REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: FHO04

Product name(s): Prothioconazole/Sulphur (50+625) SC, / Patton Supra

Chemical active substance(s): Prothioconazole 50 g/L, Sulphur 625 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorisation)

Applicant: UPL Holdings Coöperatief U.A.

Submission date: 30/05/2024

MS Finalisation date: October 2024 (initial Core Assessment)

January 2025, update September 2025 (final Core Assessment)

Version history

When	What
May 2024	Applicant version.
October 2024	The additional calculations of missing chronic adult bee and larva risk assessment following EFSA (2013) performed by the Applicant.
October 2024	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.
January 2025	Final report (Core Assessment following the commenting period). No additional information or assessments after the commenting period.
September 2025	Final report (update Core Assessment following the comments recived from Ministry of Polish Agriculture) Additional information/assessments included by the zRMS in the report in response to comments received from Polish Ministry of Agriculture are highlighted in yellow in the Points 9.1.1.2, 9.5.3 and 9.13. Not agreed or not relevant information are ~~struck through~~ and shaded for transparency.

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9 Ecotoxicology (KCP 10)

This draft registration report (dRR) is submitted for the registration of the new product Prothioconazole/Sulphur (50+625) SC, 'Patton Supra' (code FHO04) under Article 33 of Regulation (EC) 1107/2009. FHO04 is a suspension concentrate (SC) formulation containing 50 grams of prothioconazole per litre and 625 grams of sulphur per litre, for use as a fungicide on wheat and rye in Poland.

This document reviews the ecotoxicological studies for the product Patton Supra containing prothioconazole and sulphur, which were included into Annex I of Directive 91/414 (Commission Directive 2010/39/EU of 22 June 2010) and in the Regulation 1107/2009 with the Commission implementing Regulation (EU) No 737/2007 of 27 June 2007, as regards the conditions of approval of the active substances prothioconazole and sulphur.

Where appropriate this document refers to the conclusions of the EU review of the active substances prothioconazole and sulphur. This will be where:

- the active substances data are relied upon in the risk assessment of the formulation; or when
- the EU review concluded that additional data/information should be considered at national re-registration.

FHO04 was not the representative formulation for the EU review of prothioconazole or sulphur. The EFSA Journal (2007) 106 1-98 (Conclusion on the peer review of the pesticide risk assessment of the active substance prothioconazole) and EFSA Journal (2008) 221, 1-70 (Conclusion on the peer review of the pesticide risk assessment of the active substance sulphur) and the Sulphur Addendum to the DAR Volume 3 B9 for Confirmatory Data (April 2012) are considered to provide the relevant review information or a reference to where such information can be found.

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 a.s./ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms	Non-target plants
Zonal uses (field or outdoor uses, certain types of protected crops)																				
1	PL	Winter wheat (TRZAW), Spring wheat (TRZAS), Durum wheat† (TRZDU), Spelt† (TRZSP), Winter triticale (TTLWI), Spring triticale (TTLSO)	F	Septoria (*Zymoseptoria tritici*) SEPTTR Yellow rust (*Puccinia striiformis*) PUCCST Brown rust (*Puccinia triticina*) PUCCRT	Foliar spray	27 - 69	a) 1 b) 2	14	a) 4 L/ha b) 8 L/ha	a) 0.2 + 2.5 kg/ha b) 0.4 +5.0 kg/ha	100 / 400	35	-	A	A	R	A	A	A	A

† Minor crop according yo Article 51.

* 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 chronic risk to birds following the proposed use of Patton Supra were performed in accordance with EFSA (2009) guidelines. The risk from the active substances prothioconazole was acceptable at the screening level, however, the risk from sulphur and the metabolite prothioconazole-desthio was acceptable at Tier 1.

The risk to the mixture was calculated using the Finney's equation for acute toxicity and the risk quotient approach for chronic toxicity. The acute and chronic risks to the mixture (including metabolite prothioconazole-desthio) was acceptable at Tier 1.

The risk from exposure to contaminated drinking water and secondary poisoning was also considered acceptable following the proposed use of Patton Supra.

Mammals
Acute and chronic risk to mammals following the proposed use of Patton Supra were performed in accordance with EFSA (2009) guidelines. The acute risks for prothioconazole, prothioconazole-desthio and sulphur, and the long-term risks for prothioconazole and sulphur were acceptable at the screening assessment, indicating a low risk to mammals. However, the long-term risks for the metabolite prothioconazole-desthio did not pass the screening and first tier assessments. As such, additional refinement options were required to show an acceptable level of risk for the vole scenario. Three refinement options were presented, which all resulted in a safe use of Patton Supra to voles.

The risk to the mixture was calculated using the Finney's equation for acute toxicity and the risk quotient approach for chronic toxicity. The acute and chronic risks to the mixture (including metabolite prothioconazole-desthio) was acceptable at the screening level.

The risk from exposure to contaminated drinking water and secondary poisoning was also considered acceptable following the proposed use of Patton Supra.

9.1.1.2 Effects on aquatic organisms (KCP 10.2)

An acceptable risk is demonstrated for prothioconazole, its relevant metabolites, sulphur, and the formation Patton Supra.

The active substance prothioconazole and two metabolites (prothioconazole S-methyl and 1,2,4-triazole) pass spring and winter applications at BBCH 27 and BBCH 69 at FOCUS Step 2, requiring no mitigations. In spring applications, the metabolite prothioconazole-desthio passes at FOCUS Step 3, for the relevant scenarios for Poland. However, to ensure an acceptable risk for prothioconazole-desthio in winter cereals for Poland, a mitigation of a 20 m combined no-spray buffer zone and VFS is required with VFSmod, mitigation can be reduced overall to a 5 m combined no-spray buffer zone and VFS.
The risk from R scenarios not defined for spring cereals is covered by the risk assessment performed for these scenarios available for winter cereals.
Therefore finally, taking into account all relevant scenarios for Poland including D3, D4 and R1 for spring cereals and winter cereals - **a 5 m combined no-spray buffer zone and VFS is required**.
For the active substance sulphur, the sediment dwelling risk assessment was concluded as low risk. Finally, the formulation Patton Supra passes the aquatic risk assessment with no mitigations needed.
Overall, mitigations are required to ensure there is an acceptable risk to aquatic organisms, when using Patton Supra according to the GAP uses.

9.1.1.3 Effects on bees (KCP 10.3.1)

Acute oral and contact risk to bees following the proposed use of Patton Supra were performed in accordance with SANCO (2002) guidelines and show no risk.

Based on the EFSA guidance document 2013, the chronic risk to adult and larvae honeybees fails the screening assessment. However, both the chronic risk to adults and larvae pass the first-tier assessment for all scenarios, with the exception of the risk from foraging on weeds in the treated field. This risk can be mitigated with the following label restriction: "*Do not apply when flowering weeds are present./Remove weeds before flowering.*" It is also noted that risks via weeds may be unrealistic in specific MS situations, since large amounts of flowering weeds are not compatible with profitable agriculture in many crops. The decision of mitigation risk applied to bees are left at MSs level. For Poland the chronic risk is not required yet according to EFSA GD 2013.

Overall, both the acute and chronic risks from the active substances prothioconazole and sulphur, and the formulation Patton Supra were acceptable at Tier 1 following the proposed use of Patton Supra.

9.1.1.4 Effects on arthropods other than bees (KCP 10.3.2)

In-field and off-field HQ Higher tier and Tier I values based on laboratory studies with the formulation Patton Supra and the test organisms *Aphidius rhopalosiphi* and *Typhlodromus pyri* were below relevant trigger values indicating that the risk to in-field and off-field non-target arthropods is acceptable following the use of formulation Patton Supra according to the proposed use pattern.

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

TER values for sulphur and formulation Patton Supra are above the trigger value of 5 indicating there is acceptable chronic risk to earthworms, meso-, and macrofauna at the proposed GAP. TER values for prothioconazole and its relevant metabolite are below the trigger value of 5 for earthworm, however a field study indicate that the chronic risk to earthworms is acceptable at the proposed GAP.

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

The risk to non-target terrestrial plants following the proposed use of Patton Supra were performed in accordance with SANCO (2002) guidelines. The risk from the formulation was acceptable at Tier 2. No mitigation measures need to be applied.

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

Not required.

9.1.2 Grouping of intended uses for risk assessment

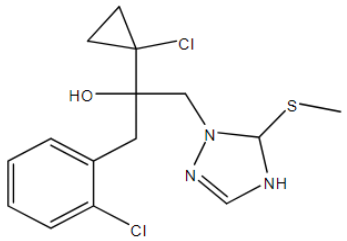
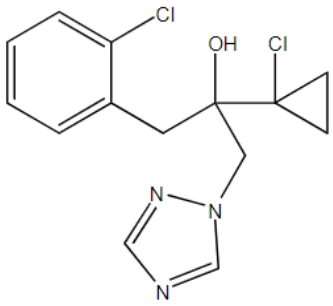
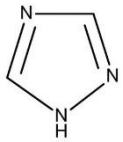
Not required.

9.1.3 Consideration of metabolites

A list of metabolites found in environmental compartments is provided below. The need for conducting a metabolite-specific risk assessment in the context of the evaluation of FHO04 is indicated in the table.

There are no metabolites for sulphur.

Table 9.1-2 Metabolites of prothioconazole

Metabolite	Molar mass	Chemical structure	~~Maximum observed occurrence in compartments~~	Exposure assessment required due to
M01: JAU 6476-S-methyl	358.3	2-(1-chlorocyclopropyl)-1-(2-chlorophenyl)-3-(4,5-dihydro-5-methylthio-1,2,4-triazolyl-1)-propan-2-ol 	~~Soil: 14.6 %AR (lab) Water/Sediment: Not observed~~	Soil
M04: JAU 6476-desthio	312.2	2-(1-chlorocycloproyl)1-(2-chlorophenyl)-3-(1,2,4-triazol-1-yl)-propan-2-ol 	~~Soil: 57.1 %AR (field) Water: 32.3 %AR Sediment: 26.9 %AR~~	Aquatic Soil
1,2,4-triazole	67.07		~~Soil: <2 %AR (lab) Water/Sediment: 37.2 %AR (water only)~~	Aquatic

zRMS comments:

Metabolites relevant for soil and water compartment listed in Table 9.1-2 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 9.1-2.

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

Avian toxicity studies have been carried out with prothioconazole, sulphur and prothioconazole's relevant metabolites. Full details of these studies are provided in the related documents.

Effects on birds of FHO04 were not evaluated as part of the EU assessment of prothioconazole or sulphur. However, the provision of further data on the formulation is not considered essential, because the toxicity

of the formulation can be read across from the data on the active substance and in order to minimise unnecessary vertebrate testing. The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process.

