0.021	0.066	75.4
D5 / pond	0.001	0.030	95.4
D5 / stream	0.023	0.096	80.5
R1 / pond	0.002	0.120	98.7
R1 / stream	0.016	1.838	99.1
R3 / stream	0.023	1.222	98.1

^a Values > 90% are highlighted in **bold**

Table 9.5-28: Contribution of metabolite M04 to risk for fish long-term compared to the parent (winter OSR, single application)

FOCUS Scenario	PEC/RAC Prothioconazole	PEC/RAC M04	Contribution metabolite (%) ^a
Step 1	0.494	82.1	99.4
Step 2			
Northern Europe, March-May	0.042	3.22	98.7
Northern Europe, June-September	0.042	3.22	98.7
Southern Europe, March-May	0.042	5.23	99.2
Southern Europe, June-September	0.042	4.22	99.0
Step 3			
D3 / ditch	0.029	0.108	78.9
D4 / pond	0.001	0.021	95.4
D4 / stream	0.025	0.078	75.8
D5 / pond	0.001	0.018	94.7
D5 / stream	0.027	0.108	80.1
R1 / pond	0.001	0.168	99.3
R1 / stream	0.019	0.518	96.5
R3 / stream	0.027	0.647	96.0

^a Values > 90% are highlighted in **bold**

Table 9.5-29: Contribution of metabolite M04 to risk for fish long-term compared to the parent (spring OSR, multiple application)

FOCUS Scenario	PEC/RAC Prothioconazole	PEC/RAC M04	Contribution metabolite (%) ^a
Step 1	0.987	164	99.4
Step 2			
Northern Europe, March-May	0.049	5.20	99.1
Northern Europe, June-September	0.049	5.20	99.1
Southern Europe, March-May	0.049	8.39	99.4
Southern Europe, June-September	0.049	6.79	99.3
Step 3			
D3 / ditch	0.025	0.141	84.8
D4 / pond	0.001	0.030	95.4
D4 / stream	0.021	0.066	75.4
D5 / pond	0.001	0.030	95.4
D5 / stream	0.023	0.096	80.5
R1 / pond	0.002	0.377	99.5
R1 / stream	0.016	1.554	99.0

^a Values > 90% are highlighted in **bold**

Table 9.5-30: Contribution of metabolite M04 to risk for fish long-term compared to the parent (spring OSR, single application)

FOCUS Scenario	PEC/RAC Prothioconazole	PEC/RAC M04	Contribution metabolite (%) ^a
Step 1	0.494	82.1	99.4
Step 2			
Northern Europe, March-May	0.042	3.22	98.7
Northern Europe, June-September	0.042	3.22	98.7
Southern Europe, March-May	0.042	5.23	99.2
Southern Europe, June-September	0.042	4.22	99.0
Step 3			
D3 / ditch	0.029	0.129	81.7
D4 / pond	0.001	0.027	96.4
D4 / stream	0.025	0.078	75.8
D5 / pond	0.001	0.018	94.7
D5 / stream	0.027	0.108	80.1
R1 / pond	0.001	0.156	99.1
R1 / stream	0.019	0.620	97.0

^a Values > 90% are highlighted in **bold**

Table 9.5-17: Contribution of metabolite M04 to risk for fish long-term compared to the parent (sunflower, multiple application)

FOCUS Scenario	PEC/RAC Prothioconazole	PEC/RAC M04	Contribution metabolite (%) ^a
Step 1	0.987	164	99.4
Step 2			
Northern Europe, March-May	0.049	5.20	99.1
Northern Europe, June-September	0.049	5.20	99.1
Southern Europe, March-May	0.049	8.39	99.4
Southern Europe, June-September	0.049	6.79	99.3
Step 3			
D3 / ditch	0.021	0.087	80.8
D4 / pond	0.001	0.027	95.7
D4 / stream	0.018	0.081	81.5
D5 / pond	0.001	0.045	97.1
D5 / stream	0.020	0.087	81.3
R1 / pond	0.002	0.284	99.3
R1 / stream	0.014	1.629	99.1
R3 / stream	0.020	1.260	98.4
R4 / stream	0.014	2.027	99.3

^a Values > 90% are highlighted in **bold**

Table 9.5-18: Contribution of metabolite M04 to risk for fish long-term compared to the parent (sunflower, single application)

FOCUS Scenario	PEC/RAC Prothioconazole	PEC/RAC M04	Contribution metabolite (%) ^a
Step 1	0.494	82.1	99.4
Step 2			
Northern Europe, March-May	0.042	3.22	98.7
Northern Europe, June-September	0.042	3.22	98.7
Southern Europe, March-May	0.042	5.23	99.2
Southern Europe, June-September	0.042	4.22	99.0
Step 3			
D3 / ditch	0.024	0.099	80.6
D4 / pond	0.001	0.090	98.9
D4 / stream	0.019	0.045	70.2
D5 / pond	0.001	0.027	96.5
D5 / stream	0.023	0.102	81.4
R1 / pond	0.001	0.042	97.7
R1 / stream	0.016	0.491	96.8
R3 / stream	0.023	0.665	96.6
R4 / stream	0.016	1.383	98.8

^a Values > 90% are highlighted in **bold**

Invertebrates acute: Endpoints are available for the two actives and the three Prothioconazole metabolites. Only metabolite **M01** is of higher or comparable toxicity as the parent (step 1); thus, the remaining metabolites do not need to be considered further in the mixture toxicity assessment. Based on FOCUS Step 1/2 PEC_{sw} values, M01 is contributing to the risk with less than 90% compared to the parent (step 2, **Table 9.5-33, 34**). Since it is not formed only in water (step 3), mixture toxicity risk assessment would be based only on the two actives according to step 8 of the AGD.

Table 9.5-33: Contribution of metabolite M01 to risk for invertebrates acute compared to the parent (winter and spring OSR and sunflower, multiple application)

FOCUS Scenario	PEC/RAC Prothioconazole	PEC/RAC M01	Contribution metabolite (%)
Step 1	2.339	0.116	4.72
Step 2			
Northern Europe, March-May	0.116	0.004	3.14
Northern Europe, June-September	0.116	0.004	3.14
Southern Europe, March-May	0.116	0.007	6.06
Southern Europe, June-September	0.116	0.006	4.62

Table 9.5-34: Contribution of metabolite M01 to risk for invertebrates acute compared to the parent (winter and spring OSR and sunflower, single application)

FOCUS Scenario	PEC/RAC Prothioconazole	PEC/RAC M01	Contribution metabolite (%)
Step 1	1.170	0.058	4.72
Step 2			
Northern Europe, March-May	0.099	0.002	2.39
Northern Europe, June-September	0.099	0.002	2.39
Southern Europe, March-May	0.099	0.005	4.67
Southern Europe, June-September	0.099	0.004	3.55

Since for invertebrates acute there is also a product endpoint available, MDR calculation according to step 2 of the AGD is done to check if CA (concentration addition) holds or if antagonism or synergism is indicated (**Table 9.5-35**).

Table 9.5-35: MDR calculation for invertebrates acute

Species	Substance	Concentration (C _i) in formulation (g a.s./L)	Fraction in product (P _i)	EC _{xi} (mg a.s./L)	EC _{xmix-CA} (mg sum a.s. /L)	EC _{xPPP} (mg sum a.s. /L)	MDR
<i>D. magna</i>	Boscalid	350	0.64	5.33	2.51	2.76 ^a	0.91
	Prothioconazole	200	0.36	1.3			

^a based on nominal contents of active substances

The MDR is between 0.2 and 5. No synergism or antagonism is indicated. Thus, the “concentration addition” concept holds and step 8 of the AGD is deemed appropriate.

Invertebrates long-term: Endpoints are available for the two actives and Prothioconazole metabolite M04. Metabolite **M04** is of higher toxicity compared to the parent (step 1). Based on FOCUS Step 1 and 2 PEC_{sw} values, it is contributing to the risk with more than 90% compared to the parent for some scenarios (step 2, **Table 9.5-36, 37**). Thus, mixture toxicity risk assessment for these scenarios would be based on Boscalid and metabolite M04 according to step 8 of the AGD for the relevant scenarios. For the other scenarios and since it is not formed only in water (step 3), mixture toxicity risk assessment would be based on the two actives according to step 8 of the AGD.

Table 9.5-36: Contribution of metabolite M04 to risk for invertebrates long-term compared to the parent (winter and spring OSR and sunflower, multiple application)

FOCUS Scenario	PEC/RAC Prothioconzaole	PEC/RAC M04	Contribution metabolite (%) ^a
Step 1	0.543	5.485	91.0
Step 2			
Northern Europe, March-May	0.027	0.174	86.6
Northern Europe, June-September	0.027	0.174	86.6
Southern Europe, March-May	0.027	0.280	91.2
Southern Europe, June-September	0.027	0.227	89.4

^a Values > 90% are highlighted in **bold**

Table 9.5-37: Contribution of metabolite M04 to risk for invertebrates long-term compared to the parent (winter and spring OSR and sunflower, single application)

FOCUS Scenario	PEC/RAC Prothioconzaole	PEC/RAC M04	Contribution metabolite (%) ^a
Step 1	0.272	2.742	91.0
Step 2			
Northern Europe, March-May	0.023	0.108	82.4
Northern Europe, June-September	0.023	0.108	82.4
Southern Europe, March-May	0.023	0.175	88.4
Southern Europe, June-September	0.023	0.141	86.0

^a Values > 90% are highlighted in **bold**

Algae: Endpoints are available for the two actives and the three Prothioconazole metabolites. Only metabolite **M04** is of higher or comparable toxicity as the parent (step 1); thus, the remaining metabolites do not need to be considered further in the mixture toxicity assessment. Based on FOCUS Step 1 and 2 PEC_{sw} values, M04 is contributing to the risk with less than 90% compared to the parent (step 2, **Tables 9.5-38, 39**). Since it is not formed only in water (step 3), mixture toxicity risk assessment should be based only on the two actives according to step 8 of the AGD.

Table 9.5-38: Contribution of metabolite M04 to risk for algae compared to the parent (winter and spring OSR and sunflower, multiple application)

FOCUS Scenario	PEC/RAC Prothioconzaole	PEC/RAC M04	Contribution metabolite (%)
Step 1	0.139	0.997	87.7
Step 2			
Northern Europe, March-May	0.007	0.032	82.1
Northern Europe, June-September	0.007	0.032	82.1
Southern Europe, March-May	0.007	0.051	88.1
Southern Europe, June-September	0.007	0.041	85.7

Table 9.5-39: Contribution of metabolite M04 to risk for algae compared to the parent (winter and spring OSR and sunflower, single application)

FOCUS Scenario	PEC/RAC Prothioconazole	PEC/RAC M04	Contribution metabolite (%)
Step 1	0.070	0.499	87.7
Step 2			
Northern Europe, March-May	0.006	0.020	76.8
Northern Europe, June-September	0.006	0.020	76.8
Southern Europe, March-May	0.006	0.032	84.3
Southern Europe, June-September	0.006	0.026	81.3

Since for algae there is also a product endpoint available, MDR calculation according to step 2 of the AGD is done to check if CA (concentration addition) holds or if antagonism or synergism is indicated (Table 9.5-40).

Table 9.5-40: MDR calculation for algae

Species	Substance	Concentration (C _i) in formulation (g a.s./L)	P _i	EC _{xi} (mg a.s./L)	EC _{xmix-CA} (mg sum a.s./L)	EC _{xPPP} (mg sum a.s./L)	MDR
<i>P. subcapitata</i>	Boscalid	350	0.64	3.75	2.97	3.49	0.85
	Prothioconazole	200	0.36	2.18			

The MDR is between 0.2 and 5. No synergism or antagonism is indicated. Thus, the “concentration addition” concept holds and step 8 of the AGD is deemed appropriate.

Sediment-dwellers long-term: Endpoints are available for the two actives and Prothioconazole metabolite M04. **M04** is of higher toxicity compared to the parent (step 1). Based on FOCUS Step 1 and 2 PEC_{sw} values, M04 is contributing to the risk with less than 90% compared to the parent (step 2, **Tables 9.5-41, 42**). Since it is not formed only in water (step 3), mixture toxicity risk assessment should be based only on the two actives according to step 8 of the AGD.

Table 9.5-41: Contribution of metabolite M04 to risk for sediment-dwellers long-term compared to the parent (winter and spring OSR and sunflower, multiple application)

FOCUS Scenario	PEC/RAC Prothioconazole	PEC/RAC M04	Contribution metabolite (%)
Step 1	0.033	0.274	89.2
Step 2			
Northern Europe, March-May	0.002	0.009	84.1
Northern Europe, June-September	0.002	0.009	84.1
Southern Europe, March-May	0.002	0.014	89.5
Southern Europe, June-September	0.002	0.011	87.3

Table 9.5-42: Contribution of metabolite M04 to risk for sediment-dwellers long-term compared to the parent (winter and spring OSR and sunflower, multiple application)

FOCUS Scenario	PEC/RAC Prothioconazole	PEC/RAC M04	Contribution metabolite (%)
Step 1	0.017	0.137	89.2
Step 2			
Northern Europe, March-May	0.001	0.005	79.2
Northern Europe, June-September	0.001	0.005	79.2
Southern Europe, March-May	0.001	0.009	86.1
Southern Europe, June-September	0.001	0.007	83.4

In summary, mixture toxicity assessment should be based on the combined toxicity of the two parents as well as on the combined toxicity of Boscalid and metabolite Prothioconazole-desthio (M04). Combined toxicity from Boscalid and Prothioconazole is relevant for all organism groups (for fish long-term and invertebrates long-term only for certain scenarios). Combined toxicity from Boscalid and metabolite M04 is relevant fish long-term and invertebrates long-term (remaining scenarios).

Combined toxicity of Boscalid and Prothioconazole

Since no synergistic effects are indicated and all ETR values of the individual active substances contained in the formulation are below the relevant trigger value (corresponding to a PEC/RAC ratio < 1) up to FOCUS Step 3 calculations, a simplified approach according to the aquatic guidance document can be chosen for all organism groups and scenarios where the decision tree indicates that the mixture tox assessment should be based on the two actives: *“If all $ETR_i \leq ETR_{trigger}/n$ ($n = \text{number of a.s.}$) the mixture also fulfils the authorisation criteria and the procedure can be stopped.”*

Expressed in PEC/RAC ratios, this means that none of the individual PEC/RAC ratios for the active substances should be above 0.5. In fact, considering FOCUS Step 3 calculations for Boscalid, PEC/RAC for the individual a.s. does not exceed 0.5 for any relevant scenario. For Prothioconazole, all PEC/RAC ratios are considerably below 0.5 already at FOCUS Step 2.

Thus, the simplified approach indicates no risk from the combined toxicity of Boscalid and Prothioconazole for all organism groups and scenarios where relevant.

zRMS comments:

The combined toxicity of the two parents, a simplified approach according to the aquatic guidance document was chosen for all organism groups and scenarios where the decision tree indicates that the mixture tox assessment should be based on the two actives, since no synergistic effects are indicated and all ETR values of the individual active substances contained in the formulation are below the relevant trigger value (corresponding to a PEC/RAC ratio < 1) up to FOCUS Step 3 calculations.

The simplified approach indicates no risk from the combined toxicity of Boscalid and Prothioconazole.

Combined toxicity of Boscalid and metabolite M04

Regarding the combined toxicity of Boscalid and metabolite Prothioconazole-desthio (M04), a mixture toxicity risk assessment for the intended uses of FGE03 in oilseed rape according to step 8 of the AGD based on ETR_{mix-CA} calculations for Boscalid and metabolite M04 is presented below. The assessment is performed for fish chronic only. This is deemed appropriate since PEC/RAC ratios for both Boscalid and metabolite M04 are clearly worst case for this organism group covering all other organism groups. FOCUS Step 1 and 2 calculations are skipped.

ETR_{mix-CA} is calculated according to equation 13 of the AGD:

$$ETR_{mix-CA} = PEC_{mix} / NOEC_{mix-CA}$$

ETR_{mix-CA} trigger for chronic endpoints is ≤ 0.1 .

PEC_{mix} is the sum of the individual PEC values of the mixture components to be considered for each scenario (equation 16 of the AGD).

$NOEC_{mix-CA}$ for fish long-term is calculated according to Equation 13 of the AGD:

$$NOEC_{mix-CA} = 1 / \sum (p_i / NOEC_i)$$

with $p_i = PEC_i / PEC_{mix}$ (equation 19)

$NOEC_{mix-CA}$ and ETR_{mix-CA} calculations at FOCUS step 3 for fish long-term based on Boscalid and Prothioconazole-desthio (M04) according to step 8 of the AGD are presented in **Tables 9.5-43 to 48**.

Table 9.5-43: $NOEC_{mix-CA}$ and ETR_{mix-CA} calculations for fish long-term based on Boscalid ($NOEC = 125 \mu\text{g/L}$) and Prothioconazole-desthio (M04; $NOEC = 3.34 \mu\text{g/L}$) – winter oilseed rape, multiple application

FOCUS Scenario ^a	PEC gl-max ($\mu\text{g/L}$) Boscalid	PEC gl-max ($\mu\text{g/L}$) M04	PEC mix gl-max ($\mu\text{g/L}$)	$NOEC_{mix}$ ($\mu\text{g/L}$)	ETR_{mix-CA} Trigger 0.1
Step 3					
D3 / ditch	1.362	0.044	1.406	58.4	0.024
D4 / pond	0.533	0.010	0.543	74.8	0.007
D4 / stream	1.202	0.022	1.224	75.5	0.016
D5 / pond	0.597	0.010	0.607	78.1	0.008
D5 / stream	1.252	0.032	1.284	65.5	0.020
R1 / pond	0.275	0.040	0.315	22.2	0.014
R1 / stream	3.144	0.614	3.758	18.0	0.209
R3 / stream	2.666	0.408	3.074	21.4	0.143

ETR values above the relevant trigger of 0.1 are shown in **bold**

^a Scenarios covered by the mixture toxicity assessment for Boscalid and Prothioconazole (simplified approach according to the aquatic guidance document) are shaded in grey.

Table 9.5-44: NOEC_{mix-CA} and ETR_{mix-CA} calculations for fish long-term based on Boscalid (NOEC = 125 µg/L) and Prothioconazole-desthio (M04; NOEC = 3.34 µg/L) – winter oilseed rape, single application

FOCUS Scenario ^a	PEC _{gl-max} (µg/L) Boscalid	PEC _{gl-max} (µg/L) M04	PEC _{mix gl-max} (µg/L)	NOEC _{mix} (µg/L)	ETR _{mix-CA} Trigger 0.1
Step 3					
D3 / ditch	1.555	0.036	1.591	68.5	0.023
D4 / pond	0.152	0.007	0.159	48.0	0.003
D4 / stream	1.341	0.026	1.367	73.8	0.019
D5 / pond	0.206	0.006	0.212	61.5	0.003
D5 / stream	1.447	0.036	1.483	66.3	0.022
R1 / pond	0.161	0.056	0.217	12.0	0.018
R1 / stream	1.128	0.173	1.301	21.4	0.061
R3 / stream	1.442	0.216	1.658	21.8	0.076

^a Scenarios covered by the mixture toxicity assessment for Boscalid and Prothioconazole (simplified approach according to the aquatic guidance document) are shaded in grey.

Table 9.5-45: NOEC_{mix-CA} and ETR_{mix-CA} calculations for fish long-term based on Boscalid (NOEC = 125 µg/L) and Prothioconazole-desthio (M04; NOEC = 3.34 µg/L) – spring oilseed rape, multiple application

FOCUS Scenario ^a	PEC _{gl-max} (µg/L) Boscalid	PEC _{gl-max} (µg/L) M04	PEC _{mix gl-max} (µg/L)	NOEC _{mix} (µg/L)	ETR _{mix-CA} Trigger 0.1
Step 3					
D3 / ditch	1.363	0.047	1.410	56.5	0.025
D4 / pond	0.730	0.010	0.740	83.8	0.009
D4 / stream	1.433	0.022	1.455	80.6	0.018
D5 / pond	0.515	0.010	0.525	73.8	0.007
D5 / stream	1.251	0.032	1.283	65.5	0.020
R1 / pond	0.675	0.126	0.801	18.6	0.043
R1 / stream	2.483	0.519	3.002	17.1	0.175

ETR values above the relevant trigger of 0.1 are shown in bold

^a Scenarios covered by the mixture toxicity assessment for Boscalid and Prothioconazole (simplified approach according to the aquatic guidance document) are shaded in grey.

Table 9.5-46: NOEC_{mix-CA} and ETR_{mix-CA} calculations for fish long-term based on Boscalid (NOEC = 125 µg/L) and Prothioconazole-desthio (M04; NOEC = 3.34 µg/L) at FOCUS Step 3 – spring oilseed rape, single application

FOCUS Scenario ^a	PEC _{gl-max} (µg/L) Boscalid	PEC _{gl-max} (µg/L) M04	PEC _{mix gl-max} (µg/L)	NOEC _{mix} (µg/L)	ETR _{mix-CA} Trigger 0.1
Step 3					
D3 / ditch	1.556	0.043	1.599	63.1	0.025
D4 / pond	0.205	0.009	0.214	49.4	0.004
D4 / stream	1.341	0.026	1.367	73.8	0.019
D5 / pond	0.386	0.006	0.392	80.3	0.005
D5 / stream	1.446	0.036	1.482	66.3	0.022
R1 / pond	0.304	0.052	0.356	19.8	0.018
R1 / stream	1.287	0.207	1.494	20.7	0.072

^a Scenarios covered by the mixture toxicity assessment for Boscalid and Prothioconazole (simplified approach according to the aquatic guidance document) are shaded in grey.

For all FOCUS Step 3 scenarios, the mixture toxicity assessment for Boscalid and Prothioconazole-desthio (M04) indicates no risk. Therefore, Step 4 calculations considering risk mitigation measures are not required.

Table 9.5-19: NOEC_{mix-CA} and ETR_{mix-CA} calculations for fish long-term based on Boscalid (NOEC = 125 µg/L) and Prothioconazole-desthio (M04; NOEC = 3.34 µg/L) – sunflower, multiple application

FOCUS Scenario ^a	PEC _{gl-max} (µg/L) Boscalid	PEC _{gl-max} (µg/L) M04	PEC _{mix gl-max} (µg/L)	NOEC _{mix} (µg/L)	ETR _{mix-CA} Trigger 0.1
Step 3					
D3 / ditch	1.115	0.029	1.144	65.0	0.018
D4 / pond	0.925	0.009	0.934	92.5	0.010
D4 / stream	1.607	0.027	1.634	78.0	0.021
D5 / pond	0.278	0.015	0.293	43.6	0.007
D5 / stream	1.081	0.029	1.110	64.0	0.017
R1 / pond	0.535	0.095	0.630	19.3	0.033
R1 / stream	3.090	0.544	3.634	19.4	0.188
R3 / stream	2.599	0.421	3.020	20.6	0.147
R4 / stream	3.917	0.677	4.594	19.6	0.234

ETR values above the relevant trigger of 0.1 are shown in **bold**

^a Scenarios covered by the mixture toxicity assessment for Boscalid and Prothioconazole (simplified approach according to the aquatic guidance document) are shaded in grey.

Table 9.5-20: NOEC_{mix-CA} and ETR_{mix-CA} calculations for fish long-term based on Boscalid (NOEC = 125 µg/L) and Prothioconazole-desthio (M04; NOEC = 3.34 µg/L) at FOCUS Step 3 – sunflower, single application

FOCUS Scenario ^a	PEC _{gl-max} (µg/L) Boscalid	PEC _{gl-max} (µg/L) M04	PEC _{mix gl-max} (µg/L)	NOEC _{mix} (µg/L)	ETR _{mix-CA} Trigger 0.1
Step 3					
D3 / ditch	1.285	0.033	1.318	65.4	0.020
D4 / pond	0.225	0.030	0.255	23.7	0.011
D4 / stream	1.031	0.015	1.046	82.1	0.013
D5 / pond	0.082	0.009	0.091	27.2	0.003
D5 / stream	1.256	0.034	1.290	63.8	0.020
R1 / pond	0.205	0.014	0.219	37.6	0.006
R1 / stream	1.271	0.164	1.435	24.2	0.059
R3 / stream	1.544	0.222	1.766	22.4	0.079
R4 / stream	2.294	0.462	2.756	17.6	0.157

ETR values above the relevant trigger of 0.1 are shown in **bold**

a Scenarios covered by the mixture toxicity assessment for Boscalid and Prothioconazole (simplified approach according to the aquatic guidance document) are shaded in grey.

For most FOCUS Step 3 scenarios, the mixture toxicity assessment for Boscalid and Prothioconazole-desthio (M04) indicates no risk.

However, calculated ETR_{mix-CA} for the most sensitive organism group fish prolonged exceeds the trigger of 0.1 for some scenarios indicating that risk cannot be excluded. Therefore, further ETR_{mix-CA} were calculated based on FOCUS Step 4 PEC_{SW} considering reduced exposure of surface water bodies:

- **Table 9.5-49:** two applications of 0.7 kg FGE03/ha in winter oilseed rape - Prothioconazole-desthio (M04)
- **Table 9.5-50:** two applications of 0.7 kg FGE03/ha in spring oilseed rape - Prothioconazole-desthio (M04)
- **Table 9.5-51:** two applications of 0.7 kg FGE03/ha in sunflower - Prothioconazole-desthio (M04)
- **Table 9.5-52:** one application of 0.7 kg FGE03/ha in sunflower - Prothioconazole-desthio (M04)

Table 9.5-21: NOEC_{mix-CA} and ETR_{mix-CA} calculations for fish long-term based on Boscalid (NOEC = 125 µg/L) and Prothioconazole-desthio (M04; NOEC = 3.34 µg/L) – winter oilseed rape, multiple application – FOCUS Step 4, mitigation of run-off

FOCUS Scenario	PEC _{gl-max} (µg/L) Boscalid	PEC _{gl-max} (µg/L) M04	PEC _{mix gl-max} (µg/L)	NOEC _{mix} (µg/L)	ETR _{mix-CA} Trigger 0.1
Step 4					
Vegetated filter strip (m)	10				
R1 / stream	1.421	0.278	1.699	18.0	0.095
R3 / stream	1.209	0.182	1.391	21.7	0.064

ETR values above the relevant trigger of 0.1 are shown in **bold**

Table 9.5-22: NOEC_{mix-CA} and ETR_{mix-CA} calculations for fish long-term based on Boscalid (NOEC = 125 µg/L) and Prothioconazole-desthio (M04; NOEC = 3.34 µg/L) – spring oilseed rape, multiple application – FOCUS Step 4, mitigation of run-off

FOCUS Scenario	PEC _{gl-max} (µg/L) Boscalid	PEC _{gl-max} (µg/L) M04	PEC _{mix gl-max} (µg/L)	NOEC _{mix} (µg/L)	ETR _{mix-CA} Trigger 0.1
Step 4					
Vegetated filter strip (m)	10				
R1 / stream	1.131	0.237	1.368	17.1	0.080

ETR values above the relevant trigger of 0.1 are shown in **bold**

Table 9.5-23: NOEC_{mix-CA} and ETR_{mix-CA} calculations for fish long-term based on Boscalid (NOEC = 125 µg/L) and Prothioconazole-desthio (M04; NOEC = 3.34 µg/L) – sunflower, multiple application – FOCUS Step 4, mitigation of run-off

FOCUS Scenario	PEC _{gl-max} (µg/L) Boscalid	PEC _{gl-max} (µg/L) M04	PEC _{mix gl-max} (µg/L)	NOEC _{mix} (µg/L)	ETR _{mix-CA} Trigger 0.1
Step 4					
Vegetated filter strip (m)	10				
R1 / stream	1.402	0.247	1.649	19.4	0.085
R3 / stream	1.186	0.192	1.378	20.6	0.067
R4 / stream	1.781	0.307	2.088	19.7	0.106
Vegetated filter strip (m)	15				
R4 / stream	1.367	0.242	1.609	19.3	0.083

ETR values above the relevant trigger of 0.1 are shown in **bold**

Table 9.5-24: NOEC_{mix-CA} and ETR_{mix-CA} calculations for fish long-term based on Boscalid (NOEC = 125 µg/L) and Prothioconazole-desthio (M04; NOEC = 3.34 µg/L) – sunflower, single application – FOCUS Step 4, mitigation of run-off

FOCUS Scenario	PEC _{gl-max} (µg/L) Boscalid	PEC _{gl-max} (µg/L) M04	PEC _{mix gl-max} (µg/L)	NOEC _{mix} (µg/L)	ETR _{mix-CA} Trigger 0.1
Step 4					
Vegetated filter strip (m)	10				
R4 / stream	2.294	0.184	0.462	1.383	88.3

ETR values above the relevant trigger of 0.1 are shown in **bold**

Risk from combined exposure to Boscalid and metabolite Prothioconazole-desthio (M04) for the most sensitive organism group fish prolonged is acceptable when the following risk mitigation measures for the relevant scenarios are considered:

- two applications of 0.7 kg FGE03/ha in winter oilseed rape – 10 m vegetated buffer (scenarios R1 stream, R3 stream)
- two applications of 0.7 kg FGE03/ha in spring oilseed rape - 10 m vegetated buffer (scenario R1)
- two applications of 0.7 kg FGE03/ha in sunflower - 10 m vegetated buffer (scenarios R1 stream, R3 stream), 15 m vegetated buffer (scenario R4 stream)
- one application of 0.7 kg FGE03/ha in sunflower - 10 m vegetated buffer (scenario R4)

No risk mitigation measures are required for single application in winter/spring oilseed rape.

zRMS comments:

zRMS agrees with mixture toxicity assessment for **Boscalid and metabolite Prothioconazole-desthio (M04)** for the most sensitive organism group fish prolonged is acceptable when the following risk mitigation measures for the relevant scenarios are considered:

- two applications of 0.7 kg FGE03/ha in winter oilseed rape – 10 m vegetated buffer (scenarios **R1** stream, **R3** stream)
- two applications of 0.7 kg FGE03/ha in spring oilseed rape - 10 m vegetated buffer (scenario **R1**)
- two applications of 0.7 kg FGE03/ha in sunflower - 10 m vegetated buffer (scenarios **R1** stream, **R3** stream). It should be noted that according to the comment given by zRMS in e-Fate Section **for R4 scenario - 15-meter vegetated buffer is not accepted. For this reason, further calculation of PEC_{sw} considering 20 -meter vegetated buffer zone should be calculated by the Applicant for MSs that needs this scenario for their national level.**
- one application of 0.7 kg FGE03/ha in sunflower - 10 m vegetated buffer (scenario **R4**)

No risk mitigation measures are required for single application in winter/spring oilseed rape.

9.5.3 Overall conclusions

For the intended uses in oilseed rape and sunflower, calculated PEC/RAC ratios at Tier 1 for the individual active substances Boscalid and Prothioconazole as well as the relevant metabolites Prothioconazole-S-methyl (M01) and 1,2,4-Triazole (M13) indicate acceptable risk for all groups of aquatic organisms up to FOCUS Step 3.

However, for the relevant metabolite Prothioconazole-desthio (M04), calculated PEC/RAC ratios at Tier 1 based on the RAC of 0.334 µg/L for the most sensitive organism group fish prolonged exceed the trigger of 1 for some scenarios indicating that risk cannot be excluded. Therefore, step 4 calculations considering reduction of run-off were performed for the relevant scenarios.

For the intended uses in winter/spring oilseed rape (two applications) and sunflowers (one or two applications), calculated PEC/RAC ratios for metabolite Prothioconazole-desthio (M04) indicate an acceptable risk for the most sensitive group of aquatic organism group fish prolonged (based on a RAC of 0.334 µg/L) in all relevant FOCUS Step 4 scenarios considering 10 m vegetated buffer. Therefore, no further assessment for the individual active substances/metabolites is necessary.

For the assessment of the combined toxicity of Boscalid, Prothioconazole and metabolites, the step-wise approach according to the aquatic guidance document as well as the decision tree for metabolites according to document FAQ Aquatic MixTox Tool_v2 is considered. The assessment is performed separately for each organism group.

In summary, mixture toxicity assessment should be based on the combined toxicity of the two parents as well as on the combined toxicity of Boscalid and metabolite Prothioconazole-desthio (M04). Combined toxicity from Boscalid and Prothioconazole is relevant for all organism groups (for fish long-term and invertebrates long-term only for certain scenarios). Combined toxicity from Boscalid and metabolite M04 is relevant fish long-term and invertebrates long-term (remaining scenarios).

Regarding the combined toxicity of the two parents, a simplified approach according to the aquatic guidance document was chosen for all organism groups and scenarios where the decision tree indicates that the mixture tox assessment should be based on the two actives, since no synergistic effects are indicated and all ETR values of the individual active substances contained in the formulation are below the relevant trigger value (corresponding to a PEC/RAC ratio < 1) up to FOCUS Step 3 calculations. The simplified approach indicates no risk from the combined toxicity of Boscalid and Prothioconazole.

Regarding the combined toxicity of Boscalid and metabolite Prothioconazole-desthio (M04), a mixture toxicity risk assessment for the intended uses of FGE03 in oilseed rape according to step 8 of the AGD based on ETR_{mix-CA} calculations for Boscalid and metabolite Prothioconazole-desthio (M04) was presented. The assessment was performed for fish chronic only. This is deemed appropriate since PEC/RAC

ratios for both Boscalid and metabolite M04 are clearly worst case for this organism group covering all other organism groups.

For most FOCUS Step 3 scenarios, the mixture toxicity assessment for Boscalid and Prothioconazole-desthio (M04) indicates no risk.

However, calculated ETR_{mix-CA} for the most sensitive organism group fish prolonged exceeds the trigger of 0.1 for some scenarios indicating that risk cannot be excluded. Therefore, step 4 calculations considering reduction of run-off were performed for the relevant scenarios.

Risk from combined exposure to Boscalid and metabolite Prothioconazole-desthio (M04) for the most sensitive organism group fish prolonged is acceptable when the following risk mitigation measures for the relevant scenarios are considered:

- two applications of 0.7 kg FGE03/ha in winter oilseed rape – 10 m vegetated buffer (scenarios R1 stream, R3 stream)
 - two applications of 0.7 kg FGE03/ha in spring oilseed rape - 10 m vegetated buffer (scenario R1)
 - two applications of 0.7 kg FGE03/ha in sunflower - 10 m vegetated buffer (scenarios R1 stream, R3 stream). **It should be noted that according to the comment given by zRMS e-fate experts in e Fate Section for R4 scenario - 15-meter vegetated buffer is not accepted. For this reason, further calculation of PEC_{sw} considering 20 -meter vegetated buffer zone should be calculated by the Applicant for MSs that needs this scenario for their national level.**
- one application of 0.7 kg FGE03/ha in sunflower - 10 m vegetated buffer (scenario R4)

No risk mitigation measures are required for single application in winter/spring oilseed rape.

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 Boscalid, Prothioconazole and the formulated products for Annex 1 inclusion. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on bees of FGE03 were not evaluated as part of the EU assessment of Boscalid and Prothioconazole, respectively. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

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

Species	Substance	Exposure System	Results	Reference
<i>Apis mellifera</i>	Boscalid	Oral	LD₅₀ > 100 µg a.s./bee	Review report, SANCO/3919 /2007-rev. 5, 21 January 2008 and DAR (2002)
<i>Apis mellifera</i>	Boscalid	Contact	LD₅₀ > 100 µg a.s./bee	
<i>Apis mellifera</i>	Boscalid (formulated)	Oral	LD ₅₀ > 166 µg a.s./bee	Review report, SANCO/3919 /2007-rev. 5, 21 January 2008 and DAR (2002)
		Contact	LD ₅₀ > 200 µg a.s./bee	
<i>Apis mellifera</i>	Prothioconazole	Oral	LD₅₀ > 71 µg a.s./bee	EFSA Scientific Report (2007) 106, 1-98, and DAR (2005)
<i>Apis mellifera</i>	Prothioconazole	Contact	LD₅₀ > 200 µg a.s./bee	
<i>Apis mellifera</i>	Prothioconazole (formulated)	Oral	LD ₅₀ > 48.7 µg a.s./bee	EFSA Scientific Report (2007) 106, 1-98, and DAR (2005)
		Contact	LD ₅₀ > 200 µg a.s./bee	
<i>Apis mellifera</i>	FGE03 (35.22 % Boscalid w/w, 20.33 % Prothioconazole w/w)	Oral 48 h	LD₅₀ > 335.1 µg/bee corresponding to 186.1 µg a.s./bee ^a	New endpoint Knautz (2023) Report No. 176531035, UPL/2023/1236 KCP 10.3.1.1.1/01
		Contact 48 h	LD₅₀ > 300 µg/bee corresponding to 166.7 µg a.s./bee ^a	
<i>Bombus terrestris</i>	FGE03 (35.22 % Boscalid w/w, 20.33 % Prothioconazole w/w)	Oral 48 h	LD₅₀ > 671.7 µg/bee corresponding to 373.1 µg a.s./bee ^a	New endpoint Chwiesko & Horstmann (2023) Report No. 176531105, UPL/2023/1279 KCP 10.3.1.1.1/02
		Contact 48 h	LD₅₀ > 600 µg/bee corresponding to 333.3 µg a.s./bee ^a	
<i>Apis mellifera</i>	FGE03 (35.22 % Boscalid w/w, 20.33 % Prothioconazole w/w)	Oral Adult 10 d	LDD₅₀ > 114 µg/bee/d corresponding to 63.3 µg a.s./bee/d ^a NOEDD = 61.2 µg/bee/d corresponding to 34.0 µg a.s./bee/d ^a LDD₁₀ = 74.5 µg/bee/d corresponding to 41.4 µg a.s./bee/d ^a	New endpoint Knautz & Horstmann (2023) Report No. 176531136, UPL/2023/1209 KCP 10.3.1.2/01

Species	Substance	Exposure System	Results	Reference
<i>Apis mellifera</i>	FGE03 (35.22 % Boscalid w/w, 20.33 % Prothioconazole w/w)	Oral Larvae 22 d	NOED = 19.05 µg/larva corresponding to 10.58 µg a.s./larva ^a ED₁₀ = 18.93 [6.27 - 57.17] µg/larva corresponding to 10.52 [3.48 – 31.76] µg a.s./larva^a	New endpoint Venturi (2023) Report No. BT073/23, UPL/2023/1235 KCP 10.3.1.3/01
Higher-tier studies (tunnel test, field studies)				
Not required.				

a sum of active substances

Endpoints used for risk assessment are highlighted **in bold**.

zRMS comments:

Acute bee toxicity data for boscalid and prothioconazole provided in Table 9.6-1 has been confirmed that they are in line with EU agreed endpoints reported in Review report, SANCO/3919 /2007-rev. 5, 21 January 2008 and DAR (2002) and EFSA Scientific Report (2007) 106, respectively.

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

Studies on effects of the formulated product to bees listed in Table above were evaluated by the zRMS and considered acceptable. The reported endpoints are confirmed.

Summary of the performed studies together with zRMS evaluation may be found in Appendix 2.

9.6.1.1 Justification for new endpoints

Acute, chronic adult and larvae toxicity studies as well as acute bumble bee studies with FGE03 are submitted with the present dossier to cover the data requirements for the product.

For honeybee larvae, the determined ED₁₀ endpoint is considered for the risk assessment as it is slightly lower than the NOED. To justify the use of the ED₁₀ endpoint, and, therewith, to consider the conclusions set in the peer review meeting on recurring issues in ecotoxicology (EFSA supporting publication 2015:EN-924)⁶, a reliability assessment of the ED₁₀ endpoint based on the reliability indicators “Normalised width of confidence interval” and “Relationship between EC₁₀ and EC₂₀/EC₅₀ confidence intervals” is presented here. An overview of the NOED and ED_x values of the chronic larvae toxicity study (Venturi, 2024; KCP 10.3.1.3/01) along with the respective confidence intervals is presented in the following table.

Table 9.6-2: Chronic larvae toxicity endpoints and confidence intervals

Endpoint	µg t.i./larva
NOED	19.05
ED₁₀ [95 % C.L.]	18.93 [6.27 - 57.17]
ED₂₀ [95 % C.L.]	32.00 [14.36 - 71.29]
ED₅₀ [95 % C.L.]	70.66 [44.40 - 112.47]

t.i.: test item (FGE03); C.L.: Confidence Limits

Assessment of normalised width of confidence interval (NW)

The normalised width of confidence interval (NW) is an indicator based on the relative width of the 95 %

⁶ EFSA (European Food Safety Authority), 2015. Technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology. EFSA supporting publication 2015:EN-924. 62 pp.

confidence interval around the EC_{10} value. It is calculated as the ratio between the width of the EC_{10} confidence interval and the median value of EC_{10} :

$$NW = (EC_{10,upp} - EC_{10,low}) / EC_{10,med}$$

NW = normalised width

$EC_{10,upp}$ = 95 %- upper confidence limit

$EC_{10,low}$ = 95 %- lower confidence limit

$EC_{10,med}$ = median value of EC_{10}

With an $ED_{10, med}$ of 18.93 $\mu\text{g t.i./larva}$ and 95% confidence upper and lower limits of 57.17 and 6.27 $\mu\text{g t.i./larva}$, respectively, the calculated normalised width of the ED_{10} confidence interval is 2.7.

According to the NW based reliability classification given in EFSA supporting publication 2015:EN-924⁶, EC_{10} values with a $NW > 2$ are rated as “bad”.

Relationship between EC_{10} and EC_{20}/EC_{50} confidence intervals

The classification rating for the certainty of the protection level of the EC_{10} value is presented in the following table:

Table 9.6-3: Classification based on the relationship between EC_{10} and EC_{20}/EC_{50} confidence intervals

Condition	Rating (certainty of the protection level)
$EC_{10} < EC_{20,low}$	High
$EC_{20,low} < EC_{10} < EC_{50,low}$	Medium
$EC_{10} > EC_{50,low}$	Low

The ED_{10} value (18.93 $\mu\text{g t.i./larva}$) of FGE03 is above the lower CL of the ED_{20} (14.36 $\mu\text{g t.i./larva}$), but well below the lower limit of ED_{50} (44.40 $\mu\text{g t.i./larvae}$) and, hence, the certainty of protection level for the ED_{10} value for the larval toxicity is considered as “medium”.

Since the reliability assessment based on the NW parameters and on the relationship between ED_{10} and ED_{20}/ED_{50} shows a poor reliability of the ED_{10} value for larval toxicity, in a worst-case approach the **95% lower limit ED_{10} value of 6.27 $\mu\text{g t.i./larva}$ corresponding to 3.48 $\mu\text{g a.s./larva}$** is used in the risk assessment for honeybee larvae.

It should be noted that this approach is very conservative since the median ED_{10} is only slightly below the NOED and mortality rate at the NOED (11%) is within control mortality (< 30%) indicating that an end-point in the range of the NOED or median ED_{10} , respectively, is biologically plausible. The width of the confidence interval indicating bad reliability is probably due to the extraordinarily low control mortality and certain variability in the lower test item doses, whereas the relationship between ED_{10} and ED_{20}/ED_{50} shows medium reliability of the ED_{10} . Therefore, larval risk assessments are also presented for median ED_{10} and NOED.

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). In the absence of any implemented guidance, for chronic risk assessments The Nordic calculator tool for chronic bee risk assessment⁷ following EPPO (2010)⁸ and EPPO as modified by ECPA (2017)⁹ schemes were used. This approach is deemed reasonable

⁷ https://eng.mst.dk/media/cpjhqzhz/nordic-calculator-tool-for-bees-acc-nz-gd-2023_ver3b-1.xlsx

⁸ EPPO (2010): Side effects for honeybees. OEPP/EPPO Bulletin 40, 323–331.

since both EFSA (2013)¹⁰ and EFSA (2023)¹¹ have not yet been taken note of in the Standing Committee on Plants, Animals, Food and Feed (SCoPAFF). There is no final decision on protection goals for bumble/solitary bees and future guidance for risk assessment is still open. Nevertheless, for the sake of completeness, a risk assessment based on EFSA (2013) is also performed for illustrative purposes.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group oilseed rape covers the risk for bees from all intended uses in terms of maximum single application rate (see 9.1.2).

⁹ ECPA (2017): Proposal for a protective and workable regulatory European bee risk assessment scheme based on the EFSA bee guidance and other new data and available approaches. POS/17/LO/28028. 09 June 2017.

¹⁰ European Food Safety Authority, 2013. EFSA Guidance Document on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees). EFSA Journal 2013;11(7):3295, 268 pp., doi:10.2903/j.efsa.2013.3295

¹¹ EFSA (European Food Safety Authority), Adriaanse P, Arce A, Focks A, Ingels B, Jölli D, Lambin S, Rundlöf M, Süßenbach D, Del Aguila M, Ercolano V, Ferilli F, Ippolito A, Szentes Cs, Neri FM, Padovani L, Rortais A, Wassenberg J and Auteri D, 2023. Revised guidance on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees). EFSA Journal 2023;21(5):7989, 133 pp. <https://doi.org/10.2903/j.efsa.2023.7989>

9.6.2.1 Hazard quotients for bees

Tier-1 acute bee risk assessment according to SANCO/10329/2002 rev.2 (final), October 17, 2002

Table 9.6-4: First-tier assessment of the risk for bees due to the use of FGE03 in oilseed rape

Table 010-11: First tier assessment of the risk for bees due to the use of FGE03 in oilseed rape			
Intended use		Oilseed rape and sunflower	
Active substance		Boscalid	
Application rate (g/ha)		2 × 245	
Honeybees			
Test design	LD ₅₀ (lab.) (µg/bee)	Single application rate (g/ha)	Q _{HO} , Q _{HC} criterion: Q _H ≤ 50
Oral toxicity	> 100	245	< 2.45
Contact toxicity	> 100		< 2.45
Intended use		Oilseed rape and sunflower	
Active substance		Prothioconazole	
Application rate (g/ha)		2 × 140	
Honeybees			
Test design	LD ₅₀ (lab.) (µg/bee)	Single application rate (g/ha)	Q _{HO} , Q _{HC} criterion: Q _H ≤ 50
Oral toxicity	> 71	140	< 1.97
Contact toxicity	> 200		< 0.70
Product		FGE03	
Application rate (g/ha)		2 × 700	
Test design	LD ₅₀ (lab.) (µg a.s./bee)	Single application rate (g/ha)	Q _{HO} , Q _{HC} criterion: Q _H ≤ 50
Oral toxicity	> 335.1	700	< 2.09
Contact toxicity	> 300		< 2.33
Bumble bees ^a			
Product		FGE03	
Application rate (g a.s./ha)		2 × 700	
Test design	LD ₅₀ (lab.) (µg/bee)	Single application rate (g/ha)	Q _{HO} , Q _{HC} criterion: Q _H ≤ 50
Oral toxicity	> 671.7	700	< 1.04
Contact toxicity	> 600		< 1.17

Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure.

a risk assessment for bumble bees not included in SANCO/10329/2002 rev.2 (final), October 17, 2002, but shown here based on honeybee trigger value for illustrative purposes since endpoint is available

The acute contact and oral HQ values for honeybees and bumble bees are below the relevant trigger values indicating acceptable risk to bees following the intended uses of FGE03. Further refined risk assessment is therefore not required.

zRMS comments:

The acute risk assessment for bees presented in Table Table 9.6-1 is agreed by the zRMS. HQ_{Oral}, contact values for the active substances and the formulated product are below the trigger of 50, indicating a low acute risk for bees. Please note that the evaluation has been performed in line with SANCO/10329/2002 rev 2 final. Overall, acceptable risk to bees may be concluded from the intended uses of FGE03. In addition, acute risk assessment has been performed according to EFSA GD 2013 and presented below:

Tier 1 chronic honeybee risk assessment for larvae according to EPPO (2010)

Table 9.6-5: First tier assessment of the chronic risk for bee larvae due to the use of FGE03 in oilseed rape

Intended use	Oilseed rape		
Active substances	Boscalid + Prothioconazole		
Test design	LLED₁₀/ED₁₀/NOED (µg a.s./larva/d)^a	Daily dose^b (µg a.s./larva/d)	TER criterion: TER > 1
Oral toxicity, larvae, 22-d	3.48 / 10.52 / 10.58	0.0151	230 / 697 / 701

TER: Toxicity exposure ratio. TER values shown in bold breach the relevant trigger.

a—sum of active substances, lower limit of ED₁₀ for larvae is used in a worst-case approach

b—Daily dose: generic worst-case exposure of 0.0151 µg a.s./larva/day based on a worst-case residue value of 1 mg a.s./kg plant matrix and the worst-case sugar intake of drone larvae of 15.1 mg sugar/larva/day (according to Rortais et al., 2005). The sugar content of nectar and product specific application rate is not included in the risk assessment.

The chronic oral TER value for honeybees exceeds the relevant trigger value indicating acceptable chronic risk to honeybee larvae following the intended uses of FGE03. Further refined risk assessment is therefore not required.

Tier 1 chronic honeybee risk assessment for adults and larvae according to EPPO (2010)/ECPA (2017)

Table 9.6-6: First tier assessment of the chronic risk for bee adults and larvae due to the post-emergence use of FGE03 in oilseed rape

Intended use	Oilseed rape		
Product	FGE03		
Application rate (g a.s./ha)^a	2 × 385		
Test design	Endpoint	Daily dose (µg/bee/d)	TER criterion: TER > 1
Oral toxicity, adults, 10-d	NOEDD: 34 µg a.s./bee/d ^a	Nectar: 0.4764 ^b	71.4 128
Oral toxicity, larvae, 22-d	LLED ₁₀ : 3.48 µg a.s./larva/d ^a ED ₁₀ : 10.52 µg a.s./larva/d NOED ₁₀ : 10.58 µg a.s./larva/d	Nectar: 0.2211 Pollen: 0.00470 Sum: 0.226 ^c	15.4 46.5 46.8

TER: Toxicity exposure ratio. TER values shown in bold breach the relevant trigger.

a—sum of active substances, lower limit of ED₁₀ for larvae is used in a worst-case approach

b—Daily dose for adults: A.R. × (0.128 g/(1000 × 0.3)) × RUD × 1000; based on the worst-case sugar need of 128 mg/bee/day (according to Rortais et al., 2005) of a foraging bee feeding exclusively of nectar containing a more representative 30% sugar (according to EFSA, 2013); RUD for nectar 2.9 mg a.s./kg (foliar spray; according to EFSA, 2013)

c—Daily dose for larvae A.R. × (0.0594 g/(1000 × 0.3)) × RUD × 1000 for nectar: based on the worst-case sugar need of 59.4 mg/larvae/d.p., nectar sugar content of 30% and RUD for nectar of 2.9 mg a.s./kg (all according to EFSA, 2013); Daily dose for larvae A.R. × RUD × 2 for pollen: based on the pollen consumption of 2 mg/5 days and RUD for pollen of 6.1 mg a.s./kg (obtained from EFSA 2013)

The chronic oral TER values for adult honeybee and honeybee larvae, respectively, exceed the relevant trigger values indicating acceptable chronic risk to adult and larval honeybees following the intended uses of FGE03. Further refined risk assessment is therefore not required.

Risk assessment according to EFSA (2013)

Table 9.6-7: Screening assessment of the acute contact risk for honeybees and bumble bees* due to the post-emergence use of FGE03 in oilseed rape

Intended use	Oilseed rape		
Active substance	Boscalid		
Application rate (g/ha)	2 × 245		
Organism	LD₅₀ (µg/bee)	Single application rate (g/ha)	HQ (trigger value)
Honeybees	> 100	245	< 2.5 (42)
Intended use	Oilseed rape		
Active substance	Prothioconazole		
Application rate (g/ha)	2 × 140		
Organism	LD₅₀ (µg/bee)	Single application rate (g/ha)	HQ (trigger value)
Honeybees	> 200	140	< 0.7 (42)
Intended use	Oilseed rape		
Product	FGE03		
Application rate (g/ha)	2 × 700		
Organism	LD₅₀ (µg/bee)	Single application rate (g/ha)	HQ (trigger value)
Honeybees	> 300	700	< 2.3 (42)
Bumble bees	> 600		< 1.2 (7)

HQ: Hazard quotient. HQ values shown **in bold** breach the relevant trigger.

* In the absence of agreed and validated test guidelines, no risk assessment for solitary bees is presented. For bumble bees, risk assessment is presented only for the formulation where endpoint is available. Considering that EFSA/2013/3295 is not taken note of yet, further risk assessment is not considered necessary here.

Acute contact screening HQ values for honeybees and bumble bees are below the relevant trigger values for the use in oilseed rape indicating acceptable risk to honeybees and bumble bees following all intended uses.

Table 9.6-8: Screening assessment of the acute oral risk for honeybees and bumble bees* due to the post-emergence use of FGE03 in oilseed rape

Intended use	Oilseed rape		
Product	Boscalid		
Application rate (g/ha)	2 × 245		
Organism	LD₅₀ (µg/bee)	Single application rate (g/ha)	ETR (trigger value, downward spraying)
Honeybees	> 100	245	< 0.02 (0.2)
Intended use	Oilseed rape		
Product	Prothioconazole		
Application rate (g/ha)	2 × 140		
Organism	LD₅₀ (µg/bee)	Single application rate (g/ha)	ETR (trigger value, downward spraying)
Honeybees	> 71	140	< 0.01 (0.2)
Intended use	Oilseed rape		
Product	FGE03		
Application rate (g/ha)	2 × 700		
Organism	LD₅₀ (µg/bee)	Single application rate (g/ha)	ETR (trigger value, downward spraying)
Honeybees	> 335.1	700	< 0.02 (0.2)
Bumble bees	> 671.7		< 0.01 (0.036)

ETR: Exposure Toxicity Ratio. ETR values shown **in bold** breach the relevant trigger.

* In the absence of agreed and validated test guidelines, no risk assessment for solitary bees is presented. For bumble bees, risk assessment is presented only for the formulation where endpoint is available. Considering that EFSA/2013/3295 is not taken note of yet, further risk assessment is not considered necessary here.

The acute oral screening ETR values for honeybees and bumble bees are below the relevant trigger values for the use in oilseed rape indicating acceptable risk to honeybees and bumble bees following all intended uses.

zRMS comments:

The acute risk assessment for bees and bumble bees presented in Tables 9.6-7 and Table 9.5-8 is agreed by the zRMS. HQoral, contact values for the active substances and the formulated product are below the trigger of 50, indicating a low acute risk for bees.

Please note that the evaluation has been performed in line with EFSA GD 2013.

Overall, acceptable acute risk to bees and bumble bees may be concluded from the intended uses of FGE03.

Table 9.6-9: Screening assessment of the chronic risk for honeybees* (adult and larvae, respectively) due to the post-emergence use of FGE03 in oilseed rape

Intended use	Oilseed rape		
Product	FGE03		
Application rate (g/ha)	2 × 700		
Organism	Endpoint (µg/bee/day or developmental period)	Short-cut value (Ef x SV)	ETR (trigger value, downward spraying)
Honeybees (adult)	114	7.6	0.047 (0.03)
Honeybees (larvae)	6.27 (LLED ₁₀) ^a 18.93 (median ED ₁₀) 19.05 (NOED)	4.4	0.49 (0.2) 0.16 (0.2) 0.16 (0.2)

ETR: Exposure Toxicity Ratio. ETR values shown **in bold** breach the relevant trigger.

* chronic risk assessments for bumble bees and solitary bees are not required according to the risk assessment scheme

a lower limit of ED₁₀ for larvae is used in a worst-case approach

The chronic adult and larvae (based on LLED₁₀) screening ETR values for honeybees exceeds the relevant trigger value for the use in oilseed rape indicating that chronic risk to honeybees cannot be excluded. Hence, a Tier-1 assessment is required to address the chronic risk to adult and larval honeybees from exposure to FGE03.

Table 9.6-10: Tier-1 assessment of the chronic adult risk for honeybees* due to the post-emergence use of FGE03 in oilseed rape

Category	Scenario	BBCH	ETR	Trigger
chronic	treated crop	40 - 69	0.0256	0.03
chronic	treated crop	≥ 70	0.0000	0.03
chronic	weeds	40 - 69	0.0032	0.03
chronic	weeds	≥ 70	0.0032	0.03
chronic	field margin	40 - 69	0.0001	0.03
chronic	field margin	≥ 70	0.0001	0.03
chronic	adjacent crop	40 - 69	0.0001	0.03
chronic	adjacent crop	≥ 70	0.0001	0.03
chronic	next crop	40 - 69	0.0024	0.03
chronic	next crop	≥ 70	0.0024	0.03

ETR: Exposure Toxicity Ratio. ETR values shown **in bold** breach the relevant trigger.

* chronic risk assessments for bumble bees and solitary bees are not required according to the risk assessment scheme

Table 9.6-11: Tier-1 assessment of the chronic larval risk for honeybees* due to the post-emergence use of FGE03 in oilseed rape (based on LLED₁₀)

Category	Scenario	BBCH	ETR	Trigger
larva	treated crop	40 - 69	0.4175	0.2
larva	treated crop	≥ 70	0.0000	0.2
larva	weeds	40 - 69	0.0522	0.2
larva	weeds	≥ 70	0.0522	0.2
larva	field margin	40 - 69	0.0019	0.2
larva	field margin	≥ 70	0.0019	0.2
larva	adjacent crop	40 - 69	0.0014	0.2
larva	adjacent crop	≥ 70	0.0014	0.2
larva	next crop	40 - 69	0.0380	0.2
larva	next crop	≥ 70	0.0380	0.2

ETR: Exposure Toxicity Ratio. ETR values shown **in bold** breach the relevant trigger.

* chronic risk assessments for bumble bees and solitary bees are not required according to the risk assessment scheme

The chronic adult Tier-1 ETR values for honeybees are below the relevant trigger value for the use in oilseed rape indicating acceptable risk to honeybees following all intended uses.

The chronic larval Tier-1 ETR values for honeybees are also below the relevant trigger value for the use in oilseed rape, except for one scenario. For “treated crop” scenario (BBCH 61-69), chronic larval Tier-1 ETR exceeds the trigger when the very conservative lower limit of the ED₁₀ is used for risk assessment. It should be noted that risk is acceptable already at the screening step if the 3-fold higher median ED₁₀ or NOED, respectively, from the larval study are used. In addition, considering that EFSA/2013/3295 is not taken note of yet, further risk assessment is not considered necessary here.

zRMS comments:

The chronic and larvae risk assessment is not required according to SANCO/10329/2002 rev 2 final. Due to the fact that the chronic tests are available for adult bee and larvae, the screening step and Tier 1 risk assessment in line with EFSA (2013) and according to recommendations given during harmonisation meeting in Central Zone in Warsaw in 2023 has been evaluated by zRMS with consideration of endpoints from submitted studies.

The chronic adult Tier-1 ETR values for honeybees are below the relevant trigger value for the use in oilseed rape indicating acceptable risk to honeybees following all intended uses.

The chronic larval Tier-1 ETR values for honeybees are also below the relevant trigger value for the use in oilseed rape, except for one scenario. For “treated crop” scenario (BBCH 61-69), chronic larval Tier-1 ETR exceeds the trigger when the very conservative lower limit of the ED₁₀ is used for risk assessment. It should be noted that risk is acceptable already at the screening step if the 3-fold higher median ED₁₀ or NOED, respectively, from the larval study are used.

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

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

Not relevant.

9.6.3 Effects on bumble bees

Acute oral and contact toxicity data on bumble bees are available for FGE03 and considered in the above risk assessment. No risk is indicated following the intended uses according to SANCO/10329/2002 rev.2 (final), October 17, 2002 and screening assessment according to EFSA/2013/3295. Further risk assessment is not considered necessary here.

9.6.4 Effects on solitary bees

There is currently no validated methodology for the assessment of toxicity to solitary bees. Consequently, such studies are not considered necessary for the time being. Risk assessment is not considered necessary here.

9.6.5 Overall conclusions

Based on the acute Tier-1 risk assessment (honeybees and bumble bees) according to SANCO/10329/2002 rev.2 (final), October 17, 2002, ~~and the chronic Tier-1 risk assessment (honeybees) according to the modified EPPO (2010)/ECPA (2017) scheme, respectively,~~ acceptable risk to bees following the intended uses of FGE03 is indicated. ~~Risk is also acceptable considering screening/Tier-1 risk assessment for honeybees according to EFSA/2013/3295.~~

Based on the acute (honeybees and bumble bees) and chronic adult (honeybees) risk assessment according to EFSA/2013/3295, no risk is indicated following all intended uses. The chronic larval Tier-1 ETR values for honeybees are also below the relevant trigger value for the use in oilseed rape for most scenarios. However, for “treated crop” scenario (BBCH 61-69), chronic larval Tier-1 ETR exceeds the

trigger when the very conservative lower limit of the ED₁₀ is used for risk assessment. It should be noted that risk is acceptable already at the screening step if the 3-fold higher median ED₁₀ or NOED, respectively, from the larval study are used. ~~In addition, considering that EFSA/2013/3295 is not taken note of yet, further risk assessment is not considered necessary here.~~

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

There is currently no validated methodology for the assessment of toxicity to bumble bees (chronic) and solitary bees (both acute and chronic). Consequently, such studies are not considered necessary for the time being. Further risk assessment is not considered necessary here.

9.7 Effects on arthropods other than bees (KCP 10.3.2)

9.7.1 Toxicity data

Studies on the toxicity to non-target arthropods have been carried out with the formulated products for Annex 1 inclusion of the two active substances. Full details of these studies are provided in the respective EU DAR and related documents. However, they are not considered for the present product registration.

Effects on non-target arthropods of FGE03 were not evaluated as part of the EU assessment of Boscalid and Prothioconazole, respectively. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

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

Species	Substance	Exposure System	Results	Reference
<i>Aphidius rhopalosiphi</i> (adults)	FGE03 (35.22 % Boscalid w/w, 20.33 % Prothioconazole w/w)	Laboratory test glass plates (2D)	LR₅₀ / ER₅₀ > 1700 g/ha	New endpoint Leopold (2023a) Report No. 176531001, UPL/2023/1053 KCP 10.3.2.1/01
<i>Typhlodromus pyri</i> (protonymphs)	FGE03 (35.22 % Boscalid w/w, 20.33 % Prothioconazole w/w)	Laboratory test glass plates (2D)	LR ₅₀ = 503.1 g/ha ER₅₀ > 272 g/ha	New endpoint Leopold (2023a) Report No. 176531063, UPL/2023/1054 KCP 10.3.2.1/02
<i>Typhlodromus pyri</i> (protonymphs)	FGE03 (35.22 % Boscalid w/w, 20.33 % Prothioconazole w/w)	Extended laboratory test Bean leaves (2D)	LR ₅₀ > 1700 g/ha ER₅₀ > 1700 g/ha	New endpoint Leopold (2024) Report No. 176531062, UPL/2024/0298 KCP 10.3.2.2/01
<i>Poecilus cupreus</i> (adults)	FGE03 (35.22 % Boscalid w/w, 20.33 % Prothioconazole w/w)	Extended laboratory test LUFA soil (2D)	LR ₅₀ > 7600 g/ha NOER ≥ 7600 g/ha	New endpoint Knautz (2024) Report No. 176531007, UPL/2024/0337 KCP 10.3.2.2/02
Field or semi-field tests				
Not available, not needed.				

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

zRMS comments:

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

9.7.1.1 Justification for new endpoints

Endpoints from studies with FGE03 are used for risk assessment since these are considered most relevant for the proposed GAP.

9.7.2 Risk assessment

The evaluation of the risk for non-target arthropods was performed in accordance with the recommenda-

tions of the “Guidance Document on Terrestrial Ecotoxicology”, as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002), and in consideration of the recommendations of the guidance document ESCORT 2.

9.7.2.1 Risk assessment for in-field exposure

Since according to the GAP, FGE03 should be applied on established crop stages only foliar-dwelling arthropods are considered in the in-field risk assessment.

Table 9.7-2: First/Higher-tier assessment of the in-field risk for non-target arthropods due to the use of FGE03 in oilseed rape at 2 × 700 g/ha (use 2)

Intended use	Oilseed rape		
Product	FGE03		
Application rate (g/ha)	2 × 700		
MAF	1.7		
Test species Tier I	LR₅₀ / ER₅₀ (lab.) (g/ha)	PER_{in-field} (g/ha)	HQ_{in-field} criterion: HQ ≤ 2
<i>Aphidius rhopalosiphi</i>	> 1700	1190	< 0.700
<i>Typhlodromus pyri</i>	503.1 / > 272		2.37 / < 4.38
Test species Higher-Tier	ER₅₀ (extended lab.) (g/ha)	PER_{in-field} (g/ha)	PER_{in-field} < ER₅₀ (extended lab.)
<i>Typhlodromus pyri</i>	> 1700	1190	yes
<i>Poecilus cupreus</i>	≥ 7600		yes

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient. Criteria values shown in **bold** breach the relevant trigger.

Table 9.7-3: First/Higher-tier assessment of the in-field risk for non-target arthropods due to the use of FGE03 in oilseed rape at 1 × 700 g/ha (use 1)

Intended use	Oilseed rape		
Product	FGE03		
Application rate (g/ha)	1 × 700		
MAF	1		
Test species Tier I	LR₅₀ / ER₅₀ (lab.) (g/ha)	PER_{in-field} (g/ha)	HQ_{in-field} criterion: HQ ≤ 2
<i>Aphidius rhopalosiphi</i>	> 1700	700	< 0.412
<i>Typhlodromus pyri</i>	503.1 / > 272		1.39 / < 2.57
Test species Higher-Tier	ER₅₀ (extended lab.) (g/ha)	PER_{in-field} (g/ha)	PER_{in-field} < ER₅₀ (extended lab.)
<i>Typhlodromus pyri</i>	> 1700	700	yes
<i>Poecilus cupreus</i>	≥ 7600		yes

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient. Criteria values shown in **bold** breach the relevant trigger.

For all intended uses, Tier-1 in-field HQ values are below the relevant trigger value for the indicator species *Aphidius rhopalosiphi*. For *Typhlodromus pyri*, Tier-1 in-field HQ values based on the ER₅₀ are below the relevant trigger value. However, extended lab studies with *Typhlodromus pyri* and *Poecilus cupreus* indicate no in-field risk; thus, additional testing and/or risk mitigation measures are not required to exclude risk to the in-field.

9.7.2.2 Risk assessment for off-field exposure

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

use group oilseed rape (2 × 0.7 kg product/ha; use 2) covers the risk for non-target arthropods from all intended uses (see 9.1.2).

Table 9.7-4: First/Higher-tier assessment of the off-field risk for non-target arthropods due to the use of FGE03 in oilseed rape

Intended use	Oilseed rape				
Product	FGE03				
Application rate (g/ha)	2 × 700				
MAF	1.7				
vdf	5 ^a				
Test species Tier I	LR₅₀ / ER₅₀ (lab.) (g a.s./ha)	Drift factor	PER_{off-field} (g a.s./ha)	CF	HQ_{off-field} criterion: HQ ≤ 2
<i>Aphidius rhopalosiphi</i>	> 1700	0.0238	2.832	10	< 0.033
<i>Typhlodromus pyri</i>	503.1 / > 272				0.113 / < 0.208
Test species Higher-Tier	ER₅₀ (extended lab.) (g/ha)	Drift factor	PER_{off-field} (g a.s./ha)	CF	Corr. PER_{off-field} < ER₅₀ (extended lab.)
<i>Typhlodromus pyri</i>	> 1700	0.0238	2.832	5	yes
<i>Poecilus cupreus</i>	≥ 7600				yes

MAF: Multiple application factor; vdf: Vegetation distribution factor; (corr.) PER: (corrected) Predicted environmental rate; CF: Correction factor; HQ: Hazard quotient.

^a according to EFSA (2015¹², 2019¹³)

For all intended uses, Tier-1 and higher-Tier off-field HQ values are below the relevant trigger value for both indicator species. Thus, higher-Tier risk assessment and/or risk mitigation measures are not required to protect non-target arthropods in the off-field.

zRMS comments:

The risk assessment presented in Tables 9.7-3 and 9.7-4 has been validated by the zRMS.

As a worst case the VDF of 5 has been considered, since available investigations indicate that VDF of 10 recommended by ESCORT 2 guidance document is not appropriate and may lead to underestimation of the exposure.

It should be, however, noted that according to EFSA Supporting publication 2019:EN-1673, VDF of 5 should be considered as the interim solution that will be reflected in the SANCO/10329/2002 rev 2 final with its implementation considered further.

Since use of VDF of 5 was not reflected in the current SANCO terrestrial guidance, its use is not yet mandatory. Nevertheless, the risk assessment performed with VDF of 5 is more protective and is thus agreed by among experts in Central Zone.

Based on calculations performed with consideration of the Tier I laboratory data acceptable and higher tier the in-field and off-field risk to non-target arthropods from all intended uses of may be concluded with no need for risk mitigation measures.

9.7.2.3 Additional higher-tier risk assessment

Not relevant.

¹² EFSA (European Food Safety Authority), 2015. Technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology. EFSA supporting publication 2015:EN-924. 62 pp.

¹³ EFSA (European Food Safety Authority), 2019. Technical report on the outcome of the Pesticides Peer Review Meeting on general recurring issues in ecotoxicology. EFSA supporting publication 2019:EN-1673. 117 pp. doi:10.2903/sp.efsa.2019.EN-1673.

9.7.2.4 Risk mitigation measures

No risk mitigation needed.

9.7.3 Overall conclusions

For all intended uses, Tier-1 in-field HQ values are below the relevant trigger value for the indicator species *Aphidius rhopalosiphi*. For *Typhlodromus pyri*, Tier-1 in-field HQ values based on the ER₅₀ are below the relevant trigger value. However, extended lab studies with *Typhlodromus pyri* and *Poecilus cupreus* indicate no in-field risk; thus, additional testing and/or risk mitigation measures are not required to exclude risk to the in-field.

For all intended uses, Tier-1 and higher-Tier off-field HQ values are below the relevant trigger value for both indicator species. Thus, higher-Tier risk assessment and/or risk mitigation measures are not required to protect non-target arthropods in the off-field.

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

9.8.1 Toxicity data

Studies on the toxicity to non-target soil meso- and macrofauna have been carried out with Boscalid, Prothioconazole and their formulated products for Annex 1 inclusion. Additional studies were performed with the relevant Prothioconazole 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 FGE03 were not evaluated as part of the EU assessment of Boscalid. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

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

Species	Substance	Exposure System	Results	Reference
<i>Eisenia fetida</i>	Boscalid	Mixed into substrate 14 d, acute	LC ₅₀ > 1000 mg/kg dw soil LC _{50 corr.} > 500 mg/kg dw soil	Review report, SANCO/3919 /2007-rev. 5, 21 January 2008 and DAR (2002)
<i>Eisenia fetida</i>	BAS 500 01 F (nominal 500 g/kg Boscalid)	Mixed into substrate 14 d, acute	LC ₅₀ > 1000 mg/kg dw soil LC _{50 corr.} > 500 mg/kg dw soil	Review report, SANCO/3919 /2007-rev. 5, 21 January 2008 and DAR (2002)
<i>Eisenia foetida</i>	BAS 500 01 F (nominal 500 g/kg Boscalid)	Mixed into substrate 56 d, chronic	NOEC = 3.6 kg/ha NOEC _{corr.} = 1.8 kg/ha corresponding to 1.197 mg a.s./kg dw soil	Review report, SANCO/3919 /2007-rev. 5, 21 January 2008 and DAR (2002) ^a
<i>Eisenia fetida</i>	Boscalid	Mixed into substrate 56 d, chronic 10 % peat content	NOEC_{corr.} = 12.5 mg/kg dw soil	Friedrich (2014) Report No. 2014/1083454 RAR (2018) ^b
<i>Eisenia fetida</i>	Boscalid 500 WG = FGG01 (51.33 % a.s. w/w)	Mixed into substrate 56 d, chronic 10 % peat content	NOEC = 296.3 mg/kg dw soil EC ₁₀ = 41.21 mg/kg dw soil corresponding to 21.15 mg a.s./kg dw soil EC_{10 corr.} = 10.58 mg a.s./kg dw soil	Rana (2023) Report No. 522-3-08-31232, UPL/2022/21325 EFSA-2022-00011613 Submitted with Article 33 dossiers for new product registrations in the Central and Southern Zones (2024) ^c
<i>Eisenia fetida</i>	Prothioconazole	Mixed into substrate 14 d, acute	LC ₅₀ > 1000 mg/kg dw soil LC _{50 corr.} > 500 mg/kg dw soil	EFSA Scientific Report (2007) 106, 1-98, and DAR (2005)
<i>Eisenia fetida</i>	Prothioconazole (formulated)	Mixed into substrate 14 d, acute	LC ₅₀ > 249.3 mg a.s./kg dw soil LC _{50 corr.} > 124.65 mg a.s./kg dw soil	EFSA Scientific Report (2007) 106, 1-98, and DAR (2005)
<i>Eisenia fetida</i>	Prothioconazole (formulated)	Mixed into substrate 56 d, chronic	NOEC = 1.33 mg a.s./kg dw soil NOEC_{corr.} = 0.665 mg a.s./kg dw soil	EFSA Scientific Report (2007) 106, 1-98, and DAR (2005)
<i>Folsomia candida</i>	Prothioconazole	Mixed into substrate 28 d, chronic	NOEC = 64 mg a.s./kg dw soil NOEC_{corr.} = 32 mg a.s./kg dw	EFSA Scientific Report (2007) 106, 1-98, and DAR

Species	Substance	Exposure System	Results	Reference
			soil	(2005)
<i>Hypoaspis aculeifer</i>	Prothioconazole	Mixed into substrate 14 d, chronic	NOEC = 100 mg a.s./kg dw soil^d	EFSA Scientific Report (2007) 106, 1-98, and DAR (2005)
<i>Eisenia fetida</i>	Prothioconazole-desthio (M04)	14 d, acute	LC ₅₀ > 1000 mg/kg dw soil LC _{50 corr.} > 500 mg/kg dw soil	EFSA Scientific Report (2007) 106, 1-98, and DAR (2005)
<i>Eisenia fetida</i>	Prothioconazole-desthio (M04)	56 d, chronic	NOEC = 1 mg/kg dw soil NOEC_{corr.} = 0.5 mg/kg dw soil	EFSA Scientific Report (2007) 106, 1-98, and DAR (2005)
<i>Folsomia candida</i>	Prothioconazole-desthio (M04)	Mixed into substrate chronic	NOEC = 62.5 mg/kg dw soil NOEC_{corr.} = 31.25 mg/kg dw soil	EFSA Scientific Report (2007) 106, 1-98, and DAR (2005)
<i>Eisenia fetida</i>	Prothioconazole-S-methyl (M01)	14 d, acute	LC ₅₀ > 1000 mg/kg dw soil LC _{50 corr.} > 500 mg/kg dw soil	EFSA Scientific Report (2007) 106, 1-98, and DAR (2005)
<i>Eisenia fetida</i>	Prothioconazole-S-methyl (M01)	56 d, chronic	NOEC = 100 mg/kg dw soil NOEC_{corr.} = 50 mg/kg dw soil	EFSA Scientific Report (2007) 106, 1-98, and DAR (2005)
<i>Folsomia candida</i>	Prothioconazole-S-methyl (M01)	Mixed into substrate chronic	NOEC = 31.6 mg/kg dw soil NOEC_{corr.} = 15.8 mg/kg dw soil	EFSA Scientific Report (2007) 106, 1-98, and DAR (2005)
<i>Eisenia andrei</i>	FGE03 (35.22 % Boscalid w/w, 20.33 % Prothioconazole w/w)	Mixed into substrate 56 d, chronic 10 % peat content	NOEC = 10 mg/kg dw soil EC ₁₀ > 10 mg/kg dw soil ^e NOEC_{corr.} = 5 mg/kg dw soil corresponding to 2.78 mg a.s./kg dw soil^f	New endpoint Hübner (2023a) Report No. 176531022, UPL/2023/2769 KCP 10.4.1.1/01
<i>Folsomia candida</i>	FGE03 (35.22 % Boscalid w/w, 20.33 % Prothioconazole w/w)	Mixed into substrate 28 d, chronic 5 % peat content	NOEC = 1000 mg/kg dw soil EC ₁₀ : n.d. ^d NOEC_{corr.} = 500 mg/kg dw soil corresponding to 277.8 mg a.s./kg dw soil^f	New endpoint Hübner (2023b) Report No. 176531016, UPL/2023/2772 KCP 10.4.2.1/01
<i>Hypoaspis aculeifer</i>	FGE03 (35.22 % Boscalid w/w, 20.33 % Prothioconazole w/w)	Mixed into substrate 14 d, chronic 5 % peat content	NOEC = 1000 mg/kg dw soil EC ₁₀ > 1000 mg/kg dw soil NOEC_{corr.} = 500 mg/kg dw soil corresponding to 277.8 mg a.s./kg dw soil^f	New endpoint Hübner (2022c) Report No. 176531089, UPL/2023/1436 KCP 10.4.2.1/02
Field studies				
Not available, not needed.				
Litter bag test				
Not relevant.				

Acute endpoints shaded in grey are not used for risk assessment since more relevant chronic endpoints are available.

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

- a Endpoint from the Review Report (2008). Please note that the study (Krieg, 2000) from the DAR was not conducted according to current guideline (OECD 222) and only 4 test item concentrations were tested. It was performed with the representative formulation for Annex 1 inclusion; thus, toxicity might also be due to co-formulants. Endpoints under b and c are therefore considered more appropriate for the current risk assessment.
- b A.s. endpoint considered acceptable for the risk assessment for the a.s. in the Boscalid RAR (2018). The study was performed according to current guidance (OECD 222). Please refer to 9.8.1.1.
- c Endpoint from a study according to OECD 222 with the UPL formulation FGG01 = Boscalid 500 WG submitted with Article 33 dossiers for new product registration in the Central and Southern Zones (2024). Please refer to 9.8.1.1.
- d Lufa 2.1 soil, ca. 0.9 % organic carbon therefore no need to correct toxicity endpoint (according to EFSA Conclusion 2007)
- e estimated value
- f sum of active substances
- g not determined since there was no adequate dose response

Hypoaspis data on Prothioconazole metabolites is not available from the list of endpoints and not considered required given the high margin of safety of the risk assessment of the parent and the formulation.

9.8.1.1 Justification for new endpoints

New data on soil mesofauna with FGE03 is now available to cover the data requirement for the product. The new endpoints are used in the risk assessment since test design and formulation are considered most relevant for the intended GAP and to account for the toxicity of the mixture.