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

Species	Substance	Exposure System	Results	Reference
Bobwhite quail (*Colinus virginianus*)	Prothioconazole	Acute	**LD_{50} > 2000 mg a.s./kg bw**	EFSA Conclusion 2007
Bobwhite quail (*Colinus virginianus*)		Short-term	LC_{50} > 5000 mg a.s./kg bw/d Calculated LD_{50} = > 1413 mg a.s./kg bw/d	
Mallard duck (*Anas platyrhynchos*)		Short-term	LC_{50} > 5000 mg a.s./kg bw/d LD_{50} = > 2457 mg a.s./kg bw/d	
Bobwhite quail (*Colinus virginianus*)		Reproductive	NOEC > 1000 mg a.s./kg diet Calculated NOEL > 86 mg a.s./kg bw/d (reproduction)	
Mallard duck (*Anas platyrhynchos*)		Reproductive	NOEC = 700 mg a.s./kg diet **Calculated NOEL = 78 mg a.s./kg bw/d (reproduction)**	
Bobwhite quail (*Colinus virginianus*)	Prothioconazole-desthio (JAU 6476-desthio)	Acute	**LD_{50} > 2000 mg p.m./kg bw**	EFSA Conclusion 2007
Bobwhite quail (*Colinus virginianus*)		Short-term (5d dietary)	LC_{50} = 4090 mg p.m./kg bw/d Calculated LD_{50} = > **297 mg a.s./kg bw/d**	
Bobwhite quail (*Colinus virginianus*)		Reproductive	NOEC = 173 mg p.m./kg diet **Calculated NOEL = 14.8 mg p.m./kg bw/d (reproduction)**	
Mallard duck (*Anas platrhynchus*)		Reproductive	NOEC > 500 mg p.m./kg diet Calculated NOEL = 63 mg p.m./kg bw/d (reproduction)	
Japanese quail (*Coturnix japonica*)	Sulphur Dust	Acute	LD_{50} > 2000 mg a.s./kg bw	EFSA Conclusion 2008
			LD_{50} > 3500 mg a.s./kg bw	EFSA Confirmatory data 2012
Bobwhite Quail (*Colinus virginianus*)	Sulfur	Short-term	LD_{50} > 1334.75 mg/kg bw	EFSA 2008[1]
Bobwhite quail	Sulphur dust	Short-term	LD_{50} > 3633.5 mg	EFSA Confirmatory

Species	Substance	Exposure System	Results	Reference
(*Colinus virginianus*)			a.s./kg bw	data 2012
Japanese quail (*Coturnix coturnix japonica*)	Sulphur Dust	Long-term (LD_{50}/10)	200 mg a.s./kg bw	EFSA Conclusion 2008 (acute LD_{50} value, calculated long-term value)
Japanese quail (*Coturnix coturnix japonica*)	Sulphur Dust	Long-term (LD_{50}/10)	**350 mg a.s./kg bw**	EFSA Confirmatory data 2012 (acute LD_{50} value, calculated long-term value)

zRMS comments:

Avian toxicity data for sulphur, prothioconazole and prothioconazole metabolite JAU 6476-desthio provided in Table 9.2-1 above were verified by zRMS and then confirmed that they are in line with EU agreed endpoints reported in EFSA Scientific Report (2008) 221, 1-70, Confirmatory data 2012 and EFSA Scientific Report (2007) 106, respectively.
It is noted that for the acute risk assessment the Applicant selected acute toxicity endpoints for active substance - sulphur, is considered acceptable by zRMS.
In zRMS's opinion as no treatment related mortalities were observed in the short-term toxicity study for the a.s prothioconazole indicating that the dietary exposure has not resulted with increased mortality of tested birds and the acute LD_{50}>2000 kg a.s./kg bw is sufficiently protective to use in the risk assessment.
However, from zRMS-PL experience it follows that some of MSs prefers lower endpoint LD_{50} >1413 mg /kg bw in the acute risk assessment. For this reason, the additional calculations are presented in the relevant Tables.
Based on the Confirmatory data for sulphur (2012) higher endpoint for short term toxicity is estimated than for acute toxicity. Therefore, the acute risk is based on lower value LD_{50}>3500 mg a.s./kg b w.
In case of the acute risk for metabolite JAU 6476-desthio acute LD_{50} >2000 mg pm/kg bw is used by the Applicant, while short-term dietary studies with this compound with lower LD_{50} of 297 mg pm/kg bw/d should be considered as treatment related mortalities were observed in these short-term dietary studies.

9.2.1.1 Justification for new endpoints

The endpoints used in the risk assessment are those reported in the EFSA conclusion 2007 for prothioconazole, and the confirmatory data released in 2012 for sulphur.

The chronic risk assessment requires the use of the acute LD_{50} / 10 if this value is lower than the reproductive toxicity NOAEL or the chronic endpoint is missing. This is the case for sulphur, where an acute LD_{50} of 3500 mg/kg bw / 10 is calculated to produce a reproductive endpoint of 350 mg/kg bw/d. This is not applicable to prothioconazole and prothioconazole-desthio, as the reproductive endpoints for both these substances are lower than their respective LD_{50} / 10.

zRMS comment:

zRMS agrees with the long-term endpoint of 350 g a.s./kg for the risk assessment for Sulphur.

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).

Prothioconazole metabolite JAU-desthio (M4) was considered to be the only major metabolite in crop foliage (EFSA Scientific Report (2007) 106) and acute and chronic toxicity studies are available to assess the risk. A total conversion of prothioconazole to the desthio metabolite (an absolute worst-case approach) was

assumed at the screening level and in the Tier-1 assessment.

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

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

Table 9.2-2: Prothioconazole - Screening assessment of the acute and long-term/reproductive risk for birds due to the use of FHO04 in cereals (use 1)

Intended use		**Cereals**			
Active substance		Prothioconazole			
Application rate (g/ha)		2 × 200 (14d)			
Acute toxicity (mg/kg bw)		~~2000~~ 1413*			
TER criterion		10			
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Cereals	Small omnivorous bird	158.8	1.2	38.1	~~52.48~~ 37.08*
Reprod. toxicity (mg/kg bw/d)		78			
TER criterion		5			
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Cereals	Small omnivorous bird	64.8	0.742	9.6	8.12 ~~72.79~~

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.
*Lower endpoint has been used by zRMS.

Table 9.2-3: Prothioconazole-desthio - Screening assessment of the acute and long-term/reproductive risk for birds from FHO04 following the use of prothioconazole- in cereals (use 1)

Intended use		**Cereals**			
Metabolite		Prothioconazole-desthio (M4)			
Application rate (g/ha)		2 × 200 (14d)			
Acute toxicity (mg/kg bw)		~~2000~~ >297			
TER criterion		10			
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Cereals	Small omnivorous bird	158.8	1.2	38.1	~~52.48~~ **7.8**
Reprod. toxicity (mg/kg bw/d)		14.8			
TER criterion		5			
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Cereals	Small omnivorous bird	64.8	0.742	9.6	**1.54**

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

Table 9.2-4: Sulphur - Screening assessment of the acute and long-term/reproductive risk for birds due to the use of FHO04 in cereals (use 1)

Intended use		**Cereals**			
Active substance		Sulphur			
Application rate (g/ha)		2 × 2500 (14d)			
Acute toxicity (mg/kg bw)		3500			
TER criterion		10			
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Cereals	Small omnivorous bird	158.8	1.2	476.4	**7.35**
Reprod. toxicity (mg/kg bw/d)		350			
TER criterion		5			
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Cereals	Small omnivorous bird	64.8	0.742	120.2	**2.91**

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.

For prothioconazole, the calculated TERa and TERlt are above the trigger values of 10 and 5 respectively, indicating a low risk to birds. For the metabolite prothioconazole-desthio (M04), the TERa and TER_{LT} is below ~~also above~~ the trigger of 10 and 5, respectively. ~~5 indicating low risk when LD_{50} >2000 mg a.s./kg has been considered. For lower LD_{50} of 1413 mg a.s./kg bw is taken into account~~ and. For this reason, the Tier 1 is required.

~~, however,~~ The ~~TER_{lt} is below the trigger value of 5 indicating an unresolved risk to birds under chronic exposure. As such, additional higher tier risk assessment is required for acute and the long term risk to metabolite prothioconazole desthio (M04).~~ This is also required for sulphur (sulphur dust), as both the TERa and TERlt are below the trigger values of 10 and 5 respectively.

zRMS comments:

Based on the calculations presented in the Table 9.2-2 to 9.2-4 it was concluded that the Tier 1 risk assessment is required for acute and long - term risk for sulphur and metabolite prothioconazole-desthio (M04).
The Tier I calculations are presented in the Point 9.2.2.2.

9.2.2.2 Tier I risk assessment

The results of the reproductive Tier-1 risk assessments for prothioconazole-desthio, and the acute and reproductive Tier-1 risk assessments for sulphur are summarised in the following tables.

Table 9.2-5: Prothioconazole-desthio - First tier assessment of the long-term/reproductive risk for birds due to the use of FHO04 in cereals (use 1)

Intended use		**Cereals**			
Metabolite		Prothioconazole-desthio (M04)			
Application rate (g/ha)		2 × 200 (14d)			
Reprod. toxicity (mg/kg bw/d)		14.8			
TER criterion		5			
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
BBCH 10-29	Large herbivorous bird "goose"	16.2	0.742*	2.40	6.16
BBCH 10 - 29	Small omnivorous bird “lark”	10.9	0.742*	1.62	9.15

BBCH 30 -39	Small omnivorous bird “lark”	5.4	0.742*	0.80	18.47
BBCH ≥ 40	Small omnivorous bird “lark”	3.3	0.742*	0.49	30.22

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.
*Calculated as MAF_m of 1.4 (2 applications, 14 days apart) times F_{TWA} factor of 0.53

Table 9.2-5-1: First tier assessment of the acute risk for birds due to the use of FHO04 in cereals (use 1)

Intended use	**Cereals**				
Active substance	Prothioconazole-desthio (M04)				
Application rate (g/ha)	2 × 200 (14d)				
Acute toxicity (mg/kg bw)	>297				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
BBCH 10-29	Large herbivorous bird "goose"	30.5	1.2	7.3	40.7
BBCH 10 - 29	Small omnivorous bird “lark”	24	1.2	5.8	51.2
BBCH 30 -39	Small omnivorous bird “lark”	12	1.2	2.9	102.4
BBCH ≥ 40	Small omnivorous bird “lark”	7.2	1.2	2.5	119

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.*Calculated as MAF_m of 1.4 (2 applications, 14 days apart) times F_{TWA} factor of 0.53

Table 9.2-6: Sulphur - First tier assessment of the acute and long-term/reproductive risk for birds due to the use of FHO04 in cereals (use 1)

Intended use	**Cereals**				
Active substance	Sulphur				
Application rate (g/ha)	2 × 2500 (14d)				
Acute toxicity (mg/kg bw)	3500				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
BBCH 10-29	Large herbivorous bird "goose"	30.5	1.2	91.50	38.25
BBCH 10 - 29	Small omnivorous bird “lark”	24	1.2	72.00	48.61
BBCH 30 -39	Small omnivorous bird “lark”	12	1.2	36.00	97.22
BBCH ≥ 40	Small omnivorous bird “lark”	7.2	1.2	21.60	162.04
Reprod. toxicity (mg/kg bw/d)	350				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
BBCH 10-29	Large herbivorous bird "goose"	16.2	0.742*	30.05	11.65
BBCH 10 - 29	Small omnivorous bird “lark”	10.9	0.742*	20.22	17.31
BBCH 30 -39	Small omnivorous bird “lark”	5.4	0.742*	10.02	34.94
BBCH ≥ 40	Small omnivorous bird “lark”	3.3	0.742*	6.12	57.18

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.
*Calculated as MAF_m of 1.4 (2 applications, 14 days apart) times F_{TWA} factor of 0.53

The TERa and TER_{lt} for all scenarios relevant to the proposed GAP are above the trigger value of 5 indicating an acceptable risk from prothioconazole-desthio and sulphur.

zRMS comments:

TER_A and TER_{LT} values for the exposure to prothioconazole, its metabolite prothioconazole-desthio and sulphur 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 metabolite JAU 6476-desthio was performed by the Applicant with consideration of the acute LD_{50} of >2000 mg pm/kg bw, while the toxicity endpoint from dietary study was more relevant for purposes of the acute risk assessment for this metabolite (please see in the commenting boxes under Table 9.2-1). The evaluations presented in Tables 9.2-3 and 9.2-5-1 above were amended accordingly with consideration of the LD_{50} of 297 mg pm/kg bw/d value.