Endpoints from Annex I inclusion for the two actives and corresponding risk assessments are only presented for sake of completeness since studies were performed on the respective representative formulations and are not considered relevant for the assessment of FGE03.

In addition, the Boscalid endpoint for earthworms ($\text{NOEC}_{\text{corr.}} = 1.197 \text{ mg a.s./kg dw soil}$) from the Review Report (2008) was derived from a DAR study (Krieg, 2000) not conducted according to current guideline (OECD 222) and with only 4 test item concentrations. It was performed with the representative formulation for Annex 1 inclusion; thus, toxicity might also be due to co-formulants. Therefore, further endpoints were considered in the earthworm risk assessment for Boscalid:

- **$\text{NOEC}_{\text{corr.}} = 12.5 \text{ mg a.s./kg dw soil}$** from the a.s. study (Friedrich, 2014 listed in LoEP 2018) submitted for Annex-1 renewal and considered acceptable for the risk assessment for the a.s. in the Boscalid RAR (2018). The study was performed according to current guidance (OECD 222).
- **$\text{EC}_{10 \text{ corr.}} = 10.58 \text{ mg a.s./kg dw soil}$** from a study according to OECD 222 with the UPL formulation FGG01 = Boscalid 500 WG (Rana, 2024) submitted with the Article 33 dossiers for new product registration in the Central and Southern Zones (2024).

Please note that both endpoints are in the same range and both endpoints are approximately 10 times higher than the endpoint from the Annex-1 inclusion providing evidence that – together with the FGE03 formulation endpoint - they are considered more appropriate for the risk assessment for earthworms from exposure to FGE03 according to the intended GAP.

zRMS comments:

Soil meso- and macrofauna data provided in Table 9.8-1 have been confirmed that they are in line with EU agreed endpoints reported in Review report, SANCO/3919 /2007-rev. 5, 21 January 2008 and DAR (2002) and EFSA Scientific Report (2007) 106 and DAR 2005, respectively.

To fulfil the data requirements as set by Commission Regulation (EU) No 284/2013, studies on soil meso and macrofauna were submitted with the formulated product.

zRMS agrees with new endpoints are used in the risk assessment since test design and formulation are considered most relevant for the intended GAP and to account for the toxicity of the mixture.

Studies on effects of the formulated product listed in Table above were evaluated by the zRMS and considered acceptable. The reported endpoints are confirmed.

Summary of the performed studies together with zRMS evaluation may be found in Appendix 2.

9.8.2 Risk assessment

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

9.8.2.1 First-tier risk assessment

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

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group oilseed rape (multiple application) covers the risk for earthworms and other non-target soil organisms (meso- and macrofauna) from all intended uses in terms of maximum soil loading (see 9.1.2).

Table 9.8-2: First-tier assessment of the acute and chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of FGE03 in oilseed rape

Intended use	Oilseed rape		
Chronic effects on earthworms			
Product/active substance	NOEC/EC ₁₀ corr. (mg/kg dw soil)	PEC _{soil} (mg/kg dw soil)	TER _{it} (criterion TER ≥ 5)
FGE03	5.0	0.373	13.4
FGE03 (sum of a.s.)	2.78	0.197 ^a	14.1
		0.326 ^b	8.53
Boscalid (in BAS 510 01 F; Review Report 2008) ^c	1.197	0.158 ^d	7.58
		0.287 ^e (0.143 ^{ef})	4.17 (8.37)
Boscalid (RAR 2018 LoEP)	12.5	0.158 ^d	79.1
		0.287 ^e	43.6
Boscalid (in FGG01)	10.58	0.158 ^d	70.0
		0.287 ^e	36.9
Prothioconazole (formulated) ^c	0.665	0.039	17.1
M01	50	0.009	5556
M04	0.50	0.033	15.2
Chronic effects on <i>Folsomia</i>			
Product/active substance	NOEC/EC ₁₀ corr. (mg/kg dw soil)	PEC _{soil} (mg/kg dw soil)	TER _{it} (criterion TER ≥ 5)
FGE03	500	0.373	1340
FGE03 (sum of a.s.)	277.8	0.197 ^a	1410
		0.326 ^b	852
Prothioconazole	32	0.039	821
M01	15.8	0.009	1756
M04	31.25	0.033	947
Chronic effects on <i>Hypoaspis</i>			
Product/active substance	NOEC/EC ₁₀ corr. (mg/kg dw soil)	PEC _{soil} (mg/kg dw soil)	TER _{it} (criterion TER ≥ 5)
FGE03	500	0.373	1340
FGE03 (sum of a.s.)	277.8	0.197 ^a	1410
		0.326 ^b	852
Prothioconazole ^g	50 ~~100~~	0.039	1282

TER values shown in **bold** fall below the relevant trigger.

a sum of PEC_{initial} Prothioconazole + PEC_{accumulation} (PEC_{act} + PEC_{soil plateau}) Boscalid – FOCUS approach

b sum of PEC_{initial} Prothioconazole + PEC_{accumulation} (PEC_{act} + PEC_{soil plateau}) Boscalid – additional approach

c Endpoints from Annex I inclusion for the two actives and corresponding risk assessments are only presented for sake of completeness since studies were performed on the respective representative formulations and are not considered relevant for the assessment of FGE03.

- d $PEC_{\text{accumulation}} (PEC_{\text{act}} + PEC_{\text{soil plateau}})$ Boscalid – FOCUS approach
e $PEC_{\text{accumulation}} (PEC_{\text{act}} + PEC_{\text{soil plateau}})$ Boscalid – additional approach
f PEC for single application
g *Hypoaspis* data on Prothioconazole metabolites is not available from the list of endpoints and not considered required given the high margin of safety of the risk assessment of the parent and the formulation.

Table 9.8-3: First-tier assessment of the acute and chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of FGE03 in sunflower

Intended use	Sunflower		
Chronic effects on earthworms			
Product/active substance	NOEC/EC ₁₀ corr. (mg/kg dw soil)	PEC _{soil} (mg/kg dw soil)	TER _{lt} (criterion TER ≥ 5)
FGE03	5.0	0.467	10.7
FGE03 (sum of a.s.)	2.78	0.246 ^a	11.3
		0.364 ^b	7.64
Boscalid (in BAS 510 01 F; Review Report 2008) ^c	1.197	0.198 ^d	6.05
		0.316 ^e (0.160 ^{ef})	3.79 (7.48)
Boscalid (RAR 2018 LoEP)	12.5	0.198 ^d	63.13
		0.316 ^e	39.56
Boscalid (in FGG01)	10.58	0.198 ^d	53.43
		0.316 ^e	33.48
Prothioconazole (formulated) ^c	0.665	0.048	13.85
M01	50	0.011	4545
M04	0.50	0.034	14.71
Chronic effects on <i>Folsomia</i>			
Product/active substance	NOEC/EC ₁₀ corr. (mg/kg dw soil)	PEC _{soil} (mg/kg dw soil)	TER _{lt} (criterion TER ≥ 5)
FGE03	500	0.467	1070
FGE03 (sum of a.s.)	277.8	0.246 ^a	1129
		0.364 ^b	763
Prothioconazole	32	0.048	666
M01	15.8	0.011	1436
M04	31.25	0.034	919
Chronic effects on <i>Hypoaspis</i>			
Product/active substance	NOEC/EC ₁₀ corr. (mg/kg dw soil)	PEC _{soil} (mg/kg dw soil)	TER _{lt} (criterion TER ≥ 5)
FGE03	500	0.467	1070
FGE03 (sum of a.s.)	277.8	0.246 ^a	1129
		0.364 ^b	763
Prothioconazole ^g	50100	0.048	1041.52083

TER values shown in **bold** fall below the relevant trigger.

- a sum of PEC_{initial} Prothioconazole + $PEC_{\text{accumulation}} (PEC_{\text{act}} + PEC_{\text{soil plateau}})$ Boscalid – FOCUS approach
b sum of PEC_{initial} Prothioconazole + $PEC_{\text{accumulation}} (PEC_{\text{act}} + PEC_{\text{soil plateau}})$ Boscalid – additional approach
c Endpoints from Annex I inclusion for the two actives and corresponding risk assessments are only presented for sake of completeness since studies were performed on the respective representative formulations and are not considered relevant for the assessment of FGE03.
d $PEC_{\text{accumulation}} (PEC_{\text{act}} + PEC_{\text{soil plateau}})$ Boscalid – FOCUS approach
e $PEC_{\text{accumulation}} (PEC_{\text{act}} + PEC_{\text{soil plateau}})$ Boscalid – additional approach
f PEC for single application

- g *Hypoaspis* data on Prothioconazole metabolites is not available from the list of endpoints and not considered required given the high margin of safety of the risk assessment of the parent and the formulation.

For the intended uses in oilseed rape and sunflower, all Tier-1 TER values for earthworms and other non-target soil organisms (meso- and macrofauna) exceed the relevant trigger values indicating no risk for all intended uses except for the Boscalid endpoint from the Annex-I inclusion for the intended multiple application when the additional approach for PEC calculation is considered (no risk for single application and/or considering FOCUS approach). However, endpoints from Annex I inclusion for the two actives and corresponding risk assessments are only presented for sake of completeness since studies were performed on the respective representative formulations and are not considered relevant for the assessment of FGE03. The risk assessment with the FGE03 study endpoint supersedes the assessment for the Boscalid endpoint. Risk is also acceptable when the two additional Boscalid endpoints (a.s. endpoint from the RAR and endpoint from study with FGG01, respectively) are used. As explained under Point 9.8.1.1, together with the FGE03 formulation endpoint, they are potentially more appropriate for the risk assessment for earthworms from exposure to FGE03 according to the intended GAP. Therefore, higher-Tier risk assessment is not required.

zRMS comments:

The risk assessment for soil macro- and meso-fauna has been validated by the zRMS.
In case a.s.- prothioconazole the NOEC value for *Hypoaspis* acc. has been amended by zRMS. according to recommendation given in Technical EFSA GD , December 2015.

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

9.8.2.2 Higher-tier risk assessment

Not relevant.

9.8.3 Overall conclusions

All Tier-1 TER values for earthworms and other non-target soil organisms (meso- and macrofauna) exceed the relevant trigger values indicating no risk for all intended uses except for the Boscalid endpoint from the Annex-I inclusion for the intended multiple application when the additional approach for PEC calculation is considered (no risk for single application and/or considering FOCUS approach). However, endpoints from Annex I inclusion for the two actives and corresponding risk assessments are only presented for sake of completeness since studies were performed on the respective representative formulations and are not considered relevant for the assessment of FGE03. The risk assessment with the FGE03 study endpoint supersedes the assessment for the Boscalid endpoint. Risk is also acceptable when the two additional Boscalid endpoints (a.s. endpoint from the RAR and endpoint from study with FGG01, respectively) are used. These endpoints are potentially more appropriate for the risk assessment for earthworms from exposure to FGE03 according to the intended GAP. Therefore, higher-Tier risk assessment is not required.

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 the formulated products for Annex 1 inclusion and Prothioconazole metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on soil microorganisms of FGE03 were not evaluated as part of the EU assessment of Boscalid and Prothioconazole, respectively. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

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

Endpoint	Substance	Exposure System	Results	Reference
C-mineralisation	BAS 500 01 F (nominal 500 g/kg Boscalid)	28 d	< 25% effects at 12 kg/ha corresponding to 6 kg a.s./ha corresponding to 8 mg a.s./kg d.w. soil	Review report, SANCO/3919 /2007-rev. 5, 21 January 2008 and DAR (2002)
N-mineralisation	BAS 500 01 F (nominal 500 g/kg Boscalid)	28 d	< 25% effects at 12 kg/ha corresponding to 6 kg a.s./ha corresponding to 8 mg a.s./kg d.w. soil	Review report, SANCO/3919 /2007-rev. 5, 21 January 2008 and DAR (2002)
C-mineralisation	Prothioconazole	28 d	< 25% effects at 2 kg/ha corresponding to 2.67 mg/kg d.w. soil	EFSA Scientific Report (2007) 106, 1-98, and DAR (2005)
N-mineralisation	Prothioconazole	28 d	< 25% effects at 2 kg/ha corresponding to 2.67 mg/kg d.w. soil	EFSA Scientific Report (2007) 106, 1-98, and DAR (2005)
N-mineralisation	Prothioconazole-desthio (M04)	28 d	< 25% effects at 1 kg/ha corresponding to 1.33 mg/kg d.w. soil	EFSA Scientific Report (2007) 106, 1-98, and DAR (2005)
C-mineralisation	Prothioconazole-S-methyl (M01)	28 d	< 25% effects at 2 kg/ha corresponding to 2.67 mg/kg d.w. soil	EFSA Scientific Report (2007) 106, 1-98, and DAR (2005)
N-mineralisation	Prothioconazole-S-methyl (M01)	28 d	< 25% effects at 2 kg/ha corresponding to 2.67 mg/kg d.w. soil	EFSA Scientific Report (2007) 106, 1-98, and DAR (2005)
N-mineralisation	Boscalid 350 g/kg + Prothioconazole 200 g/kg WG (35.88% Boscalid w/w, 19.99% Prothioconazole w/w)	28 d	< 25% effects at 10 kg/ha corresponding to 13.33 mg/kg d.w. soil corresponding to 7.45 mg a.s./kg d.w. soil ^a	New endpoint Raithatha (2023) Report No. 608-3-15-33022, UPL/2022/21328 KCP 10.5/01

C-mineralisation endpoints shaded in grey are not used for risk assessment since they are no longer considered relevant.

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

^a sum of active substances

zRMS comments:

Data for soil microorganism for boscalid and prothioconazole and their metabolites provided in Table 9.9-1 are in line with EU agreed endpoints reported in Review report, SANCO/3919 /2007-rev. 5, 21 January 2008 and DAR (2002) and EFSA Scientific Report (2007) 106, respectively.

Information regarding effects on carbon mineralisation is no longer a data requirement and for this reason is struck

through in tables above.

9.9.1.1 Justification for new endpoints

C-mineralisation endpoints from the EU review are no longer considered relevant for risk assessment. New data on N-mineralisation with FGE03 is now available to cover the data requirement for the product. The new endpoint is used in the risk assessment since test design and formulation are considered most relevant for the intended GAP and to account for mixture toxicity. Endpoint from Annex I inclusion for Boscalid and corresponding risk assessment is only presented for sake of completeness since study was performed on the respective representative formulation and is not considered relevant for the assessment of FGE03.

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, and were already used in the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) (see 9.8).

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group oilseed rape (multiple application) covers the risk for soil microorganisms from all intended uses in terms of maximum soil loading (see 9.1.2).

Table 9.9-2: Assessment of the risk for effects on soil micro-organisms due to the use of FGE03 in oilseed rape

Oilseed rape			
Intended use	Oilseed rape		
N-mineralisation			
Product/active substance	Max. conc. with effects ≤ 25 % (mg/kg dw soil)	PEC _{soil} (mg/kg dw soil)	Risk acceptable?
FGE03	13.33	0.373	yes
FGE03 (sum of a.s.)	7.45	0.197 ^a	yes
		0.326 ^b	yes
Boscalid (formulated) ^c	8	0.158 ^d	yes
		0.287 ^e	yes
Prothioconazole	2.67	0.039	yes
M01	2.67	0.009	yes
M04	1.33	0.033	yes

a sum of $PEC_{initial}$ Prothioconazole + $PEC_{accumulation}$ (PEC_{act} + $PEC_{soil\ plateau}$) Boscalid – FOCUS approach

b sum of $PEC_{initial}$ Prothioconazole + $PEC_{accumulation}$ (PEC_{act} + $PEC_{soil\ plateau}$) Boscalid – additional approach

c Endpoint from Annex I inclusion for Boscalid and corresponding risk assessment is only presented for sake of completeness since studies were performed on the respective representative formulation and are not considered relevant for the assessment of FGE03.

d $PEC_{accumulation}$ (PEC_{act} + $PEC_{soil\ plateau}$) Boscalid – FOCUS approach

e $PEC_{accumulation}$ (PEC_{act} + $PEC_{soil\ plateau}$) Boscalid – additional approach

Table 9.9-3: Assessment of the risk for effects on soil micro-organisms due to the use of FGE03 in sunflower

Sunflower			
Intended use	Sunflower		
N-mineralisation			
Product/active substance	Max. conc. with effects ≤ 25 % (mg/kg dw soil)	PEC _{soil} (mg/kg dw soil)	Risk acceptable?
FGE03	13.33	0.467	yes
FGE03 (sum of a.s.)	7.45	0.246 ^a	yes
		0.364 ^b	yes
Boscalid (formulated) ^c	8	0.198 ^d	yes
		0.316 ^e	yes
Prothioconazole	2.67	0.048	yes
M01	2.67	0.0011	yes
M04	1.33	0.034	yes

a sum of PEC_{initial} Prothioconazole + PEC_{accumulation} (PEC_{act} + PEC_{soil plateau}) Boscalid – FOCUS approach

b sum of PEC_{initial} Prothioconazole + PEC_{accumulation} (PEC_{act} + PEC_{soil plateau}) Boscalid – additional approach

c Endpoint from Annex I inclusion for Boscalid and corresponding risk assessment is only presented for sake of completeness since studies were performed on the respective representative formulation and are not considered relevant for the assessment of FGE03.

d PEC_{accumulation} (PEC_{act} + PEC_{soil plateau}) Boscalid – FOCUS approach

e PEC_{accumulation} (PEC_{act} + PEC_{soil plateau}) Boscalid – additional approach

The maximum concentrations with effects on micro-organisms ≤ 25% exceed the respective maximum PEC_{soil} values indicating no risk for all intended uses.

zRMS comments:

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

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

Overall, no unacceptable effects on soil microbial activity are expected following application of FGE03.

9.9.3 Overall conclusions

The maximum concentrations with effects on micro-organisms ≤ 25% exceed the respective maximum PEC_{soil} values indicating no risk for all intended uses.

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

9.10.1 Toxicity data

Studies on the toxicity to non-target terrestrial plants have been carried out with the formulated products for Annex 1 inclusion of the two active substances. Full details of these studies are provided in the respective EU DAR and related documents. However, they are not considered for the present product registration.

Effects on non-target terrestrial plants of FGE03 were not evaluated as part of the EU assessment of Boscalid and Prothioconazole, respectively. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

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

Species	Substance	Exposure System	Results	Reference
6 species	FGE03 (35.22 % Boscalid w/w, 20.33 % Prothioconazole w/w)	21 d Seedling emergence	NOER = 1900 g/ha	New endpoint Stürtz & Horstmann (2023a) Report No. 176531087, UPL/2023/0743 KCP 10.6.2/01
6 species	FGE03 (35.22 % Boscalid w/w, 20.33 % Prothioconazole w/w)	21 d Vegetative vigour	NOER = 1900 g/ha	New endpoint Stürtz & Horstmann (2023b) Report No. 176531086, UPL/2023/0742 KCP 10.6.2/02

m: monocotyledonous; d: dicotyledonous

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

zRMS comments:

Studies on toxicity of FGE03 to non-target terrestrial plants were evaluated by the zRMS and are considered acceptable. For details of evaluation, please refer to Appendix 2. The endpoints reported in Table 9.10-1 are confirmed to be correct.

9.10.1.1 Justification for new endpoints

Endpoints from studies with FGE03 are used for risk assessment since these are considered most relevant for the intended GAP.

9.10.2 Risk assessment

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

Risk is considered acceptable since data on seedling emergence and vegetative vigour from six species do not indicate phytotoxic effects > 50% at the maximum intended application rate. Therefore, a Tier-2 risk assessment based on dose-response data is not required.

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

Not required.

9.10.2.3 Higher-tier risk assessment

Not relevant.

9.10.2.4 Risk mitigation measures

No risk mitigation needed.

9.10.3 Risk assessment

9.10.3.1 Tier-1 risk assessment (based on screening data)

Risk is considered acceptable since data on seedling emergence and vegetative vigour from six species do not indicate phytotoxic effects > 50% at the maximum intended application rate. Therefore, a Tier-2 risk assessment based on dose-response data is not required.

zRMS comments:

zRMS agrees with approach provided by the Applicant.

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

ER₅₀ > 1900 g product/ha from seedling emergence and vegetative vigour tests are above the maximum test rates, covering the maximum single application rate of 1900 g product/ha indicating an acceptable risk to non-target crops.

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

Not required.

9.10.3.3 Higher-tier risk assessment

Not relevant.

9.10.3.4 Risk mitigation measures

No risk mitigation needed.

9.10.4 Overall conclusions

Risk is considered acceptable since data on seedling emergence and vegetative vigour from six species do not indicate phytotoxic effects > 50% at the maximum intended application rate. Therefore, a Tier-2 risk assessment based on dose-response data is not required.

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

Not available and not required.

9.12 Monitoring data (KCP 10.8)

Not available and not required.

9.13 Classification and Labelling

Based on the summation method and classification of Boscalid as Aquatic Chronic 2 and Prothioconazole as Aquatic Acute 1 (M=10) and Aquatic Chronic 1 (M=1), respectively, FGE03 is classified as Aquatic Acute 1, H400, and Aquatic Chronic 2, H411.

The following labelling is than proposed from an ecotoxicological point of view:

Pictogram: GHS09

Signal word: Warning

H-statements:

H410 Very toxic to aquatic life with long lasting effects.

P-statements:

P273 Avoid release to the environment.

P391 Collect spillage.

P501 Dispose of contents/container to hazardous or special waste collection point, in accordance with local/regional/national and/or international regulation.

zRMS comments:

We agree with H400 (acute), H410 (chronic) classification of the product.

Following phrases must be included in the label:

Hazard statement: H410

Signal word: Warning

Pictogram: GHS09

Safety phrases: P391, P501

Hazard pictograms:	GHS09 
Signal word:	Warning
Hazard statement(s):	H410 - Very toxic to aquatic life with long lasting effects
Precautionary statement(s):	P391: Collect spillage P501: Dispose of contents/container to hazardous or special waste collection point, in accordance with local, regional, national and/or international regulation

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.2.1/01	■■■■	2025 ^a	Acute toxicity study of boscalid 350 g/kg + prothioconazole 200 g/kg wg (equivalent to boscalid 35.0% + prothioconazole 20.0% w/w wg) to rainbow trout, <i>oncorhynchus mykiss</i> . Report No. 503-3-07-38626, UPL/2024/1451 ■■■■ GLP Unpublished	Y	UPL Europe
KCP 10.2.1/02	Kamle, M.K.	2023a	Acute Immobilisation Study of Boscalid 350 g/kg + Prothioconazole 200 g/kg WG to <i>Daphnia magna</i> Report No. 502-3-07-33000, UPL/2022/2968 Jai Research Foundation, Valvada, Gujarat, India GLP Unpublished	N	UPL Europe
KCP 10.2.1/03	Kamle, M.K.	2023b	Alga (<i>Pseudokirchneriella subcapitata</i>), Growth Inhibition Test with Boscalid 350 g/kg + Prothioconazole 200 g/kg WG Report No. 501-3-07-32999, UPL/2022/2965 Jai Research Foundation, Valvada, Gujarat, India GLP Unpublished	N	UPL Europe
KCP 10.3.1.1.1/01 & KCP 10.3.1.1.2/01	Knautz, T.	2023	Boscalid 500 g/kg + Prothioconazole 200 g/kg WG: Effects (Acute Contact and Oral) on Honey Bees (<i>Apis mellifera</i> L.) in the Laboratory Report No. 176531035, UPL/2023/1236 ibacon GmbH, Rossdorf, Germany GLP Unpublished	N	UPL Europe
KCP 10.3.1.1.1/02 & KCP 10.3.1.1.2/02	Chwiesko, D. & Horstmann, C.	2023	Boscalid + Prothioconazole (350 g/kg + 200 g/kg WG): Acute Contact and Oral Toxicity to Bumblebees (<i>Bombus terrestris</i> L.) in the Laboratory Report No. 176531105, UPL/2023/1279 ibacon GmbH, Rossdorf, Germany GLP Unpublished	N	UPL Europe
KCP 10.3.1.2/01	Knautz, T. & Horstmann, C.	2023	Boscalid + Prothioconazole (350 g/kg + 200 g/kg WG): Chronic Oral Toxicity Test on the Honey Bee (<i>Apis mellifera</i> L.) in the Laboratory	N	UPL Europe

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
			Report No. 176531136, UPL/2023/1209 ibacon GmbH, Rossdorf, Germany GLP Unpublished		
KCP 10.3.1.3/01	Venturi, S.	2022	Effects of Boscalid + Prothioconazole (350 g/kg + 200 g/kg WG) on honeybees (<i>Apis mellifera</i> L.) 22-day larval toxicity test with repeated exposure Report No. BT073/23, UPL/2023/1235 BioTecnologie BT S.r.l., Todi (PG), Italy GLP Unpublished	N	UPL Europe
KCP 10.3.2.1/01	Leopold, J.	2023a	Boscalid + Prothioconazole (350 g/kg + 200 g/kg WG): Effects on the Parasitoid <i>Aphidius rhopalosiphi</i> (Hymenoptera: Braconidae) in the Laboratory. A Dose Response Test on Glass Plates Report No. 176531001, UPL/2023/1053 ibacon GmbH, Rossdorf, Germany GLP Unpublished	N	UPL Europe
KCP 10.3.2.1/02	Leopold, J.	2023b	Boscalid + Prothioconazole (350 g/kg + 200 g/kg WG): Effects on the Predatory Mite <i>Typhlodromus pyri</i> (Acari: Phytoseiidae) in the Laboratory. A Dose Response Test on Glass Plates Report No. 176531063, UPL/2023/1054 ibacon GmbH, Rossdorf, Germany GLP Unpublished	N	UPL Europe
KCP 10.3.2.2/01	Leopold, J.	2024	Boscalid + Prothioconazole (350 g/kg + 200 g/kg WG): Effects on the Predatory Mite <i>Typhlodromus pyri</i> (Acari: Phytoseiidae), Extended laboratory study - Dose Response Test Report No. 176531062, UPL/2024/0298 ibacon GmbH, Rossdorf, Germany GLP Unpublished	N	UPL Europe
KCP 10.3.2.2/02	Knautz, T.	2024	Boscalid + Prothioconazole (350 g/kg + 200 g/kg WG): Effects on the Carabid Beetle <i>Poecilus cupreus</i> L. - Extended laboratory study - Dose Response Study Report No. 176531007, UPL/2024/0337 ibacon GmbH, Rossdorf, Germany GLP Unpublished	N	UPL Europe

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 10.4.1.1/01	Hübner, S.	2023a	Boscalid + Prothioconazole (350 g/kg + 200 g/kg WG): Effects on Reproduction and Growth of the Earthworm (<i>Eisenia fetida</i>) in Artificial Soil Report No. 176531022, UPL/2023/2769 ibacon GmbH, Rossdorf, Germany GLP Unpublished	N	UPL Europe
KCP 10.4.2.1/01	Hübner, S.	2023b	Boscalid + Prothioconazole (350 g/kg + 200 g/kg WG): Effects on Reproduction of Collembola (<i>Folsomia candida</i>) in Artificial Soil Report No. 176531016, UPL/2023/2772 ibacon GmbH, Rossdorf, Germany GLP Unpublished	N	UPL Europe
KCP 10.4.2.1/02	Hübner, S.	2023c	Boscalid + Prothioconazole (350 g/kg + 200 g/kg WG): Effects on Reproduction of the Predatory Mite <i>Hypoaspis aculeifer</i> in Artificial Soil Report No. 176531089, UPL/2023/1436 ibacon GmbH, Rossdorf, Germany GLP Unpublished	N	UPL Europe
KCP 10.5/01	Raithatha, A.	2023	Effect of Boscalid 350 g/kg + Prothioconazole 200 g/kg WG (GPF515) on Soil Microorganisms: Nitrogen Transformation Test Report No. 608-3-15-33022, UPL/2022/21328 Jai Research Foundation, Valvada, Gujarat, India GLP Unpublished	N	UPL Europe
KCP 10.6.2/01	Stürtz, S.	2023a	Boscalid + Prothioconazole (350 + 200 g/kg – WG): Effects on Terrestrial (Non-Target) Plants: Vegetative Vigour Test Report No. 176531087, UPL/2023/0743 ibacon GmbH, Rossdorf, Germany GLP Unpublished	N	UPL Europe
KCP 10.6.2/02	Stürtz, S.	2023b	Boscalid + Prothioconazole (350 + 200 g/kg – WG): Effects on Terrestrial (Non-Target) Plants: Seedling Emergence and Seedling Growth Report No. 176531086, UPL/2023/0742 ibacon GmbH, Rossdorf, Germany GLP	N	UPL Europe

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
			Unpublished		

List of data submitted by the applicant and not relied on

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

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

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

Appendix 2 Detailed evaluation of the new studies

A 2.1 KCP 10.1 Effects on birds and other terrestrial vertebrates

A 2.1.1 KCP 10.1.1 Effects on birds

A 2.1.1.1 KCP 10.1.1.1 Acute oral toxicity

A 2.1.1.2 KCP 10.1.1.2 Higher tier data on birds

A 2.1.2 KCP 10.1.2 Effects on terrestrial vertebrates other than birds

A 2.1.2.1 KCP 10.1.2.1 Acute oral toxicity to mammals

A 2.1.2.2 KCP 10.1.2.2 Higher tier data on mammals

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

A 2.2 KCP 10.2 Effects on aquatic organisms

A 2.2.1.1 XXXXXXXXXX KCP 10.2.1/01

Comments of zRMS:	The study was conducted in line with OECD 203 (2019) with no minor deviations. Test media was analysed for concentration of both active substances and stability at 0 h and every 24 h renewal during the study and was within the acceptable limit ($\pm 20\%$ of nominal concentration). All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: $LC_{50} = 8.329$ mg product/L
-------------------	---

Reference: KCP 10.2.1/01

Report: Acute toxicity study of boscalid 350 g/kg + prothioconazole 200 g/kg WG (equivalent to boscalid 35.0% + prothioconazole 20.0% w/w wg) to rainbow trout, *Oncorhynchus mykiss*, XXXXXXXXXX, Report no. 502-3-07-338626, UPL/2024/1451

Guideline(s): OCSPP 850.1075 (2016), OECD 203 (2019)

Deviations: No

GLP: Yes

Acceptability: Yes

Executive summary

This study was performed to assess the potential toxic effects of Boscalid 350 g/kg + Prothioconazole 200 g/kg WG to *Oncorhynchus mykiss*, under semi-static conditions.

Based on results of the preliminary range finding studies, concentration levels selected for the main study were 02.7, 4.4, 7.0, 11.3 and 18.0 mg Boscalid 350 g/kg + Prothioconazole 200 g/kg WG/L,

along with control. In the main study, 96-h semi-static toxicity test procedure was followed, comprising six groups. Each group consisted of seven fish per treatment.

The stability test of test item in the test media was performed during the method validation (JRF Study N° 228-2-13-38625). Test item was found to stable in the test media up to 96 h ($\pm 20\%$ of the nominal concentration). Test media was analysed for concentration and stability at 0 and every 24 h renewal during the main study and was within the acceptable limit ($\pm 20\%$ of the nominal concentration).

The highest tested concentration level of Boscalid 350 g/kg + Prothioconazole 200 g/kg WG causing no mortality was 4.4 mg/L and the lowest tested concentration causing mortality within the 96-h test period was 7.0 mg/L. The concentration showed 100% immobility was 18.0 mg/L.

The 24, 48, 72 and 96 h LC_{50} of Boscalid 350 g/kg + Prothioconazole 200 g/kg WG was determined to be 8.329 mg/L.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:** Boscalid 350 g/kg + Prothioconazole 200 g/kg WG (GPF515/FGE03)
Batch no.: BDPT/WG/240607/550/23/PP
Purity: Boscalid: 357.8 g/kg ($35.78 \pm 0.11\%$ w/w)
Prothioconazole: 199.0 g/kg ($19.90 \pm 0.07\%$ w/w)
Date of expiry: 06 June 2026
2. **Test concentrations:** Control: Reconstituted water
Test item: 2.7, 4.4, 7.0, 11.3 and 18.0 mg test item/L
3. **Test organism:**
Species: Rainbow trout (*Oncorhynchus mykiss*)
Source: Kaka Aqua and Agro Associates, Himachal Pradesh, India
Age: The fish were the same age and fish mean length were 5.6 ± 0.18 cm.
Housing: Test vessels of 25 L capacity, filled with 20 L of water (0.6 g wet weight fish/L loading)
Test medium: Reverse Osmosis (RO) water with a total hardness and alkalinity of 63 and 60 mg/L as $CaCO_3$, respectively and conductivity of 85.6 $\mu S/cm$
Feeding: No feeding during exposure
4. **Environmental conditions:**
Dissolved oxygen: 80.1 – 95.5 %
pH: 7.28 – 8.00
Water temperature: 12.1 – 12.6 °C
Water hardness: 63 mg/L as $CaCO_3$
Alkalinity: 60 mg/L as $CaCO_3$
Conductivity: 85.6 $\mu S/cm$
Light: 717 lux, 16 hours light / 8 hours dark

B. STUDY DESIGN AND METHODS

5. **Dates of work:** 06 November 2024 – 03 March 2025

6. **Test organism assignment and treatment**

First instar *Oncorhynchus mykiss* were exposed to the test item at 5 concentrations of 2.7, 4.4, 7.0, 11.3 and 18.0 mg test item/L and a control of water for 96 hours. 42 fish were divided into six groups (25 L beakers with 20 L of test media) were tested.

[illegible]

G4	7.0	7	1(7)	1(7)	2(2), 1(5)	1(5)	1(5)	1(5)	1(5)	1(5)	1(5)
G5	11.3	7	1(7)	1(7)	2(6), 3(1)	3(1)	3(1)	3(1)	3(1)	3(1)	3(1)
G6	18.0	7	1(7)	9(1), 1(6)	2(7)	*	*	*	*	*	*
Key: h = Hour, G = Group, Conc. = Concentration, * = Data are not available due to mortality											
Behavioral Responses: 1 = Normal, 2 = Dead, 3 = Loss of equilibrium, 9 = Lying on the bottom											

Note: Figures presented outside the parentheses refer to the behavioral symptoms and inside the parentheses refer to the total number of fish.

D. ANALYTICAL RESULTS

The stability test of Boscalid 350 g/kg + Prothioconazole 200 g/kg WG (Equivalent to Boscalid 35.0% + Prothioconazole 20.0% w/w WG) in test media was performed during the method validation (JRF Study N° 228-2-13-38625). Boscalid and Prothioconazole in the test media was found stable for up to 96 h ($\pm 20\%$ of the nominal concentration). The test media was analysed for the active ingredient concentration and stability of the test solution at 0 and every 24 h renewal during the main study.

Table A 2.2.1.1-3: Active ingredient concentration and stability of Boscalid in Reconstituted water

Test item concentrations (mg/L)	0 day, 0 h	0 day, 24 h	1 day, 0 h	1 day, 24 h	2 day, 0 h	2 day, 0 h	3 day, 0 h	3 day, 24 h	Mean
	Recovery (%)								
0.0	-					-	-	-	
2.7	100.86	99.10	99.95	109.32	95.06	102.07	92.26	99.87	99.81
4.4	103.58	103.72	103.26	111.87	99.48	104.27	98.94	104.82	103.74
7.0	99.08	98.19	98.30	98.85	93.79	93.55	91.19	98.20	96.39
11.3	100.42	100.41	100.03	96.45	94.43	93.20	94.30	100.64	97.49
18.0	101.81	101.06	NA*	NA*	NA*	NA*	NA*	NA*	101.44

Table A 2.2.1.1-4: Active ingredient concentration and stability of Prothioconazole in Reconstituted water

Test item concentrations (mg/L)	0 day, 0 h	0 day, 24 h	1 day, 0 h	1 day, 24 h	2 day, 0 h	2 day, 24 h	3 day, 0 h	3 day, 24 h	Mean
	Recovery (%)								
0.0	-					-	-	-	
2.7	99.14	100.56	100.08	105.59	99.98	108.82	104.80	95.29	101.78
4.4	100.76	98.99	98.72	101.13	96.42	96.53	94.87	92.41	97.48
7.0	98.97	99.19	97.80	98.60	96.81	101.47	99.67	96.99	98.69
11.3	99.17	98.45	99.23	97.35	96.48	99.13	99.06	88.71	97.2
18.0	99.02	98.99	NA*	NA*	NA*	NA*	NA*	NA*	99.0

E. VALIDITY CRITERIA

The study was considered valid since the following criteria were met:

Table A 2.2.1.1-5: Study validity criteria

Validity Criteria (OECD 203)	Required	Observed
In the control(s), the mortality should not exceed 10% at the end of the exposure.	≤ 10%	0%
Dissolved oxygen concentration	≥ 60 %	80.1%
Analytical measurement of test concentrations should be performed.	-	Yes

Validity Criteria (OCSPP 850.1075)	Required	Observed
All test vessels should be identical	-	Yes, all identical
Treatments should be randomly or indiscriminately assigned to individual test vessel locations or individual test organisms should be randomly or indiscriminately assigned to test vessels.	-	Yes, treatments was randomly assigned
Control (and vehicle (solvent) control, if a vehicle was used) should be included in the test.	-	Yes, control included
Organisms showed signs of disease, stress and/or death	>10%	0%
Fish should not be fed during the test	-	Yes, no fish were fed
A surfactant or dispersant should not be used in the preparation of a stock or test solution.	-	Yes, surfactant and dispersant was not used.

F. TOXICITY ENDPOINTS

The results are presented in table below:

Table A 2.2.1.1-6: LC₅₀ value and the associated 95% confidence limits

Period (hours)	LC ₅₀ value (mg/L)	Confidence limits	
		Lower	Upper
		(mg/L)	(mg/L)
24	8.329	6.427	10.810
48			
72			
96			

The highest tested concentration level of Boscalid 350 g/kg + Prothioconazole 200 g/kg WG causing no mortality was 4.4 mg/L and the lowest tested concentration causing mortality within the 96-h test period was 7.0 mg/L. The concentration showed 100% immobility was 18.0 mg/L.

III. CONCLUSION

The acute exposure of Boscalid 500 WG to the *Oncorhynchus mykiss* has been investigated using a semi-static method for 96 hours. The 24, 48, 72 and 96 h LC₅₀ value of Boscalid 350 g/kg + Prothioconazole 200 g/kg WG were 8.329 mg/L, respectively for immobilization of *Oncorhynchus mykiss*.

Mortality in the control was 0% at the end of the test. No sign of disease or stress, e.g., discolouration or unusual behaviour, such as, trapping at the surface of water was observed in the control group. Dissolved oxygen concentration was $\geq 60\%$ in the control and test vessels, at the end of the test. Thus, the validity criteria were met.

Test media was analysed for concentration and stability at 0 h and every 24 h renewal during the main study and was within the acceptable limit ($\pm 20\%$ of nominal concentration).

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

A 2.2.1.1 Kamle, 2023a (KCP 10.2.1/01)

Comments of zRMS:	The study was conducted in line with OECD 202 with no minor deviations. Boscalid and Prothioconazole in the test media was stable up to 48 h ($\pm 20\%$ of the nominal concentration). Test media was analysed for concentration and stability at 0 and 48 h during the main study and was within the acceptable limit ($\pm 20\%$ of the nominal concentration). Therefore, results were expressed in nominal concentration. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: EC₅₀ = 5.012 mg product/L
-------------------	---

Reference: KCP 10.2.1/01

Report: Acute Immobilisation Study of Boscalid 350 g/kg + Prothioconazole 200 g/kg WG to *Daphnia magna*, Kamle, M.K., 2023, Report no. 502-3-07-33000, UPL/2022/2968

Guideline(s): OCSPP 850.1010 (2016), OECD 202 (2004)

Deviations: No

GLP: Yes

Acceptability: Yes

Executive summary

This study was performed to assess the potential toxic effects of Boscalid 350 g/kg + Prothioconazole 200 g/kg WG to *Daphnia magna*, under static conditions.

Based on results of the preliminary range finding studies, concentration levels selected for the main study were 0.8, 1.8, 3.9, 8.5, 18.7 and 41.2 mg Boscalid 350 g/kg + Prothioconazole 200 g/kg WG/L, along with control. In the main study, 48-h static toxicity test procedure was followed, comprising seven groups. Each group consisted of four replicates with 5 daphnids per replicate.

The stability test of test item in the test media was performed during the method validation (JRF Study N° 228-2-13-33021). Test item was found to stable in the test media up to 48 h ($\pm 20\%$ of the nominal concentration). Test media was analysed for concentration and stability at 0 and 48 h during the main study and was within the acceptable limit ($\pm 20\%$ of the nominal concentration).

The highest tested concentration level of Boscalid 350 g/kg + Prothioconazole 200 g/kg WG causing no immobilisation was 1.8 mg/L and the lowest tested concentration causing immobilisation within the 48-h test period was 3.9 mg/L. The concentration showed 100% immobility was 41.2 mg/L.

The 24 h and 48 h EC_{50} of Boscalid 350 g/kg + Prothioconazole 200 g/kg WG was determined to be 12.794 and 5.012 mg/L.

IV. MATERIALS AND METHODS

G. MATERIALS

- | | |
|---------------------------------|---|
| 11. Test material: | Boscalid 350 g/kg + Prothioconazole 200 g/kg WG (GPF515/FGE03) |
| Batch no.: | BDPT/WG/220819/550/19 |
| Purity: | Boscalid: 358.8 ± 0.7 g/kg ($35.88 \pm 0.07\%$ w/w)
Prothioconazole: 199.9 ± 0.8 g/kg ($19.99 \pm 0.08\%$ w/w) |
| Date of expiry: | 18 August 2024 |
| 12. Test concentrations: | <u>Control:</u> Reconstituted water
<u>Test item:</u> 0.8, 1.8, 3.9, 8.5, 18.7 and 41.2 mg test item/L |
| 13. Toxic reference: | Potassium dichromate |
| CAS No.: | 7778-50-9 |
| Study No.: | JRF 502-3-07-32856 |
| 14. Test organism: | |
| Species: | Daphnids (<i>Daphnia magna</i>) |
| Source: | in-house culture, origin from MicroBioTests Inc., Kleimoer 15, 9030 Mariakerke (Gent), Belgium |
| Age: | Less than 24 hours old, second progeny daphnids |
| Housing: | Test vessels of 250 mL capacity which was loosely covered with transparent polythene to minimize the entry of dust and other particulates into solution |
| Test medium: | Reconstituted water with a total hardness and alkalinity of 210 and 60 mg/L as $CaCO_3$, respectively and conductivity of 356 $\mu S/cm$ |
| Feeding: | No feeding during exposure |

15. Environmental conditions:

Dissolved oxygen:	95.6 – 99.9 %
pH:	7.15 – 7.33
Water temperature:	20.4 – 20.9 °C
Water hardness:	210 mg/L as CaCO ₃
Alkalinity:	60 mg/L as CaCO ₃
Conductivity:	356 µS/cm
Light:	1020 - 1050 lux, 16 hours light / 8 hours dark

H. STUDY DESIGN AND METHODS

16. Dates of work: 28 January – 02 March 2023

17. Test organism assignment and treatment

First instar *Daphnia magna* were exposed to the test item at 6 concentrations of 0.8, 1.8, 3.9, 8.5, 18.7 and 41.2 mg test item/L and a control of reconstituted water for 48 hours. 4 replicates with 5 daphnids per treatment (250 mL beakers with 120 mL of test media) were tested.

18. Dose preparation

Reconstituted water was used as vehicle to dissolve the test item. Six test concentrations of 0.8, 1.8, 3.9, 8.5, 18.7 and 41.2 mg Boscalid 350 g/kg + Prothioconazole 200 g/kg WG/L were prepared. A control was tested in parallel.

19. Measurements and observations

Test daphnids were observed for immobility and abnormal behaviour or appearance, at 0, 3, 24 and 48 h of exposure. Mobility of the daphnids was assessed by gently agitating each test container for 15 seconds and observing their swimming behaviour. Daphnids unable to swim, during the agitation of the test container, were recorded as immobile and removed from the test vessels at the time of observation.

20. Analytical verification

10 mL of test solution from each replicate were collected using a micropipette into 50 mL test tubes. The collected amount was 40 mL for each group at 0 and 48 h. The samples were divided into two portions. One portion, 20 mL of each group was sent to the Department of Chemistry, JRF for active substance concentration analysis and the second portion was stored as back up sample at -20 ± 5 °C until the study completion. Active substance concentration in each sample was determined using the validated analytical method.

21. Statistics

The 24 h and 48 h EC₅₀ values and the associated 95% confidence limits were calculated following the Probit analysis method (Finney, 1971).

V. RESULTS AND DISCUSSION

I. IMMOBILIZATION AND SUB-LETHAL EFFECTS

No immobility daphnids or any unusual signs were observed in the control group throughout the test. The highest tested concentration level of Boscalid 350 g/kg + Prothioconazole 200 g/kg WG causing no immobilisation was 1.8 mg/L and the lowest tested concentration causing 100% immobilisation within the 48h test period was 41.2 mg/L. Detailed data is presented in the table below.

Table A 2.2.1.1-1: Cumulative Immobility Data at 0, 3, 24 and 48 h Exposure Period

Test concentration (mg/L)	Number of Daphnia/group	Immobility of <i>Daphnia</i>								Cumulative immobility at 48 h (%)
		0 h	%	3 h	%	24 h	%	48 h	%	
Control	20	0	0	0	0	0	0	0	0	0
0.8	20	0	0	0	0	0	0	0	0	0
1.8	20	0	0	0	0	0	0	0	0	0
3.9	20	0	0	0	0	0	0	7	35	35
8.5	20	0	0	0	0	7	35	9	45	80
18.7	20	0	0	1	5	11	55	6	30	90
41.2	20	0	0	6	30	13	65	1	5	100

J. ANALYTICAL RESULTS

The stability test of the Boscalid 350 g/kg + Prothioconazole 200 g/kg WG in the test media was performed during the method validation (JRF Study N° 228-2-13-33021). Boscalid and Prothioconazole in the test media was stable up to 48 h ($\pm 20\%$ of the nominal concentration). Test media was analysed for concentration and stability at 0 and 48 h during the main study and was within the acceptable limit ($\pm 20\%$ of the nominal concentration). Therefore, results were expressed in nominal concentration.

Table A 2.2.1.1-2: Active ingredient concentration and stability of Boscalid in Reconstituted water

Concentrations (mg/L)	Boscalid concentration based on purity of test item (µg/L)	0 h		48 h	
		Calculated conc. (µg/L)	Recovery (%)	Calculated conc. (µg/L)	Recovery (%)
0.0	0.00	-	-	-	-
0.8	287.04	307.905	107.27	303.027	105.57
1.8	645.84	669.149	103.61	651.432	100.87
3.9	1399.32	1404.687	100.38	1367.634	97.74
8.5	3049.80	3088.911	101.28	3016.777	98.92
18.7	6709.56	6942.533	103.47	6718.542	100.13
41.2	14782.56	16345.775	110.57	15575.209	105.36

Table A 2.2.1.1-3: Active ingredient concentration and stability of Prothioconazole in Reconstituted water

Concentrations (mg/L)	Prothioconazole concentration based on purity of test item (µg/L)	0 h		48 h	
		Calculated conc. (µg/L)	Recovery (%)	Calculated conc. (µg/L)	Recovery (%)
0.0	0.00	-	-	-	-
0.8	159.92	171.767	107.41	182.496	114.12
1.8	359.82	374.197	104.00	392.182	108.99
3.9	779.61	802.653	102.96	820.691	105.27
8.5	1699.15	1779.989	104.76	1804.653	106.21
18.7	3738.13	3977.607	106.41	4080.678	109.16
41.2	8235.88	9319.225	113.15	9503.779	115.39

K. VALIDITY CRITERIA

The study was considered valid since the following criteria were met:

Table A 2.2.1.1-4: Study validity criteria

Validity Criteria (OECD 202)	Required	Observed
Immobilization in the control	≤ 10%	0%
Dissolved oxygen concentration	≥ 3 mg/L	≥ 8.38 mg/L

Validity Criteria (OCSPP 850.1010)	Required	Observed
All test vessels should be identical	-	Yes, all identical
Treatments should be randomly or indiscriminately assigned to individual test vessel locations or individual test organisms should be randomly or indiscriminately assigned to test vessels.	-	Yes, treatments was randomly assigned
Control (and vehicle (solvent) control, if a vehicle was used) should be included in the test.	-	Yes, control included
Organisms showed signs of disease, stress and/or death	>10%	0%
Daphnids should not be fed during the test	-	Yes, no daphnids were fed
A surfactant or dispersant should not be used in the preparation of a stock or test solution.	-	Yes, surfactant and dispersant was not used.

L. TOXICITY ENDPOINTS

The results are presented in table below:

Table A 2.2.1.1-5: EC₅₀ value and the associated 95% confidence limits

Period (hours)	EC ₅₀ value (mg/L)	Confidence limits	
		Lower	Upper
		(mg/L)	(mg/L)
24	12.794	9.247	17.701
48	5.012	3.289	7.638

The highest tested concentration level of Boscalid 350 g/kg + Prothioconazole 200 g/kg WG causing no immobilisation was 1.8 mg/L and the lowest tested concentration causing 100% immobilisation within the 48h test period was 41.2 mg/L.

VI. CONCLUSION

The acute immobilization of Boscalid 500 WG to the *Daphnia magna* has been investigated using a static method for 48 hours. The 24 h and 48 h EC₅₀ value of Boscalid 350 g/kg + Prothioconazole 200 g/kg WG were 12.794 and 5.012 mg/L, respectively for immobilization of *Daphnia magna*.

Immobilisation in the control was 0% at the end of the test. No sign of disease or stress, e.g., discolouration or unusual behaviour, such as, trapping at the surface of water was observed in the control group. Dissolved oxygen concentration was ≥8.38 mg/L in the control and test vessels, at the end of the test. Thus, the validity criteria were met.

Test media was analysed for concentration and stability at 0 and 48 h during the main study and was within the acceptable limit (±20% of nominal concentration).

A 2.2.1.2 Kamle, 2023b (KCP 10.2.1/02)

Comments of zRMS:	The study was conducted in line with OECD with following deviations: -As per OCSPP 850.4500, for microscopic counting during the main study, two samples should be taken from each test vessel and two counts should be made of each sample, but for this study cells were counted as per OECD 201, one cell count were made per replicate.
-------------------	--

	-As per OCSPP 850.4500, illumination should be maintained between 4300 lux, but in this study it was maintained as per OECD 201 between 4440 to 8880 lux. As per OCSPP 850.4500, for main study, there should be four replicates for treatment groups and control group(s) but in this study, replicates were used as per OECD 201, i.e. three replicates for treatment group(s) and six replicates for control group(s) and for limit test, six replicates for treatment and control group(s). The test item in the test media was stable up to 96 h ($\pm 20\%$ of nominal concentration). The test media was analysed for active ingredient concentration and stability at 0, 72, and 96 h, during the main study and was within the acceptable limit ($\pm 20\%$ of nominal concentration). All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: 96 h ErC50 = 6.353 mg/L nom
--	---

Reference:	KCP 10.2.1/02
Report:	Alga (<i>Pseudokirchneriella subcapitata</i>), Growth Inhibition Test with Boscalid 350 g/kg + Prothioconazole 200 g/kg WG, Kamle, M.K., 2023b, Study code 501-3-07-32999, UPL/2022/2965
Guideline(s):	OECD Test Guideline 201, 'Freshwater Alga and Cyanobacteria, Growth Inhibition Test' adopted March 23, 2006, Annex 5, corrected July 28, 2011. EPC Ecological Effects Test Guidelines, OCSPP 850.4500, "Algal Toxicity", EPA 712-C-006, January 2012
Deviations:	As per OCSPP 850.4500, for microscopic counting during the main study, two samples should be taken from each test vessel and two counts should be made of each sample, but for this study cells were counted as per OECD 201, one cell count were made per replicate. As per OCSPP 850.4500, illumination should be maintained between 4300 lux, but in this study it was maintained as per OECD 201 between 4440 to 8880 lux. As per OCSPP 850.4500, for main study, there should be four replicates for treatment groups and control group(s) but in this study, replicates were used as per OECD 201, i.e. three replicates for treatment group(s) and six replicates for control group(s) and for limit test, six replicates for treatment and control group(s).
GLP:	Yes
Acceptability:	Yes

Executive summary

This study was performed to assess the inhibitory effect of Boscalid 350 g/kg + Prothioconazole 200 g/kg WG on the growth of alga *Pseudokirchneriella subcapitata* under static conditions.

Exponentially growing cultures of *P. subcapitata* were exposed to the test concentration levels of 0.0 (control), 0.6, 1.3, 2.5, 5.0, and 10.0 mg Boscalid 350 g/kg + Prothioconazole 200 g/kg WG/L. Algal cultures were assessed for the growth by visual cell count at 24 h, 48 h, 72 h, and 96 h.

Recovery phase was completed for the control and the highest test concentration (10.0 mg/L) on day 6, as the normal growth was observed for the control as the well as the treatment group at 10.0 mg/L. This indicates an algistatic effect of Boscalid 350 g/kg + Prothioconazole 200 g/kg WG at the test concentration 10.0 mg/L on *Pseudokirchneriella subcapitata*.

The stability test of Boscalid 350 g/kg + Prothioconazole 200 g/kg WG in the test media was performed during the method validation study (JRF Study N° 228-2-13-33021), Boscalid 350 g/kg + Prothioconazole 200 g/kg WG in the test media was stable up to 96 h ($\pm 20\%$ of nominal concentration). The test media was analysed for active ingredient concentration and stability of Boscalid 350 g/kg + Prothioconazole 200 g/kg WG at 0, 72, and 96 h, during the main study. The active ingredients concentration of Boscalid 350 g/kg + Prothioconazole 200 g/kg WG in the test media was within the acceptable limit ($\pm 20\%$ of nominal concentration).

A concentration-effect relationship was observed and statistically analysed to obtain effect concentrations at 72 and 96 h were as follows:

Nominal concentration (mg/L)						
Endpoint	NOEC	LOEC	IC ₀₅	IC ₁₀	IC ₂₀	IC ₅₀
0-72 h Growth Rate (95% CI)	0.6	1.3	1.866 (1.227 – 2.838)	2.472 (1.758 – 3.475)	3.475 (2.661 – 4.539)	6.653 (5.129 – 8.630)
0-72 h Biomass (95% CI)			1.102 (0.649 – 1.871)	1.371 (0.873 – 2.153)	1.786 (1.236 – 2.582)	2.972 (2.198 – 4.018)
0-72 h Yield (95% CI)			1.191 (0.667 – 2.128)	1.476 (0.902 – 2.415)	1.910 (1.276 – 2.858)	3.126 (2.259 – 4.325)
0-96 h Growth Rate (95% CI)	1.3	2.5	1.600 (1.211 – 2.113)	2.168 (1.734 – 2.710)	3.141 (2.661 – 3.707)	6.353 (5.534 – 7.295)
0-96 h Biomass (95% CI)	0.6	1.3	1.072 (0.916 – 1.253)	1.300 (1.135 – 1.489)	1.644 (1.472 – 1.837)	2.570 (2.360 – 2.799)
0-96 h Yield (95% CI)			1.084 (0.830 – 1.416)	1.288 (1.023 – 1.622)	1.589 (1.312 – 1.923)	2.377 (2.061 – 2.742)

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material** (GPF515/FGE03)

Boscalid	350 g/kg	+	Prothioconazole	200 g/kg	WG
Batch no.:	BDPT/WG/220819/550/19				
Purity:	Boscalid: 358.8 ± 0.7 g/kg ($35.88 \pm 0.07\%$ w/w) Prothioconazole: 199.9 ± 0.8 g/kg ($19.99 \pm 0.08\%$ w/w)				
Date of expiry:	18 August 2024				
2. **Test concentrations:**

<u>Control:</u>	0.0 mg/L
<u>Test item:</u>	0.6, 1.3, 2.5, 5.0 and 10.0 mg/L
3. **Toxic reference:** Potassium dichromate

CAS No.:	7778-50-9
Study No.:	501-3-07-32855 (separate study)
4. **Test organism:**

Species:	<i>Pseudokirchneriella subcapitata</i> , strain ATCC 22662
Source:	Origin culture obtained from American Type Culture Collection, (ATCC), Manassas, USA; Alga subculture maintained at Jai Research Foundation, Ecotoxicology Section, Valvada, District Valsad, Gujarat under static condition. Pre-culture was prepared prior to commencement of study at temperature between 22°C – 23°C.
Test duration:	96 hours
Test chambers:	250 mL capacity sterile conical flasks
5. **Environmental conditions**

Temperature:	22°C – 23°C
Test medium:	22.1°C – 22.2°C
pH:	7.11 – 7.73
Light:	6720 to 6820 lux, continuous illumination

B. STUDY DESIGN AND METHODS

6. **Dates of work:** 27 January – 09 March 2023
7. **Test organism assignment and treatment**

The fresh water, unicellular green alga *Pseudokirchneriella subcapitata* was exposed to the test item at five concentrations from 0.6 to 10.0 mg/L and a control for 96 hours with three replicates (sterile conical flasks of 250 mL volume with 100 mL test medium) per test concentration and six replicates for control. A static test was chosen. At the end of the main study, determination of algistatic and algicidal effects was performed with the following method. Aliquots of 0.5 mL from each replicate of G1 and G6 were taken and mixed. These aliquots of 1.5 mL were transferred into a new test vessel with 118.5 mL of nutrient medium and final volume was made up to 120 mL. These cultures were incubated under the environmental conditions used in the main study for a period of 6 Days and observed for algal growth on Day 0, 2, 4 and 6 as the growth occurred by a factor of more than 100 times. Potassium dichromate is recommended as a positive control in a separate study.

8. Dose preparation

Five test concentrations of 0.6, 1.3, 2.5, 5.0 and 10.0 mg test item /L were prepared in 1X AAP media as vehicle. A negative control without test item was tested in parallel. The test solutions were analysed for active ingredient and stability analysis using the validated analytical method. For the main study, three replicates for treatment and six replicates for control group were prepared, respectively. 10 mL of test samples from each replicate were drawn at 0, 72, and 96 h interval and

mixed together for each group for analytical verification.

9. Measurements and observations

Cell count and cell appearance observation were made at 24 hours interval up to 96 hours by manual cell count using a haemocytometer and a microscope. During recovery phase, the cell concentration of control and highest group were determined once at Day 2, Day 4 and Day 6. Microscopic observation was performed to verify the normal and healthy appearance of the inoculum culture and to observe and record any abnormal appearance of the algae.

10. Analytical verification

The stability test of Boscalid 350 g/kg + Prothioconazole 200 g/kg WG in the test media was performed during the method validation study (JRF Study N° 228-2-13-33021) and the test item in the test media was stable up to 96 h ($\pm 20\%$ of nominal concentration). The test media was analysed for active ingredient concentration and stability at 0, 72, and 96 h, during the main study and was within the acceptable limit ($\pm 20\%$ of nominal concentration).

11. Statistics

NOEC and LOEC were determined by a statistical procedure for multi sample comparison. Data of biomass, specific growth rate and yield were subjected to Brown-Forsythe test to meet the homogeneity of variance before conducting Analysis of Variance (ANOVA) and Dunnett's Test through in-house developed, validated computer software.

The IC05, IC10, IC20, and IC50 were calculated using the Probit analysis method (Finney, 1971) for 72 h and 96 h.

II. RESULTS AND DISCUSSION

A. GROWTH AND YIELD

In the microscopic observation, the inoculum culture appeared normal and healthy. No size, shape, and color difference were observed in any test concentration when compared to control during preliminary range finding study as well as in the main study.

The cell concentration in the control cultures was increased by a factor of 138.4 times within the three-day test period. In treated groups, biomass decreased with increased concentration of test item, during the 72-h exposure period.

At 72 h, statistically no significant difference in the mean of biomass, growth rate and yield, were observed at concentration 0.6 mg/L, while statistically significant decrease was observed at concentrations 1.3, 2.5, 5.0 and 10.0 mg/L.

The cell concentration in the control cultures was increased by a factor of 705.6 times within the four day test period. In treated groups, biomass was decreased with increased concentration of test item, during the 96-h exposure period.

At 96 h, statistically no significant difference in the mean of biomass and yield was observed at concentration 0.6 mg/L, while statistically significant decrease was observed at concentrations 1.3, 2.5, 5.0 and 10.0 mg/L. Hence based on statistically significant effect, NOEC and LOEC were determined as 1.3 and 2.5, respectively for growth rate, and 0.6 and 1.3 mg/L, respectively for biomass and yield.

Details are presented in the tables below.

Table A 2.2.1.2-1: Percentage inhibition of biomass, growth rate and yield

Concentration (mg/L)	0.0	0.6	1.3	2.5	5.0	10.0
Inhibition of biomass (%) at 0-72 h	0.0	0.63	16.76	18.02	88.58	98.73
Inhibition of biomass (%) at 0-96 h	0.0	0.23	9.27	48.66	91.45	98.71

Inhibition of growth rate (%) at 0-72 h	0.0	0.15	3.50	2.77	45.69	66.13
Inhibition of growth rate (%) at 0-96 h	0.0	0.00	0.88	15.67	39.24	69.11
Inhibition of yield (%) at 0-72 h	0.0	0.29	15.69	12.84	90.11	96.85
Inhibition of yield (%) at 0-96 h	0.0	0.11	5.90	64.31	92.52	99.06

B. ANALYTICAL RESULTS

The stability test of Boscalid 350 g/kg + Prothioconazole 200 g/kg WG in the test media was performed during the method validation study (JRF Study N° 228-2-13-33021) and the test item in the test media was stable up to 96 h ($\pm 20\%$ of nominal concentration). The test media was analysed for active ingredient concentration and stability at 0, 72, and 96 h, during the main study and was within the acceptable limit ($\pm 20\%$ of nominal concentration).

Table A 2.2.1.2-2: Active ingredients Concentration and Stability - Boscalid

Concentrations (mg/L)	Boscalid concentration based on purity of test item (µg/L)	0 day, 0 h		0 day, 72 h		0 day, 96 h	
		Calculated conc. (µg/L)	Recovery (%)	Calculated conc. (µg/L)	Recovery (%)	Calculated conc. (µg/L)	Recovery (%)
0.0	0.00	-	-	-	-	-	-
0.6	215.28	205.128	95.28	203.581	94.57	203.472	94.52
1.3	466.44	465.083	99.71	468.895	100.53	460.724	98.77
2.5	897.00	874.234	97.46	873.514	97.38	868.063	96.77
5.0	1794.00	1794.216	100.01	1787.168	99.62	1778.336	99.13
10.0	3588.00	3593.151	100.14	3594.345	100.18	3551.853	98.99

Table A 2.2.1.2-3: Active ingredients Concentration and Stability - Prothioconazole

Concentrations (mg/L)	Prothioconazole concentration based on purity of test item (µg/L)	0 day, 0 h		0 day, 72 h		0 day, 96 h	
		Calculated conc. (µg/L)	Recovery (%)	Calculated conc. (µg/L)	Recovery (%)	Calculated conc. (µg/L)	Recovery (%)
0.0	0.00	-	-	-	-	-	-
0.6	119.94	121.555	101.35	120.066	100.11	118.989	99.21
1.3	259.87	272.223	104.75	257.893	99.24	257.837	99.22
2.5	499.75	522.139	104.48	507.280	101.51	503.385	100.73
5.0	999.50	1057.705	105.82	1066.840	106.74	1042.560	104.31
10.0	1999.00	2164.770	108.29	2165.287	108.32	2179.129	109.01

C. VALIDITY CRITERIA

The study was considered valid since validity criteria were met:

Table A 2.2.1.2-4: Study validity criteria

Validity Criteria OECD 201	Required	Observed
Biomass increase at 72 h in the control within three day test	At least 16 times	138.4 factor times
Mean coefficient of variation for section-by-section growth rate for the control cultures over the test period (for 72h)	$\leq 35\%$	2.94%
Coefficient of variation of average specific growth rate in the control (for 72h)	$\leq 7\%$	0.00%
Validity Criteria OCSPP 850.4500		

All test vessels and closures should be identical	-	Yes, all identical
Treatments should be assigned randomly to test vessels, and test vessels should be assigned randomly to positions in the incubator.	-	Yes, randomly assigned
An untreated control should be included in the test	-	Yes, control is included
The concentration of solvent in the range used should not affect growth of the test species	-	Yes, no solvent was used.
Cell Density increase in Control Cultures (for 96h)	At least 100 times	705.6 times
Coefficient of variation of average yield and growth rate control	≤ 15%	0.52% for yield and 0.00% average specific growth rate
Test concentrations used in definitive test	≥ 5	5 test concentrations
Control should not be contaminated with the test item	-	Yes, not contaminated
Lowest test concentration level should be less than the 96-hour yield, averagespecific growth rate and area under curve IC ₅₀ values based on cell density	-	Yes
Temperature and light intensity recorded during the test	-	Yes

D. TOXICITY ENDPOINTS

The results based on nominal test concentrations are presented in the following Table.

Table A 2.2.1.2-5: Effective concentrations based on inhibition of biomass, inhibition of growth rate and inhibition of yield during 72- and 96-hours exposure of Boscalid 350 g/kg + Prothioconazole 200 g/kg WG to *Pseudokirchneriella subcapitata*

Response variable based on % inhibition (0-72 h)		Effective concentration (mg/L)	95% confidence limits		NOEC	LOEC
			Lower limit	Upper limit		
			(mg/L)	(mg/L)		
Biomass	I _b C ₀₅	1.102	0.649	1.871	0.6	1.3
	I _b C ₁₀	1.371	0.873	2.153		
	I _b C ₂₀	1.786	1.236	2.582		
	I _b C ₅₀	2.972	2.198	4.018		
Growth rate	I _r C ₀₅	1.866	1.227	2.838	0.6	1.3
	I _r C ₁₀	2.472	1.758	3.475		
	I _r C ₂₀	3.475	2.661	4.539		
	I _r C ₅₀	6.653	5.129	8.630		
Yield	I _y C ₀₅	1.191	0.667	2.128	0.6	1.3
	I _y C ₁₀	1.476	0.902	2.415		
	I _y C ₂₀	1.910	1.276	2.858		
	I _y C ₅₀	3.126	2.259	4.325		

Response variable based on % inhibition (0-96 h)		Effective concentration (mg/L)	95% confidence limits		NOEC	LOEC
			Lower limit	Upper limit		
			(mg/L)	(mg/L)		
Biomass	I _b C ₀₅	1.072	0.916	1.253	0.6	1.3
	I _b C ₁₀	1.300	1.135	1.489		
	I _b C ₂₀	1.644	1.472	1.837		
	I _b C ₅₀	2.570	2.360	2.799		
Growth rate	I _r C ₀₅	1.600	1.211	2.113	1.3	2.5
	I _r C ₁₀	2.168	1.734	2.710		
	I _r C ₂₀	3.141	2.661	3.707		
	I _r C ₅₀	6.353	5.534	7.295		
Yield	I _y C ₀₅	1.084	0.830	1.416	0.6	1.3
	I _y C ₁₀	1.288	1.023	1.622		
	I _y C ₂₀	1.589	1.312	1.923		
	I _y C ₅₀	2.377	2.061	2.742		

NOEC: No observed effect concentration was 0.6 mg/L of test item at 72 h and at 96 h for inhibition of biomass, growth rate and yield, except inhibition of growth rate at 96 h was 1.3 mg/L

LOEC: Lowest observed effect concentration was 1.3 mg/L of test item at 72 h and at 96 h for inhibition of biomass, growth rate and yield except, inhibition of growth rate at 96 h was 2.5 mg/L.

III. CONCLUSION

The results of the study show that Boscalid 350 g/kg + Prothioconazole 200 g/kg WG at tested concentrations has inhibitory effects on the growth rate and yield of *Pseudokirchneriella subcapitata* during the 72- and 96- hours exposure period. The validity criteria according to the OECD guideline 201 have been met in this study. The 72 hours and 96-hours Biomass IC₅₀ were 2.972 mg/L and 2.570 mg/L, respectively. The 72 hours and 96 hours Yield IC₅₀ were 3.126 mg/L and 2.377 mg/L, respectively. The 72 hours and 96 hours Growth Rate IC₅₀ were 6.653 mg/L and 6.353 mg/L, respectively. No observed effect concentration (NOEC) is 0.6 mg/L of test item for 72 h inhibition of biomass, growth rate and yield, 96 h inhibition of biomass and yield; Lowest observed effect concentration (LOEC) is 1.3 mg/L of test item for inhibition of biomass, growth rate and yield, 96 h inhibition of biomass and yield. NOEC and LOEC for 96 h inhibition of growth rate were 1.3 and 2.5 mg/L, respectively. Based on the result of recovery phase, it can be concluded that test item at test concentration 10.0 mg/L has algistatic effect on *Pseudokirchneriella subcapitata*.

All criteria were met. The stability test of Boscalid 350 g/kg + Prothioconazole 200 g/kg WG in the test media was performed during the method validation study (JRF Study N° 228-2-13-33021) and the test item in the test media was stable up to 96 h ($\pm 20\%$ of nominal concentration). The test media was analysed for active ingredient concentration and stability at 0, 72, and 96 h, during the main study and was within the acceptable limit ($\pm 20\%$ of nominal concentration).

A 2.2.2	KCP 10.2.2	Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms
A 2.2.3	KCP 10.2.3	Further testing on aquatic organisms
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 2.3.1.1.1.1	Knautz, 2023 (KCP 10.3.1.1.1/01)	

Comments of zRMS:	The study was conducted in line with OECD 213 with minor deviations. A 5 µL droplet was chosen in deviation to the guideline recommendation of a 1 µL droplet, since a higher volume ensured a more reliable dispersion of the test item; laboratory experience has proven that higher volumes are suitable and no adverse effects on the outcome of the study are to be expected. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: The contact LD₅₀ (48 h) > 300 µg product/bee. The oral LD₅₀ (48 h) = 335.1 µg product/bee.
-------------------	---

Reference: KCP 10.3.1.1.1/01

Report:	Boscalid 350 g/kg + Prothioconazole 200 g/kg WG: Effects (Acute Contact and Oral) on Honey Bees (<i>Apis mellifera</i> L.) in the Laboratory, Knautz, T., 2023, Study code 176531035, UPL/2023/1236
Guideline(s):	OECD 213: 'Honeybees, Acute Oral Toxicity Test', adopted on September 21, 1998 OECD 214: 'Honeybees, Acute Contact Toxicity Test', adopted on September 21, 1998
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Executive summary

An acute toxicity test for the effects of test item, FGE03 (Boscalid 350 g/kg + Prothioconazole 200 g/kg WG) on honeybee, *Apis mellifera* was performed following OECD Guideline No 213 and 214 (1998).

Acute toxicity test was conducted as limit test with a nominal dose of 300 µg product/bee along with a control group using pure sucrose solution 50% w/v (oral test) and water with 0.1% Triton x-100 (contact test). For oral test, based on the diet consumed, the actual dose consumed was determined as 335.1 µg product/bee.

The acute oral and contact test treated groups and control groups consisted of 5 replicates, each replicate containing 10 bees per cage. The food was provided as syringes and weighed before and after the feeding duration. The bees were kept in the dark with temperature between 25°C to 27°C and relative humidity at 61 - 68 % during the test period of 48 hours with *ad libitum* sucrose solution 50% w/v (after end of dosing). Observations for mortality and abnormal behaviour were made at 4 hours after start of dosing and at 24 hours and 48 hours after end of dosing.

The oral toxicity test showed 2.0% mortality in control but no mortality in the actual consumed dose of 335.1 µg product/bee (target oral dose 300 µg product/bee) during the 48 hours test period. No abnormal behaviour was observed in test dose during the test period.

The validation test was performed using Dimethoate as positive control. The bees were treated with Dimethoate at the doses of 0.34, 0.17, 0.08 and 0.06 µg a.i./bee based on the actual consumed dose. This positive control test was conducted with 10 bees and 3 replicates. The mortalities observed were 100.0 % at 24 hours and 48 hours after end of dosing. Abnormal behaviours were observed in the actual consumed dose of 0.17 and 0.34 µg a.i./bee.

The contact toxicity test showed 8.0% mortality in control but no mortality in the test dose of 300 µg product/bee during the 48 hours test period. No abnormal behaviour was observed in test dose during the test period.

The bees were treated with Dimethoate at the doses of 0.10, 0.15, 0.20 and 0.30 µg a.i./for positive control. This positive control test was conducted with 10 bees and 3 replicates. The mortalities observed were 53.3% and 66.7% at 24 hours and 48 hours after end of dosing, respectively. Abnormal behaviours were observed in the control.

The oral and contact LD₅₀ value of the positive control was determined statistically by Trimmed Spearman-Kärber Procedure and probit analysis, respectively. The 95% confidence limits were calculated. From the mortality results of validation test and based on the actual consumed dose, the 48 hours oral LD₅₀ of Dimethoate was 0.13 µg a.i./bee with 95% confidence limits between 0.12 and 0.14 µg a.i./bee. The 48 hours contact LD₅₀ of Dimethoate was 0.24 µg a.i./bee with 95% confidence limits between 0.21 and 0.28 µg a.i./bee. The result were within the validity criteria as required by OECD No. 213 and 214 guideline.

The contact LD50 value (24 and 48 h) of FGE03 were determined to be > 300 µg product/bee. The oral LD50 value (24 and 48 h) were determined to be > 335.1 µg product/bee. The contact and oral NOED values (24 and 48 h) were ≥ 300 and ≥ 335.1 µg product/bee, respectively.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material**
Batch no.: Boscalid 350 g/kg + Prothioconazole 200 g/kg WG (FGE03)
Purity: BDPT/WG/220915/550/20
Boscalid: 352.2 ± 0.2 g/kg (35.22 ± 0.02 % w/w)
Prothioconazole: 203.3 ± 0.1 g/kg (20.33 ± 0.01 % w/w)
Date of expiry: September 14, 2024
2. **Test concentrations:** Control: 50% w/v aqueous sucrose solution (oral);
tap water with 0.1 % Triton X-100 (contact)
Test item: 300 µg product/bee target dose for both oral and contact test, 335.1 µg product/bee as actual consumed dose for oral test
Reference item:
Oral test: 0.05, 0.08, 0.15 and 0.30 µg dimethoate/bee, actual consumed dose, 0.06, 0.08, 0.17 and 0.34 µg dimethoate/bee
Contact test 0.10, 0.15, 0.20 and 0.30 µg dimethoate/bee
3. **Toxic reference**
Batch no.: Dimethoate 400 g/L (DANADIM PROGRESS)
Purity: 10214034
413 g/L (41.3% w/v)
4. **Test organism:**
Species: Worker honeybees *Apis mellifera* L.
Source: Honeybee colonies, disease-free and queen-right, bred by test facility.
Age: Adult female worker bees
Housing: Stainless steel chambers with size ca. 8.2 cm × 5.9 cm × 4.2 cm with removable glass sheet in front and lined with filter paper in the inner walls. 10 bees/chamber
Feeding: 50% sucrose solution *ad libitum*. The feeding solution was provided in syringes that were inserted into the cages via an opening in the top of the test units and from which bees accessed the food directly.
5. **Environmental conditions**
Temperature: 25°C – 27°C
Relative humidity: 61 – 68 %
Light: 24 hours dark, except during observations

B. STUDY DESIGN AND METHODS

1. **Dates of work:** 19-22 June 2023
2. **Test organism assignment and treatment**

All bees used in the test derived from healthy, disease free, queen-right bee colonies and bred by test facility. Bees were collected with plastic tubes from the outer honeycombs without the use of smoke and without anesthetics. 10 bees were randomly allocated to each labelled stainless-steel chambers, lined with filter paper in inner walls and removable glass sheet at the front. The chambers were placed under dark conditions in environmental test chambers.

Oral test application:

The test item and reference item were applied in 50 % w/v sucrose solution, which was used as carrier (food) in the oral test. For the control pure 50 % w/v sucrose solution was offered to the bees.

The treated food was offered in syringes, which were weighed before and after introduction into the cages (duration of uptake was 3 hours 40 minutes for the test item treatment). After 3 hours 40 minutes, the syringes containing the treated food were removed, weighed and replaced by ones containing fresh, untreated food.

The mean target dose levels (e.g. 300.0 µg product/bee nominal) would have been obtained if exactly 20 mg/bee of the treated food were ingested. In practice, uptake of the treated sugar solutions differed slightly from the nominal 20 mg/bee and results are given based on the measured consumption.

Contact test application:

A single 5 µL droplet of FGE03 in an appropriate carrier (tap water + 0.1 % Triton-X 100*) was placed on the dorsal bee thorax using a calibrated pipette (Multipette©, Eppendorf).

For the control one 5 µL droplet of tap water containing 0.1 % Triton-X 100* was used. The reference item was also applied in 5 µL tap water (dimethoate made up in tap water containing 0.1 % Triton-X 100*).

A 5 µL droplet was chosen in deviation to the guideline recommendation of a 1 µL droplet, since a higher volume ensured a more reliable dispersion of the test item; ibacon experience has proven that higher volumes are suitable and no adverse effects on the outcome of the study are to be expected.

3. Dose preparation

The limit test for both oral and contact test were conducted with dose of 300 µg a.i./bee.

For oral test, the test solution was applied in sucrose solution 50% w/v, the actual consumed dose level was 335.1 µg product/bee. A positive control test was conducted at 0.05, 0.08, 0.15 and 0.30 µg dimethoate/bee, actual consumption dose were 0.06, 0.08, 0.17 and 0.34 µg dimethoate/bee. The positive control solutions containing dimethoate were prepared in sucrose solution 50% w/v.

For contact test, the test solution was applied with tap water containing 0.1% Triton X-100. A positive control test was conducted at 0.10, 0.15, 0.20 and 0.30 µg dimethoate/bee. The positive control solutions containing dimethoate were prepared in tap water containing 0.1% Triton X-100.

4. Measurements and observations

Mortality was measured with the number of dead bees after 4 (± 0.5) hours (first day); 24 and 48 (± 2) hours.

Behavioral abnormalities were assessed after 4 (± 0.5) hours (first day); 24 and 48 (± 2) hours.

Sub-lethal effects such as symptoms of poisoning or any abnormal behaviour in comparison to the control.

5. Statistics

As no test item treatment group showed mortality above 10.0 %, no statistical evaluation on the LD_x has been carried out.

The contact and oral NOED values of the test item were determined using Fisher's Exact Binomial Test with pairwise comparison (one-sided greater, $\alpha = 0.05$).

The contact and oral LD50 values of the reference item were determined according to Probit Analysis and Trimmed Spearman-Kärber Procedure, respectively).