Overall, acceptable acute and long-term risk may be concluded for birds exposed to prothioconazole, metabolite JAU 6476-desthio and sulphur in FHO04.

9.2.2.3 Mixture toxicity

As Patton Supra contains two active substances, a mixture risk assessment is required. For the acute risk assessment, the Finney's equation is used to calculate a mixture toxicity ($LD_{50, mix}$) based on the toxicity of each substance and their proportions within the product. For the long-term mixture risk assessment, the cumulative risk quotient approach (Trigger/TER) is used, with a resultant RQ_{mix} less than one resulting in an acceptable risk.

First, the prothioconazole-desthio metabolite relative application rate was calculated, as this metabolite needs to be included alongside the two active substances in the mixture risk assessment (Table 9.2-7).

Table 9.2-7: First tier assessment of the acute and long-term/reproductive risk for birds due to the use of prothioconazole in cereals

Metabolite	Metabolite molecular weight (g/mol)	Parent molecular weight (g/mol)	Metabolite formulation fraction	Coefficient [a)]	Metabolite relative concentration (g/L) [b)]
Prothioconazole-desthio	312.2	344.3	0.571	0.518	25.88

a) Coefficient = (metabolite molecular weight / parent molecular weight) * metabolite formulation fraction
b) Metabolite relative concentration = coefficient * parent formulation concentration (i.e. 50 g/L)

Acute mixture toxicity

For the assessment of acute effects (mortality), a surrogate LD_{50} can be calculated. The EFSA Guidance Document indicates that the following equation should be used for deriving a surrogate LD_{50} for a mixture of active substances with known toxicity assuming dose additivity:

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

where:
$X\,(a.s._i)$ = fraction of active substance (i) in the formulation mixture
$LD_{50}\,(a.s._i)$ = acute toxicity for the active substance (i)

The LD_{50} of the mix is summarised in the table below.

Table 9.2-8: Acute LD_{50} for the mixture of active substances and relevant metabolites

Test substance	Concentration of active substance in the formulation mixture (g/L)	Fraction of active substance in the formulation mixture, X(a.s.) [a)]	Acute toxicity endpoint (mg/kg bw/d)	Fraction of active substance/LD_{50} for the active substance	LD_{50} mix (mg/kg bw)
Prothioconazole	24.11*	0.04	2000	0.00002	**3315.79**
Prothioconazole-desthio	25.89	0.04	2000	0.00002	
Sulphur	625.00	0.93	3500	0.00026	
Total	-	1	-	0.000302	

*Parent concentration (50 g/L) – metabolite relative concentration (25.89 g/L)
a) Concentration of an active substance in the formulation, divided by, the total concentration of all active substances in the formulation.

To achieve a basis for a comparison of single active substance and mixture toxicity in terms of potential risk, a "tox per fraction" is calculated for each active substance and compared to the corresponding quotient for the mixture using the following equation, according to the EFSA guidance:

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

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

Table 9.2-9: "Tox per fraction" quotient for acute toxicity

Active substance or metabolite	Fraction of active substance in the formulation mixture, X(a.s.) [a)]	Acute toxicity endpoint (mg/kg bw/d)	Tox per fraction (a.s)	Tox per fraction of the formulation mixture	Tox fraction (mix)/tox fraction (as)	Deviation (%) [b)]
Prothioconazole	0.04	2000	55989.20	3315.79	0.059	5.92
Prothioconazole-desthio	0.04	2000	52147.29		0.064	6.36
Sulphur Dust	0.93	3500	3780.00		0.877	87.72

a) Concentration of an active substance in the formulation, divided by, the total concentration of all active substances in the formulation.
b) Deviation % = Tox per fraction (mix) / Tox per fraction (a.s.) × 100

As neither the two active substances nor the metabolite contributes > 90 % to the mixture toxicity, a combined assessment is necessary.

Table 9.2-10: Screening and first-tier assessment of the acute combined risk for birds due to the use of FHO04 in cereals (use 1)

Intended use	**Cereals**				
Product	FHO04				
Application rate (g/ha)	2 x 2900 (14 d) (200 g/ha prothioconazole + 200 g/ha prothioconazole-desthio + 2500 g/ha sulphur)				
Acute toxicity (mg/kg bw)	~~3315.79~~ 2326				
TER criterion	10				
Crop scenario **Growth stage**	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**

Screening					
Cereals	Small omnivorous bird	158.8	1.2	552.62	~~6.00~~ **4.2**
First-tier					
Cereals, BBCH 10-29	Large herbivorous bird "goose"	30.5	1.2	106.14	~~31.24~~ 21.20
Cereals, BBCH 10-29	Small omnivorous bird “lark”	24	1.2	83.52	~~39.70~~ 27.85
BBCH 30-39	Small omnivorous bird “lark”	12	1.2	41.76	~~79.40~~ 55.7
BBCH ≥ 40	Small omnivorous bird “lark”	7.2	1.2	25.06	~~132.34~~ 92.8

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

The TER values for each generic focal species were greater than the trigger value of 10, showing a safe use can be concluded using the formulation Patton Supra.

~~Reproductive mixture toxicity~~

~~**Table 9.2-11: Chronic LD_{50} for the mixture of active substances and relevant metabolites**~~

~~Test substance~~	~~Concentration of active substance in the formulation mixture (g/L)~~	~~Fraction of active substance in the formulation mixture, X(a.s.) [a)]~~	~~Chronic toxicity endpoint (mg/kg bw/d)~~	~~Fraction of active substance/LD_{50} for the active substance~~	~~LD_{50} mix (mg/kg bw)~~
~~Prothioconazole~~	~~24.11*~~	~~0.04~~	~~78~~	~~0.000458~~	~~**175.60**~~
~~Prothioconazole-desthio~~	~~25.89~~	~~0.04~~	~~14.8~~	~~0.002591~~	
~~Sulphur Dust~~	~~625.00~~	~~0.93~~	~~350~~	~~0.002646~~	
~~Total~~	~~–~~	~~1~~	~~–~~	~~0.005695~~	

~~* Parent concentration (50 g/L) – metabolite relative concentration (25.89 g/L)~~
~~a) Concentration of an active substance in the formulation, divided by, the total concentration of all active substances in the formulation.~~

~~**Table 9.2-12: “Tox per fraction” quotient for chronic toxicity**~~

~~Active substance or metabolite~~	~~Fraction of active substance in the formulation mixture, X(a.s.) [a)]~~	~~Chronic toxicity end-point (mg/kg bw/d)~~	~~Tox per fraction (a.s)~~	~~Tox per fraction of the formulation mixture~~	~~Tox fraction (mix)/tox fraction (as)~~	~~Deviation (%) [b)]~~
~~Prothioconazole~~	~~0.04~~	~~78~~	~~2183.58~~	~~175.60~~	~~0.080~~	~~8.04~~
~~Prothioconazole-desthio~~	~~0.04~~	~~14.8~~	~~385.89~~		~~0.455~~	~~45.50~~
~~Sulphur~~	~~0.93~~	~~350~~	~~378.00~~		~~0.465~~	~~46.45~~

~~a) Concentration of an active substance in the formulation, divided by, the total concentration of all active substances in the formulation.~~
~~b) Deviation % = Tox per fraction (mix) / Tox per fraction (a.s.) * 100~~

~~As neither the two active substances nor the metabolite contributes > 90 % to the mixture toxicity, a combined assessment is necessary for chronic risk.~~

Table 9.2-13: Screening and first-tier assessment of the reproductive combined risk for birds due to the use of FHO04 in cereals (use 1)

Product	**FHO04**							
Application rate (g/ha)	2 x 2900 (14 d) (200 g/ha prothioconazole + 200 g/ha prothioconazole-desthio + 2500 g/ha sulphur)							
TER criterion	**5**							
Crop scenario Growth stage	**Indicator/generic focal species**	**TER**			**RQ**			**RQ Sum**
		Prothioconazole	**Prothioconazole-desthio**	**Sulphur**	**Prothioconazole**	**Prothioconazole-desthio**	**Sulphur**	
Screening								
Cereals		8.11	1.54	2.91	0.62	0.40	0.14	**1.15**
First-tier								
Cereals	BBCH 10-29 Large herbivorous bird "goose"	8.11*	6.16	11.65	0.62	0.10	0.03	0.75
	BBCH 10-29 Small omnivorous bird "lark"	8.11*	9.15	17.31	0.62	0.07	0.02	0.71
	BBCH 30-39 Small omnivorous bird "lark"	8.11*	18.47	34.94	0.62	0.03	0.01	0.66
	BBCH ≥ 40 Small omnivorous bird "lark"	8.11*	30.22	57.18	0.62	0.02	0.01	0.64

* Worst case Screening level TER

The RQsum for mixture toxicity to prothioconazole, prothioconazole-desthio and sulphur was above the trigger value of 1 at the screening assessment. However, in the first-tier assessment the RQsum values were below the trigger value of 1 for all indicator/generic focal species in the first-tier assessment, indicating a low risk to birds from the combined active substances and metabolite.

zRMS comments:

Combined acute risk assessment

LD_{50mix} for the mixture of calculated by the Applicant in the Table 9.2-8 should be considered the lower toxicity endpoint LD_{50} of 297 pm./kg bw for metabolite obtained from dietary study for birds.
For this reason, LD_{50mix} has been recalculated by zRMS with consideration of this endpoint and a total conversion of prothioconazole to the JAU 6476-desthio metabolite as the worst-case approach.

zRMS calculations are presented below.