The software used to perform the statistical analysis was ToxRat Professional, Version 3.2.1, ® Tox-Rat Solutions GmbH.

II. RESULTS AND DISCUSSION

A. FOOD CONSUMPTION AND MORTALITY

The mean feed consumption and mortality after exposure of bees to 300 µg Boscalid 350 g/kg + Prothioconazole 200 g/kg WG per bee and the positive control with dimethoate are presented in the table below.

No mortality occurred.

Table A 2.3.1.1.1-1: Food consumption and mortality of honeybees treated with control and test item FGE03 (Boscalid 350 g/kg + Prothioconazole 200 g/kg WG) in oral toxicity test

Treatment code	Target dose	Actual consumed dose	Mortality mean (%)			Behavioural abnormalities mean (%)		
			4 h	24 h	48 h	4 h	24 h	48 h
Water control	0.0	-	2.0	2.0	2.0	0.0	0.0	0.0
Test item µg product/bee	300	335.1	0.0	0.0	0.0	0.0	0.0	0.0
Reference item µg a.i./bee	0.34	0.34	6.7	100.0	100.0	30.0	0.0	0.0
	0.17	0.17	0.0	86.7	90.0	0.0	13.3	10.0
	0.08	0.08	0.0	0.0	0.0	0.0	0.0	0.0
	0.06	0.06	3.3	3.3	3.3	0.0	0.0	0.0

In the positive control performed with dimethoate, mortalities between 86.7% and 100.0 % were observed at 24 hours and 48 hours after end of dosing, respectively. There was no mortality and behavioural abnormalities for the test item at test end.

Table A 2.3.1.1.1-2: Mortality and behavioural abnormalities of honeybees treated with control and test item FGE03 (Boscalid 350 g/kg + Prothioconazole 200 g/kg WG) in contact toxicity test

Treatment code	Exposed dose	Mortality mean (%)			Behavioural abnormalities mean (%)		
		4 h	24 h	48 h	4 h	24 h	48 h
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Test item (µg product/bee)	300	0.0	6.0	8.0	8.0	2.0	0.0
Reference item (µg a.i./bee)	0.30	0.0	53.3	66.7	0.0	0.0	0.0
	0.20	0.0	26.7	40.0	0.0	0.0	0.0
	0.15	0.0	6.7	16.7	0.0	0.0	0.0
	0.10	0.0	0.0	0.0	0.0	0.0	0.0

In the positive control performed with dimethoate, mortalities of 53.3% and 66.7% was observed at 24 hours and 48 hours after end of dosing. At 48-hour showed 8.0% mortality at test end.

B. VALIDITY CRITERIA

The test was considered valid because the following criteria were met:

Table A 2.3.1.1.1-3: Study validity criteria

Parameter	Required	Observed oral test	Observed contact test
Control mortality	≤10%	2.0%	0%
LD ₅₀ -value of reference	0.10 to 0.35 µg a.i./bee (oral) 0.10 to 0.30 µg a.i./bee (contact)	0.13 µg a.i./bee	0.28 µg a.i./bee

C. TOXICITY ENDPOINTS

The oral LD₅₀ value for Boscalid 350 g/kg + Prothioconazole 200 g/kg WG was determined to be >335.1 µg product/bee. The single tested concentration 300 µg product/bee (actual dose consumption was 335.1 µg a.i./bee) showed no mortality and no observed abnormal behaviour. Abnormal behaviours were observed in the actual consumed dose of 0.17 and 0.34 µg dimethoate/bee. LD₅₀ values were 0.13 µg dimethoate/bee for 24-hour and 48-hour.

The contact LD₅₀ value for Boscalid 350 g/kg + Prothioconazole 200 g/kg WG was determine to be > 300 µg product/bee. The single tested concentration 300.0 µg product/bee showed 8.0% mortality at 48-hour and 8% observed abnormal behaviours at 4-hour. There was no abnormal behaviours observed for the reference item. LD₅₀ values were 0.28 and 0.24 µg dimethoate/bee for 24-hour and 48-hour, respectively.

Table A 2.3.1.1.1-4: LD₅₀ values for positive control (dimethoate)

Parameter	Oral test result (µg a.i./bee)	Contact test result (µg a.i./bee)
24 hours LD ₅₀ of positive control	0.13	0.28
95% confidence (lower limit)	0.12	0.25
95% confidence (upper limit)	0.14	0.35
48 hours LD ₅₀ of positive control	0.13	0.24
95% confidence (lower limit)	0.12	0.21
95% confidence (upper limit)	0.14	0.28

III. CONCLUSION

The acute toxicity of FGE03 on adult honeybees (*Apis mellifera* L.) was investigated an acute contact and an acute oral, limit study under laboratory conditions.

The contact LD₅₀ value (24 and 48 h) of FGE03 were determined to be > 300 µg product/bee.

The oral LD₅₀ value (24 and 48 h) were determined to be > 335.1 µg product/bee.

The contact and oral NOED values (24 and 48 h) were ≥ 300 and ≥ 335.1 µg product/bee, respectively.

A 2.3.1.1.1.2 Chwiesko & Horstmann, 2023 (KCP 10.3.1.1.1/02)

Comments of zRMS:	The study was conducted in line with OECD 246 with minor deviations. Application 5µL droplets was chosen in deviation to the guideline recommendation of 2µL as higher volume ensures a more reliable dispersion of the test item. No adverse effects on the outcome of the study are to be expected. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: The contact LD₅₀ (48 h) > 671.7 µg product/bumble bee. The oral LD₅₀ (48 h) = 671.7 µg product/bumblebee.
-------------------	--

Reference: KCP 10.3.1.1.1/02

Report: Boscalid + Prothioconazole (350 g/kg + 200 g/kg WG): Acute Contact and Oral Toxicity to Bumblebees (*Bombus terrestris* L.) in the Laboratory, Chwiesko, D. & Horstmann, C., 2023, Study code 176531105, UPL/2023/1279

Guideline(s): OECD 246: 'Bumblebees, Acute Contact Toxicity Test', adopted on 9 October 2017
OECD 247: 'Bumblebees, Acute Oral Toxicity Test', adopted on 9 October 2017

Deviations:	Yes. Application 5µL droplets was chosen in deviation to the guideline recommendation of 2µL as higher volume ensures a more reliable dispersion of the test item. No adverse effects on the outcome of the study are to be expected.
GLP:	Yes
Acceptability:	Yes

Executive summary

An acute oral and contact toxicity test for the effects of test item, Boscalid + Prothioconazole (350 + 200 g/kg – WG), FGE03 on bumblebee, *Bombus terrestris* L. was performed following OECD Guideline No 246 and 247 adopted on October 2022 with test period of 48 hours.

Oral and contact tests were conducted with nominal doses of 57, 103, 185, 333 and 600 µg product/bumblebee along with a control group using sucrose solution 50 % w/v (oral control) and tap water containing 0.1% Triton X-100 (contact control). The treated groups and control groups consisted of 30 replicates and 35 replicates for contact and oral toxicity test, respectively. Each replicate containing 1 bees. For the oral test, the treatment replicates were offered a volume of 40 µL test solution and control replicates were offered a volume of 40 µL sucrose solution 50% w/v for a diet consumption period of 4 hours. Only the bumblebees consumed at least 80% of the mean food uptake per treatment group were considered for the evaluation. Based on the diet consumed, the mean actual dose consumed was determined to be 65.0, 117.3, 205.6, 357.3 and 671.7 µg product/bumblebee.

The recovery rates of Boscalid and Prothioconazole in the test item concentration of the contact and oral test were within ± 20 % of the nominal concentrations.

The bees were kept in the dark with temperature between 25°C to 27°C and relative humidity at 59 – 65 % during the test period of 48 hours with *ad libitum* sucrose solution 50% w/v. Observations for mortality and abnormal behaviour were made at 4 hours, at 24 hours and 48 hours.

As the test item treatment groups in the contact test did not show mortality above 50.0 %, no statistical evaluation of the LD50 values were carried out, the contact LD50 (48 h) value was estimated to be >600 µg product/bumblebee. The contact NOED (48 h) value was calculated to be ≥ 600 µg product/bumblebee.

As the test item treatment groups in the oral test did not show mortality above 50.0 %, no statistical evaluation of the LD50 values were carried out, the oral LD50 (48 h) value was estimated to be >671.7 µg product/bumblebee. The oral NOED (48 h) value was calculated to be ≥ 671.7 µg product/bumblebee.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material	Boscalid 350 g/kg + Prothioconazole 200 g/kg WG (FGE03)
Batch no.:	BDPT/WG/220915/550/20
Purity:	<u>Boscalid</u> : 352.2 ± 0.2 g/kg (35.22 ± 0.02 % w/w) <u>Prothioconazole</u> : 203.3 ± 0.1 g/kg (20.33 ± 0.01 % w/w)
Date of expiry:	September 14, 2024

- 2. Test concentrations:** Control: 50% w/v aqueous sucrose solution (oral);
tap water with 0.1 % v/v Triton X-100 (contact)
Contact toxicity test item nominal dose: 57, 103, 185, 333 and 600 µg product/bumblebee
Mean consumed dose level of test item: 65.0, 117.3, 205.6, 357.3 and 671.7 µg product/bumblebee for oral test
Reference item: Oral test 4.0 µg dimethoate/bumblebee; Contact test 10 µg dimethoate/bumblebee
- 3. Toxic reference** Dimethoate 400 g/L (Danadim Progress)
Batch no.: 10214034
Purity: 413 g/kg (41.3 % w/w) analysed, according to Certificate of Analysis
- 4. Test organism:**
Species: Bumblebees *Bombus terrestris* L.
Source: Medium sized bumblebee colonies, healthy and queen-right, having all brood stages, sourced from a commercial bumblebee breeding company, Koppert Deutschland GmbH, Zeppellinstr. 32, 47638 Straelen, Germany.
Age: Adult female worker bumblebees, 10-12 weeks old
Housing: Cylindrical, latticed plastic cages (Nicot queen cages) with size approx. 7.3 cm length x 2.2 cm diameter and 1.7 cm at the small opening. The test units were placed on a plate, the small opening closed by a rubber plug holding a syringe containing the feeding solution. 1 bumblebee/cage, contact test 30 replicates for test treatments, control and reference item, oral test 35 replicates for test treatments, control and reference item.
Feeding: 50% w/v sucrose solution *ad libitum*; was given directly after treatment using syringes
- 5. Environmental conditions**
Temperature: 25°C – 27°C
Relative humidity: 59 - 65 %
Light: 24 hours dark, except during dosing and observations

B. STUDY DESIGN AND METHODS

- 1. Dates of work:** 01 – 29 August 2023 (including analytical and biological phase)
- 2. Test organism assignment and treatment**

All bumblebees used in the test derived from healthy and queen-right bee colonies. The bumblebee colonies were sourced from Koppert Deutschland GmbH, Zeppellinstr. 32, 47638 Straelen, Germany. Bee colony was arranged in a plastic box, which was packed in cardboard. The bees were collected with plastic cages within one week after delivery. The bumblebees were collected from 9 colonies and were randomly allocated to the different treatment groups to avoid any colony effect within a treatment group. 1 bee per cage. The test units were placed on a plate, the small opening closed by a rubber plug holding a syringe containing the feeding solution and placed under dark conditions in environmental test chambers during 48 hours exposure period.

Contact application was conducted outside of the test unit. 5 µL of each test treatments group was placed on the dorsal thorax using a calibrated pipette.

The treated/untreated food was provided *ad libitum*. Approximately 40 µL of food solution per bumblebee was provided in syringes. Syringes were weighed before and after introduction into the cages to feed the bumblebees during test period. The uptake period was 4 hours, after 4 hours the syringes containing the remaining food were removed, weighed and replaced by syringes containing fresh, untreated food. The calculation of the target dose was based on 40 mg food uptake. The ingested consumed oral doses were calculated based on the measured consumption.

The uptake of the treated sugar solutions differed from the nominal 40 mg/bumblebee. For this reason, individual bumblebees which did not take up at least 80% of the mean food uptake per treatment group were excluded from the evaluation of mortality and behavioural abnormalities, as well as from the calculation of the dose in the test item treatment group.

3. Dose preparation

The acute oral and contact test were conducted with dose of 57, 103, 185, 333 and 600 µg product/bumblebee. The test solution was prepared in sucrose solution 50% w/v before application for oral test and tap water containing 0.1 % v/v Triton X-100 for contact test.

The nominal target dose would have been obtained if exactly 40 mg/bumblebee of the treated food had been consumed in acute oral test. But the treated sugar solutions uptake differed from the nominal 40 mg/bumblebee. For this reason, only those bumblebees were considered which consumed at least 80% of the mean food uptake per treatment group. The actual mean consumed dose levels of the test item in the oral test were 65.0, 117.3, 205.6, 357.3 and 671.7 µg product/bumblebee.

The nominal dose of reference item was 10 µg dimethoate/bumblebee for contact test and 4 µg dimethoate/bumblebee for oral test. The positive control solutions containing dimethoate were prepared in sucrose solution 50% v/v and tap water with 0.1 % v/v Triton X-100 for oral and contact test, respectively.

4. Analytical Determination

Duplicate specimens were taken on the day which the highest and lowest dose of test item stock solutions were freshly prepared. Approximately 10 mL per specimen was sampled and stored deep frozen until analysis. HPLC-UV method was used for determination. Boscalid and Prothioconazole were determined in each sample.

5. Measurements and observations

Mortality and abnormal behaviour were recorded at different timepoints, at 4-hour (± 0.5) and thereafter at 24-hour (± 2) and at 48-hour (± 2). Sublethal effects such as symptoms of poisoning, cannot walk, feeble movements of legs and antennae and weak response to stimulation were recorded as abnormal behaviours.

The amount of diet consumption was assessed from weight difference of the syringes with feeding solution before and after loading treated diet and after the 4-h exposure period.

6. Statistics

As the test item treatment groups in the contact and oral test did not show mortality above 50.0 %, no statistical evaluation of the LD₅₀ values was carried out. The contact and oral LD₅₀ values are considered to be higher than the highest tested dose rate. The contact and oral NOED values of the test item were determined using Fisher's Exact Binomial Test with Bonferroni Correction (one-sided greater, $\alpha = 0.05$).

The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ToxRat Solutions GmbH.

II. RESULTS AND DISCUSSION

A. FOOD CONSUMPTION AND MORTALITY

The mean feed consumption and mortality after exposure of bumblebees to a single concentration of test item and the positive control with dimethoate are presented in the table below.

No mortality occurred in the test item oral and contact toxicity test results.

Table A 2.3.1.1.1.2-1: Food consumption, mortality and behavioural abnormalities of bumblebees treated with control, reference item and test item FGE03 (Boscalid 350 g/kg + Prothioconazole 200 g/kg WG) in oral toxicity test

Treatment code	Nominal dose	Actual mean consumed dose	Mortality mean (%)			Behavioural abnormalities mean (%)		
			4 h	24 h	48 h	4 h	24 h	48 h
Water Control	0.0	-	0.0	0.0	0.0	0.0	0.0	0.0
Test item (µg product/ bee)	600	671.7	0.0	0.0	0.0	0.0	14.8	14.8
	333	357.3	0.0	0.0	4.0	4.0	16.0	16.7
	185	205.6	0.0	0.0	0.0	0.0	0.0	0.0
	103	117.3	0.0	0.0	0.0	0.0	0.0	0.0
	57	65.0	0.0	0.0	0.0	0.0	0.0	0.0
Reference item (µg dimethoate/bee)	-	4.2	34.5	100.0	100.0	100.0	-	-

No mortality showed in the control, 4.0% mean mortality showed in the 357.3 µg product/bee at 48-hour. In the positive control performed with dimethoate, mortalities of 100 % were observed at 24 hours after end of dosing. Abnormal behaviour was observed in 357.3 and 671.7 µg product/bee after 24 h and 48 h exposure.

Table A 2.3.1.1.1.22-2: Mortality and behavioural abnormalities of bumblebees treated with control, reference item and test item FGE03 (Boscalid 350 g/kg + Prothioconazole 200 g/kg WG) in contact toxicity test

Treatment code	Exposed dose	Mortality mean (%)			Behavioural abnormalities mean (%)		
		4 h	24 h	48 h	4 h	24 h	48 h
Control	0.0	0.0	3.3	3.3	0.0	0.0	0.0
Test item (µg product/ bee)	600	0.0	0.0	6.7	40.0	60.0	75.0
	333	0.0	0.0	0.0	6.7	13.3	16.7
	185	0.0	0.0	0.0	0.0	0.0	0.0
	103	0.0	0.0	0.0	0.0	0.0	3.3
	57	0.0	0.0	3.3	0.0	0.0	0.0
Reference item (µg dimethoate/bee)	10	0.0	86.7	93.3	100.0	100.0	100.0

Mean mortality of 6.7% and 3.3% showed in the 600 and 57 µg product/bee after 48-hour exposure. In the positive control performed with dimethoate, mortalities between 86.7% and 93.3% were observed at 24 hours and 48 hours after end of dosing. Abnormal behaviour was observed in the test concentrations of 333 and 600 µg product/bee and positive control after 4-hour of exposure.

B. ANALYTICAL VERIFICATION

The analytical recovery rates of the active substance Boscalid and Prothioconazole for the lowest and highest concentrations were between 93% to 104%, it is within the acceptable range of 70 – 120%.

Table A 2.3.1.1.1.22-3: Analysis of concentration for oral and contact test

Dose/ bumblebee	Nominal concentration in solution	Recovery of the nominal value in the solution	
		Boscalid	Prothioconazole
Contact Test: Application solution 600 µg product/ bumblebee *,**	120 g product/L application solution	104 %	103 %
Contact Test: Application solution 57 µg product/ bumblebee *	11.4 g product/L application solution	92 %	91 %
Oral Test: Feeding solution 600 µg product/ bumblebee *	15 g product/kg feeding solution	94 %	95 %

Oral Test: Feeding solution 57 µg product/ bumblebee *, **	1.43 g product/kg feeding solution	93 %	94 %
--	---------------------------------------	------	------

* The dose levels of the test item were based on the product without taking into consideration active substance content.

** stock solution

C. VALIDITY CRITERIA

The test was considered valid because the following criteria were met:

Table A 2.3.1.1.2-4: Study validity criteria

Parameter	Required	Observed
Control mortality (test end)	≤10%	0% (oral) 3.3% (contact)
Mortality of reference (oral) Mortality of reference (contact)	≥50% at test end	100.0% 93.3%

D. TOXICITY ENDPOINTS

The LD₅₀ values for test item treatment groups have not carried out statistical evaluation as the test item treatment groups in oral and contact tests did not show mortality above 50.0%.

The tested concentrations of contact toxicity test of 600 µg product/bumblebee showed 6.7% mean mortality at test end. Abnormal behaviours of the bumblebees were showed in the test concentration 333 and 600 µg product/bumblebee.

The oral LD₅₀ were estimated to be >671.7 µg product/bumblebee and NOED was calculated to be ≥671.7 µg product/bumblebee. The result was based on the actual mean consumed oral tested dose.

The contact LD₅₀ were estimated to be >600 µg product bumblebee and NOED was calculated to be ≥600 µg product/bumblebee.

III. CONCLUSION

The toxicity of Boscalid + Prothioconazole (350 + 200 g/kg – WG) was tested in an acute contact and oral toxicity test using bumblebees.

As the test item treatment groups in the contact test did not show mortality above 50.0 %, no statistical evaluation of the LD₅₀ values were carried out, the contact LD₅₀ (48 h) value was estimated to be >600 µg product/bumblebee. The contact NOED (48 h) value was calculated to be ≥600 µg prout/bumblebee.

As the test item treatment groups in the oral test did not show mortality above 50.0 %, no statistical evaluation of the LD₅₀ values were carried out, the oral LD₅₀ (48 h) value was estimated to be >671.7 µg product/bumblebee. The oral NOED (48 h) value was calculated to be ≥671.7 µg product/bumblebee.

A 2.3.1.1.2 KCP 10.3.1.1.2 Acute contact toxicity to bees

For acute contact toxicity studies in honeybees and bumble bees, please refer to Points A 2.3.1.1.1.1 (Knautz, 2023a; KCP 10.3.1.1.1/02) and A 2.3.1.1.1.2 (Chwiesko & Horstmann, 2023; KCP 10.3.1.1.1/02), respectively.

A 2.3.1.2 KCP 10.3.1.2 Chronic toxicity to bees

A 2.3.1.2.1 Knautz & Horstmann, 2023 (KCP 10.3.1.2/01)

Comments of zRMS:	The study was conducted in line with OECD 245 with no deviation. The concentrations of the active ingredients in the applied test item feeding solutions were within the required range of ± 20 % of the nominal concentrations.
-------------------	---

	All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: LDD₅₀ = 114 µg product/bee/day NOEDD = 61.2 µg product/bee/day
--	--

Reference:	KCP 10.3.1.2/01
Report:	Boscalid + Prothioconazole (350 + 200 g/kg – WG): Chronic Oral Toxicity Test on the Honey Bee (<i>Apis mellifera</i> L.) in the Laboratory. Knautz, T. & Horstmann, C., 2023, Report no. 176531136, UPL/2023/1209
Guideline(s):	OECD (2017), Test No. 245: Honey Bee (<i>Apis Mellifera</i> L.), Chronic Oral Toxicity Test (10-Day Feeding)
Deviations:	None
GLP:	Yes
Acceptability:	Yes

Executive summary

A chronic oral toxicity test for the effects of test item, Boscalid + Prothioconazole (350 + 200 g/kg – WG), FGE03 on Honeybee, *Apis mellifera* L. was performed following OECD Guideline No 245 adopted on year 2017.

Under laboratory conditions, 30 freshly hatched female worker bees (*Apis mellifera* L.) per treatment group were exposed for 10 days to 5 concentrations. The test item was administered daily to the bees in sugar solution at the following concentrations: 15000, 8333, 4630, 2572 and 1429 mg product/kg feeding solution. These concentrations resulted in actual mean dose of 176, 122, 88.2, 61.2 and 31.8 µg product/bee/day after 10 days. An untreated control and a reference item were included in this study.

Mortality occurred in test item treated dose levels, 61.2, 88.2, 121.9 and 175.5 ranging from 6.7 to 96.7 % at test end (10 days following the start of chronic exposure). There was no mortality in the water control (50 % w/v sucrose solution).

Test item related behavioural abnormalities of 6.7% were observed on day 10 in a single test item treatment 4630 mg product/kg feeding solution.

The reference item (dimethoate) at a concentration of 1 mg dimethoate/kg feeding solution corresponding to an actual consumed dose of 0.015 µg dimethoate/bee/day caused 100 % mortality on day 8.

The analytical verification showed ±20% recovery rate of nominal concentration confirmed the correct preparation of all the dosing solutions of the test item.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item	Boscalid + Prothioconazole (350 + 200 g/kg – WG), FGE03
Batch No.:	BDPT/WG/220915/550/20
Actual content of active ingredients:	Boscalid: 35.22 ± 0.02 % w/w, 352.2 ± 0.2 g/kg Prothioconazole: 20.33 ± 0.01 % w/w, 203.3 ± 0.1 g/kg according to the certificate of analysis
Description:	Solid, brown and beige

Expiry date: September 14, 2024

2. Reference Item

Toxic reference: Dimethoate 400 g/L (Danadim Progress)

Batch no.: 10214034

Purity: 390 g/kg (39.0 % w/w)

3. Test organisms

Species: *Apis mellifera* L.

Age: Young adult female worker bees (2 days old)

Source: Honeybee colonies, disease-free and queen-right, bred by ibacon

Food: 50% w/v aqueous sugar solution

Collection: Brood combs with sealed brood from four different hives were used in the test in which bees visibly started to emerge. The combs contained pollen which was used as a first feeding source for the freshly hatched bees. The combs were taken from the hive two days before start of the exposure and adult bees were swept off.

B. STUDY DESIGN AND METHODS

1. Experimental dates: Biological
Phase: June 13 - 23, 2023
Analytical
Phase: August 15, 2023

2. Test design

Test cage description: Stainless steel chambers 8.2 cm x 5.9 cm x 4.2 cm (length x height x width)

Replication: 3 per test item dose level, controls and reference item dose

No. of individuals: 10 per test unit

Duration of test: 10 days

3. Environmental test conditions

Temperature: 33 – 34 °C

Humidity: 50 – 70 %; mean relative humidity was 69 %

Photoperiod: Darkness (except during observation)

4. Treatments

Test concentration: Nominal: 15000, 8333, 4630, 2572 and 1429 mg product/kg feeding solution
This corresponded to:
5283, 2935, 1631, 906 and 503 mg Boscalid/kg feeding solution
3050, 1694, 941, 523 and 290 mg Prothioconazole/kg feeding solution

Actual Dose Level: 95.9, 80.2, 55.1, 41.9 and 25.1 µg a.i./bee/day
(based on the actual intake of treated food)

Actual Mean Dose Level: 176, 122, 88.2, 61.2 and 31.8 µg product/bee/day

This corresponded to:

61.8, 42.9, 31.1, 21.5 and 11.2 µg Boscalid/bee/day

35.7, 24.8, 17.9, 12.4 and 6.46 µg Prothioconazole /bee/day

Controls: 50% w/v aqueous sugar solution

Analysis Determination: Duplicate samples were taken from the highest and lowest test item concentration on Day 9, 10 mL per sample. After sampling, the samples were stored deep frozen. Method of analysis was HPLC-method.

5. Measurement and Observation

Number of dead bees and abnormal behaviours were assessed daily until test end, ten days following start of exposure. Sub-lethal effects such as symptoms of poisoning or any abnormal behaviours (cramps, apathy, cannot walk, feeble movements of legs and antennae, weak response and etc) in comparison to the control were recorded.

6. Statistic

The $LC_{50/20/10}$ and $LDD_{50/20/10}$ values of the test item were determined with Probit Analysis (Finney 1971). The NOEC/NOEDD of the test item was determined using Fisher's Exact Binomial Test with onferroni-Holm correction (pairwise comparison, one-sided greater, $\alpha = 0.05$), which is a distribution-free test and does not require testing for normality or homogeneity prior to analysis.

It was not necessary to correct the test item, since no control mortality occurred.

The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, © Tox-Rat Solutions GmbH.

II. RESULTS AND DISCUSSION

A. MORTALITY

Mortality data for the test material are summarised in the table below.

Table A 2.3.1.2.1-1: 10 days Chronic Oral Toxicity of Boscalid 500 WG to young adult honeybees – Mortality and Abnormal Behaviors

Treatment Group	Concentration [mg/kg feeding solution]	Mean dose * per/bee/day [µg /bee]	Mortality at day 10 [% Mean]	Behavior abnormalities at day 10 [% Mean]
Test item (mg product/kg)	15000	176	96.7	0.0
	8333	122	46.7	0.0
	4630	88.2	20.0	6.7
	2572	61.2	6.7	0.0
	1429	31.8	0.0	0.0
Untreated control	0.0	0.0	0.0	0.0
Reference Item	1.0	0.015	100.0	0.0

Results are average from three replicates (ten bees each) per concentration or control

* At the test end, 10 days following application

B. ANALYTICAL RESULTS

The analytical recovery rates of the active ingredient Boscalid and Prothioconazole in the feeding solutions were as follows:

Boscalid Feeding Solution 1429 DAA9	95 %
Boscalid Feeding Solution 15000 DAA9	97 %
Prothioconazole Feeding Solution 1429 DAA9	92 %
Prothioconazole Feeding Solution 15000 DAA9	95 %

The analytical verification within 92 – 97 % of recovery rate of nominal values confirmed the correct preparation of the dosing solutions of the test item. It is within the $\pm 20\%$ of nominal concentration.

C. VALIDITY CRITERIA

Table A 2.3.1.2.1-2: Validity criteria

Parameter	Required	Observed
Control mortality (test end)	$\leq 15\%$	0.0%
Mortality of reference	$\geq 50\%$ at test end	100.0%

D. TOXICITY ENDPOINTS

Table A 2.3.1.2.1-3: Summary of endpoints

Endpoint at test termination (day 10)		
LC ₅₀	7336 mg product/kg feeding solution	95 % C.I.: 6256 – 8695
LDD ₅₀	114 µg product/bee/day	95 % C.I.: 104 – 126
LC ₂₀	4566 mg product/kg feeding solution	95 % C.I.: 3588 – 5415
LDD ₂₀	86.3 µg product/bee/day	95 % C.I.: 74.1 – 95.6
LC ₁₀	3563 mg product/kg feeding solution	95 % C.I.: 2601 – 4363
LDD ₁₀	74.5 µg product/bee/day	95 % C.I.: 61.0 – 84.3
NOEC	2572 mg product/kg feeding solution	-
NOEDD	61.2 µg product/bee/day	-

C.I. = Confidence limit

III. CONCLUSION

The chronic oral toxicity of Boscalid + Prothioconazole (350 + 200 g/kg – WG) on young adult honey bees (*Apis mellifera* L.) was investigated in a 10-day chronic, dose-response feeding study under laboratory conditions.

All validity criteria for the study were met. After 10 days of continuous exposure, mortality in the control was 0.0 % and thus below the threshold of 15 %. Mortality in the reference treatment group was 100 % and thus above the threshold of 50 %.

The recovery rates of Boscalid and Prothioconazole in the highest and lowest test item concentration of Day 9 were within $\pm 20\%$ of the nominal concentrations.

The LDD₅₀, LDD₂₀ and LDD₁₀ were determined to be 114, 86.3 and 74.5 µg product/bee/day, respectively. Based on Boscalid doses this corresponds to a LDD₅₀, LDD₂₀ and LDD₁₀ of 40.2, 30.4 and 26.2 µg Boscalid /bee/day. Based on Prothioconazole doses this corresponds to a LDD₅₀, LDD₂₀ and LDD₁₀ of 23.2, 17.5 and 15.1 µg Prothioconazole /bee/day.

The LC₅₀, LC₂₀ and LC₁₀ were determined to be 7336, 4566 and 3563 product/kg feeding solution, respectively. Based on Boscalid concentrations this corresponds to a LC₅₀, LC₂₀ and LC₁₀ of 2584, 1608 and 1255 mg Boscalid/kg feeding solution. Based on Prothioconazole concentrations this corresponds to a LC₅₀, LC₂₀ and LC₁₀ of 1491, 928 and 724 mg Prothioconazole /kg feeding solution.

The NOEDD was determined to be 61.2 µg product/bee/day (corresponding to 21.6 µg Boscalid/bee/day and to 12.4 µg Prothioconazole /bee/day) and the NOEC was 2572 mg product/kg feeding solution (corresponding to 906 mg Boscalid /kg feeding solution and to 523 mg Prothioconazole /kg feeding solution).

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

A 2.3.1.3.1 Venturi, 2024 (KCP 10.3.1.3/01)

Comments of zRMS	The study was conducted in line with OECD 239 with the following deviations. - The mortality assessment on D7 was not carried out for the following reason: On D7 there are no treatments or feeding of the larvae and performing a mortality assessment would have involved opening the dryer with a consequent decrease of temperature and humidity. Therefore, to avoid stress to the larvae (in a very delicate stage such as the
------------------	--

	transition to pre-pupa) the assessment was performed directly on D8. The content of active substances (Boscalid and Prothioconazole) were analysed in the lowest and highest test item concentrations of the stock solutions (prepared on D3) used to treat the diets and was determined to be within 20% of the nominal values for all test item treatment groups, therefore the endpoints were calculated based on nominal concentrations and doses. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: NOED for adult emergence on D22 =19.05 µg test item/larva (6.71 µg Boscalid/larva and 3.87 µg Prothioconazole/larva). ED₅₀=70.66 µg test item/larva (44.40 – 112.47)
--	--

Reference:	KCP 10.3.1.3/01
Report:	Effects of Boscalid + Prothioconazole (350 + 200 g/kg – WG) on honey-bees (<i>Apis mellifera</i> L.) 22-day larval toxicity test with repeated exposure, Venturi, S., 2024, Study code BT073/23, UPL/2023/1235
Guideline(s):	OECD 239: ‘Honey bee (<i>Apis mellifera</i> L.) larval toxicity test, repeated exposure’, adopted on 7 July 2021
Deviations:	The mortality assessment on D7 was not carried out for the following reason: On D7 there are no treatments or feeding of the larvae and performing a mortality assessment would have involved opening the dryer with a consequent decrease of temperature and humidity. Therefore, to avoid stress to the larvae (in a very delicate stage such as the transition to pre-pupa) the assessment was performed directly on D8.
GLP:	Yes
Acceptability:	Yes

Executive summary

The 22-day larval toxicity test with repeated exposure in the laboratory was performed as a dose-response test based on OECD 239. The test item was dissolved in ultra-pure water and then in the larval food (aqueous sugar solution mixed with royal jelly) and administered daily to the larvae from day 3 (D3) to 6 (D6) of the test.

The stock solutions and the treated diet were prepared freshly each day of administration. The reference item Dimethoate was tested at a cumulative dose of 7.39 µg/larva. An untreated control was run in parallel with the royal jelly-based diet.

For each test item treatment group, for the control group and the reference item treatment group, 3 replicates of 12 larvae each were set up.

The validity criteria of the OECD No. 239 (2021) with regards to control larval mortality on D8 (< 15% from D3 to D8 across replicates), control adults’ emergence on D22 (≥ 70% across replicates) and toxicity on the reference item (mortality > 50% on D8) were met. Thus, the study is valid.

The content of active substances was analysed in the lowest and highest test item concentrations of the stock solutions (prepared on D3) used to treat the diets and was determined to be within 20% of the nominal values for all samples, therefore the endpoints were calculated based on nominal concentrations and doses. The control was also analysed, and no contamination was detected (<LOD).

Regarding the effects on larvae on D8 (developmental period), the test item Boscalid + Prothioconazole (350 + 200 g/kg – WG) (FGE03) caused statistically significant mortality at the three highest tested doses.

Therefore, the NOED for larvae on D8 was determined to be 34.29 µg test item/larva (12.08 µg Boscalid/larva and 6.97 µg Prothioconazole/larva) equivalent to a NOEC of 222.69 mg test item/kg diet (78.43 mg Boscalid/kg diet and 45.27 mg Prothioconazole/kg diet).

Regarding the effects on adult emergence on D22, the test item Boscalid + Prothioconazole (350 + 200 g/kg – WG) (FGE03) caused statistically significant reduction in emergence rate respect to the control at every tested dose except at the lowest dose.

The NOED and the NOEC for adult emergence on D22 was determined to be 19.05 µg test item/larva (6.71 µg Boscalid/larva and 3.87 µg Prothioconazole/larva) and 123.71 mg test item/kg diet (43.57 mg Boscalid/kg diet and 25.15 mg Prothioconazole/kg diet), respectively

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:** Boscalid 350 g/kg + Prothioconazole 200 g/kg WG (FGE03)
Batch no.: BDPT/WG/220915/550/20
Purity: Boscalid: 352.2 g/kg (35.22% w/w)
Prothioconazole: 203.3 g/kg (20.33% w/w)
Date of expiry: 14 September 2024
2. **Test doses:** Control: ultra-pure water
Test item: 19.05, 34.29, 61.73, 111.11 and 200.00 µg test item/larva
(corresponding to 6.71, 12.08, 21.74, 39.13 and 70.44 µg Boscalid/larva and 3.87, 6.97, 12.55, 22.59 and 40.66 µg Prothioconazole/larva)

- 3. Toxic reference:** Dimethoate
Batch no.: BCCF3993
Purity: 99.4%
- 4. Test organism:**
Species: *Apis mellifera ligustica*
Source: Healthy colony maintained at BioTecnologie BT S.r.l. (colonies no. 1, 4, 11)
Age: 3 days old bee larvae
Test units: Larvae were reared in crystal polystyrene grafting cells with an internal diameter of 9 mm and a depth of 8 mm. The cells were sterilized. Each cell was placed into a well of a 48-well plate. The top of the grafting cell was maintained at the level of the plate by placing below it a piece of dental roll wetted with 500 µL of sterilizing solution enhanced with 15% weight/volume glycerol. Emerging bees were fed with syrup/sucrose solution dispensed *ad libitum* using 2.5 mL syringes.
Feeding: Diet A (D1): 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 2% weight of yeast extract, 12% weight of glucose and 12% weight of fructose.
Diet B (D3): 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 3% weight of yeast extract, 15% weight of glucose and 15% weight of fructose.
Diet C (from D4 to D6): 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 4% weight of yeast extract, 18% weight of glucose and 18% weight of fructose
- 5. Environmental conditions**
Temperature: 29.1 – 35.1 °C (average measured during the test 34.2 °C)
Relative humidity: 85.2 – 92.9%, average 92.2% (D1 to D8)
64.4 – 83.1%, average 80.5% (D8 to D15)
46.0 – 66.6%, average 61.0% (D15 – D22)
Light: 24 h darkness, except during observation

B. STUDY DESIGN AND METHODS

- 1. Experimental phase:** 20 June – 10 July 2023
- 2. Experiment design**

The 22-day larval toxicity test with repeated exposure in the laboratory was performed as dose-response test. Twelve larvae from each of three colonies were allocated to the same plate, each plate corresponded to a treatment group. All larvae were fed once per day from D1 to D6 (except D2), and food was added even if the previous food was not totally consumed. The test item was dissolved in water and then in the larval food (aqueous sugar solution mixed with royal jelly) and administered daily to the larvae from day 3 (D3) to 6 (D6) of the test.

3. Application

The stock solutions and the treated diet were prepared daily and warmed in an incubator before use. The reference item Dimethoate was tested at a cumulative dose of 7.39 µg/larva. An untreated control was run in parallel with the royal jelly-based diet.

For each test item treatment group, for the control group and the reference item treatment group, 3 replicates of 12 larvae each were set up.

Five doses of test item, 19.05, 34.29, 61.73, 111.11 and 200.0 µg/larva were test along an untreated control and a reference item used for the test.

4. Analytical Determination

For analytical determination, the test items were analyzed by HPLC with DAD detector in double replicates. The analytical samples were sampled on D3 from the test solution in the test system at lowest and highest concentration.

5. Measurements and observations

Assessments of mortality and any developmental/behavioral abnormality were performed daily from D4 to D8 (except on D7) and on D15. On D15, larvae that had not transformed into pupae were recorded as dead and removed. Hatched adults were recorded on D22. Adult emergence rate was calculated as percentage by comparing the number of bees emerged on D22 with the number of larvae on D3 when dosing started.

Pupal mortality and the emergence rate of adults were also assessed on D22. Pupal mortality was evaluated on D15 and on D22. On D15 as a percentage by comparing the number of dead pupae from D8 to D15 with the number of larvae entering in pre-pupa stage on D8. On D22 as a percentage by comparing the number of pupae that failed to emerge, including those bees without emergence on D22 and dead pupae removed during pupa stage with the number of larvae entering in pre-pupa stage on D8.

6. Statistics

The software ToxRatPro Version 3.3.0 was used for the statistics. To evaluate the statistical significance of the D8 and D22 data and the NOED/NOEC, the Step-down Cochran Armitage test was applied. To evaluate the LD/LCx and ED/ECx values a Weibull analysis using linear max. likelihood regression was performed.

The results of the Weibull analysis on D22 were classified according to Appendix E of EFSA Technical report 2019: EN-1673.

II. RESULTS AND DISCUSSION

A. MORTALITY

The table below presents the mean mortalities on D8 and D22, the pupal mortality and the effects on adult emergence on D8 and D22.

Table A 2.3.1.3.1-1: Mortality (M) and corrected mortality (CM) of *Apis mellifera* larvae on D8 exposure

Treatment group	Cumulative dose [µg test item/larva]	Concentration [mg test item/kg diet]	Larvae mortality on DB		
			Mortality mean [%]	Corr. Mortality mean [%]	Significant
Control	0	0	0	n.a.	n.a.
Test item (T1)	19.05	123.71	0	0	-
Test item (T2)	34.29	222.69	0	0	-
Test item (T3)	61.73	400.83	5.56	5.56	+
Test item (T4)	111.11	721.50	16.67	16.67	+
Test item (T5)	200.00	1298.70	97.22	97.22	+

n.a. = not applicable

+ = significant; - = non-significant (Step-down Cochran-Armitage test – $\alpha = 0.05$, one-sided greater)

Table A 2.3.1.3.1-2: Pupal Mortality

Treatment group	Cumulative dose [µg test item/larva]	Concentration [mg test item/kg diet]	Pupal mortality from D8 to D15*	Pupal mortality from D8 to D22**
			Mean [%]	Mean [%]
Control	0	0	0	2.78
Test item (T1)	19.05	123.71	2.78	11.11
Test item (T2)	34.29	222.69	0	36.11
Test item (T3)	61.73	400.83	3.03	35.61
Test item (T4)	111.11	721.50	21.21	53.79
Test item (T5)	200.00	1298.70	100	100

*calculated as a percentage comparing the number of dead pupae from D8 to D15 with the number of alive pupae on D8

**calculated as a percentage comparing the number of dead pupae from D8 to D22 with the number of alive pupae on D8

Table A 2.3.1.3.1-3: Total mortality (M) and corrected mortality (CM) from D3 to D22 and emergence (E) on D22

Treatment group	Cumulative dose [µg test item/larva]	Concentration [mg test item/g diet]	Pupal mortality from D8 to D15*			Pupal mortality from D8 to D22**	
			M – Mean [%]	CM – Mean [%]	Sign.	E - Mean [%]	Sign.
Control	0	0	2.78	n.a.	n.a.	97.22	n.a.
Test item (T1)	19.05	123.71	11.11	8.57	-	88.89	-
Test item (T2)	34.29	222.69	36.11	34.29	+	63.89	+
Test item (T3)	61.73	400.83	38.89	37.14	+	61.11	+
Test item (T4)	111.11	721.50	63.89	62.86	+	36.11	+
Test item (T5)	200.00	1298.70	100	100	+	0	+

n.a. = not applicable

+ = significant; - = non-significant (Step-down Cochran-Armitage test – $\alpha = 0.05$, one-sided greater)

Table A 2.3.1.3.1-4: Reference Item (Dimethoate) – mean mortality

Treatment group	Dose [µg a.s./larva]	Concentration [mg a.s./kg diet]	Mortality on D8 Mean [%]
Reference item	7.39	48.00	100.00

B. ANALYTICAL RESULTS

The content of active substances (Boscalid and Prothioconazole) were analysed in the lowest and highest test item concentrations of the stock solutions (prepared on D3) used to treat the diets and was determined to be within 20% of the nominal values for all test item treatment groups, therefore the endpoints were calculated based on nominal concentrations and doses. The highest concentration sample was named S1 and the lowest concentration sample was S5.

The untreated control (ultrapure water) was also analysed, and no contamination was detected (<LOD).

Table A 2.3.1.3.1-5: Analytical Results of Boscalid content in test item

Samples Name	Nominal Concentration		Measured a.s. conc. [mg/L]		Mean measured [mg/L]	Mean recovery [%]
	[g test item/L]	[g a.s./L]	Diluted sample	Undiluted sample		
S1D3-r001	1380.00	486.04	91.8022	524.58	522.86	107.58
S1D3-r002			91.2005	521.15		
S5D3-r001	14440.00	5085.77	132.8110	5312.44	5314.48	104.50
S5D3-r002			132.9129	5316.52		

Table A 2.3.1.3.1-6: Analytical Results of Prothioconazole content in test item

Samples Name	Nominal Concentration		Measured a.s. conc. [mg/L]		Mean measured [mg/L]	Mean recovery [%]
	[g test item/L]	[g a.s./L]	Diluted sample	Undiluted sample		
S1D3-r001	1380.00	280.55	51.0886	291.93	288.70	102.90
S1D3-r002			49.9568	285.47		
S5D3-r001	14440.00	2935.65	74.2677	2970.71	2979.00	101.48
S5D3-r002			74.6823	2987.29		

The analytical results demonstrate that the Boscalid and Prothioconazole content in the water solutions prepared on D3 at the highest and lowest concentration was in the range of $\pm 20\%$ of nominal concentrations and consequently that the procedure for the preparation of the treated diet was corrected.

C. VALIDITY CRITERIA

All required validity criteria were met. Accordingly, the study is regarded as valid.

Table A 2.3.1.3.1-7: Study validity criteria

Parameters	Required	Observed
Cumulative larval mortality from D3 to D8 in control	$\leq 15\%$	0%
Adult emergence rate on D22 in control	$\geq 70\%$	97.22%
Larval mortality on D8 in reference item treatment	$\geq 50\%$	100%

D. TOXICITY ENDPOINTS

Regarding the effects on larvae on D8 (developmental period), the test item Boscalid 350 g/kg + Prothioconazole 200 g/kg WG caused statistically significant mortality at three highest tested doses.

Therefore, the NOED for larvae on D8 was determined to be 34.29 μg test item/larva (12.08 μg Boscalid/larva and 6.97 μg Prothioconazole/larva) equivalent to a NOEC 222.69 mg test item/kg diet (78.43 mg Boscalid/kg diet and 45.27 mg Prothioconazole/kg diet).

Regarding the effects on adult emergence on D22, the test item Boscalid 350 g/kg + Prothioconazole 200 g/kg WG caused statistically significant reduction in emergence rate respect to the control at every tested dose except the lowest dose.

The NOED and the NOEC for adult emergence on D22 was determined to be 19.05 μg test item/larva (6.71 μg Boscalid/larva and 3.87 μg Prothioconazole/larva) and 123.71 mg test item/kg diet (43.57 mg Boscalid/kg diet and 25.15 mg Prothioconazole/kg diet), respectively at the end of the test.

Table A 2.3.1.3.1-8: Adult emergence on D22 after repeated exposure– summary of endpoints

Endpoint dose	Adult Emergence on D22 [µg test item/larva]	Adult Emergence on D22 [µg a.s./larva]
ED ₁₀	18.93 (6.27 – 57.17)	Boscalid: 6.67 (2.21 – 20.14) Prothioconazole: 3.85 (1.27 – 11.62)
ED ₂₀	32.00 (14.36 – 71.29)	Boscalid: 11.27 (5.06 – 25.11) Prothioconazole: 6.51 (2.92 – 14.49)
ED ₅₀	70.66 (44.40 – 112.47)	Boscalid: 24.89 (15.64 – 39.61) Prothioconazole: 14.37 (9.03 – 22.87)
NOED	19.05	Boscalid: 6.71 Prothioconazole: 3.87
Endpoint concentration	Adult Emergence on D22 [mg test item/kg diet]	Adult Emergence on D22 [mg a.s./kg diet]
EC ₁₀	122.92 (40.71 – 371.23)	Boscalid: 43.29 (14.34 – 130.75) Prothioconazole: 24.99 (8.28 – 75.47)
EC ₂₀	207.79 (93.25 – 462.92)	Boscalid: 73.18 (32.84 – 163.04) Prothioconazole: 42.24 (18.96 – 94.11)
EC ₅₀	458.83 (288.31 – 730.32)	Boscalid: 161.60 (101.54 – 257.22) Prothioconazole: 93.28 (58.61 – 148.48)
NOEC	123.71	Boscalid: 43.57 Prothioconazole: 25.15

ED/EC_{10/20/50} evaluated by Weibull analysis; effect concentrations given with 95% confidence limits in brackets.

III. CONCLUSION

The effects of the test item Boscalid + Prothioconazole (350 + 200 g/kg – WG) (FGE03) on the larval development and subsequent adult emergence of honeybees (*Apis mellifera* L.), were tested in a GLP compliant laboratory study.

The validity criteria of the GD OECD No. 239 (2021) with regards to control larval mortality on D8 (< 15% from D3 to D8 across replicates), control adults' emergence on D22 (≥ 70% across replicates) and toxicity on the reference item (mortality > 50% on D8) were met. Thus, the study is valid.

The content of active substances was analysed in the lowest and highest test item concentrations of the stock solutions (prepared on D3) used to treat the diets and was determined to be within 20% of the nominal values for all samples, therefore the endpoints were calculated based on nominal concentrations and doses. The control was also analysed, and no contamination was detected (<LOD).

Regarding the effects on larvae on D8 (developmental period), the test item Boscalid + Prothioconazole (350 + 200 g/kg – WG) (FGE03) caused statistically significant mortality at the three highest tested doses.

Therefore, the NOED for larvae on D8 was determined to be 34.29 µg test item/larva (12.08 µg Boscalid/larva and 6.97 µg Prothioconazole/larva) equivalent to a NOEC of 222.69 mg test item/kg diet (78.43 mg Boscalid/kg diet and 45.27 mg Prothioconazole/kg diet).

Regarding the effects on adult emergence on D22, the test item Boscalid + Prothioconazole (350 + 200 g/kg – WG) (FGE03) caused statistically significant reduction in emergence rate respect to the control at every tested dose except at the lowest dose.

The NOED and the NOEC for adult emergence on D22 was determined to be 19.05 µg test item/larva (6.71 µg Boscalid/larva and 3.87 µg Prothioconazole/larva) and 123.71 mg test item/kg diet (43.57 mg Boscalid/kg diet and 25.15 mg Prothioconazole/kg diet), respectively.

A 2.3.1.4	KCP 10.3.1.4	Sub-lethal effects
A 2.3.1.5	KCP 10.3.1.5	Cage and tunnel tests
A 2.3.1.6	KCP 10.3.1.6	Field tests with honeybees

A 2.3.2 KCP 10.3.2 Effects on arthropods other than bees
A 2.3.2.1 KCP 10.3.2.1 Standard laboratory testing for non-target arthropod
A 2.3.2.1.1 Leopold, 2023a (KCP 10.3.2.1/01)

Comments of zRMS:	The study was conducted in line with the respective guideline with no deviations. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: LR₅₀ > >1700 g product/ha ER₅₀ reproduction > 1700 g product/ha
-------------------	---

Reference: KCP 10.3.2.1/01

Report: Boscalid + Prothioconazole (350 + 200 g/kg – WG): Effects on the Parasitoid *Aphidius rhopalosiphi* (Hymenoptera: Braconidae) in the Laboratory. A Dose Response Test on Glass Plates, Leopold, J., 2023a, Study code 176531001, UPL/2023/1053

Guideline(s): Mead-Briggs *et al.*: A laboratory test for evaluating the effects of plant protection products on the parasitic wasp, *Aphidius rhopalosiphi* (DeStephani-Perez) (Hymenoptera, Braconidae). (2000)
Mead-Briggs *et al.*: An extended laboratory test for evaluating the effects of plant protection products on the parasitic wasp, *Aphidius rhopalosiphi* (Hymenoptera, Braconidae). (2010)

Deviations: No

GLP: Yes

Acceptability: Yes

Executive summary

An acute toxicity study, on mortality of the parasitoid, *Aphidius rhopalosiphi*, was conducted in the laboratory based on Mead-Briggs et al. 2000 and Mead-Briggs et al. 2010. Four replicates of ten adult parasitoids (7 females and 3 males) each were exposed for 48-hours to an untreated control, the reference item Danadim Progress (nominal: 400 g dimethoate/L) and to dried residues of Boscalid 350 g/kg + Prothioconazole 200 g/kg WG applied to glass plates at five test item application rates of 21, 63, 189, 567 and 1700 g product/ha in 200 L spray volume/ha.

Under worst case laboratory condition, the LR₅₀ value of Boscalid + Prothioconazole (350 + 200 g/kg – WG) is estimated to be higher than 1700 g product/ha in 200 L water/ha. The NOER (no observed effect rate) for mortality is equal to or higher than 1700 g product/ha and LOER (lowest observed effect rate) is higher than 1700 g product/ha in 200 L water/ha.

Reproduction of *Aphidius rhopalosiphi* was assessed in the control and in all test item application rates. Reproduction was not affected up to and including the highest test item application rate of 1700 g product/ha. The ER₅₀ value of Boscalid + Prothioconazole (350 +200 g/kg - WG) for reproduction is estimated to be higher than 1700 g product/ha in 200 L water/ha. The NOER for effects on reproduction is equal to or higher than 1700 g product/ha and the LOER is higher than 1700 g product/ha in 200 L water/ha.

All validity criteria were met. The study is considered valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:** Boscalid + Prothioconazole (350 + 200 g/kg – WG)
Product Code: FGE03
Batch no.: BDPT/WG/220915/550/20
Purity: Boscalid: 352.2 ± 0.2 g/kg ($35.22 \pm 0.02\%$ w/w)
Prothioconazole: 203.3 ± 0.1 g/kg ($20.33 \pm 0.01\%$ w/w)
Date of expiry: 14 September 2024
2. **Test concentrations:** Control: 200 L deionised water/ha
Test item: 21.0, 63.0, 189.0, 567.0 and 1700.0 g product/ha
(equivalent to concentration 0.105, 0.315, 0.945, 2.84 and 8.50 g product/L)
3. **Toxic reference:** Danadim Progress (dimethoate)
Batch no.: 10214034
Purity: 400 g/L (nominal), 413 g/L (analyzed)
Study number: 147505103

4. Test organism:

Species:	<i>Aphidius rhopalosiphi</i> Parasitoids (Hymenoptera, Braconidae)
Source:	Katz Biotech AG, Baruth, Germany
Age:	<48 hours, adults
Test units:	Exposure: Comprising of two treated glass plates (13 cm x 13 cm) which were held apart by an untreated aluminum frame (13 cm x 1.5 cm x 1 cm per side) and held together with at least 2 clamps. 3 sides of the frame had 6 ventilation holes covered with a cloth. The 4 th side of the frame had 1 small hole for inserting and feeding the test organisms. Post-exposure units: Untreated pots (13 cm in diameter) with barley seedlings (<i>Hordeum vulgare</i> ‘Sunshine’; 14 - 26 seedlings, 10 days old) infested with 100 - 200 host aphids of all developmental stages (<i>Rhopalosiphum padi</i> ; number of aphids was estimated) were enclosed within a clear polyacrylic cylinder (30 cm high and 10 cm in diameter). The cylinder had two holes (70 x 195 mm) which were closed with a fine gauze to improve the ventilation and another hole (approximately 2 cm in diameter) closed with cotton wool for the introduction of the parasitoids. The top of the cylinder was closed with a fine gauze. The soil surface was covered with a thin layer of quartz sand.
Test design:	Exposure: 7 treatment group, 4 replicates per treatment group, 10 per unit, approximately 48 hours exposure period Post-exposure: 20 replicates per treatment group, 1 female per unit, 11- 12 days exposure observation
Feeding:	A solution of fructose (10 %),

5. Environmental conditions

Temperature:	19 to 21 °C
Relative humidity:	73 to 76 % (acclimatization, exposure period) 74 to 82 % (post-exposure period; within the test units)
Light:	16:08 light-dark cycles, 700 to 1760 lux (acclimatization, exposure, parasitisation period), 11250 to 17150 lux (post-parasitisation period)
Ventilation:	Exposure units were ventilated with a small pump

B. STUDY DESIGN AND METHODS

1. Experimental phase: 30 May 2023 – 14 June 2023

2. Experiment treatment

Dilutions were prepared in 200 L deionised water/ha. Treatments were applied to glass plates with spraying scheme which were left to dry and then used to construct the test units. The parasitoids were introduced to these units and their behaviour and mortality was assessed 2, 24 and 48 h after test initiation. Newly hatched wasps of suitable age were kept in glass tubes under test conditions until application. Wasps were fed with aqueous fructose solution. During exposure period, 4 replicates per treatment group with 10 wasps per unit (7 females and 3 males) were prepared. During parasitisation period (post-exposure period), 20 replicates per treatment group with 1 female per unit were prepared. Moribund insects were not included in these assessments. Parasitisation period was 24 hours, at the end of the 24-h parasitisation period, the females were removed from the post-exposure units.

3. Measurements and observations

Observations of mortality were recorded approx. 2, 24 and 48 hours after test initiation. The parasitoids alive, affected, moribund and dead was recorded. Moribund parasitoids were counted as dead.

Number of aphid mummies was counted 11 – 12 days after the 24 hours parasitisation period in all replicates where the females were alive after the 24 hours parasitisation period. The number of live, dead or not found females after the 24 hours parasitisation period was documented. The reproduction assessment was performed for the control and for those test item groups where the corrected mortality (M_{corr}) was $\leq 50\%$.

No reproduction testing was performed with the reference item.

4. Statistics

The LR_{50} and ER_{50} value could not be calculated as no mortality or effects on reproduction above 50% were noted.

Mortality data obtained from the control and test item treatments were analysed for significance using the Bonferroni-Holm Fisher's Exact Binomial Test (one-sided greater, $\alpha = 0.05$), which is a distribution-free test and does not require testing for normality or homogeneity prior to analysis. However, a qualitative trend analysis by contrasts ($\alpha = 0.05$) and the Tarone's Test ($\alpha = 0.01$) had to be carried out previously to check for the presence of linear or quadratic trends and extra-binomial variance.

The two-sample comparison between the reference item and control was analysed using the Fisher's Exact Binomial Test (one-sided greater, $\alpha = 0.05$).

Reproduction data were tested for normal distribution, homogeneity of variance and the presence of linear trends using the Shapiro-Wilk's Test ($\alpha = 0.01$), the Levene's Test ($\alpha = 0.01$) and a trend analysis by contrasts ($\alpha = 0.05$). Because reproduction data were normally distributed and homogeneous and no linear trend was revealed, the Dunnett's t-Test (multiple comparison, one-sided smaller, $\alpha = 0.05$) was used.

The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ToxRat Solutions GmbH).

II. RESULTS AND DISCUSSION

A. MORTALITY, REPRODUCTIVE OUTPUT AND BEHAVIOUR

The mean mortality of *Aphidius rhopalosiphi* was 0.0 % in the control treatment and was between 0.0 and 2.5 % in the test item treatments, corresponding to corrected mortalities of 0.0 to 2.5 %. Mortality was not statistically significantly increased compared to the control up to and including the highest application rate of 1700 g product/ha (Bonferroni-Holm Fisher's Exact Binomial Test, one-sided greater, $\alpha = 0.05$).

The reference item applied at a rate of 0.3 mL Danadim Progress/ha produced a statistically significant corrected mortality of 100.0 % after 48 hours (Fisher's Exact Binomial Test, one-sided greater, $\alpha = 0.05$).

No behavioural abnormalities were observed at any test item application rate after 2, 24 and 48 hours.

Reproduction of *A. rhopalosiphi* was assessed in the control and at all test item application rates. The mean reproduction of *A. rhopalosiphi* was 34.8 mummies per female in the control treatment and was between 34.0 and 42.0 mummies per female in the test item treatments, corresponding to a reduction of reproduction of 2.4 to -20.5 %. Reproduction was not statistically significantly reduced compared to the control up to and including the highest application rate of 1700 g product/ha (Dunnett's t-Test, one-sided smaller, $\alpha = 0.05$).

Table A 2.3.2.1.1-1: Mortality and parasitisation efficiency of *Aphidius rhopalosiphi* adults after a 48-hour exposure to of Boscalid + Prothioconazole (350 + 200 g/kg WG) (FGE03)

Treatment group	Rates [g product/ha]	Mortality [%]		Corr. Mortality [%]	Parasitisation Rate [mummies/female]		Effect on reproduction [%]
Control	0	0.0 ± 0.0	-	-	34.8 ± 13.7	-	-
Test item	21.0	0.0 ± 0.0	n.s.	0.0	42.0 ± 1603	n.s.	-20.5
	63.0	2.5 ± 5.0	n.s.	2.5	38.6 ± 14.4	n.s.	-10.9
	189.0	0.0 ± 0.0	n.s.	0.0	36.8 ± 15.0	n.s.	-5.7
	567.0	0.0 ± 0.0	n.s.	0.0	35.1 ± 12.9	n.s.	-0.6
	1700.0	0.0 ± 0.0	n.s.	0.0	34.0 ± 15.8	n.s.	2.4
Reference item	0.3 mL product/ha	100.0 ± 0.0	*	100.0	-	-	-

n.s. = not significant; * = significant

B. VALIDITY CRITERIA

All required validity criteria were met. Accordingly, the study is regarded as valid.

Table A 2.3.2.1.1-2: Study validity criteria

Parameters	Required	Observed
Mean control mortality	≤ 13%	0.0%
Mean Reference Item Mortality	> 50%	100.0%
Mean control reproduction rate	≥ 5.0	34.8
Number of females failed to produce mummies in the control	≤ 2	0

C. TOXICITY ENDPOINTS

The median lethal rate (LR₅₀) for mortality and the median effect rate on reproductive output (ER₅₀) were determined and are presented in the following table.

Table A 2.3.2.1.1-3: Mortality and parasitisation efficiency of *Aphidius rhopalosiphi* of exposure to Boscalid + Prothioconazole (350 + 200 g/kg - WG) (FGE03) – summary of endpoints

Endpoints	Rate
	Test item [g product/ha]
Mortality LR ₅₀	>1700
NOER for Mortality	≥1700
LOER for Mortality	>1700
Reproduction ER ₅₀	>1700
NOER for Reproduction	≥1700
LOER for Reproduction	>1700

LR₅₀ and ER₅₀ value could not be calculated as no mortality or effects on reproduction above 50% were noted.

III. CONCLUSION

Under worst case laboratory conditions, the LR₅₀ value of Boscalid + Prothioconazole (350 + 200 g/kg - WG) is estimated to be higher than 1700 g product/ha in 200 L water/ha. The NOER (no observed effect rate) for mortality is equal to or higher than 1700 g product/ha and the LOER (lowest observed effect rate) is higher than 1700 g product/ha in 200 L water/ha.

Reproduction of *Aphidius rhopalosiphi* was assessed in the control and in all test item application rates. Reproduction was not affected up to and including the highest test item application rate of 1700 g product/ha. The ER₅₀ value of Boscalid + Prothioconazole (350 +200 g/kg - WG) for reproduction is estimated to be higher than 1700 g product/ha in 200 L water/ha. The NOER for effects on repro-

duction is equal to or higher than 1700 g product/ha and the LOER is higher than 1700 g product/ha in 200 L water/ha..

All validity criteria were met. The study is considered valid.

A 2.3.2.1.2 Leopold, 2023b (KCP 10.3.2.1/02)

Comments of zRMS:	The study was conducted in line with the respective guideline with no deviations. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: LR₅₀=503.1 g product/ha ER₅₀ > 272 g product/ha
-------------------	---

Reference: KCP 10.3.2.1/02

Report: Boscalid + Prothioconazole (350 + 200 g/kg – WG): Effects on the Predatory Mite *Typhlodromus pyri* (Acari: Phytoseiidae) in the Laboratory. A Dose Response Test on Glass Plates, Leopold, J., 2023b, Study code 176531063, UPL/2023/1054

Guideline(s): Blümel *et al.*, Laboratory residual contact test with the predatory mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) for regulatory testing of plant protection products. (2000)

Deviations: No

GLP: Yes

Acceptability: Yes

Executive summary

An acute 7-day toxicity study, on the predatory mite, *Typhlodromus pyri*, was conducted in the laboratory according to Blümel et al. 2000. Three replicates of 20 predatory mites each were exposed for 7 days to an untreated control, the reference item Danadim Progress (nominal: 400 g Dimethoate/L) and to dried residues of Boscalid + Prothioconazole (350 + 200 g/kg – WG) applied to glass plates at five test item application rates of 43.5, 109, 272, 680 and 1700 g product/ha, in 200 L water/ha.

Under worst case laboratory conditions, the LR₅₀ value of Boscalid + Prothioconazole (350 +200 g/kg - WG) for mortality is 503.1 g product/ha in 200 L water/ha (95 % confidence limits: 290.7 to 870.7 g product/ha). The NOER (no observed effect rate) for mortality is 109 g product/ha and the LOER (lowest observed effect rate) is 272 g product/ha in 200 L water/ha.

Reproduction of *Typhlodromus pyri* was assessed in the control and at 43.5, 109 and 272 g product/ha. Reproduction was not statistically significantly affected up to and including the application rate of 272 g product/ha. The ER₅₀ value of Boscalid + Prothioconazole (350 +200 g/kg - WG) for reproduction is estimated to be higher than 272 g product/ha in 200 L water/ha since no effects above 50 % were recorded up to the test item application rate of 272 g product/ha. The NOER for reproduction is equal to or higher than 272 g product/ha and the LOER is higher than 272 g product/ha in 200 L water/ha.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:** Boscalid + Prothioconazole (350 + 200 g/kg - WG)
Product Code: FGE03
Batch no.: BDPT/WG/220915/550/20
Purity: Boscalid: 352.2 ± 0.2 g/kg (35.22 ± 0.02% w/w)
Prothioconazole: 203.3 ± 0.1 g/kg (20.33 ± 0.01% w/w)
Date of expiry: 14 September 2024
2. **Test concentrations:** Control: 200 L deionised water/ha
Test item: 43.5, 109.0, 272.0, 680.0 and 1700.0 g product/ha
3. **Toxic reference:** 9 mL Danadim Progress, dimethoate/ha
Batch no.: 10214034
Purity: 400 g/L (nominal), 413 g/L (analyzed)
Study number: 147505103
4. **Test organism:**
Species: *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae)
Source: Katz Biotech AG, An der Birkenpfuhlheide 10, D-15837 Baruth
Age: Protonymphs, not older than 24 hours
Test units: Two cover slides (glass, 24 mm x 60 mm) fixed by gluing small cover slides (glass, 20 mm x 20 mm) to both side-ends. A glue barrier was placed on the test unit to keep the mites on this test arena. Plastic trays (11 cm x 11 cm x 6 cm) half-filled with water, with a foam rubber and a glass-plate on top covered by tissue paper, tissue paper in contact with the water. The test units were placed on the tissue paper.
Test design: 3 replicates per treatment group, 20 per unit, 14 days for the control and all test item treatment groups where reproduction was assessed after 7 days. Introduction of mites 35 – 55 mins after application (after drying of test units).
Feeding: Pine (*Pinus sp.*) and birch (*Betula sp.*) pollen (3:1) *ad libitum* on the test start and each assessment day except for the last one *resp.* at least every four days.

5. Environmental conditions

Temperature: 24 - 26 °C
Relative humidity: 65 – 73 %
Light: 16:08 light-dark cycles, 290 – 380 lux

B. STUDY DESIGN AND METHODS

1. Experimental phase: 30 June – 14 July 2023

2. Experiment treatment

Spray solutions were prepared in 200 L deionised water/ha. Treatments were applied to glass plates which were left to dry and then used to construct the test arenas. 3 replicates per treatment group with 20 mites per unit.

The weight of the glass plate was determined immediately before and after application and the amount of spray deposit per cm² was calculated as the difference between the weight before and after spraying. The procedure was repeated 5 times in a row without changing the adjustment and every time the application rate was within 200 L/ha $\pm$ 10 %. The uniformity of the deposit distribution was checked visually.

Mites were then introduced to the arenas with fine brush and spraying scheme, then their survival assessed over a 7-day period. Duration of exposure was 14 days.

3. Measurements and observations

The number of living, dead and escaped mites as well as mites trapped by the glue barrier was counted on day 3 and on day 7 after test initiation. Dead mites were removed. Escaped mites were considered as dead.

Reproduction was assessed for all test item rates between day 7 and day 14 with a maximum interval of 3 days after test initiation. Sex ratio for reproduction testing was 1 male:5 females at a minimum on day 7. All eggs laid until day 7 inclusive were removed and not counted. Three assessments were carried out on day 10, 13 and 14 after test initiation. At each assessment, eggs and juveniles were counted and removed afterwards. The mean number of eggs produced per female between 7 and 14 days after test initiation was calculated.

4. Statistics

The LR₅₀ value of the mortality was calculated using the Logit-Analysis. The ER₅₀ value for reproduction could not be calculated as no effects on reproduction above 50 % were noted.

Mortality data obtained from the control and test item treatments were analysed for significance using the Step-down Cochran-Armitage Test ($\alpha = 0.05$, one-sided greater), which is a distribution-free test and does not require testing for normality or homogeneity prior to analysis. However, a qualitative trend analysis by contrasts ($\alpha = 0.05$) and the Tarone's Test ($\alpha = 0.01$) had to be carried out previously to check for the presence of linear or quadratic trends and extra-binomial variance.

The two-sample comparison between the reference item and control was analysed using the Fisher's Exact Binomial Test (one-sided greater, $\alpha = 0.05$).

Reproduction data were tested for normal distribution, homogeneity of variance and the presence of linear trends using the Shapiro-Wilk's Test ($\alpha = 0.01$), the Levene's Test ($\alpha = 0.01$) and a trend analysis by contrasts ($\alpha = 0.05$). Because reproduction data were normally distributed and homogeneous and no linear trend was revealed and the number of degrees of freedom were insufficient to perform the Dunnett's t-Test, the Multiple Sequentially-rejective t-Test after Bonferroni-Holm (multiple comparison, one-sided smaller, $\alpha = 0.05$) was used.

The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ® ToxRat

Solutions GmbH.

II. RESULTS AND DISCUSSION

A. MORTALITY, REPRODUCTIVE OUTPUT AND BEHAVIOUR

In the control group, the mean mortality was 8.3%.

Boscalid + Prothioconazole (350 + 200 g/kg – WG) ranged from 10.0 to 83.3 % in the test item treatments, corresponding to corrected mortalities of 1.8 to 81.8 %. Mortality was not statistically significantly increased compared to the control up to and including the application rate of 109 g product/ha (Step-down Cochran-Armitage Test, one-sided greater, $\alpha = 0.05$).

The reference item applied at a rate of 9.0 mL Danadim Progress/ha produced a statistically significant mortality of 100 % (corrected mortality 100 %) after 7 days (Fisher's Exact Binomial Test, one-sided greater, $\alpha = 0.05$).

Table A 2.3.2.1.2-1: Mortality and reproduction of mites (*Typhlodromus pyri*) after exposure to of Boscalid + Prothioconazole (350 + 200 g/kg – WG)

Treatment group	Rates ¹ [g product/ha]	Mortality ² [%]		Corrected mortality ³ [%]	Reproduction ⁴ [eggs/female]		Effect on reproduction ⁵ [%]
Control	0	8.3 ± 7.6		-	5.9 ± 0.9		-
Test item	43.5	10.0 ± 5.0	n.s.	1.8	6.0 ± 0.3	n.s.	-1.8
	109	11.7 ± 5.8	n.s.	3.6	5.6 ± 0.4	n.s.	6.2
	272	28.3 ± 14.4	n.s.	21.8	6.0 ± 0.9	n.s.	-0.5
	680	78.3 ± 16.1	n.s.	76.4	-		-
	1700	83.3 ± 7.6	n.s.	81.8	-		-
Reference item	9.0 mL product/ha	100 ± 0.0	*		-		-

¹ Application rate in 200 L spray volume/ha

² Mortality: after 7 days of exposure to spray residues on glass plates,
(Step-down Cochran-Armitage Test, one-sided greater, $\alpha = 0.05$; n.s. = not significant, * = significant)

³ Corrected mortality according to Abbott and improvements by Schneider-Orelli

⁴ Reproduction: mean number of eggs/female, (Multiple Sequentially-rejective t-Test after Bonferroni-Holm; one-sided smaller; $\alpha = 0.05$; n.s. = not significant)

⁵ Calculated on the exact raw data; negative values indicate better reproductive performance compared to the control

B. VALIDITY CRITERIA

All required validity criteria were met. Accordingly, the study is regarded as valid.

Table A 2.3.2.1.2-2: Study validity criteria

Parameters	Required	Observed
Mean mortality in the control treatment	≤ 20%	8.3%
Mean mortality in the reference item	> 50%	100 %
Mean control reproduction rate after 14 days	≥ 4.0	5.9

C. TOXICITY ENDPOINTS

The median lethal rate (LR₅₀) for mortality and the median effect rate on reproductive output (ER₅₀) were determined and are presented in the following table.

Table A 2.3.2.1.2-3: Mortality and reproductive output of mite *Typhlodromus pyri* of exposure to Boscalid + Prothioconazole (350 + 200 g/kg – WG)– summary of endpoints

Endpoints	Rate
	Test item [g product/ha]
Mortality LR ₅₀ (95% CL)	503.1 (290.7 – 870.7)

NOER for Mortality	109
LOER for Mortality	272
Reproduction ER₅₀	> 272
NOER for Reproduction	≥ 272
LOER for Reproduction	> 272

LR₅₀ was calculated with Logit Analysis; ER₅₀ value for reproduction could not be calculated as no effects on reproduction above 50% were noted; CL = confidence limits

III. CONCLUSION

Under worst case laboratory conditions, the LR₅₀ value of Boscalid + Prothioconazole (350 +200 g/kg - WG) for mortality is 503.1 g product/ha in 200 L water/ha (95 % confidence limits: 290.7 to 870.7 g product/ha). The NOER (no observed effect rate) for mortality is 109 g product/ha and the LOER (lowest observed effect rate) is 272 g product/ha in 200 L water/ha.

Reproduction of *Typhlodromus pyri* was assessed in the control and at 43.5, 109 and 272 g product/ha. Reproduction was not statistically significantly affected up to and including the application rate of 272 g product/ha. The ER₅₀ value of Boscalid + Prothioconazole (350 +200 g/kg - WG) for reproduction is estimated to be higher than 272 g product/ha in 200 L water/ha since no effects above 50 % were recorded up to the test item application rate of 272 g product/ha. The NOER for reproduction is equal to or higher than 272 g product/ha and the LOER is higher than 272 g product/ha in 200 L water/ha.

All validity criteria were met. The study is considered valid.

A 2.3.2.2 KCP 10.3.2.2 Extended laboratory testing for non-target arthropods

A 2.3.2.2.1 Leopold, 2024 (KCP 10.3.2.2/01)

Comments of zRMS:	The study was conducted in line with the respective guideline with no deviations. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: LR₅₀/ER₅₀> 1700 g product/ha
-------------------	---

Reference: KCP 10.3.2.2/01

Report: Boscalid + Prothioconazole (350 + 200 g/kg – WG): Effects on the Predatory Mite *Typhlodromus pyri* (Acari: Phytoseiidae), Extended Laboratory study. Dose Response Test, Leopold, J., 2024, Study code 176531062, UPL/2024/0298

Guideline(s): Blümel *et al.*, 2000: Laboratory residual contact test with the predatory mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) for regulatory testing of plant protection products.
Oomen 1988: Guideline for the evaluation of side-effects of pesticides *Phytoseiulus persimilis* A.-H

Deviations: No

GLP: Yes

Acceptability: Yes

Executive summary

An acute 7-day toxicity study, on the predatory mite, *Typhlodromus pyri*, was conducted in the extended laboratory condition according to Blümel et al. 2000. Six replicates of 10 predatory mites each were exposed for 7 days to an untreated control, the reference item Danadim Progress (nominal: 400 g Dimethoate/L) and to dried residues of Boscalid + Prothioconazole (350 + 200 g/kg – WG) applied to leaf disc containers at five test item application rates of 43.5, 109, 272, 680 and 1700 g product/ha, in 200 L water/ha.

Under extended laboratory conditions, the LR₅₀ value of Boscalid + Prothioconazole (350 + 200 g/kg - WG) is estimated to be higher than 1700 g product/ha. The NOER (no observed effect rate) for mortality effects was < 43.5 g product/ha and the LOER (lowest observed effect rate) for mortality effects was 43.5 g product/ha.

Reproduction of *Typhlodromus pyri* was assessed in the control and at all application rates. There were no effects on reproduction up to and including 1700 g product/ha. Therefore, the ER₅₀ value was estimated to be higher than 1700 g product/ha. The NOER for reproduction effects was equal to or higher than 1700 g product/ha and the LOER for reproduction effects was higher than 1700 g product/ha.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:** Boscalid + Prothioconazole (350 + 200 g/kg - WG)
Product Code: FGE03
Batch no.: BDPT/WG/220915/550/20
Purity: Boscalid: 352.2 ± 0.2 g/kg ($35.22 \pm 0.02\%$ w/w)
Prothioconazole: 203.3 ± 0.1 g/kg ($20.33 \pm 0.01\%$ w/w)
Date of expiry: 14 September 2024
2. **Test concentrations:** Control: 200 L deionised water/ha
Test item: 43.5, 109.0, 272.0, 680.0 and 1700.0 g product/ha
3. **Toxic reference:** 40.0 mL Danadim Progress, dimethoate/ha
Batch no.: 10214034
Purity: 400 g/L (nominal), 402 g/L (analyzed)
Study number: 147506103
4. **Test organism:**
Species: *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae)
Source: Katz Biotech AG, An der Birkenpfuhlheide 10, D-15837 Baruth
Age: Protonymphs, not older than 24 hours
Test units: Detached primary leaves of bean plants (*Phaseolus vulgaris* L., 27 days old) were cut to discs with a diameter of about 55 mm. the leaf cuts were treated on their upper surface. The test unit was placed with its treated side upward on a wet cotton wool pad in a petri dish. The petri dish was filled with some tap water to keep the cotton wool pad wet. A small strip of filter paper (approximately 5 mm x 25 mm) was partly laid onto the leaf (approximately 5 mm distance to the leaf margin) and extended onto the cotton wool pad (with approximately 20 mm contact to the pad).
Test design: 6 replicates per treatment group, 10 per unit, 14 days for the control and all test item treatment groups where reproduction was assessed after 7 days. Introduction of mites 35 – 60 mins after application (after drying of test units).
Feeding: Pine (*Pinus* sp.) and birch (*Betula* sp.) pollen (3:1) *ad libitum* on the test start and each assessment day except for the last one *resp.* at least

every four days.

5. Environmental conditions

Temperature: 25 - 26 °C
Relative humidity: 66 – 69 %
Light: 16:08 light-dark cycles, 230 – 470 lux

B. STUDY DESIGN AND METHODS

1. Experimental phase: 19 February – 26 March 2024

2. Experiment treatment

Spray solutions were prepared in 200 L deionised water/ha. Single application on the upper surface of the detached leaves according to agriculture practice with a laboratory spraying equipment. 6 replicates per treatment group with 10 mites per unit.

The weight of the glass plate was determined immediately before and after application and the amount of spray deposit per cm² was calculated as the difference between the weight before and after spraying. The procedure was repeated 5 times in a row without changing the adjustment and every time the application rate was within 200 L/ha $\pm$ 10 %. The uniformity of the deposit distribution was checked visually.

Mites were then introduced to the arenas with fine brush and spraying scheme, then their survival assessed over a 7-day period. Duration of exposure was 14 days.

3. Measurements and observations

The number of living, dead and escaped mites was counted twice in the first week on day 2 and on day 7 after test initiation. Dead mites were removed. Escaped mites were considered as dead.

Reproduction was assessed for all test item rates between day 7 and day 14 with a maximum interval of 3 days after test initiation. Sex ratio for reproduction testing was less than 1 male:5 females on day 7. All eggs laid until day 7 inclusive were removed and not counted. At each assessment, eggs and juveniles were counted and removed afterwards. The mean number of eggs produced per female between 7 and 14 days after test initiation was calculated.