Avian LD_{50} (mix)
(step 1 in EFSA GD 2009, Appendix B)

	Prothioconazole	JAU 6476-desthio	Sulphur
Relative amount of a.s. (%)	24.11	25.89	625
Fraction in the a.s. mixture	0.04	0.04	0.93
LD_{50} of a.s. or met[mg/kg bw]	1413	>297	3500
Fraction / LD_{50}	0.00003	0.00013	0.00027
Sum	0.00043		
1/ sum = predicted LD_{50} (mix)	2326		

Avian "tox per fraction"
(step 1 in EFSA GD 2009, Appendix B)

	Prothioconazole	JAU 6476-desthio	Sulphur	"mix"
Content in the formulation	24.11	25.89	625	
Fraction in mixture	0.04	0.04	0.93	
LD_{50} (mg/kg bw)	1413	>297	3500	LD_{50mix} =333.3
Tox per fraction the formulation mixture	35 325	7425	3763.50	
LD_{50} mix (Tox per fraction of the formulation mixture)	2326			
Tox fraction (mix)/tox fraction (as)	0.065	0.31	0.62	
Deviation (%) [b)]	6.6	31	62.4	

[a)] Concentration of an active substance in the formulation, divided by, the total concentration of all active substances in the formulation.
[b)] Deviation % = Tox per fraction (mix) / Tox per fraction (a.s.) * 100

As neither the two active substances nor the metabolite contributes > 90 % to the mixture toxicity, a combined assessment is necessary for acute risk.

Screening and first-tier assessment of the acute combined risk for birds due to the use of FHO04 in cereals

Intended use	**Cereals**
Product	FHO04
Application rate (g/ha)	2 x 2900 (14 d) (200 g/ha prothioconazole + 200 g/ha prothioconazole-desthio + 2500 g/ha sulphur)
Acute toxicity (mg/kg bw)	2326
TER criterion	10

Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Screening					
Cereals	Small omnivorous bird	158.8	1.2	552.62	**4.2**
First-tier					
Cereals, BBCH 10-29	Large herbivorous bird "goose"	30.5	1.2	106.14	21.20

Cereals, BBCH 10-29	Small omnivorous bird "lark"	24	1.2	83.52	27.85
BBCH 30-39	Small omnivorous bird "lark"	12	1.2	41.76	55.7
BBCH ≥ 40	Small omnivorous bird "lark"	7.2	1.2	25.06	92.8

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

Combined long-term toxicity:

zRMS agrees that for the approach assuming dose additivity of the active substances, reliable results would only be expected for combinations of effect levels with a defined x, e.g. a LD_{50}, but not for NOELs since the latter effect indicators may represent varying risk or response levels for different compounds depending on dose-spacing according to GD B&M, EFSA Journal 2009; 7(12): 1438, Appendix B. Therefore, for the risk assessment based on long-term effects it is not recommended to consider the use of predicted toxicity values.
Therefore, the calculated $NOEl_{mix}$ provided by the Applicant was not considered by zRMS in the current risk assessment.
Simplified approach (TER_{mix}), agreed in Central Zone with respective calculations based on the lowest TER_{LT} values is presented below by zRMS.

TER_{mix} approach:

The calculated TER_{LT} values for the single active substances and the representative indicator species (screening and Tier 1) included in the tables above are summarised in below.

TER_{LT} values for active substances and metabolite JAU 6476-desthio

Intended use	BBCH 30–65		
Crop scenario, Growth stage and Indicator species	**TER_{LT}**		
	Prothioconazole	**JAU-desthio (M4)**	**sulphur**
BBCH 10-29 Large herbivorous bird "goose"	26.92*	40.7 [1)]	6.16 [1)]

*Screening step
[1)] The lowest Tier 1 value

Prothioconazole		**JAU-desthio (M4)**		**Sulphur**		**Σ1/TER**	**$Σ1/TER^{-1}$**	**Trigger**
26.92	0.037	40.7	0.0245	11.65	0.085	1.15	6.67	5

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

9.2.2.4 Higher-tier risk assessment

Not required.

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

Puddle scenario

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

With an arithmetic mean K(f)oc of 1765 mL/g for prothioconazole and 3615.3 mL/g for sulphur, both active substances belong to the group of more sorptive substances. Prothioconazole-desthio has an arithmetic mean K(f)oc of 575.4 mL/g so also belongs to the group of more sorptive substances.

Table 9.2-14: Assessment of the risk for birds due to exposure via contaminated drinking water in puddles: consideration of ratio between application rate and toxicity endpoint

Prothioconazole			
Effective application rate (g/ha)	400 (200*2)		
Acute toxicity (mg/kg bw)	~~>2000~~ >1413	quotient	~~0.20~~ 0.28
Reprod. toxicity (mg/kg bw/d)	78	quotient	5.13
Prothioconazole-desthio (M04)			
Effective application rate (g/ha)	400 (200*2)		
Acute toxicity (mg/kg bw)	~~>2000~~ > 297	quotient	~~0.20~~ 1.34
Reprod. toxicity (mg/kg bw/d)	14.8	quotient	27.03
Sulphur			
Effective application rate (g/ha)	5000 (2500*2)		
Acute toxicity (mg/kg bw)	3500	quotient	1.43
Reprod. toxicity (mg/kg bw/d)	350	quotient	14.29

All acute and chronic ratios are below the threshold of 50 for all substances, demonstrating that the risk to birds from contaminated drinking water is acceptable following the proposed use of Patton Supra.

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.

9.2.2.6 Effects of secondary poisoning

The log P_{ow} of prothioconazole amounts to 4.16 and thus exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is required. Two prothioconazole metabolites also have log Pow values higher than the trigger value of 3 (prothioconazole-desthio 3.04 and prothioconazole-methyl 4.19).

Risk assessment for earthworm-eating birds via secondary poisoning

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

Table 9.2-15: Assessment of the risk for earthworm-eating birds due to exposure to prothioconazole via bioaccumulation in earthworms (secondary poisoning) for the intended use in cereals (use 1)

Parameter	Prothioconazole	Comments
PEC_{soil} (mg/kg soil)	0.22	PECmax (dRR Part B8, Section 8.7, Table 8.7-3)
log P_{ow} / P_{ow}	3.82/6607 ~~4.16 / 14454~~	EFSA (2007)
Koc	1765	EFSA (2007)
Foc	0.02	Default
BCF_{worm}	2.72 ~~4.937~~	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.12 \times P_{ow}) / foc \times Koc$
PEC_{worm}	0.60 ~~1.086~~	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.63 ~~1.141~~	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	78	
TER_{lt}	124 ~~68~~	

TER values shown in bold fall below the relevant trigger.

Table 9.2-16: Assessment of the risk for earthworm-eating birds due to exposure to prothioconazole-desthio via bioaccumulation in earthworms (secondary poisoning) following the intended use in cereals (use 1)

Parameter	Prothioconazole-desthio	Comments
PEC_{soil} (mg/kg soil)	0.221	PECmax (dRR Part B8, Section 8.7, Table 8.7-7)
log P_{ow} / P_{ow}	3.04 / 1096	EFSA (2007)
Koc	575.40	Arithmetic mean (n=4), EFSA (2007)
Foc	0.02	Default
BCF_{worm}	1.220	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.12 \times P_{ow}) / foc \times Koc$
PEC_{worm}	0.270	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.283	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	14.8	
TER_{lt}	52	

TER values shown in bold fall below the relevant trigger.

Table 9.2-17: Assessment of the risk for earthworm-eating birds due to exposure to prothioconazole-methyl via bioaccumulation in earthworms (secondary poisoning) following the intended use in cereals (use 1)

Parameter	Prothioconazole- S-methyl	Comments
PEC_{soil} (mg/kg soil)	0065	PECmax (dRR Part B8, Section 8.7, Table 8.7-5)
log P_{ow} / P_{ow}	4.19 / 15488	
Koc	2556.3	Arithmetic mean (n=4)
Foc	0.02	Default
BCF_{worm}	3.696	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.12 \times P_{ow}) / foc \times Koc$
PEC_{worm}	0.2402	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.2522	$DDD = PEC_{worm} \times 1.05$

Parameter	Prothioconazole- S-methyl	Comments
NOEL (mg/kg bw/d)	7.80	Parent endpoint / 10
TER_{lt}	31	

TER values shown in bold fall below the relevant trigger.

TER >5 for both prothioconazole and its relevant metabolites, hence no further refinements are required.

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 and prothioconazole-desthio in water.

Due to the low predicted environmental concentrations of JAU 6476-S-methyl in surface water no fish-BCF-study was conducted even though this metabolite has a log Pow of 4.19. The BCF for this metabolite can be estimated from its log Pow and the BCF and the log Pow of the parent compound with a sufficient degree of precision. The chemical structure of JAU 6476-S-methyl is very similar to the one of parent compound prothioconazole since the only modification of the parent molecule during the formation of JAU 6476-S-methyl is the methylation of the sulphur atom. This methylation results in an increase of the Pow of JAU 6476-S-methyl that is 20000 (log Pow = 4.30) in comparison to 3981 (log Pow = 3.60) of prothioconazole. Therefore, it can be anticipated that the BCF for S-methyl is not more than 5.02 times higher (20000/3981 = 5.02) than the BCF_{parent} (whole fish, normalised to 6% fat) for prothioconazole that has been determined to be 18.8. An estimated BCF for JAU 6476-S-methyl of 94.4 (18.8 x 5.02 = 94.4) is also in accordance with the results of the fish BCF study that has been conducted for the parent compound. In this study, JAU 6476-S-methyl was detected as a minor metabolite in edibles and viscera of fish. No significant increase of the level and the portion of the total radioactive residue (TRR) was observed for the metabolite JAU 6476-S-methyl between day 7 and day 14. Therefore, it can be concluded that formation and degradation/depuration of this metabolite, which had been formed in the fish, were in balance. If the metabolite had a much higher bioaccumulation potential than the parent compound, a further increase of its level and portion of the TRR (total radioactive residue) would have to be expected from day 7 to day 14 in the BCF study. Hence a BCF of 94.4 seems to be a realistic estimate for the BCF of JAU 6476-S-methyl in fish.

As worst-case the BCF estimate (94.4) has been used for risk assessment of prothioconazole-methyl.

Table 9.2-18: Assessment of the risk for fish-eating birds due to exposure to prothioconazole via bioaccumulation in fish (secondary poisoning) for the intended use in cereals (use 1)

Parameter	Prothioconazole	Comments
PEC_{sw} (mg/L)	0.043	PECsw max (Step 1)
BCF_{fish}	19.7	EFSA (2007)
BMF	-	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	0.856	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.136	$DDD = PEC_{fish} \times 0.159$
NOEL (mg/kg bw/d)	78	
TER_{lt}	573	

TER values shown in bold fall below the relevant trigger.

Table 9.2-19: Assessment of the risk for fish-eating birds due to exposure to prothioconazole-desthio via bioaccumulation in fish (secondary poisoning) following the intended use in cereals (use 1)

Parameter	Prothioconazole-desthio	Comments
PEC_{sw} (mg/L)	0.082	PECsw max (Step 1)
BCF_{fish}	65	EFSA (2007)
BMF	-	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	5.30	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.843	$DDD = PEC_{fish} \times 0.159$
NOEL (mg/kg bw/d)	14.80	
TER_{lt}	17.54	

TER values shown in bold fall below the relevant trigger.

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

Parameter	Prothioconazole- S -methyl	Comments
PEC_{sw} (mg/L)	0.0094	PECsw max (Step 1)
BCF_{fish}	94.4	BFC estimate (see above)
BMF	-	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	0.85 ~~0.438~~	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.13 ~~0.070~~	$DDD = PEC_{fish} \times 0.159$
NOEL (mg/kg bw/d)	7.8	Parent / 10
TER_{lt}	60 ~~113~~	

TER values shown in bold fall below the relevant trigger.

All long-term TER values were above the relevant trigger of 5, demonstrating that the risk to birds from exposure to prothioconazole and its metabolites via bioaccumulation are acceptable following the proposed use of Patton Supra.

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.

Consideration of the maximum soil and surface water exposure is agreed by the zRMS as it represents worst case comparing to 21-d TWA concentrations. Some additional explanations were added in tables above.
Despite all corrections of the zRMS, acceptable risk of secondary exposure from all relevant compounds could be concluded for birds.

9.2.2.7 Biomagnification in terrestrial food chains

Not relevant.

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

Not relevant.

9.2.4 Overall conclusions

Acute and chronic risk to birds following the proposed use of Patton Supra were performed in accordance with EFSA (2009) guidelines. The risk from the active substances prothioconazole was acceptable at the screening level, while the risk from sulphur and the metabolite prothioconazole-desthio was acceptable at Tier 1.
The risk to the mixture was calculated using the Finney's equation for acute toxicity and the risk quotient approach for chronic toxicity. The acute and chronic risks to the mixture (including metabolite prothioconazole-desthio) were acceptable at Tier 1.

The risk from exposure to contaminated drinking water and secondary poisoning was also considered acceptable following the proposed use of Patton Supra.

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

9.3.1 Toxicity data

Mammalian toxicity studies have been carried out with prothioconazole, sulphur and their relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents (EFSA Conclusion 2007, EFSA Conclusion 2008 and EFSA Confirmatory data 2012).

Effects on mammals of Patton Supra were not evaluated as part of the EU assessment of prothioconazole or sulphur. However, the provision of further data on the formulation Patton Supra is not considered essential, because the toxicity of the formulation can be read across from the data on the active substance and also in order to minimise unnecessary vertebrate testing. The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process.

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

Species	Substance	Exposure System	Results	Reference
Rat	Prothioconazole	Oral Acute	**LD_{50} > 6200 mg a.s./kg bw/d**	EFSA 2007
		Long term Developmental	NOAEL = 9.7 (parental), **95.6 (reproduction) mg/kg bw/d**	
Rat	Prothioconazole-desthio (JAU 6476-desthio)	Oral Acute	LD_{50} = 2506 (females), 2806 (males), 2652 (geomean) mg p.m./kg bw/d	
Mouse		Oral Acute	LD_{50} = 3459 (females), **2235 (males) mg p.m./kg bw/d**	
Rat		Oral Developmental	NOAEL = 2.5 (parental), **10 (reproduction) mg/kg bw/d**	
		Oral Short term	NOEL = 1000 mg a.s./kg bw/d	
Rat	Sulphur	Oral Acute	LD_{50} > 1760 mg a.s./kg bw	EFSA Conclusion 2008
		Oral Short term	NOEL > 1000 mg a.s./kg bw/d	
		Oral Acute	**LD_{50} >35000 mg/kg bw**	EFSA Confirmatory data 2012

Species	Substance	Exposure System	Results	Reference
		Long term	**3500 mg/kg bw**	Calculated LD_{50}/10 from EFSA Confirmatory data 2012

Bold values indicate endpoints used in the risk assessment.

zRMS comments:

Mammalian toxicity data for sulphur, prothioconazole and prothioconazole metabolite JAU 6476-desthio provided in Table 9.3-1 above is validated by zRMS and then confirmed that they are in line with EU agreed endpoints reported in EFSA Scientific Report (2008) 221, 1-70, Confirmatory data 2012 and EFSA Scientific Report (2007) 106, respectively.

9.3.1.1 Justification for new endpoints

All endpoints are as agreed in the EFSA conclusions on prothioconazole (2007), with no changes expected to prothioconazole or its metabolite and the confirmatory data released in 2012 for sulphur.

9.3.2 Risk assessment for spray applications

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

Prothioconazole-desthio (M4) was considered to be the only major metabolite in crop foliage (EFSA Scientific Report (2007) 106) and acute and reproductive toxicity studies are available to assess the risk. A total conversion of prothioconazole to the prothioconazole-desthio metabolite was assumed as worst-case approach at the screening level and in the Tier-1 assessment.

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

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

Table 9.3-2: Prothioconazole - Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of FHO04 in cereals (use 1)

Intended use	Cereals				
Active substance	Prothioconazole				
Application rate (g/ha)	2 × 200 (14d)				
Acute toxicity (mg/kg bw)	> 6200				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Cereals	Small herbivorous mammal	118.4	1.2	28.4	218.19
Reprod. toxicity (mg/kg bw/d)	95.60				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Cereals	Small herbivorous mammal	48.3	0.742*	7.2	13.34

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.
*Calculated as MAF_m of 1.4 (2 applications, 14 days apart) times F_{TWA} factor of 0.53

The TERa and TERlt for prothioconazole are above the trigger values of 10 and 5 respectively, indicating that the risk to mammals is low.

zRMS comments:

The screening step risk assessment for prothioconazole is validated by the zRMS. Acceptable acute and long-term risk may be concluded for mammals exposed to prothioconazole in FHO04.

Table 9.3-3: Prothioconazole-desthio - Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of FHO04 in cereals (use 1)

Intended use	Cereals				
Metabolite	Prothioconazole-desthio				
Application rate (g/ha)	2 × 200 (14d)				
Acute toxicity (mg/kg bw)	2235				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Cereals	Small herbivorous mammal	118.4	1.2	28.4	78.65
Reprod. toxicity (mg/kg bw/d)	10				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Cereals	Small herbivorous mammal	48.3	0.742*	7.2	**1.40**

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.
*Calculated as MAF_m of 1.4 (2 applications, 14 days apart) times F_{TWA} factor of 0.53

The TERlt for the metabolite prothioconazole-desthio is below the trigger values of 5, indicating an unresolved risk to mammals under chronic exposure. As such, a complete Tier I assessment is required.

zRMS comments:

The screening step risk assessment for prothioconazole-desthio is validated by the zRMS. Acceptable acute risk may be concluded for mammals exposed to prothioconazole in FHO04.

The TER_{LT} for the metabolite prothioconazole-desthio is below the trigger values of 5, indicating an unresolved risk to mammals under long-term exposure. Therefore, Tier 1 risk assessment is required.

Table 9.3-4: Sulphur - Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of FHO04 in cereals (use 1)

Intended use	Cereals				
Active substance	Sulphur				
Application rate (g/ha)	2 × 2500 (14d)				
Acute toxicity (mg/kg bw)	35000				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Cereals	Small herbivorous mammal	118.4	1.2	355.2	98.54
Reprod. toxicity (mg/kg bw/d)	3500				
TER criterion	5				

Crop scenario Growth stage	Indicator/generic focal species	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{lt}
Cereals	Small herbivorous mammal	48.3	0.742*	89.6	39.06

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.
*Calculated as MAF_m of 1.4 (2 applications, 14 days apart) times F_{TWA} factor of 0.53

The TERa and TERlt for sulphur are above the trigger values of 10 and 5 respectively, indicating that the risk to mammals is low.

zRMS comments:

The screening step risk assessment for sulphur is validated by the zRMS. Acceptable acute and long-term risk may be concluded for mammals exposed to sulphur in FHO04.

9.3.2.2 Tier I risk assessment

Table 9.3-5: Prothionconazole-desthio - First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of FHO04 in cereals (use 1)

Intended use	Cereals
Metabolite	Prothioconazole-desthio
Application rate (g/ha)	2 × 200 (14d)
Reprod. toxicity (mg/kg bw/d)	10
TER criterion	5

Crop scenario Growth stage	Indicator/generic focal species	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{lt}
BBCH ≥ 20	Small insectivorous mammal "shrew"	1.9	0.742*	0.28	35.47
BBCH ≥ 40	Small herbivorous mammal "vole"	21.7	0.742*	3.22	**3.11**
BBCH 10-29	Small omnivorous mammal "mouse"	7.8	0.742*	1.16	8.64
BBCH 30-39	Small omnivorous mammal "mouse"	3.9	0.742*	0.58	17.28
BBCH ≥ 40	Small omnivorous mammal "mouse"	2.3	0.742*	0.34	29.30

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.
*Calculated as MAF_m of 1.4 (2 applications, 14 days apart) times F_{TWA} factor of 0.53

The TERlt values for prothioconazole-desthio are above the trigger value of 5, indicating safe use, except for the TERlt for the small herbivorous mammal “vole”. As such, additional higher tier risk assessment is required for the long-term risk from the metabolite prothioconazole-desthio in cereals.

zRMS comments:

Based on the calculations presented in the Table 9.3-5 higher tier risk assessment is required for the long-term risk from the metabolite prothioconazole-desthio in cereals for Small herbivorous mammal "vole" at BBCH>40.

Higher-tier risk assessment

A refinement options for the “vole” scenario for crop growth stage BBCH ≥ 40 is presented in the following table considering a refinement of the deposition factor and a more realistic application rate for the metabolite prothioconazole-desthio (M04).

The EFSA (2009) value for deposition factor is 0.3, however since the publication of this guidance document the crop interception values have been revised (EFSA 2014;12(5):3662). In this revision the crop interception of cereals relevant to the proposed GAP (BBCH 30-69) was increased from 70% to 80%. As

such, the deposition factor can be reduced from 0.3 to 0.2. This refinement is possible because i) the crop is suitably established so there is confidence in the crop interception values at these growth stages and ii) the grasses on which the voles feed are below the level of the cereal crop so interception occurs before contact with the foodstuff relevant to the scenario.

However, using this refinement option only results in a TER value of 4.67, which just fails the risk assessment. Therefore, a more realistic application rate for the metabolite prothioconazole-desthio was calculated, rather than using the parent application rate (which was an absolute worst-case approach). Two different approaches are considered to calculate the metabolite application rate:

- The molecular conversion factor approach: the molecular weight conversion of 0.907 (metabolite molecular weight of 312.2 g/mol / parent molecular weight of 344.3 g/mol) and assuming 100% of the metabolite is formed, resulting in an application rate of 181 g/ha.

- The percentage of prothioconazole-desthio in cereals (35% of the total radioactive residue (TRR) according to the DAR (2005) for prothioconazole) approach: as wheat can be considered as surrogate for monocotyledonous plants, and the diet of the common vole consist of grass and cereals for the exposure scenario in cereals according to the EFSA Journal 2009; 7(12): 1438, it is deemed acceptable to refine the exposure rate for the metabolite of concern considering that the real percentage of prothioconazole-desthio in cereals is about three times lower than the parent. The resulting metabolite application rate equals 70 g/ha.

Using the reduced deposition factor, along with the calculated metabolite application rate, results in a TER > 5. These factors are implemented in the scenario together resulting in an acceptable risk for metabolite prothioconazole-desthio (Table 9.3-6)

Using this refined metabolite application rate specific for cereals, in addition to the reduced deposition factor, results in a TER > 5. However, this refinement option also results in a TER > 5 when the not-refined deposition factor is considered (i.e, 0.3 instead of 0.2).

Overall, a safe use can be concluded from the use of Patton Supra in cereals for mammals.