4. Statistics

The LR₅₀ and ER₅₀ value could not be calculated as no mortality or effects on reproduction above 50% were observed.

Mortality data obtained from the control and test item treatments were analysed for significance using the Chi² 2x2 Table Test with Bonferroni Correction (one-sided greater, α = 0.05), which is a distribution-free test and does not require testing for normality or homogeneity prior to analysis. However, a qualitative trend analysis by contrasts (α = 0.05) had to be carried out previously to check for the presence of linear or quadratic trends.

Reproduction data were tested for normal distribution, homogeneity of variance and the presence of linear trends using the Shapiro-Wilk's Test (α = 0.01), the Levene's Test (α = 0.01) and a trend analysis by contrasts (α = 0.05). Because reproduction data were normally distributed and homogeneous and a linear trend was revealed, the Williams t-Test (multiple comparison, one-sided smaller, α = 0.05) was used.

The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ® ToxRat Solutions GmbH.

II. RESULTS AND DISCUSSION

C. MORTALITY, REPRODUCTIVE OUTPUT AND BEHAVIOUR

In the control group, the mean mortality was 18.3%.

Boscalid + Prothioconazole (350 + 200 g/kg – WG) ranged from 31.7 to 45.0 % in the test item treatments, corresponding to corrected mortalities of 16.3 to 32.7 %. Mortality was statistically significantly increased compared to the control at all test item application rates (Chi² 2x2 Table Test with Bonferroni Correction, one-sided greater, $\alpha = 0.05$).

The reference item applied at a rate of 40.0 mL Danadim Progress/ha produced a statistically significant mortality of 100 % (corrected mortality 100 %) after 7 days.

Table A 2.3.2.2.1-1: Mortality and reproduction of mites (*Typhlodromus pyri*) after exposure to of Boscalid + Prothioconazole (350 + 200 g/kg – WG)

Treatment group	Rates ¹ [g product/ha]	Mortality ² [%]		Corrected mortality ³ [%]	Reproduction ⁴ [eggs/female]		Effect on reproduction ⁵ [%]
Control	0	18.3 ± 13.3		-	5.0 ± 1.7		-
Test item	43.5	40.0 ± 38.5	*	26.5	4.6 ± 1.8	n.s.	7.9
	109	38.3 ± 38.2	*	24.5	5.3 ± 2.5	n.s.	-4.4
	272	45.0 ± 20.7	*	32.7	5.7 ± 3.8	n.s.	-13.4
	680	35.0 ± 13.8	*	20.4	6.8 ± 2.6	n.s.	-35.1
	1700	31.7 ± 19.4	*	16.3	7.2 ± 1.2	n.s.	-42.1
Reference item	40.0 mL product/ha	100 ± 0.0	*	100.0	-	-	-

¹ Application rate in 200 L spray volume/ha

² Mortality: after 7 days of exposure to spray residues on leaf surfaces,

(Chi² 2x2 Table Test with Bonferroni Correction, one-sided greater, $\alpha = 0.05$; * = significant)

³ Corrected mortality according to Abbott and improvements by Schneider-Orelli

⁴ Reproduction: mean number of eggs/female, (Williams t-Test; one-sided smaller; $\alpha = 0.05$; n.s. = not significant)

⁵ Calculated on the exact raw data; negative values indicate better reproductive performance compared to the control

D. VALIDITY CRITERIA

All required validity criteria were met. Accordingly, the study is regarded as valid.

Table A 2.3.2.2.1-2: Study validity criteria

Parameters	Required	Observed
Mean mortality in the control treatment	≤ 20%	18.3%
Mean mortality in the reference item	> 50%	100 %
Mean control reproduction rate after 14 days	≥ 4.0	5.0

E. TOXICITY ENDPOINTS

The median lethal rate (LR₅₀) for mortality and the median effect rate on reproductive output (ER₅₀) were determined and are presented in the following table.

Table A 2.3.2.2.1-3: Mortality and reproductive output of mite *Typhlodromus pyri* of exposure to Boscalid + Prothioconazole (350 + 200 g/kg – WG) – summary of endpoints

Endpoints	Rate
	Test item [g product/ha]
Mortality LR ₅₀ (95% CL)	> 1700
NOER for Mortality	< 43.5

LOER for Mortality	43.5
Reproduction ER₅₀	> 1700
NOER for Reproduction	≥ 1700
LOER for Reproduction	> 1700

LR₅₀ and ER₅₀ value could not be calculated as no mortality above 50% were observed.

III. CONCLUSION

Under extended laboratory conditions, the LR₅₀ value of Boscalid + Prothioconazole (350 +200 g/kg - WG) is estimated to be higher than 1700 g product/ha. The NOER (no observed effect rate) for mortality effects was < 43.5 g product/ha and the LOER (lowest observed effect rate) for mortality effects was 43.5 g product/ha.

Reproduction of *Typhlodromus pyri* was assessed in the control and at all application rates. There were no effects on reproduction up to and including 1700 g product/ha. Therefore, the ER₅₀ value was estimated to be higher than 1700 g product/ha. The NOER for reproduction effects was equal to or higher than 1700 g product/ha and the LOER for reproduction effects was higher than 1700 g product/ha.

All validity criteria were met. The study is considered valid.

A 2.3.2.2.2 Knautz, 2024 (KCP 10.3.2.2/02)

Comments of zRMS:	The study was conducted in line with the respective guideline with no deviations. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: LR₅₀/ER₅₀> 1700 g product/ha
-------------------	---

Reference:	KCP 10.3.2.2/02
Report:	Boscalid + Prothioconazole (350 + 200 g/kg – WG): Effects on Carabid Beetle <i>Poecilus cupreus</i> L. – Extended Laboratory Study – Dose-Response Study, Knautz, T., 2024, Study No.176531007, UPL/2024/0337
Guideline(s):	GLP complaint study based on BBA guideline part VI, 23-2.1.8,1991 and Heimbach et. al, 2000
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Executive summary

A 14-days toxicity study, on the carabid beetle, *Poecilus cupreus*. L, was conducted in the extended laboratory condition according to BBA guideline part VI, 1991 and Heimbach et.al. 2000. Five replicates of 6 beetles (3 males and 3 females per unit), each were exposed for 14 days to an untreated control, the reference item Danadim Progress (nominal: 400 g Dimethoate/L) and Boscalid + Prothioconazole (350 + 200 g/kg – WG) at five test item application rates of 475, 950, 1950, 3800 and 7600 g test item/ha, in 400 L water/ha.

Under extended laboratory conditions, the LR50 value of Boscalid + Prothioconazole (350 +200 g/kg - WG) is estimated to be > 7600 g test item/ha, corresponding to > 2677 g Boscalid and > 1545 g Prothioconazole/ha. The NOER (no observed effect rate) was determined to be $\geq$ 7600 g Boscalid + Prothioconazole (350 + 200 g/kg – WG)/ha corresponding to $\geq$ 2677 g Boscalid/ ha and corresponding to $\geq$ 1545 g Prothioconazole/ha.

No test item related behavioural abnormalities occurred at any time for the duration of the experiment. The food consumption was 100 % in test item and control treatment group. Reference item group showed 25 % food consumption over the entire experimental period.

The study met the validity criteria according to Heimbach et al. (2000).

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:** Boscalid + Prothioconazole (350 + 200 g/kg - WG)
Product Code: FGE03
Batch no.: BDPT/WG/220915/550/20
Purity: Boscalid: 352.2 ± 0.2 g/kg ($35.22 \pm 0.02\%$ w/w)
Prothioconazole: 203.3 ± 0.1 g/kg ($20.33 \pm 0.01\%$ w/w)
Date of expiry: 14 September 2024
2. **Test concentrations:** Control: 400 L deionised water/ha
Test item: 7600, 3800, 1950, 950 and 475 g product/ha
Corresponding to 2677, 1338, 699, 335 and 167 g Boscalid/ha
Corresponding to 1545, 773, 386, 193 and 96.6 g Prothioconazole/ha
3. **Toxic reference:** 5.0 mL Danadim Progress, dimethoate/ha
Batch no.: 10214034
Purity: 400 g/L (nominal), 402 g/L (analyzed)
Analysis code: 147506103

4. Test organism:

Species:	<i>Peocilus cupreus</i> L. (Carabid beetles, Coleoptera: Carabidae)
Source:	Bio-Test Labor GmbH, Birkenallee 19, 18184 Sagerheide, Germany
Age at test start:	Adult, 7-9 weeks female/ male
Acclimatisation:	7 days under test conditions of which the last 4 days without food
Test units:	Plastic boxes (18.3 cm x 13.6 cm x 6 cm; length, width, height); LUFA 2.1; 250 ± 1 g dry soil, at the beginning the soil was moistened to about 55 ± 5 % of its maximum water holding capacity with deionised water (maximum water holding capacity of this LUFA 2.1 batch was 32.0 %; therefore, 44 g deionised water per test unit was added). The substrate surface was about 185 cm ² and the height of the moistened soil was approximately 1 cm. A plastic lid was perforated in order to maintain ventilation, during application the walls of the boxes were protected with an insert to avoid an increase of the concentration of the spray liquid on the soil by run off of droplets from the walls. The insert was removed after application. The natural soil, which was used as a substrate, represents a worst-case scenario with a high proportion of sand and a low content of organic matter.
Feeding:	With punctured deep frozen fly pupae (<i>Delia spec.</i>) on day 0 before application) and on days 2, 4, 7 and 11 after application, at a rate of 1 pupa per viable beetle. First feeding was done shortly before application.

5. Environmental conditions

Temperature:	20 – 21 °C (acclimatisation), 20 – 21 °C (exposure)
Relative humidity:	71 – 72 % (acclimatisation), 70 – 72 % (exposure)
Light Regime:	16:08 light-dark cycles,
Light intensity:	490 – 790 lux (acclimatisation), 500 – 800 lux (exposure)

B. STUDY DESIGN AND METHODS

1. Experimental phase: 04 – 11 March 2024

2. Experiment treatment

Spray solutions were prepared in 400 L deionised water/ha. Single application of the test units filled with the soil according to agricultural practice with a laboratory-spraying equipment onto the soil surface. The test units contained the test organisms during application. Five replicates per treatment group with 6 beetles (3 males and 3 females) per unit.

The application rates of the test item were 475, 950, 1950, 3800 and 7600 g test item/ha, corresponding to 167, 335, 699, 1338 and 2677 g Boscalid/ha; 96.6, 193, 386, 773 and 1545 g Prothioconazole/ha. Control units were sprayed with 400 L deionised water/ha and reference unit was applied at an application rate of 2000 mL Danadim Progress/ha.

Carabid beetles were introduced to the prepared test units 7 days before application, then the mortality and further sublethal symptoms were assessed at 2-hour, 1, 2, 4, 7, 11 and 14 days after application. Food consumption were assessed on day 2, 4, 7, 11 and 14 after application.

3. Test Design

The beetles were exposed to Boscalid + Prothioconazole (350 + 200 g/kg – WG) to five test item rates in a dose response design on natural soil (LUFA 2.1). The control, test item and reference item was applied directly onto the soil surface and test organisms via a laboratory spray applicator and a water amount of 400 L/ha. The control group was sprayed with deionised water and dimethoate (Danadim Progress, nominal 400 g/L) was used as reference item.

Each treatment consisted of 5 test units each containing 6 individuals (3 males and 3 females)/test unit. Assessments of mortality and behaviour were conducted ca. 2 hours, 1, 2, 4, 7, 11 and 14 days after application. Food consumption was assessed on days 2, 4, 7, 11 and 14 after application.

4. Measurements and observations

Number of living and dead beetles was counted ca. 2 hours after application and 1, 2, 4, 7, 11 and 14 days after the application; at the last assessment the soil was searched for missing beetles. Damaged beetles were placed at one corner of the trays and were counted as dead, if they were still there 24 h later.

Number of fly pupae consumed or untouched was counted on day 2, 4, 7, 11 and 14 after application; missing pupae were denoted consumed. The soil surface was searched for remaining untouched food on each feeding day.

Number of damaged beetles (e.g. behavioural abnormalities, uncoordinated movements, lying on the back) was counted ca. 2 hours and 1, 2, 4, 7, 11 and 14 days after application.

5. Statistics

NOER: Mortality Step-down Cochran-Armitage Test (one-sided greater, $\alpha = 0.05$). The LR50 was estimated to be higher than the highest tested rate. Food consumption in any test item treatment group was 100%, no statistical evaluation was conducted.

The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ® ToxRat Solutions GmbH.

II. RESULTS AND DISCUSSION

A. MORTALITY, REPRODUCTIVE OUTPUT AND BEHAVIOUR

In the control group, the mortality was 3.3%.

Boscalid + Prothioconazole (350 + 200 g/kg – WG) mortality 6.7 % (corresponding to 3.4 % corrected mortality) occurred in the second highest test item rate (3800 g test item/ha) only at the end of experiment. Traces of cannibalism were observed on all three dead organisms in the control and in the 3800 g test item/ha treatment group.

The reference item applied at a rate of 2000 mL Danadim Progress/ha was observed 100 % of mortality on day 7 of the experiment.

No test item related behavioural abnormalities occurred at any time for the duration of the experiment.

The sum of mean food consumption (mean number of consumed pupae per living beetle over the entire observation period) was 5.00 in the test item treatment group (corresponding to 100 % of the control group). The sum of mean food consumption in the reference item group was 1.2 pupae per living beetle over the entire experimental period (this corresponds to 25 % of the food consumption in the control group).

Table A 2.3.2.2.2-1: Effects of Boscalid + Prothioconazole (350 + 200 g/kg – WG) on ground beetles (*Poecilus cupreus*) in an extended laboratory trial

Treatment group	Rates [g product/ha]	Mortality ¹ [%]		Mortality corrected [%]	Pupae consumed per beetle ²		Food consumption ³ [%]
Control	0	3.3		-	5.0		-
Test item	475	0.0	0.0	5.0	100	475	0.0
	950	0.0	0.0	5.0	100	950	0.0
	1950	0.0	0.0	5.0	100	1950	0.0
	3800	6.7	3.4	5.0	100	3800	6.7
	7600	0.0	0.0	5.0	100	7600	0.0
Reference item	2000	100.0	96.7	1.2	25	2000	100.0

¹ Mortality: mean of 5 replicates with 6 beetles/replicates, 30 beetles in total

² sum of mean values over entire study period

³ compared to the control

B. VALIDITY CRITERIA

All required validity criteria were met. Accordingly, the study is regarded as valid.

Table A 2.3.2.2.2-2: Study validity criteria

Parameters	Required	Observed
Control mortality on day 14	≤ 6.7 %	3. 3%
Reference item Mortality on day 14	65 ± 35 %	100 %

C. TOXICITY ENDPOINTS

The median lethal rate (LR₅₀) for mortality and no observed effect rate (NOER) were determined and are presented in the following table.

Table A 2.3.2.2.2-3: Mortality and reproductive output of ground beetles (*Poecilus cupreus*) of exposure to Boscalid + Prothioconazole (350 + 200 g/kg – WG) – summary of endpoints

Endpoints	Rate
	Test item [g product/ha]
LR ₅₀	> 7600
NOER	≥ 7600

III. CONCLUSION

In this extended laboratory study, no effects on either mortality, food consumption or behaviour of the ground dwelling predator *Poecilus cupreus* were observed after exposure of the beetles 7600, 3800, 1950, 950 and 475 g Boscalid + Prothioconazole (350 + 200 g/kg – WG)/ha.

The LR₅₀ was estimated to be > 7600 g Boscalid + Prothioconazole (350 + 200 g/kg – WG)/ha corresponding to > 2677 g Boscalid/ ha and corresponding to > 1545 g Prothioconazole/ha.

The NOER (no observed effect rate) was determined to be ≥ 7600 g Boscalid + Prothioconazole (350 + 200 g/kg – WG)/ha corresponding to ≥ 2677 g Boscalid/ ha and corresponding to ≥ 1545 g Prothioconazole/ha.

The study met the validity criteria according to Heimbach et al. (2000).

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 Hübner, 2023a (KCP 10.4.1.1/01)

Comments of zRMS:	The study was conducted in line with OECD 222 with no deviations. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: 56d NOEC $\geq$ 10 mg prod./kg dw soil 56d EC₁₀ $\geq$ 10 mg prod./kg dw soil
-------------------	---

Reference:	KCP 10.4.1.1/01
Report:	Boscalid + Prothioconazole (350 + 200 g/kg – WG): Effects on Reproduction and Growth of Earthworm <i>Eisenia fetida</i> in Artificial Soil, Hübner, S., 2023a, Study code 176531022, UPL/2023/2769
Guideline(s):	OECD Guideline for the Testing of Chemicals, No. 222 (2016): Earthworm Reproduction Test in Soil; Adopted: 29th July 2016.
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Executive summary

The objective of the study was to determine the effect of with Boscalid + Prothioconazole (350 +200 g/kg – WG), on behaviour (sub lethal), mortality, growth and reproduction of earthworm (*Eisenia andrei*). Adult earthworms were exposed to artificial soil treated with eight nominal concentrations of 0.163, 0.294, 0.529, 0.953, 1.71, 3.09, 5.56 and 10.0 mg test item/kg soil dry weight in addition to untreated control for 56 days. Mortality and growth (body weight) of the earthworm were assessed after 28 days and the effects on reproduction (number of juveniles produced) were assessed after 56 days. Deionised water as control treatment.

In an earthworm reproduction and growth study with Boscalid + Prothioconazole (350 +200 g/kg - WG) the No Observed Effect Concentration (NOEC) for mortality, weight changes and reproduction of the earthworm *Eisenia andrei* was determined to be ≥ 10.0 mg test item/kg soil dry weight, i.e. the highest concentration tested. The Lowest Observed Effect Concentration (LOEC) was estimated to be >10.0 mg test item/kg soil dry weight. The LC₅₀ was estimated to be >10.0 mg test item/kg soil dry weight. The EC₁₀, EC₂₀ and EC₅₀ were estimated to be >10.0 mg test item/kg soil dry weight.

In the most recent test with the reference item Carbendazim (performed under the test facility Study No. 105689022 from January to March 2023), there were statistically significant effects on reproduction at a concentration of 1.00 mg a.i./kg soil dry weight and above, which is in line with the guideline OECD 222 (effects should be observed between 1 and 5 mg a.i./kg soil dry weight). The EC₅₀ for reproduction was calculated as 1.10 mg a.i./kg soil dry weight.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:** Boscalid + Prothioconazole (350 + 200 g/kg – WG)
Product Name: FGE03
Batch no.: BDPT/WG/220915/550/20
Purity: Boscalid: 352.2 ± 0.2 g/kg ($35.22 \pm 0.02\%$ w/w)
Prothioconazole: 203.3 ± 0.1 g/kg ($20.33 \pm 0.01\%$ w/w)
Date of expiry: 14 September 2024
2. **Test concentrations:** Control: deionised water
Test item: 0.163, 0.294, 0.529, 0.953, 1.71, 3.09, 5.56 and 10.0 mg test item/kg soil dry weight
3. **Toxic reference:** Carbendazim
Batch no.: Not reported
Purity: Nominal 600 g/L
Study number: 105689022
4. **Test organism:**
Species: *Eisenia andrei* (Annelida:Oligochaeta), Earthworm
Source: In-house bred in a standardized conditions.
Age: Approximately 7 – 8 months with well-developed clitellum with body weights ranging between 301 to 590 mg
Test units: Plastic boxes (18.3 cm x 13.6 cm x 6 cm, tapered towards the bottom, with a soil surface of approximately 16.5 cm x 11.5 cm = 189.75 cm²) with perforated transparent lids to enable exchange of air, to minimise evaporation from the artificial soil, and to prevent the earthworms from escaping. Each container was filled with $626.5 \text{ g} \pm 1 \text{ g}$ of the prepared soil (500 g dry weight plus deionised water). The height of the soil layer in the containers was approximately 5 cm.
Test substrate: Artificial soil prepared according to OECD 222
Feeding: Air-dried and finely ground cattle manure moisture with water
5. **Environmental conditions**
Temperature: 18 to 22 °C
Soil pH: 5.4 – 5.8 at test initiation and 6.2 – 6.3 at test termination
Moisture content: 26.1 – 27.0 % (52.1 to 54.0 % of WHC) at test initiation and 24.1 – 26.8 % (48.2 to 53.5 % of WHC) at test end
Light: 16:08 light-dark cycles, 400 to 800 lux

B. STUDY DESIGN AND METHODS

1. **Experimental phase:** 29 June – 28 August 2023
2. **Experiment treatment**

The test concentrations selected for the study were 0.163, 0.294, 0.529, 0.953, 1.71, 3.09, 5.56 and 10.0 mg test item/kg dry weight of artificial soil along with the control group. There were four replicates for the treatment group and eight replicates for the control group, with 10 earthworms in each replicate. All earthworms were rinsed with tap water, dried with dry paper towel, weighed individually and randomly assigned to batches of 10 earthworms. The earthworms were placed on the surface of the artificial soil after application. Test duration was 56 days After 28 days of exposure the artificial soil was transferred to a tray and adult earthworms were counted, removed and weighed per replicate. Another 28 days, juvenile were removed by placing the test units in a water bath at 50 – 60 °C for about 30 minutes and counting all emerging earthworms.

The toxic reference, Carbendazim, was tested in another GLP study with study number 105689022, with concentrations ranging from 0.45 – 2.26 mg a.i./kg soil dry weight. The test was performed from January to March 2023.

3. Measurements and observations

Worms were assessed for mortality, behavioral and morphological abnormalities, body weight and the amount of food added after 28 days of exposure. Body weight change (adults) was assessed at test start (Day 0) and at 28 days after application. On day 28, juveniles were counted and released into the test soil for additional 28 days under the same test conditions. The number of juveniles was counted after 56 days of application.

4. Statistics

Mortality data were analysed for significance by using the Fisher's Exact Binomial Test (multiple comparison, with Bonferroni Correction, $\alpha = 0.05$, one-sided greater). The LC_{50} at day 28 was not determined by statistical analysis as no mortality above 50% was observed.

The body weight change and reproduction data were tested for normal distribution and homogeneity of variance ($\alpha = 0.01$) using the Shapiro-Wilk's test and the Levene's test, respectively.

As the data for body weight changes and reproduction were normally distributed and homogeneous and did not follow a monotonicity trend (contrast trend), in both cases, Dunnett's test was used to compare treatment and control values (multiple comparison, two-sided for weight and one-sided smaller for reproduction, $\alpha = 0.05$).

The determination of the NOEC and LOEC values was based on the results of the statistical evaluation.

The EC_{10} , EC_{20} and EC_{50} were not determined by statistical analysis as no reduction of reproduction above 10% was observed.

The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ToxRat® Solutions GmbH.

II. RESULTS AND DISCUSSION

A. MORTALITY AND REPRODUCTIVE OUTPUT

The reference item Carbendazim (performed under the test facility Study No. 105689022 from January to March 2023) showed statistically significant treatment related effects on reproduction at a concentration of 1.00 mg Carbendazim/kg soil dry weight and above. The EC_{50} for reproduction was 1.10 mg Carbendazim/kg soil dry weight.

No mortality was observed in any test item treated group. No statistically significant differences of the test item treated group compared to the control were observed (Fisher's Exact Test, one-sided greater, $\alpha = 0.05$). Therefore, the NOEC for mortality was determined to be ≥ 10.0 mg test item/kg soil dry weight. The LOEC for mortality was estimated to be >10.0 mg test item/kg soil dry weight. The LC_{50} was estimated to be >10.0 mg test item/kg soil dry weight.

The body weight changes in the test item treated groups were not statistically significantly different compared to the control up to and including the highest test concentration of 10.0 mg test item/kg soil dry weight (Dunnett's t-test, $\alpha = 0.05$, two-sided). Therefore, the NOEC for body weight changes was determined to be ≥ 10.0 mg test item/kg soil dry weight. The LOEC for body weight changes was estimated to be >10.0 mg test item/kg soil dry weight.

The reproduction rates were not statistically significantly different compared to the control up to and including the highest test concentration of 10.0 mg test item/kg soil dry weight (Dunnett's test, $\alpha = 0.05$, one-sided smaller). Therefore, the NOEC for reproduction was determined to be ≥ 10.0 mg test item/kg soil dry weight. The LOEC for reproduction was estimated to be >10.0 mg test item/kg soil dry weight. The EC_{10} , EC_{20} and EC_{50} were estimated to be >10.0 mg test item/kg soil dry weight.

No behavioural abnormalities were observed, and all earthworms burrowed into the soil within 15 minutes after introduction. In all treatment groups, food was consumed.

The results of mortality and reproductive output are presented in the table below.

Table A 2.4.1.1.1-1: Record for reproductive assessment

Boscalid + Prothioconazole 350 + 200 g/kg WG [mg test item/kg sdw]	Control	0.163	0.294	0.529	0.953	1.71	3.09	5.56	10.0
Mortality (day 28) [%]	0	0	0	0	0	0	0	0	0
Statistical significance ¹	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Body weight change (day 28) [%]	36.7	27.3	30.7	29.2	28.6	32.2	30.3	29.1	27.8
Statistical significance ²	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Mean No. of juveniles (day 56)	125	122	114	125	128	127	124	149	117
Statistical significance ²	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
% reduction in reproductive output (day 56)	-	97	91	99	102	101	99	119	93
Food consumption	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0

sdw: soil dry weight, n.s.; not significantly different compared to the control, -; not applicable

¹ Fisher's Exact test, $\alpha = 0.05$, one-sided greater

² Dunnett's t-test, $\alpha = 0.05$, two-sided for weight changes and one-sided smaller for reproduction

B. VALIDITY CRITERIA

All required validity criteria were met. Accordingly, the study is regarded as valid.

Table A 2.4.1.1.1-2: Study validity check for parameters in the control group

Parameter	Required	Observed
Control mortality	$\leq 10\%$	0%
Control Reproduction (Juveniles per Container):	≥ 30	70 to 160
Coefficient of Variation of the Control Reproduction:	$< 30\%$	23.2%

C. TOXICITY ENDPOINTS

The Lowest Observed Effect Concentration (LOEC) and the No Observed Effect Concentration (NOEC) for mortality and reproductive output, the median Lethal Concentration (LC₅₀) for mortality, and the Effect Concentration on reproductive output (EC_{10, 20, 50}) were determined and are presented in the following table.

Table A 2.4.1.1.1-3: Summary of endpoints

Endpoints	
	mg test item/kg soil dry weight
NOEC (day 28 mortality and weight)	≥ 10.0
LOEC (day 28 mortality and weight)	> 10.0
LC ₅₀	> 10.0
NOEC (day 56 reproduction)	≥ 10.0
LOEC (day 56 reproduction)	> 10.0
EC ₁₀ Values	> 10.0
EC ₂₀ Values	> 10.0
EC ₅₀ Values	> 10.0

III. CONCLUSION

In an earthworm reproduction and growth study with Boscalid + Prothioconazole (350 +200 g/kg - WG) the No Observed Effect Concentration (NOEC) for mortality, weight changes and reproduction of the earthworm *Eisenia andrei* was determined to be ≥ 10.0 mg test item/kg soil dry weight, i.e. the highest concentration tested. The Lowest Observed Effect Concentration (LOEC) was estimated to be > 10.0 mg test item/kg soil dry weight. The LC₅₀ was estimated to be > 10.0 mg test item/kg soil dry weight. The EC₁₀, EC₂₀ and EC₅₀ were estimated to be > 10.0 mg test item/kg soil dry weight.

In the most recent test with the reference item Carbendazim (performed under the test facility Study No. 105689022 from January to March 2023), there were statistically significant effects on reproduction at a concentration of 1.00 mg a.i./kg soil dry weight and above, which is in line with the guideline OECD 222 (effects should be observed between 1 and 5 mg a.i./kg soil dry weight). The EC₅₀ for reproduction was calculated as 1.10 mg a.i./kg soil dry weight.

A 2.4.1.2 KCP 10.4.1.2 Earthworms - field studies

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

A 2.4.2.1 KCP 10.4.2.1 Species level testing

A 2.4.2.1.1 Hübner, 2023b (KCP 10.4.2.1/01)

Comments of zRMS:	The study was conducted in line with OECD 232 with no deviations All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: NOEC_{reproduction} >1000 mg prod./kg soil dw. 56d EC₁₀ = not determined as the maximum reduction was below 10 %.
-------------------	---

Reference: KCP 10.4.2.1/01

Report: Boscalid + Prothioconazole (350 + 200 g/kg – WG): Effects on Reproduction of Collembola (*Folsomia candida*) in Artificial Soil, Hübner, S., 2023b, Study code 176531016, UPL/2023/2772

Guideline(s): OECD Guideline for the Testing of Chemicals, No. 232 (2016): Collembola Reproduction Test in Soil; Adopted: 29th July 2016.

Deviations: No

GLP: Yes

Acceptability: Yes

Executive summary

The objective of the study was to determine the effect of Boscalid + Prothioconazole (350 + 200 g/kg - WG), on survival and the reproductive output of the *Folsomia candida* Willem (Collembola, Isotomidae) under exposure for 28 days at nominal concentrations of 16.3, 29.4, 52.9, 95.3, 171, 309, 556 and 1000 mg test item/kg soil dry weight. Deionised water was used for the control.

Boscalid + Prothioconazole (350 +200 g/kg - WG) caused no statistically significant effects on mortality and reproduction of *Folsomia candida* up to and including the concentration of 1000 mg test item/kg soil dry weight. Therefore, the overall No Observed Effect Concentration (NOEC) was determined to be ≥ 1000 mg test item/kg soil dry weight. The overall Lowest Observed Effect Concentration (LOEC) was estimated to be >1000 mg test item/kg soil dry weight. The LC_{50} was estimated to be >1000 mg test item/kg soil dry weight. The EC_{10} could not be determined by statistical analysis, since there was no adequate concentration response. The EC_{20} and EC_{50} values were estimated to be >1000 mg test item/kg soil dry weight.

In a separate study, the reference item Boric acid showed statistically significant effects on reproduction at concentrations of 30.5 mg/kg soil dry weight and above. The EC_{50} for reproduction was calculated to be 79.3 mg/kg soil dry weight.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:** Boscalid + Prothioconazole (350 + 200 g/kg - WG)
Product Name: FGE03
Batch no.: BDPT/WG/220915/550/20
Purity: Boscalid: 352.3 ± 0.2 g/kg, $35.22 \pm 0.02\%$ w/w (analytic)
Prothioconazole: 203.3 ± 0.1 g/kg, $20.33 \pm 0.01\%$ w/w (analytic)
Date of expiry: 14 September 2024
2. **Test concentrations:** Control: Untreated soil (moistened with deionised water)
Test item: 16.3, 29.4, 52.9, 95.3, 171, 309, 556 and 1000 mg test item/kg soil dry weight
3. **Toxic reference:** Boric acid
Batch no.: Not reported
Purity: Not reported
Study number: 136526016

4. Test organism:

Species:	<i>Folsomia candida</i> Willem (Collembola, Isotomidae)
Source:	The synchronized individuals were bred at ibacon
Age:	9-12 days old juveniles at test start, adults
Test units:	Glass vessels (Ø: 5 cm, volume: 100 mL) closed tightly to avoid water evaporation, filled with 30 ± 1.0 g of the test substrate. The height of the soil layer in the containers was 2 to 3 cm.
Test substrate:	Artificial soil prepared according to OECD 232, maximum water holding capacity of the artificial soil, 34% of the dry weight
Feeding:	granulated dry yeast until test start

5. Environmental conditions

Temperature:	18 to 22 °C
pH:	5.8 – 6.0 at test initiation and 5.5 – 5.6 at test termination
Water content:	17.6 to 18.2 % (51.7 to 53.4 % of WHC) at test initiation and 15.9 to 17.6 % of soil dry weight (46.9 to 51.7 % of WHC) at test initiation
Light:	16:08 light-dark cycles, 400 to 800 lux

B. STUDY DESIGN AND METHODS

1. Experimental phase: 31 May 2023 – 29 June 2023

2. Experiment treatment

A study was conducted to determine the effect of Boscalid + Prothioconazole (350 + 200 g/kg – WG) on the mortality and reproduction of Collembola (*Folsomia candida*). Eight replicates for the control and four replicates per test item group, containing ten Collembola each (total 80 individuals per control and 40 individuals per test item group) were each exposed for 28 days to nominal concentrations of 16.3, 29.4, 52.9, 95.3, 171, 309, 556 and 1000 mg test item/kg soil dry weight and to an untreated control (soil moistened with deionized water). A reference item (boric acid, at a concentration range of 30.5 to 200 mg/kg soil dry weight) is tested at least once a year to ensure the laboratory test conditions are adequate and to verify the response of the test organisms does not change significantly over time. The most recent test relevant to this study was performed from April to May 2023 under ibacon study number 136526016.

A stock solution was prepared by weighing 1000.1 mg of test item using an analytical balance. The test item was transferred into a glass beaker and deionised water was added to obtain a final net weight of 88.3 g. The resulting suspension contained a concentration of 11.3262 mg test item/g. A dilution series was prepared according to the dilution series.

Collembola was collected with an aspirator, put into a small glass tube and placed onto the surface of the treated artificial soil.

3. Measurements and observations

The numbers of living adult Collembola at day 28 after application were recorded. Missing adult Collembola were recorded as dead as it was assumed that missing adult Collembola had died and degraded during the test period. Surviving Collembola were observed for any abnormal behaviour or conditions at day 28 after application. Reproduction was determined as number of juvenile Collembola at day 28 after application.

4. Statistics

Mortality data were statistically analysed using Fisher's Exact Binomial Test (multiple comparison, with Bonferroni Correction, $\alpha = 0.05$, one-sided greater. The LC_{50} at day 28 was not determined by statistical analysis as no mortality above 50% was observed.

Reproduction data were tested for normal distribution and homogeneity of variance using Shapiro-Wilk's test and Levene's test ($\alpha = 0.01$).

Since the reproduction data were normally distributed and homogeneous and did follow a monotonicity trend (contrast trend) the Williams t-test (multiple comparison, $\alpha = 0.01$, one-sided smaller) was used to compare treatment and control values.

The determination of the NOEC and LOEC values was based on the results of the statistical evaluation. The EC values could not be determined by statistical analysis since there was no adequate concentration response.

The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ToxRat Solutions GmbH.

II. RESULTS AND DISCUSSION

A. MORTALITY AND REPRODUCTIVE OUTPUT

Mortality of *Folsomia candida* was not statistically significantly different compared to the control up to and including the highest test concentration of 1000 mg test item/kg soil dry weight. No abnormal behaviour was observed with the surviving Collembola.

There were no statistically significant effects on reproduction of *Folsomia candida* up to and including the highest test concentration of 1000 mg test item/kg soil dry weight.

The reference item Boric acid showed statistically significant treatment related effects on reproduction at the concentration of 30.5 mg/kg soil dry weight and above. The EC₅₀ for reproduction was 79.3 mg/kg soil dry weight (95% confidence limits of 71.8 to 87.1 mg/kg soil dry weight).

The results of mortality and reproductive output are presented in the table below.

Table A 2.4.2.1.1-1: Results of mortality and reproductive output of *Folsomia candida*

Test item concentration [mg/kg sdw]	Mean Mortality [%]	Statistical significance ¹	Mean number of juveniles	% of Control	Statistical significance ²
Control	4	-	893	-	-
16.3	0	n.s.	918	103	n.s.
29.4	0	n.s.	827	93	n.s.
52.9	5	n.s.	849	95	n.s.
95.3	5	n.s.	786	88	n.s.
171	5	n.s.	752	84	n.s.
309	3	n.s.	828	93	n.s.
556	5	n.s.	725	81	n.s.
1000	13	n.s.	837	94	n.s.

sdw: soil dry weight, n.s.; not significantly different compared to the control, *, significantly different compared to the control

¹ Fisher's Exact Test, $\alpha = 0.05$, one-sided greater.

² Williams t-test, $\alpha = 0.05$, one-sided smaller.

B. VALIDITY CRITERIA

All required validity criteria were met. Accordingly, the study is regarded as valid.

Table A 2.4.2.1.1-2: Study validity check for parameters in the control group

Parameter	Required	Observed
Control mortality	≤ 20%	4%
Control Reproduction (Juveniles per Container):	≥ 100	827 to 943
Coefficient of Variation of the Control Reproduction:	< 30%	5.2%

C. TOXICITY ENDPOINTS

The NOEC mortality was determined to be ≥1000 mg test item/kg soil dry weight. The LOEC for mortality was estimated to be >1000 mg test item/kg soil dry weight. The LC₅₀ of Boscalid + Prothioconazole (350 + 200 g/kg-WG) for *Folsomia candida* in soil was estimated to be >1000 mg test item/kg soil dry weight.

The NOEC for reproduction was determined to be ≥1000 mg test item/kg soil dry weight and the LOEC for reproduction was determined to be >1000 mg test item/kg soil dry weight. The EC₂₀ and EC₅₀ was determined to be >1000 mg test item/kg soil dry weight.

The summary of endpoints were determined and are presented in the following table.

Table A 2.4.2.1.1-3: Survival and reproductive output of the springtail *Folsomia candida* Willem after exposure to Boscalid 500 WG – Summary of endpoints

Endpoints [mg/kg soil dry weight]			
NOEC mortality	≥1000		
LOEC mortality	>1000		
LC ₅₀ mortality ³	>1000		
NOEC reproduction	≥1000		
LOEC reproduction	>1000		
EC Values reproduction ⁴	EC ₁₀ : n.d.	EC ₂₀ : >1000 ³	EC ₅₀ : >1000

³ estimated value

n.d. = not determined due to mathematical reasons

III. CONCLUSION

Boscalid + Prothioconazole (350 +200 g/kg - WG) caused no statistically significant effects on mortality and reproduction of *Folsomia candida* up to and including the concentration of 1000 mg test item/kg soil dry weight. Therefore, the overall No Observed Effect Concentration (NOEC) was determined to be ≥1000 mg test item/kg soil dry weight. The overall Lowest Observed Effect Concentration (LOEC) was estimated to be >1000 mg test item/kg soil dry weight. The LC₅₀ was estimated to be >1000 mg test item/kg soil dry weight. The EC₁₀ could not be determined by statistical analysis, since there was no adequate concentration response. The EC₂₀ and EC₅₀ values were estimated to be >1000 mg test item/kg soil dry weight.

In a separate study, the reference item Boric acid showed statistically significant effects on reproduction at concentrations of 30.5 mg/kg soil dry weight and above. The EC₅₀ for reproduction was calculated to be 79.3 mg/kg soil dry weight.

A 2.4.2.1.2 Hübner, 2023c (KCP 10.4.2.1/02)

Comments of zRMS:	The study was conducted in line with OECD 226 with no deviations. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: NOEC > 1000 mg prod./kg soil dw EC₁₀ > 1000 mg prod./kg soil dw
-------------------	--

Reference:	KCP 10.4.2.1/02
Report:	Boscalid + Prothioconazole (350 + 200 g/kg – WG): Effects on Reproduction of the Predatory Mite <i>Hypoaspis aculeifer</i> in Artificial Soil, Hübner, S., 2023c, Study code 176531089, UPL/2023/1436
Guideline(s):	OECD Guideline for the Testing of Chemicals, No. 226 (2016): Predatory mite (<i>Hypoaspis aculeifer</i>) Reproduction Test in Soil; Adopted: 29th July 2016.
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Executive summary

A study was conducted to determine the effect of Boscalid + Prothioconazole (350 +200 g/kg - WG) on the mortality and reproduction of the soil mite (*Hypoaspis aculeifer*) according to OECD 226. The soil mites were exposed for 14 days to artificial soil (prepared according to OECD 226) treated with the test item to obtain the nominal concentrations of 16.3, 29.4, 52.9, 95.3, 171, 309, 556 and 1000 mg test item/kg soil dry weight and to an untreated control with added deionized water only).