Table 9.3-6: Prothioconazole-desthio - Higher-tier assessment of the long-term/reproductive risk for mammals due to the use of FHO04 in cereals (use 1) – refined parameters

Intended use			Cereals							
Metabolite			Prothioconazole-desthio							
Application rate [AR] (g/ha)	**Parent AR**		2 × 200 (14d)							
	Mol. conversion AR		2 × 181 (14d)							
	Cereal AR		2 × 70 (14d)							
Reprod. toxicity (mg/kg bw/d)			10							
TER criterion			5							
Refinement approach	**Focal species**	**Food category, % in diet**	**FIR/bw**	**RUD_m**	**DF**	**PT**	**SVm**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Deposition factor	*Microtus arvalis* (Common vole)	Grass, 100 %	1.33	54.2	0.2*	1	14.42	0.74^	2.14	**4.67**
Deptosition factor + mol. conversion AR	*Microtus arvalis* (Common vole)	Grass, 100 %	1.33	54.2	0.2*	1	14.42	0.74^	1.94	5.16
Deptosition factor + cereal AR	*Microtus arvalis* (Common vole)	Grass, 100 %	1.33	54.2	0.2*	1	14.42	0.74^	0.75	13.35
Cereal AR	*Microtus arvalis* (Common vole)	Grass, 100 %	1.33	54.2	0.3	1	~~14.42~~ 21.62	0.74^	1.12	8.90

FIR/bw: Food intake rate per body weight; RUD: residue unit dose; DF: deposition factor (considering possible interception by

the crop); MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.
^Calculated as MAF_m of 1.4 (2 applications, 14 days apart) times F_{TWA} factor of 0.53
*Refined parameter, see text preceding table for further description and justification

zRMS comments:

zRMS agrees with refined risk assessment for common vole presented in the Table 9.3-6.
The TER_{LT} values with consideration of refined parameters such DF, molar conversion or cereal application rate 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 FHO04.

9.3.2.3 Mixture toxicity

As Patton Supra contains two active substances, a mixture risk assessment is required. For the acute risk assessment, the Finney equation is used to calculate a mixture toxicity ($LD_{50, mix}$) based on the toxicity of each substance and their proportions within the product. For the long-term mixture risk assessment, the cumulative risk quotient approach (Trigger/TER) is used, with a resultant RQ_{mix} less than one resulting in an acceptable risk.
First, the prothioconazole-desthio metabolite relative application rate was calculated, as this metabolite needs to be included alongside the two active substances in the mixture risk assessment (Table 9.3-7).

Table 9.3-7: First tier assessment of the acute and long-term/reproductive risk for birds due to the use of prothioconazole in cereals

Metabolite	Metabolite molecular weight (g/mol)	Parent molecular weight (g/mol)	Metabolite formulation fraction	Coefficient [a)]	Metabolite relative concentration (g/L) [b)]
Prothioconazole-desthio	312.2	344.3	0.571	0.518	25.88

a) Coefficient = (metabolite molecular weight / parent molecular weight) * metabolite formulation fraction
b) Metabolite relative concentration = coefficient * parent formulation concentration (i.e. 50 g/L)

Acute mixture toxicity

For the assessment of acute effects (mortality), a surrogate LD_{50} can be calculated. The EFSA Guidance Document indicates that the following equation should be used for deriving a surrogate LD_{50} for a mixture of active substances with known toxicity assuming dose additivity:

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

where:
$X\,(a.s._i)$ = fraction of active substance (i) in the formulation mixture
$LD_{50}\,(a.s._i)$ = acute toxicity for the active substance (i)

The LD_{50} of the mix is summarised in the table below.

Table 9.3-8: Acute LD_{50} for the mixture of active substances and relevant metabolites

Test substance	Concentration of active substance in the formulation mixture (g/L)	Fraction of active substance in the formulation mixture, X(a.s.) [a)]	Acute toxicity endpoint (mg/kg bw/d)	Fraction of active substance/LD_{50} for the active substance	LD_{50} mix (mg/kg bw)
Prothioconazole	24.11*	0.036	6200	0.001006	**20252.49**
Prothioconazole-desthio	25.89	0.038	2235	0.001017	
Sulphur	625.00	0.926	35000	0.001026	
Total	-	1	-	0.000049	

*Parent concentration (50 g/L) – metabolite relative concentration (25.89 g/L)
a) Concentration of an active substance in the formulation, divided by, the total concentration of all active substances in the formulation.

To achieve a basis for a comparison of single active substance and mixture toxicity in terms of potential risk, a "tox per fraction" is calculated for each active substance and compared to the corresponding quotient for the mixture using the following equation, according to the EFSA guidance:

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

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

Table 9.3-9: "Tox per fraction" quotient for acute toxicity

Active substance or metabolite	Fraction of active substance in the formulation mixture, X(a.s.) [a)]	Acute toxicity endpoint (mg/kg bw/d)	Tox per fraction (a.s)	Tox per fraction of the formulation mixture	Tox fraction (mix)/tox fraction (as)	Deviation (%) [b)]
Prothioconazole	0.04	6200	173566.53	20252.49	0.12	11.67
Prothioconazole-desthio	0.04	2235	58274.60		0.35	34.75
Sulphur	0.93	35000	37800.00		0.54	53.58

a) Concentration of an active substance in the formulation, divided by, the total concentration of all active substances in the formulation.
b) Deviation % = Tox per fraction (mix) / Tox per fraction (a.s.) × 100

As neither the two active substances nor the metabolite contributes > 90 % to the mixture toxicity, a combined assessment is necessary.

zRMS comment:

zRMS agrees with LD_{50} mix of 20252.49 mg/kg bw. As neither the two active substances nor the metabolite contributes > 90 % to the mixture toxicity, a combined acute risk assessment is necessary.

Table 9.3-10: Screening and first-tier assessment of the acute combined risk for mammals due to the use of FHO04 in cereals (use 1)

Intended use	Cereals
Product	FHO04
Application rate (g/ha)	2 x 2900 (14d) (200 g/ha prothioconazole + 200 g/ha prothioconazole-desthio + 2500 g/ha sulphur)

Acute toxicity (mg/kg bw)	20252.49				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Screening					
Cereals	Small herbivorous mammal	118.4	1.2	412.03	49.15
Tier 1					
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
BBCH ≥ 20	Small insectivorous mammal "shrew"	1.9	1.2	6.61	3062.99
BBCH ≥ 40	Small herbivorous mammal "vole"	21.7	1.2	75.52	268.20
BBCH 30-39	Small omnivorous mammal "mouse"	3.9	1.2	13.57	1492.37
BBCH ≥ 40	Small omnivorous mammal "mouse"	2.3	1.2	8.00	2530.67

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

The acute TER values were greater than the trigger value of 10 at both screening and tier-1 level, showing a safe use can be concluded using the formulation Patton Supra.

Reproductive mixture toxicity

Table 9.3-11: Chronic LD_{50} for the mixture of active substances and relevant metabolites

Test substance	Concentration of active substance in the formulation mixture (g/L)	Fraction of active substance in the formulation mixture, X(a.s.) [a)]	Chronic toxicity endpoint (mg/kg bw/d)	Fraction of active substance/LD_{50} for the active substance	LD_{50} mix (mg/kg bw)
Prothioconazole	24.11*	0.04	96	0.000374	223.54
Prothioconazole-desthio	25.89	0.04	10	0.003835	
Sulphur	625.00	0.93	3500	0.000265	
Total	-	1	-	0.004473	

* Parent concentration (50 g/L) – metabolite relative concentration (25.89 g/L)
a) Concentration of an active substance in the formulation, divided by, the total concentration of all active substances in the formulation.

Table 9.3-12: "Tox per fraction" quotient for active substances for chronic toxicity

Active substance or metabolite	Fraction of active substance in the formulation mixture, X(a.s.) [a)]	Chronic toxicity endpoint (mg/kg bw/d)	Tox per fraction (a.s)	Tox per fraction of the formulation mixture	Tox fraction (mix)/tox fraction (as)	Deviation (%) [b)]
Prothioconazole	0.04	95.6	2676.28	223.54	0.08	8.35
Prothioconazole-desthio	0.04	10	260.74		0.86	85.73
Sulphur	0.93	3500	3780.00		0.059	5.91

a) Concentration of an active substance in the formulation, divided by, the total concentration of all active substances in the formulation.
b) Deviation % = Tox per fraction (mix) / Tox per fraction (a.s.) * 100

As neither the two active substances nor the metabolite contributes > 90 % to the mixture toxicity, a combined chronic assessment is necessary.

~~**Table 9.3-13: Screening assessment of the reproductive combined risk for mammals due to the use of FHO04 in cereals (use 1)**~~

~~**Product**~~	~~**FHO04**~~						
~~**Application rate (g/ha)**~~	~~2 x 2900 (14 d)~~ ~~(200 g/ha prothioconazole + 200 g/ha prothioconazole-desthio + 2500 g/ha sulphur)~~						
~~**TER criterion**~~	~~5~~						
~~**Crop scenario**~~	~~**TER**~~			~~**RQ**~~			~~**RQ Sum**~~
	~~**Prothioconazole**~~	~~**Prothioconazole-desthio**~~	~~**Sulphur**~~	~~**Prothioconazole**~~	~~**Prothioconazole-desthio**~~	~~**Sulphur**~~	
~~**Screening**~~							
~~Cereals~~	~~13.34~~	~~1.40~~	~~39.06~~	~~0.37~~	~~0.27~~	~~0.01~~	~~0.65~~

~~The RQsum for mixture toxicity to prothioconazole, prothioconazole-desthio and sulphur are below the trigger value of 1 at the screening assessment. This shows acceptable risk to mammals from the combined active substances and metabolite.~~

zRMS comments:

Combined acute risk assessment:

Based on performed calculations in the Table 9.3-10, acceptable combined acute risk to mammals exposed to the mixture of both active compounds and metabolite JAU 6476-desthio may be concluded.

Combined long-term risk assessment

Simplified approach (TER_{mix}) and respective calculations based on the lowest TER_{LT} values is presented below.

						Σ1/TER	**$\Sigma 1/TER^{-1}$**	**Trigger**
Prothioconazole		**JAU 6476-desthio**		**Sulphur**				
13.34 [1)]	0.075	1.40[1)]	0.71	39.06 [1)]	0.025	0.81	**1.21**	5
13.34 [1)]	0.075	13.36 [2)]	0.074	39.06 [1)]	0.025	0.17	5.88	5

[1)] TER_{LT} at screening step
[2)] TER_{LT} based on the refined parameters

Based on performed zRMS's calculations, an unacceptable combined long-term risk to mammals exposed to the mixture of both active compounds and metabolite JAU 6476-desthio is below trigger of 5 with consideration the screening TER_{LT} values. Therefore, for JAU6476 -desthio refined TER_{LT} value was considered for TERmix calculations and trigger of 5 was achieved, indicating an acceptable combined long-term risk assessment.

9.3.2.4 Drinking water exposure

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

Puddle scenario

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

With a geometric K(f)oc of 1765 mL/g for prothioconazole and 3615.3 mL/g for sulphur, both active substances belong to the group of more sorptive substances. Prothioconazole-desthio has a geometric K(f)oc

of 574 mL/g so also belongs to the group of more sorptive substances.

Table 9.3-14: Assessment of the risk for birds due to exposure via contaminated drinking water in puddles: consideration of ratio between application rate and toxicity endpoint

Prothioconazole			
Effective application rate (g/ha)	400 (200*2)		
Acute toxicity (mg/kg bw)	6200.00	quotient	0.06
Reprod. toxicity (mg/kg bw/d)	95.60	quotient	4.18
Prothioconazole-desthio (M04)			
Effective application rate (g/ha)	400 (200*2)		
Acute toxicity (mg/kg bw)	2235.00	quotient	0.18
Reprod. toxicity (mg/kg bw/d)	10.00	quotient	40.00
Sulphur			
Effective application rate (g/ha)	5000 (2500*2)		
Acute toxicity (mg/kg bw)	35000	quotient	0.14
Reprod. toxicity (mg/kg bw/d)	3500	quotient	1.43

All acute and chronic ratios are below the threshold of 50 for all substances, demonstrating that the risk to mammals from contaminated drinking water is acceptable following the proposed use of Patton Supra.

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.

9.3.2.5 Effects of secondary poisoning

The log P_{ow} of prothioconazole amounts to 4.16 and thus exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is required. Two prothioconazole metabolites also have log Pow values higher than the trigger value of 3 (prothioconazole-desthio 3.04 and prothioconazole-methyl 4.19).

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.

Table 9.3-15: Assessment of the risk for earthworm-eating mammals due to exposure to prothioconazole via bioaccumulation in earthworms (secondary poisoning) for the intended use in cereals (use 1)

Parameter	Prothioconazole	comments
PEC_{soil} (mg/kg soil)	0.22	PECmax (dRR Part B8, Section 8.7, Table 8.7-5)
log P_{ow} / P_{ow}	3.82/6607 4.16 / 14454	EFSA (2007)
Koc	1765	EFSA (2007)
Foc	0.02	Default

Parameter	Prothioconazole	comments
BCF_{worm}	2.72 ~~4.937~~	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.12 \times P_{ow}) / foc \times Koc$
PEC_{worm}	0.60 ~~1.086~~	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.77 ~~1.390~~	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	95.6	
TER_{lt}	124.2 ~~69~~	

TER values shown in bold fall below the relevant trigger.

Table 9.3-16: Assessment of the risk for earthworm-eating mammals due to exposure to prothioconazole-desthio via bioaccumulation in earthworms (secondary poisoning) for the intended use in cereals (use 1)

Parameter	Prothioconazole-desthio	comments
PEC_{soil} (mg/kg soil)	0.221	PECmax (dRR Part B8, Section 8.7, Table 8.7-5)
log P_{ow} / P_{ow}	3.04 / 1096	EFSA (2007)
Koc	575.40	Arithmetic mean (n=4) (EFSA (2007))
Foc	0.02	Default
BCF_{worm}	1.216	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.12 \times P_{ow}) / foc \times Koc$
PEC_{worm}	0.269	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.344	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	10	
TER_{lt}	29	

TER values shown in bold fall below the relevant trigger.

Table 9.3-17: Assessment of the risk for earthworm-eating mammals due to exposure to prothioconazole-methyl via bioaccumulation in earthworms (secondary poisoning) for the intended use in cereals (use 1)

Parameter	Prothioconazole-methyl	comments
PEC_{soil} (mg/kg soil)	0.065	PECmax (dRR Part B8, Section 8.7, Table 8.7-5)
log P_{ow} / P_{ow}	4.19 / 15488	EFSA (2007)
Koc	2556.30	Arithmetic mean (n=4) (EFSA (2007))
Foc	0.02	Default
BCF_{worm}	3.696	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.12 \times P_{ow}) / foc \times Koc$
PEC_{worm}	0.2402	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.3075	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	9.56	
TER_{lt}	31	

TER values shown in bold fall below the relevant trigger.

Resulting TER are above the trigger value of 5 hence no further refinements are required.

Risk assessment for fish-eating mammals via secondary poisoning

According to EFSA/2009/1438, the risk for piscivorous mammals is assessed for a mammal of 3000 g body

weight with a daily food consumption of 425 g. Bioaccumulation in fish is estimated based on predicted concentrations in surface water / is based on the regulatory acceptable concentration for aquatic organisms as a limit value for admissible concentrations of prothioconazole and prothioconazole-desthio in water. Due to the low predicted environmental concentrations of JAU 6476-S-methyl in surface water no fish-BCF-study was conducted even though this metabolite has a log Pow of 4.19. The BCF for this metabolite can be estimated from its log Pow and the BCF and the log Pow of the parent compound with a sufficient degree of precision. The chemical structure of JAU 6476-S-methyl is very similar to the one of parent compound prothioconazole since the only modification of the parent molecule during the formation of JAU 6476-S-methyl is the methylation of the sulphur atom. This methylation results in an increase of the Pow of JAU 6476-S-methyl that is 20000 (log Pow = 4.30) in comparison to 3981 (log Pow = 3.60) of prothioconazole. Therefore, it can be anticipated that the BCF for S-methyl is not more than 5.02 times higher (20000/3981 = 5.02) than the BCF_{parent} (whole fish, 38ummarized to 6% fat) for prothioconazole that has been determined to be 18.8. An estimated BCF for JAU 6476-S-methyl of 94.4 (18.8 x 5.02 = 94.4) is also in accordance with the results of the fish BCF study that has been conducted for the parent compound. In this study, JAU 6476-S-methyl was detected as a minor metabolite in edibles and viscera of fish. No significant increase of the level and the portion of the total radioactive residue (TRR) was observed for the metabolite JAU 6476-S-methyl between day 7 and day 14. Therefore, it can be concluded that formation and degradation/depuration of this metabolite, which had been formed in the fish, were in balance. If the metabolite would have a much higher bioaccumulation potential than the parent compound, a further increase of its level and portion of the TRR (total radioactive residue) would have to be expected from day 7 to day 14 in the BCF study. Hence, a BCF of 94.4 seems to be a realistic estimate for the BCF of JAU 6476-S-methyl in fish.

As worst-case the BCF estimate (94.4) has been used for risk assessment of prothioconazole-methyl.

Table 9.3-18: Assessment of the risk for fish-eating mammals due to exposure to prothioconazole via bioaccumulation in fish (secondary poisoning) for the intended use in cereals (use 1)

Parameter	Prothioconazole	comments
PEC_{sw} (mg/L)	0.043	PECsw max (Step 1)
BCF_{fish}	19.70	EFSA (2007)
BMF	-	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	0.847~~56~~	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.12~~2~~	$DDD = PEC_{fish} \times 0.142$
NOEL (mg/kg bw/d)	95.6	
TER_{lt}	787	

TER values shown in bold fall below the relevant trigger.

Table 9.3-19: Assessment of the risk for fish-eating mammals due to exposure to prothioconazole-desthio via bioaccumulation in fish (secondary poisoning) for the intended use in cereals (use 1)

Parameter	Prothioconazole-desthio	comments
PEC_{sw} (mg/L)	0.082	PECsw max (Step 1)
BCF_{fish}	65	
BMF	-	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	5.3	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.753	$DDD = PEC_{fish} \times 0.142$
NOEL (mg/kg bw/d)	10	
TER_{lt}	13	

TER values shown in bold fall below the relevant trigger.

Table 9.3-20: Assessment of the risk for fish-eating mammals due to exposure to prothioconazole methyl via bioaccumulation in fish (secondary poisoning) for the intended uses in cereals

Parameter	Prothioconazole-methyl	Comments
PEC_{sw} (mg/L)	0.009 ~~6~~	PECsw max (Step 1)
BCF_{fish}	94.4	BFC estimate (see above)
BMF	-	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	0.85 ~~0.434~~	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.12 ~~0.062~~	$DDD = PEC_{fish} \times 0.142$
NOEL (mg/kg bw/d)	9.6	Parent / 10
TER_{lt}	80 ~~155~~	

TER values shown in bold fall below the relevant trigger.

All long-term TER values were above the relevant trigger of 5, demonstrating that the risk to mammals from exposure to prothioconazole and its metabolites via bioaccumulation are acceptable following the proposed use of Patton Supra.

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.

Consideration of the maximum soil and surface water exposure is agreed by the zRMS as it represents worst case comparing to 21-d TWA concentrations. Some additional explanations were added in tables above.
Despite all corrections of the zRMS, acceptable risk of secondary exposure from all relevant compounds could be concluded for mammals.

9.3.2.6 Biomagnification in terrestrial food chains

Not relevant.

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

Not relevant.

9.3.4 Overall conclusions

Acute and chronic risk to mammals following the proposed use of Patton Supra were performed in accordance with EFSA (2009) guidelines. The acute risks for prothioconazole, prothioconazole-desthio and sulphur, and the long-term risks for prothioconazole and sulphur were acceptable at the screening assessment, indicating a low risk to mammals. However, the long-term risks for the metabolite prothioconazole-desthio did not pass the screening and first tier assessments. As such, additional refinement options were required to show an acceptable level of risk for the vole scenario. Three refinement options were presented, which all resulted in a safe use of Patton Supra to voles.

The risk to the mixture was calculated using the Finney's equation for acute toxicity and the risk quotient approach for chronic toxicity. The acute and chronic risks to the mixture (including metabolite prothioconazole-desthio) was acceptable at the screening level.

The risk from exposure to contaminated drinking water and secondary poisoning was also considered acceptable following the proposed use of Patton Supra.

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

Not relevant.

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 the active substances prothioconazole and sulphur, and prothioconazole relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

In addition, new studies for sulphur were provided and assessed by RMS as confirmatory data in an Addendum to the DAR (April 2012):

- A new sediment-water toxicity study on chronic effects of sulphur (applied as sulphur dust, using spiked sediment) on the sediment-dwelling midge *Chironomus riparius*.
- A new sediment-water toxicity study on *Chironomus riparius* investigating the effects of sodium sulphate added to the water column was provided as supportive information also in the context of the evaluation of the confirmatory data.

Effects on aquatic organisms of Patton Supra were not evaluated as part of the EU assessment of active substance 1. New data submitted with this application are listed in Appendix 1 and 40ummarized in Appendix 2.