Boscalid + Prothioconazole (350 +200 g/kg - WG) caused no statistically significant effects on mortality and reproduction of *Hypoaspis aculeifer* up to and including the concentration of 1000 mg test item/kg soil dry weight. Therefore, the overall No Observed Effect Concentration (NOEC) was determined to be ≥ 1000 mg test item/kg soil dry weight. The overall Lowest Observed Effect Concentration (LOEC) was estimated to be >1000 mg test item/kg soil dry weight. The LC_{50} , EC_{10} , EC_{20} and EC_{50} values were estimated to be >1000 mg test item/kg soil dry weight.

The reference item dimethoate showed statistically significant treatment related effects on reproduction at a concentration of 2.23 mg a.i./kg soil dry weight and above. The EC_{50} for reproduction was 3.25 mg a.i./kg soil dry weight.

I. MATERIALS AND METHODS

A. MATERIALS

- Test material:** Boscalid + Prothioconazole (350 + 200 g/kg - WG)
Product Name: FGE03
Batch no.: BDPT/WG/220915/550/20
Purity: Boscalid: 352.2 ± 0.2 g/kg, $35.22 \pm 0.02\%$ w/w (analytic)
Prothioconazole: 203.3 ± 0.1 g/kg, $20.33 \pm 0.01\%$ w/w (analytic)
Date of expiry: 14 September 2024
- Test concentrations:** Control: Untreated (soil with deionized water)
Test item: 16.3, 29.4, 52.9, 95.3, 171, 309, 556 and 1000 mg test item/kg soil dry weight
- Toxic reference:** Dimethoate
Batch no.: Not reported
Purity: Not reported
Study number: 159084089

4. Test organism:

Species:	<i>Hypoaspis aculeifer</i> (Predatory mite, Acari:Laelapidae)
Source:	culture by ibacon
Age:	Adults, approximately 8 days after reaching the adult stage (29 days after placing adult females in clean rearing vessels over a period of 2 days)
Test units:	Glass vessels (Ø: 5 cm, volume: 100 mL) closed with close fitted cover. Each test unit was filled with 20 ± 1.0 g of the test substrate.
Test substrate:	Artificial soil prepared according to OECD 226, maximum water holding capacity of the artificial soil is determined to be 34%
Feeding:	Cheese mites (<i>Tyrophagus putrescentiae</i>) at day 0, 2, 5, 7, 9 and 12

5. Environmental conditions

Temperature:	18 to 22 °C
pH:	5.8 – 6.0 at test initiation 5.9 – 6.2 at test termination
Water content:	17.6 to 18.2 % of soil dry weight (51.7 to 53.4 % of WHC) at test initiation and 16.9 to 18.0 % of soil dry weight (49.6 to 53.1 % of WHC) at test end
Light:	16:08 light-dark cycles, 400 to 800 lux

B. STUDY DESIGN AND METHODS

1. Experimental phase: 31 May – 16 June 2023

2. Experiment treatment

A study was conducted to determine the effect of Boscalid + Prothioconazole (350 + 200 g/kg – WG) on the mortality and reproduction of the predatory soil mite (*Hypoaspis aculeifer*). Eight replicates for the control and four replicates per test item group, containing ten adult female mites each (total 80 individuals per control and 40 individuals per test item group) were each exposed for 14 days to nominal concentrations of 16.3, 29.4, 52.9, 95.3, 171, 309, 556 and 1000 mg test item/kg soil dry weight and to an untreated control (deionized water only). A reference item (dimethoate) is tested at least once a year to laboratory test conditions are adequate and to verify that the response of the test organisms does not change significantly over time. The most recent test relevant to this study was conducted in January 2023 and archive under ibacon Study No. 159084089.

3. Measurements and observations

Number of surviving adult female predatory mites 14 days after test initiation was recorded (counted after extraction). Missing adult predatory mites were recorded as dead as it was assumed they would have degraded after death during the test period. The living predatory mites were observed for differences in morphology or any abnormalities at experimental end. Reproduction was determined by the number of juvenile mites at day 14 after application, counted after extraction.

4. Statistics

Mortality data were statistically analysed using Fisher's Exact Binomial Test (multiple comparison, with Bonferroni Correction, $\alpha = 0.05$, one-sided greater). The LC_{50} at day 14 was not determined by statistical analysis as no mortality above 50% was observed.

Reproduction data were tested for normal distribution and homogeneity of variance using Shapiro-Wilk's test and Levene's test ($\alpha = 0.01$). Since the reproduction data were normally distributed and homogeneous and did follow a monotonicity trend (contrast trend) the Williams t-test (multiple comparison, $\alpha = 0.05$, onesided smaller) was used to compare treatment and control values.

The determination of the NOEC and LOEC values was based on the results of the statistical evaluation.

The EC values were not determined by statistical analysis since no reduction of reproduction above 10% was observed.

The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ToxRat Solutions GmbH.

II. RESULTS AND DISCUSSION

A. MORTALITY AND REPRODUCTIVE OUTPUT

Compared to the control group, statistically significant increase in mortality of *Hypoaspis aculeifer* was detected from the test item concentration 16.3 to 1000 mg test item/kg soil dry weight after 14 days of exposure.

The reference item dimethoate showed statistically significant treatment related effects on mortality and reproduction at a concentration of 2.23 mg a.i./kg soil dry weight and above. The LC₅₀ and EC₅₀ for mortality and reproduction were 2.56 and 3.25 mg a.i./kg soil dry weight, respectively.

The results of mortality and reproductive output are presented in the table below.

Table A 2.4.2.1.2-1: Results of mortality and reproductive output of *Hypoaspis aculeifer*

Boscalid 500 WG [mg/kg sdw]	Mean Mortality [%]	Statistical significance ¹	Mean Number of juveniles	% of Control	Statistical significance ²
Control	6	-	218	-	-
16.3	8	n.s.	238	109	n.s.
29.4	5	n.s.	221	101	n.s.
52.9	8	n.s.	230	105	n.s.
95.3	8	n.s.	228	105	n.s.
171	8	n.s.	240	110	n.s.
309	5	n.s.	232	106	n.s.
556	0	n.s.	258	118	n.s.
1000	3	n.s.	243	111	n.s.

sdw: soil dry weight, n.s.; not significantly different compared to the control

¹ Fisher's Exact Test, $\alpha = 0.05$, one-side greater

² Williams t-test, $\alpha = 0.05$, one-sided smaller.

- not applicable

B. VALIDITY CRITERIA

All required validity criteria were met. Accordingly, the study is regarded as valid.

Table A 2.4.2.1.2-2: Study validity check for parameters in the control group

Parameter	Required	Observed
Control mortality	≤ 20%	6%
Control Reproduction (Juveniles per Container):	≥ 50	198 to 247
Coefficient of Variation of the Control Reproduction:	≤ 30%	7.5%

C. TOXICITY ENDPOINTS

The Lowest Observed Effect Concentration (LOEC) and the No Observed Effect Concentration (NOEC) for mortality and reproductive output, the median Lethal Concentration (LC₅₀) for mortality, and the Effect Concentration on reproductive output (EC₅₀) were determined and are presented in the following table.

Table A 2.4.2.1.2-3: Survival and reproductive output of the Predatory mite *Hypoaspis aculeifer* after 14-days exposure to Boscalid + Prothioconazole (350 + 200 g/kg – WG) -Summary of endpoints

Endpoints [mg/kg soil dry weight]			
NOEC mortality	≥1000		
LOEC mortality	>1000		
LC ₅₀ mortality ³	>1000		
NOEC reproduction	≥1000		
LOEC reproduction	>1000		
EC values reproduction ³	EC ₁₀ = >1000	EC ₂₀ = >1000	EC ₅₀ = >1000

³ estimated value

The overall No Observed Effect Concentration (NOEC) was determined to be ≥1000 mg test item/kg soil dry weight. The overall Lowest Observed Effect Concentration (LOEC) was estimated to be >1000 mg test item/kg soil dry weight. The LC₅₀ and EC₅₀ values were estimated to be >1000 mg test item/kg soil dry weight. Other EC values could not be determined by statistical analysis since there was no adequate concentration response.

III. CONCLUSION

Boscalid + Prothioconazole (350 +200 g/kg - WG) caused no statistically significant effects on mortality and reproduction of *Hypoaspis aculeifer* up to and including the concentration of 1000 mg test item/kg soil dry weight. Therefore, the overall No Observed Effect Concentration (NOEC) was determined to be ≥1000 mg test item/kg soil dry weight. The overall Lowest Observed Effect Concentration (LOEC) was estimated to be >1000 mg test item/kg soil dry weight. The LC₅₀, EC₁₀, EC₂₀ and EC₅₀ values were estimated to be >1000 mg test item/kg soil dry weight.

The reference item dimethoate showed statistically significant treatment related effects on reproduction at a concentration of 2.23 mg a.i./kg soil dry weight and above. The EC₅₀ for reproduction was 3.25 mg a.i./kg soil dry weight.

A 2.4.2.2 KCP 10.4.2.2 Higher tier testing

A 2.5 KCP 10.5 Effects on soil nitrogen transformation

A 2.5.1 Raithatha, 2023 (KCP 10.5/01)

Comments of zRMS:	The study was conducted in line with OECD 216 with no deviations. All the validity criteria were met. Boscalid 350 g/kg + Prothioconazole 200 g/kg WG (GPF515), does not have any long-term influence on nitrogen transformation in soil, at its maximum predictable environmental concentration (1T), i.e., 4.0 kg/ha or 5.33 mg/kg dry weight soil and at 2.5 times of the maximum single filed application rate (2.5T), i.e., 10.0 kg/ha or 13.33 mg/kg dry weight soil.
-------------------	--

Reference:	KCP 10.5/01
Report:	Effect of Boscalid 350 g/kg + Prothioconazole 200 g/kg WG (GPF515) on Soil Microorganisms: Nitrogen Transformation Test, Raithatha, A., 2023. Study code 608-3-15-33022, UPL/2022/21328
Guideline(s):	OECD guideline for the testing of Chemicals No. 216: ‘Soil Microorganisms: Nitrogen Transformation Test’ (21st January 2000).
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Executive summary

The effect of Boscalid 350 g/kg + Prothioconazole 200 g/kg WG (GPF515) on soil microorganisms was evaluated through a nitrogen transformation test by measuring the rate of nitrate formation in the treated soil as compared to control (untreated) soil. The study was conducted at the concentrations of 4.0 kg test item/ha of soil or 5.33 mg/ha of soil (maximum application rate) and 10.0 kg test item/ha of soil or 13.33 mg/ha of soil (2.5 times of maximum application rate).

The study was conducted with test soil EFM-67 (C/N) (sand: 71.59%, silt: 9.82% and clay: 18.73%), amended with 5 g of Lucerne grass per/kg soil dry weight before application, in glass containers with three replicates per treatment. On the day of treatment and on 0th, 7th, 14th and 28th day, the soil samples were tested for nitrate formation rate.

There was no significant difference in nitrate formation and rate of nitrate formation in the test concentration of 4.0 and 10.0 kg test item/ha of soil on day 0, 7, 14 and 28 when compared to control on 28 days of exposure. In test concentration of 4.0 and 10.0 kg test item/ha of soil, the percent deviation of rate of nitrate formation when compared to control on day 28 were -3.38% and +5.41%, respectively. As the difference of nitrate formation rates compared to the control were less than 25% on 28th day, the study was terminated at this point.

Results of this study revealed that Boscalid 350 g/kg + Prothioconazole 200 g/kg WG (GPF515), does not have any long-term influence on nitrogen transformation in soil, at its maximum predictable environmental concentration (1T), i.e., 4.0 kg/ha or 5.33 mg/kg dry weight soil and at 2.5 times of the maximum single filed application rate (2.5T), i.e., 10.0 kg/ha or 13.33 mg/kg dry weight soil.

I. MATERIALS AND METHODS

A. MATERIALS

- Test material:** Boscalid 350 g/kg + Prothioconazole 200 g/kg WG
Product Name: GPF515
Batch no.: BDPT/WG/220819/550/19
Purity: Boscalid: 358.8 ± 0.7 g/kg (35.88 ± 0.07 % w/w)
Prothioconazole: 199.9 ± 0.8 g/kg (19.99 ± 0.08 % w/w)
Date of expiry: August 18, 2024
- Toxic reference:** Not reported
Study No.: Not reported

3. Test soil:

Soil type:	Pasture land
Soil code:	EFM-67 (C/N)
Source:	Bank of Par River, Village Nani Vahiyal, Taluka Dharampur, District Valsad, Gujarat, India, depth of collection is 0 to 25 cm
Soil characteristics:	Another study, 609-3-15-33438, that characterize the soil. Sand content: 71.59%; Silt content: 9.82%; Clay content: 18.73%; pH: 7.03; Maximum water holding capacity (WHC _{max}): 55.06%; Cation exchange capacity (CEC): 269.60 mmol/kg; Organic carbon content: 0.56 %; Total nitrogen content (TN): 0.03%; Microbial biomass: 652.22 mg/kg soil dry weight (11.65% of TOC); Initial nitrate content of soil: 53.60 mg nitrate/kg soil dry weight
Details of substrate:	Lucerne grass: Supplier: Isha Agro Developers Pvt. Ltd.; Content of Lucerne grass in test concentration 5 g/kg test soil dry weight; Carbon content (C): 41.68%; Total nitrogen content (TN): 3.18%; Ratio C/N: 13:11:1
Test duration:	28 days
Test units:	3 replicates per treatment, 3 different treatments: Control, 4.0 (maximum single field application) and 10.0 (2.5 times of maximum single field application) kg/ha of soil

4. Environmental conditions

Temperature:	20 ± 2 °C
Moisture:	Mean moisture content of soil: 9.55 % (WHC at 50 ± 5%)
Light:	Darkness

B. STUDY DESIGN AND METHODS

1. Dates of work: 05 January – 10 February 2023

2. Soil preparation and application

The soil was collected from the Bank of Par River, Village - Nani Vahiyal, Taluka - Dharampur, District - Valsad, Gujarat, India, from a depth of 0 to 25 cm. The soil sample was transported under temperature-controlled condition. The area from which soil collected was pastureland. There was no application of crop protection products, mineral fertilisers or biological materials within a year before sampling.

It was neither flooded nor dried. After taking the soil to the laboratory, it was manually cleared of large objects (e.g., stones and other plants material). Then it was processed by sieving without excess drying to a particle size less than or equal to 2 mm. The soil was stored in the refrigerator at 4 ± 2 °C, and an aerobic condition was ensured during the storage of soil.

A volume of 0.5 mL of the test Formulation 1T, Formulation 2.5T, and Control Formulation were added to separate pre-labelled incubated containers containing 318.83 g of moistened soil in each container to achieve the dose level 4.0 kg/ha or 5.33 mg/kg dry weight soil for treatment (1T) and 10.0 kg/ha or 13.33 mg/kg of soil for treatment (2.5T), and control, respectively.

The soil was mixed very well for a homogeneous distribution of the test item in the soil. Distilled water was added to maintain moisture level at 50 ± 5% water holding capacity. During mixing, care was taken to avoid compacting and balling of the soil. The test containers were covered with perforated polypropylene sheet to prevent water loss and maintain an aerobic condition.

3. Dose preparation

The test item formulation for the dose level of 1T was prepared by transferring 26.65 mg of Boscalid 350 g/kg + Prothioconazole 200 g/kg WG (GPF515) to 10 mL volumetric flask and the volume was made up to the mark with distilled water (Formulation 1T).

The test item formulation for the dose level of 2.5T was prepared by transferring 66.65 mg of Boscalid 350 g/kg + Prothioconazole 200 g/kg WG (GPF515) to 10 mL volumetric flask and the volume was made up to the mark with distilled water (Formulation 2.5T).

The control sample was prepared following the same procedure using distilled water without the test item (Control Formulation).

4. Measurements and observations

A quantity of 32 g of soil (on dry weight basis of 25 g) from control and the treated soil containers were transferred into reagent bottles. A quantity of 125 mL 0.1 M potassium chloride solution was added in each bottle. To optimise the extraction, the bottles containing the soil and extraction solution were not more than half full. The mixtures were shaken at 150 ± 5 rpm for 60 minutes at 20 ± 2 °C. The mixtures were centrifuged at 5000 rpm for ten minutes and filtered through Whatman number 1 filter paper. From the filtrate, 100 mL liquid phase was transferred to a pre-labelled beaker and 2 mL of 2 M ammonium sulphate was added. Then these solutions were analysed for nitrate content by pre-calibrated multipurpose bench meter with nitrate electrode using nitrate electrode. The calibration of multipurpose bench meter with nitrate electrode was performed using the potassium nitrate standard solution. The nitrate concentration was directly recorded using the multipurpose bench meter with nitrate electrode. The above procedure was repeated on each sampling.

5. Statistics

The statistical analysis of mean nitrate content data was performed by utilizing in-house developed validated statistical computer software for the data homogeneity (F test for homogeneity of variance).

II. RESULTS AND DISCUSSION

A. NITRATE CONTENT

The mean nitrate (NO_3^-) content in the control soil samples was 56.67, 68.80, 86.93 and 98.13 mg nitrate/kg dry weight soil, on day 0, 7, 14, and 28, respectively.

The mean nitrate (NO_3^-) content at the dose level 4.0 kg/ha or 5.33 mg/kg dry weight soil (1T) was 57.87, 69.47, 91.07 and 100.80 mg nitrate/kg dry weight soil, on day 0, 7, 14, and 28, respectively.

The mean nitrate (NO_3^-) content at the dose level 10.0 kg/ha or 13.33 mg/kg dry weight soil (2.5T) was 59.47, 69.33, 88.40 and 98.80 mg nitrate/kg dry weight soil, on day 0, 7, 14, and 28, respectively.

Table A 2.5.1-1: Nitrate Content

Treatment		Nitrate Content (mg/kg dry weight soil)			
		Day			
		0	7	14	28
Control	CR ₁	58.40	67.20	84.00	96.40
	CR ₂	57.20	69.20	86.40	97.60
	CR ₃	54.40	70.00	90.40	100.40
	Mean	56.67	68.80	86.93	98.13
1T	1TR ₁	57.20	71.20	94.00	101.60
	1TR ₂	58.00	69.60	92.00	99.20
	1TR ₃	58.40	67.60	87.20	101.60
	Mean	57.87	69.47	91.07	100.80
2.5T	2.5TR ₁	60.40	72.00	85.60	96.00
	2.5TR ₂	59.60	70.00	88.00	98.00
	2.5TR ₃	58.40	66.00	91.60	102.40

Treatment	Nitrate Content (mg/kg dry weight soil)			
	Day			
	0	7	14	28
Mean	59.47	69.33	88.40	98.80

B. NITRATE FORMATION

Soils treated with test concentrations of 4.0 (1T) and 10.0 (2.5T) kg test item/ha of soil were assessed for nitrogen transformation for 28 days. The difference of percent deviation of nitrate formation between the test concentration of 4.0 kg test item/ha on 0th, 7th, 14th, 28th day were +4.05%, -9.72%, -19.31% and +13.13%, respectively, whereas at the dose level 10.0 kg/ha or 13.33 mg/kg dry weight soil (2.5T), it was +18.50%, +4.17%, -5.02%, +6.88% and +5.41%, respectively.

Table A 2.5.1-2: Nitrate Formation Rate

Treatment	Nitrate Formation Rate (mg nitrate/kg dry weight soil/day)		
	0-7	0-18	0-28
Control	1.73	2.16	1.48
1T	1.66	2.37	1.53
2.5T	1.41	2.07	1.40

Table A 2.5.1-3: Percent Deviation of Mean nitrate Content

Days	Percent Deviation of Mean Nitrate Content	
	Treatment (1T)	Treatment (2.5T)
0 – 7	+4.05	+18.50
0 – 18	-9.72	+4.17
7 – 18	-19.31	-5.02
18 - 28	+13.13	+6.88
0 - 28	-3.38	+5.41

C. VALIDITY CRITERIA

The test was considered valid because the following criteria were met:

Table A 2.5.1-4: Study validity criteria

Parameter	Required	Observed
%RSD between control replicates on day 28	≤ 15%	2.09%

RSD – Relative standard deviation

D. STATISTICAL ANALYSIS

Statistically, no significant difference in the mean nitrate content of treatment (1T) was observed on day 0, 7, 14, and 28 when compared with that of the control.

Statistically, no significant difference in the mean nitrate content of treatment (2.5T) was observed on day 0, 7, 14, and 28 when compared with that of the control.

III. CONCLUSION

Results of this study revealed that Boscalid 350 g/kg + Prothioconazole 200 g/kg WG (GPF515), does not have any long-term influence on nitrogen transformation in soil, at its maximum predictable environmental concentration (1T), i.e., 4.0 kg/ha or 5.33 mg/kg dry weight soil and at 2.5 times of the maximum single filed application rate (2.5T), i.e., 10.0 kg/ha or 13.33 mg/kg dry weight soil.

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

A 2.6.1 KCP 10.6.1 Summary of screening data

A 2.6.2 KCP 10.6.2 Testing on non-target plants

A 2.6.2.1 Stürtz, 2023a (KCP 10.6.2/01)

Comments of zRMS:	The study was conducted in line with OECD 227 with minor deviations. The analytical recovery rate of the active substance Boscalid in the stock solution was 102% of the nominal value and the analytical recovery rate of the active substance Prothioconazole in the stock solution was 104% of the nominal value. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: NOER> 1900 g product/ha No phytotoxic effects were observed for any species tested.
-------------------	--

Reference: KCP 10.6.2/01

Report: Boscalid + Prothioconazole (350 + 200 g/kg – WG): Effects on Terrestrial (Non-Target) Plants: Vegetative Vigour Test, Stürtz, S., 2023a, Study code 176531087, UPL/2023/0743

Guideline(s): OECD Guidelines for the Testing of Chemicals, Guideline 227, 'Terrestrial Plant Test: Vegetative Vigour Test', adopted on July 19, 2006

Deviations: No

GLP: Yes

Acceptability: Yes

Executive summary

The study objective was to determine the effects of Boscalid + Prothioconazole (350 + 200 g/kg - WG) on the vegetative vigour of non-target terrestrial plant species. The effects Boscalid + Prothioconazole (350 + 200 g/kg – WG) on the mortality and fresh weight of six non-target plant species representing six plant families were assessed following a single application of 1.90 kg test item/ha (corresponding to 669.2 g Boscalid + 386.3 g Prothioconazole/ha) on *Brassica oleracea*, *Daucus carota*, *Glycine max*, *Lactuca sativa*, *Avena sativa* and *Allium cepa*.

The analytical recovery rate of the active ingredient Boscalid in the stock solution was 103% of the nominal value and the analytical recovery rate of the active ingredient Prothioconazole in the stock solution was 105% of the nominal value. The analytical method of recovery was developed in the performing laboratory. The HPLC-UV determination method was implemented.

No statistically significant reduction of the fresh weight was observed for any species tested.

No mortality was observed for any species tested.

No phytotoxic effects were observed for any species tested

All validity criteria were met.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:** Boscalid + Prothioconazole (350 + 200 g/kg - WG)
Product Name: FGE03
Batch no.: BDPT/WG/220915/550/20
Purity: Boscalid: 352.2 ± 0.2 g/kg (35.22 ± 0.02 % w/w)
Prothioconazole: 203.3 ± 0.1 g/kg (20.33 ± 0.01 % w/w)
Date of expiry: 14 September 2024
2. **Test concentrations:** Control: deionised water
Test item: 1.90 kg test item/ha (corresponding to 669.2 g Boscalid + 386.3 g Prothioconazole/ha) in spray volume of 100 L/ha, prepared by diluting 19.001 g test item in 1000 mL deionized water
3. **Test organisms:**
Plant species: *Brassica oleraceae*, *Daucus carota*, *Glycine max*, *Lactuca sativa*, *Avena sativa* and *Allium cepa*
Exposure time: 21 days
Test units: Commercial plastic flower pots (Ø15 cm), filled with test soil which has been steam sterilized, delivered and analyzed by the LUFA Speyer, Germany (sandy loam, LUFA 2.3, particle size ≤ 0.2 cm, with a pH of 6.0 ± 0.4 , and organic content of $0.66 \pm 0.05\%$)
4. **Environmental conditions**
Air temperature: $15.9^{\circ}\text{C} - 23.5^{\circ}\text{C}$
Relative humidity: $50.0\% - 86.0\%$
Photoperiod: 16/8 h (light/dark)
Light intensity: $300 - 400 \mu\text{E}/\text{m}^2/\text{s}$

B. STUDY DESIGN AND METHODS

1. **Dates of work:** 17 May 2023 – 25 July 2023 (including Biological and Analytical phases)
2. **Test design and treatment**

The plants were grown until they had reached the 2 to 4 true leaf stage prior to dosing. Test rates were calculated for a water amount of 100 L/ha and were administered onto the plants using laboratory spraying equipment. At least 20 plants were tested per treatment group and species. The concentration of the active ingredient in the dosing solution was verified analytical. The exposure time was 21 days. The application was done using laboratory spraying equipment and in an accurate way with reproducible coverage.

3. Dose preparation

The spray mixture was prepared by diluting 19.001 g test item to 1000 mL using deionised water, 1.90 kg test item/ha (corresponding to 669.2 g Boscalid + 386.3 g Prothioconazole/ha). This dosing solution was checked analytically using HPLC-UV-method according to criteria set forth by SANTE/2020/12830 Rev 2.

4. Measurements and observations

Six plant species, *Avena sativa*, *Allium cepa*, *Brassica oleracea*, *Daucus carota*, *Glycine max*, and *Lactuca sativa* were tested. Visual phytotoxicity observations and mortality counts were conducted 7 and 14 and 21 days after application. Mortality was considered if no living tissue could be found on the leaves or shoots. Growth stages were recorded according to BBCH-Monograph, at the application day and 21 days after application. The fresh weight of the above ground part of all survived plants of a pot (each pot was considered as a replicate) was determined 21 days after application.

5. Analytical verification

The analytical recovery rate of the active ingredient Boscalid in the stock solution was 102% of the nominal value and the analytical recovery rate of the active ingredient Prothioconazole in the stock solution was 104% of the nominal value. This dosing solution was checked analytically using HPLC-UV-method according to criteria set forth by SANTE/2020/12830 Rev 2.

6. Statistics

Fresh weight data were tested for normal distribution and homogeneity of variance using the Shapiro-Wilk's test ($\alpha = 0.01$) and the Levene's test ($\alpha = 0.01$). The data were normally distributed and homogeneous and so the Student t-test (two-sample comparison, one-sided smaller, $\alpha = 0.05$) was used.

No mortality was observed, so no statistical analysis was performed.

The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ToxRat Solutions GmbH.

II. RESULTS AND DISCUSSION

A. BIOLOGICAL RESULTS

Mortality: 1.90 kg FGE03/ha (corresponding to 669.2 g Boscalid + 386.3 g Prothioconazole/ha) showed no significant effect on the survivorship of the tested species.

Phytotoxicity: Test item 1.90 kg FGE03/ha (corresponding to 669.2 g Boscalid + 386.3 g Prothioconazole/ha) showed no phytotoxicity symptoms for any of the tested species.

Growth stage: Not much differences in growth stage could be detected between the test item groups and the controls for the six tested species at any of the rates tested.

Fresh Weight: Test item 1.90 kg FGE03/ha (corresponding to 669.2 g Boscalid + 386.3 g Prothioconazole/ha) showed no significant reduction of the fresh weight of the tested species.

B. ANALYTICAL RESULTS

The analytical recovery rate of the active ingredient Boscalid in the stock solution was 102% of the nominal value and the analytical recovery rate of the active ingredient Prothioconazole in the stock solution was 104% of the nominal value. In the control solution no test item ingredients were detected.

C. VALIDITY CRITERIA

The study was considered valid for all species; since the following criteria were met:

Table A 2.6.2.1-1: Study validity criteria

Parameter	Required	Observed
Emergence in the control	$\geq 70.0\%$	81% to 98%
Mean survival of the control plants	$\geq 90.0\%$	100%

The control plants exhibited no visible phytotoxic effects and the plants exhibited only normal variation in growth and morphology for that particular species.

D. TOXICITY ENDPOINTS

The NOER for all species were 1.90 kg test item/ha. The LOER was > 1.90 kg test item/ha for all species in the test parameters of fresh weight, mortality, phytotoxicity and growth rate.

Table A 2.6.2.1-2: Toxicity endpoints

Test species	NOER (kg test item/ha)	LOER (kg test item/ha)
<i>Brassica oleracea</i>	1.90	> 1.90
<i>Daucus carota</i>	1.90	> 1.90
<i>Glycine max</i>	1.90	> 1.90
<i>Lactuca sativa</i>	1.90	> 1.90
<i>Allium cepa</i>	1.90	> 1.90
<i>Avena sativa</i>	1.90	> 1.90

III. CONCLUSION

Boscalid + Prothioconazole (350 + 200 g/kg - WG) was tested for effects on the vegetative vigour using six plant species out of six different plant families.

The analytical recovery rate of the active ingredient Boscalid in the stock solution was 103% of the nominal value and the analytical recovery rate of the active ingredient Prothioconazole in the stock solution was 105% of the nominal value.

No statistically significant reduction of the fresh weight was observed for any species tested.

No mortality was observed for any species tested.

No phytotoxic effects were observed for any species tested.

The validity criteria were met.

A 2.6.2.2 Stürtz, 2023b (KCP 10.6.2/02)

Comments of zRMS:	The study was conducted in line with OECD 208 with following deviations: According to validity criteria, emergence rate of the control seeds should be $\geq 70\%$, but <i>Lactuca sativa</i> emergence rate of the control seeds was 67% due to sensitivity of dry soil surface. No presumed effect on the study as one emerged seed more in the control group and the criterion of 70% would have been met and then emergence of the seeds in the control and the treatment group would also not be statistically significant. The deviation is therefore considered negligible All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: NOER> 1900 g product/ha No phytotoxic effects were observed for any species tested.
-------------------	---

Reference:	KCP 10.6.2/02
Report:	Boscalid + Prothioconazole (350 + 200 g/kg - WG): Effects on Terrestrial (Non-Target) Plants: Seedling Emergence and Seedling Growth, Stürtz, S., 2023b, Study code 176531086, UPL/2023/0742
Guideline(s):	OECD Guidelines for the Testing of Chemicals, Guideline 208: 'Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test', adopted on July 2006.
Deviations:	According to validity criteria, emergence rate of the control seeds should be $\geq 70\%$, but <i>Lactuca sativa</i> emergence rate of the control seeds was 67% due to sensitivity of dry soil surface. No presumed effect on the study as one emerged seed more in the control group and the criterion of 70% would have been met and then emergence of the seeds in the control and the treatment group would also not be statistically significant. The deviation is therefore considered negligible.
GLP:	Yes
Acceptability:	Yes

Executive summary

The study objective was to determine the effects of Boscalid + Prothioconazole (350 + 200 g/kg - WG) on early growth of non-target terrestrial plant species. At least 20 seedlings of six different plant species were with Boscalid + Prothioconazole (350 + 200 g/kg - WG) at application rates of 1.90 kg test item/ha in 100 L/ha spray volume (corresponding to 669.2 g Boscalid + 386.3 g Prothioconazole/ha). Deionized water was used for the control treatment. The plants were assessed for mortality and phytotoxicity symptoms 7, 14 and 21 days after 50% seedling emergence in the respective control. Growth stages were assessed at 14 or 21 days after 50% seedling emergence in the respective control. Fresh weight was assessed 14 or 21 days after 50% seedling emergence in the respective control.

The analytical recovery rate of the active ingredient Boscalid in the stock solution was 103% of the nominal value and the analytical recovery rate of the active ingredient Prothioconazole in the stock solution was 105% of the nominal value.

No statistically significant reduction of the fresh weight and of the emergence rate was observed for any species tested.

No mortality was observed for any species tested with the test item.

No phytotoxic effects were observed for any species tested.

All validity criteria were met.

The NOER was determined to be 1.90 kg test item/ha and LOER was determined to be >1.90 kg test item/ha for all tested species. No ER_{50} was reported in this study.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	Boscalid + Prothioconazole (350 + 200 g/kg - WG)
Product Name:	FGE03
Batch no.:	BDPT/WG/220915/550/20
Purity:	Boscalid: 352.2 ± 0.2 g/kg ($35.22 \pm 0.02\%$ w/w) Prothioconazole: 203.3 ± 0.1 g/kg ($20.33 \pm 0.01\%$ w/w)
Date of expiry:	14 September 2024

2. **Test concentrations:** Control: deionized water
Test item: 1.90 kg test item/ha (corresponding to 669.2 g Boscalid + 386.3 g Prothioconazole/ha) in 100 L water/ha spray volume
3. **Test organisms:**
 - Plant species:** *Brassica oleraceae*, *Daucus carota*, *Glycine max*, *Lactuca sativa*, *Avena sativa* and *Allium cepa*
 - Exposure time:** 14 or 21 days after 50% emergence in the respective control depending on the growth of the seedling
 - Test units:** Commercial plastic flower pots (Ø15 cm), filled with steam sterilized soil (LUFA 2.3, sandy loam), particle size ≤ 0.2 cm, with a pH of 6.0 ± 0.4 , and organic content of $0.66 \pm 0.05\%$
4. **Environmental conditions:**
 - Air temperature:** $16.3 - 23.5^{\circ}\text{C}$ (mean temperature 20.8°C)
 - Relative humidity:** 50 – 90% (mean humidity 65%)
 - Photoperiod:** 16/8 h (light/dark)
 - Light intensity:** 300 - 400 $\mu\text{E}/\text{m}^2/\text{s}$ (mean light intensity 341 $\mu\text{E}/\text{m}^2/\text{s}$)

B. STUDY DESIGN AND METHODS

1. **Dates of work:** 17 May – 25 July 2022 (including Biological and Analytical phases)
2. **Test design and treatment**

The seeds were introduced manually into the soil. After sowing the pots were placed on saucers and watered. 4 to 10 pots per treatment group were sown one day before application. At least 20 seeds were tested per treatment group. Each pot represented one replicate. For a given test species, all uncoated seeds used in the test were from the same source and lot number.

On the day after sowing one rate of the test item was sprayed in 100 L/ha of deionised water onto the soil. At least 20 seeds were tested per rate and species. The exposure time was 14 or 21 days after 50% emergence in the respective control depending on the growth of the seedlings. The concentration of the active ingredient in the dosing solution was verified analytically.

3. Dose preparation

The spray mixture was prepared by diluting 19.001 g of Boscalid + Prothioconazole (350 + 200 g/kg – WG) to 1000 mL using deionised water (corresponding to 1.90 kg test item/ha in 100 L/ha, 669.2 g Boscalid + 386.3 g Prothioconazole/ha). This dosing solution was checked analytically using HPLC-UV-method.

4. Measurements and observations

Fresh weight, emergence and mortality of the plants treated with the test item were compared with the results of the control plants.

The fresh weight of the above ground part of all survived plants of a pot (each pot is considered as a replicate) was determined 14 or 21 days after 50% seedling emergence in the respective control. Emergence was checked daily on weekdays (except weekends) until 50% of the respective control plants had emerged. Further checks were done weekly. The number of living and dead plants was recorded 7 and 14 days or 7, 14 and 21 days after 50% seedling emergence in the respective control. A plant was considered dead if no living tissue could be found on the leaves or shoots. All other plants were considered living. Visual phytotoxicity (e.g. chlorosis, necrosis, deformation) was recorded weekly according to EPPO Standard PP 1/135 (4) after 50% seedling emergence in the respective control. Growth stages at day 14 or 21 after 50% seedling emergence in the respective control were reported according to BBCH-Monograph - Growth stages.

5. Analytical verification

The analytical recovery rate of the active ingredient Boscalid in the stock solution was 132% of the nominal value and the analytical recovery rate of the active ingredient Prothioconazole in the stock

solution was 105% of the nominal value. This dosing solution was checked analytically using HPLC-UV-method according to criteria set forth by SANTE/2020/12830 Rev.

6. Statistics

Fresh weight data were tested for normal distribution and homogeneity of variance using the Shapiro-Wilk's test ($\alpha = 0.01$) and the Levene's test ($\alpha = 0.01$). The data were normally distributed and homogeneous (all species except *Avena sativa*) and so the Student t-test (two-sample comparison, one-sided smaller, $\alpha = 0.05$) was used. For *Avena sativa*, the data were normally distributed but not homogeneous and so the Welch t-test (two-sample comparison, one-sided smaller, $\alpha = 0.05$) was used.

For the emergence data Fisher's Exact Binomial Test (two-sample comparison, one-sided greater, $\alpha = 0.05$) was used.

No mortality was observed, so no statistical analysis was performed.

The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ® ToxRat Solutions GmbH.

II. RESULTS AND DISCUSSION

A. BIOLOGICAL RESULTS

No statistically significant reduction of the fresh weight and of the emergence rate was observed for any species tested.

No mortality was observed for any species tested with the test item.

No phytotoxic effects were observed for any species tested.

B. ANALYTICAL RESULTS

The analytical recovery rate of the active ingredient Boscalid in the stock solution was 102% of the nominal value and the analytical recovery rate of the active ingredient Prothioconazole in the stock solution was 104% of the nominal value. In the control solution no test item ingredients were.

C. VALIDITY CRITERIA

The study was considered valid for all species, since the following criteria were met:

Table A 2.6.2.2-1: Study validity criteria

Parameter	Required	Observed
Emergence in the control	$\geq 70.0\%$	85 – 100 % except <i>Lactuca sativa</i>
Mean survival of the control plants	$\geq 90.0\%$	100%

Moreover, no phytotoxic effects were detected in the control plants and the cultivation conditions for a particular species were identical.

D. TOXICITY ENDPOINTS

The NOER was 1.90 kg a.i./ha for all test species and the LOER was >1.90 kg a.i./ha for all test species.

Table A 2.6.2.2-2: Toxicity endpoints

Test species	NOER (kg test item/ha)	LOER (kg test item/ha)
<i>Brassica oleracea</i>	1.90	> 1.90
<i>Daucus carota</i>	1.90	> 1.90
<i>Glycine max</i>	1.90	> 1.90
<i>Lactuca sativa</i>	1.90	> 1.90

<i>Allium cepa</i>	1.90	> 1.90
<i>Avena sativa</i>	1.90	> 1.90

III. CONCLUSION

Boscalid + Prothioconazole (350 + 200 g/kg – WG) was tested for effects on seedling emergence and seedling growth of six plant species out of six different plant families.

The analytical recovery rate of the active ingredient Boscalid in the stock solution was 103% of the nominal value and the analytical recovery rate of the active ingredient Prothioconazole in the stock solution was 105% of the nominal value.

No statistically significant reduction of the fresh weight and of the emergence rate was observed for any species tested.

No mortality was observed for any species tested with the test item.

No phytotoxic effects were observed for any species tested.

All validity criteria were met.

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

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

A 2.8 KCP 10.8 Monitoring data