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

Species	Substance	Exposure System	Results	Reference
Fish				
Oncorhynchus mykiss	Prothioconazole	Acute	**LC_{50} = 1.83 mg a.s./L $_{mm}$**	EFSA Conclusion 2007
Lepomis macrochirus			LC_{50} = 4.59 mg a.s./L $_{mm}$	
Cyprinus carpio			LC_{50} = 6.91 mg a.s./L $_{mm}$	
Cyprinodon variegatus			LC_{50} > 10.3 mg a.s./L $_{mm}$	
Oncorhynchus mykiss		Chronic	**NOEC = 0.308 mg a.s./L $_{mm}$**	
Oncorhynchus mykiss	Prothioconazole-desthio	Acute	**LC_{50} = 6.63 mg p.m./L $_{mm}$**	
Leuciscus idus melanotus			LC_{50} = 13.20 mg p.m./L $_{mm}$	

Species	Substance	Exposure System	Results	Reference
Oncorhynchus mykiss		Chronic	**NOEC = 3.34 µg p.m./L** mm	
Oncorhynchus mykiss	Prothioconazole methyl	Acute	**LC_{50} = 1.80 mg p.m./L** mm	
Oncorhynchus mykiss	1,2,4-triazole	Acute	**LC_{50} = 498 mg p.m./L** mm	
		Chronic	**NOErC = 3.2 mg p.m./L** mm	
Oncorhynchus mykiss	Sulfur 80% WG	Acute 96h Static	**LC_{50} > 0.063 mg a.s./L** solubility limit	EFSA 2008, EFSA Confirmatory data 2012
Oncorhynchus mykiss	Sulfur 80% WG	28 d flow-through	**LC_{50} > 0.063 mg a.s./L** solubility limit	EFSA 2008, EFSA Confirmatory data 2012
Aquatic invertebrates				
Daphnia magna	Prothioconazole	Acute	**EC_{50} = 1.30 mg a.s./L** mm	EFSA Conclusion 2007
		Chronic	**NOEC = 0.56 mg a.s./L** mm	
	Prothioconazole-desthio	Acute	**EC_{50} > 10 mg p.m./L** mm	
		Chronic	**NOEC = 0.10 mg p.m./L** mm	
	Prothioconazole methyl	Acute	**EC_{50} = 2.8 mg p.m./L** mm	
	1,2,4-triazole	Acute	**EC_{50} = 900 mg p.m./L** mm	
	Sulphur 80% WG	Acute 48h Static	**EC_{50} > 0.063 mg a.s./L** (solubility limit)	EFSA 2008, EFSA Confirmatory data 2012
Sediment organisms				
Chironomus riparius	Prothioconazole	Chronic	**NOEC = 9.14 mg a.s./L** nom	EFSA Conclusion 2007
	Prothioconazole-desthio	Chronic	**NOEC = 2.0 mg p.m./L** mm	
	Sulphur Dust	Chronic 28 d Static Sediment spiked	**NOEC = 608 mg a.s./L** nom 949 mg/kg nom	EFSA Confirmatory data 2012
	Sodium sulfate	Chronic 28 d Static Water spiked	NOEC = 100 mg/L nom	EFSA Confirmatory data 2012
Freshwater algae				
Pseudokirchneriella subcapitata	Prothioconazole	Sub-chronic	**E_rC_{50} = 2.18 mg a.s./L** mm E_bC_{50} = 1.10 mg a.s./L mm	EFSA Conclusion 2007
Scenedesmus subspicatus	Prothioconazole-desthio	Sub-chronic	**E_rC_{50} = 0.55 mg p.m./L** mm E_bC_{50} = 0.073	

Species	Substance	Exposure System	Results	Reference
			mg p.m./L $_{mm}$	
Pseudokirchneriella subcapitata	Prothioconazole methyl	Sub-chronic	**E_rC_{50} = 47.4 mg p.m./L $_{mm}$** E_bC_{50} = 3.77 mg p.m./L $_{mm}$	
Pseudokirchneriella subcapitata	1,2,4-triazole	Sub-chronic	**E_rC_{50} = 22.5 mg p.m./L $_{mm}$** E_bC_{50} = 8.2 mg p.m./L $_{mm}$	
Scenedesmus subspicatus	Sulphur dust	Acute 72h Static	E_rC_{50} and E_bC_{50} = **0.002 mg a.s./L $_{mm}$**	EFSA 2008, EFSA Confirmatory data 2012
Scenedesmus subspicatus	Sulphur 80% WG	Acute 72h Static	E_rC_{50} and E_bC_{50} >0.063 mg a.s./L (solubility limit)	

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

zRMS comments:

Endpoints presented in Table 9.5-1 are in line with the EU agreed endpoints reported in EFSA Scientific Report (2008) 221, 1-70, Confirmatory data 2012 and EFSA Scientific Report (2007) 106, respectively.

Table 9.5-2: Endpoints and effect values relevant for the risk assessment for aquatic organisms – FHO04

Species	Substance	Exposure System	Results	Reference
Daphnia magna	FHO04	48 h, s	EC_{50} = **37.584** mg/L $_{nom}$	P. D. Singh, 2023a, Report No. 502-3-07-29112 KCP 10.2.1/01
Pseudokirchneriella subcapitata	FHO04	72 h, s	E_rC_{50} = **26.363** mg/L $_{nom}$ E_yC_{50} = 8.395 mg/L $_{nom}$ E_bC_{50} = 8.395 mg/L $_{nom}$	P. D. Singh, 2023b, Report No. 501-3-07-29111 KCP 10.2.1/02

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

zRMS comments:

Studies on toxicity of FH004 to aquatic organisms were evaluated and agreed by the zRMS.
For details of the evaluation please refer to Appendix 2.

Mixture toxicity

According to EFSA Scientific Report (2008) 221, active substances with a very low water solubility limit can be considered in general as having a non-significant risk to aquatic organisms. This is relevant for the active substance sulphur, with a very low water solubility (0.063 mg a.s./L). Furthermore, no effects were observed at concentrations that exceeded the water solubility, by several orders of magnitude (table 9.5-1). Therefore, it was not considered necessary to assess the risk for aquatic organisms by calculating Toxicity Exposure Ratios (TER) as sulphur can be considered of no concern for aquatic organisms.

For this reason, a mixture toxicity considering the theoretical toxicity to each aquatic group assuming concentration addition, according to the EFSA guidance document, is not deemed necessary. As a result, the mixture toxicity is covered by the individual assessment of the active substance prothioconazole and its metabolite prothioconazole-desthio, as well the risk assessment with the formulation measured toxicity.

zRMS comments:

zRMS agrees that a mixture toxicity considering the theoretical toxicity to each aquatic group assuming concentration addition, according to the EFSA guidance document is not necessarily due to that the active substance sulphur has a very low water solubility (0.063 mg a.s./L).
It should be also taken into consideration that no effects were observed at concentrations that exceeded the water solubility, by several orders of magnitude (table 9.5-1). Therefore, it was not considered necessary to assess the risk for aquatic organisms by calculating RAC as sulphur can be considered of no concern for aquatic organisms.

9.5.1.1 Justification for new endpoints

In addition to the active substances and metabolite toxicity data, new endpoints are provided for acute toxicity of the formulated product Patton Supra to aquatic invertebrates and algae.

According to Regulation (EC) No. 284/2013, also acute formulation effects on fish should be presented if "*the acute toxicity of the plant protection product cannot be predicted on the basis of the data for the active substance*". In case of Patton Supra, the active substance sulphur has a very low water solubility (0.063 mg a.s./L) and no effects were observed at concentrations which exceeded the water solubility by several orders of magnitude. Thus. this active substance is considered to have a non-significant risk to aquatic organisms according to EFSA Scientific Report (2008)-221 and the formulation fish acute risk assessment is covered by the active substance prothioconazole. From the sulphur endpoints for acute aquatic toxicity, it appears algae is the most sensitive group (EC_{50} 0.02 mg a.s./L, *P. subcapitata*). Likewise, for prothioconazole, the acute toxicity endpoints show algae is the most sensitive group (EC_{50} 1.1 mg a.s./L, *P. subcapitata*).

Furthermore, the available data on the toxicity of the formulation product towards aquatic invertebrates and algae (Table 9.5-2) shows that the formulation is clearly less sensitive (factor of 10) than the active substance prothioconazole and its relevant metabolite prothioconazole-desthio. The same trend would be expected for the toxicity of the formulation product towards fish.

Finally, Article 62 of Regulation (EC) No 1107/2009 of 21 October 2009 directs that testing on vertebrate animals for the purpose of the regulation must only be carried out where no other rationale or methods are available. Based on this information, testing the formulated product on fish was not considered necessary.

9.5.2 Risk assessment

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

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

Relevant scenarios for Poland at Step 3-4 are D3, D4 and R1, calculation for the other scenarios are reported but greyed out.

Table 9.5-3: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prothioconazole for each organism group based on FOCUS Steps 1, 2 and 3 calculations for applications of 2 x 200 g a.s./ha in spring cereals

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**		**Sediment dwelling inv.**
Test species		*Oncorhynchus mykiss*	*Oncorhynchus mykiss*	*Daphnia magna*	*Daphnia magna*	*Pseudokirchneriella subcapitata*		*Chironomus riparius*
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	ErC_{50}		NOEC
(µg/L)		1830	308	1300	560	2180		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)**	**PEC/RAC**					**PEC $_{sed}$ [µg/kg]**	**PEC/RAC**
Step 1	43.44	**2.374**	**1.410**	**3.342**	0.776	0.199	708.61	0.775
Step 2								
N-Europe	2.12	0.116	0.069	0.163	0.038	0.010	31.07	0.034
S-Europe	2.12	0.116	0.069	0.163	0.038	0.010	27.65	0.030
Step 3								
~~D1 ditch~~	~~1.693~~	~~0.093~~	~~0.055~~	~~0.130~~	~~0.030~~	~~0.008~~	~~6.722~~	~~0.007~~
~~D1 stream~~	~~0.968~~	~~0.053~~	~~0.031~~	~~0.074~~	~~0.017~~	~~0.004~~	~~0.716~~	~~0.001~~
D3 ditch	1.271**/1.11	0.069/0.061	0.041/0.036	0.098/0.085	0.022/0.020	0.006/0.005	1.181	/0.001
D4 pond	0.063	0.003	0.002	0.005	0.001	<0.001	0.408	<0.001
D4 stream	1.096**/0.946	0.06/0.052	0.035/0.031	0.084/0.073	0.019/0.017	0.0050/0.004	0.254	/<0.001
D5 pond	0.062	0.003	0.002	0.005	0.001	<0.001	0.424	<0.001
D5 stream	1.183**/1.02	0.064/0.056	0.038/0.033	0.091/0.078	0.02/0.018	0.005/0.005	0.348	/<0.001
R4 stream	0.838**/0.723	0.045/0.040	0.027/0.023	0.064/0.056	0.015/0.013	0.0038/0.003	1.159	/0.001

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

*** Max PECsw between early (BBCH 27) and late (BBCH 69) applications, ** single application**

Table 9.5-4: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prothioconazole for each organism group based on FOCUS Steps 1, 2 and 3 calculations for for applications of 2 x 200 g a.s./ha in winter cereals

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**		**Sediment dwelling inv.**
Test species		*Oncorhynchus mykiss*	*Oncorhynchus mykiss*	*Daphnia magna*	*Daphnia magna*	*Pseudokirchneriella subcapitata*		*Chironomus riparius*
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	ErC_{50}		NOEC
(µg/L)		1830	308	1300	560	2180		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)**	**PEC/RAC**					**PEC $_{sed}$ [µg/kg]**	**PEC/RAC**
Step 1	43.44	**2.374**	**1.410**	**3.342**	0.776	0.199	708.61	0.775
Step 2								
N-Europe	2.12	0.116	0.069	0.163	0.038	0.010	31.07	0.034
S-Europe	2.12	0.116	0.069	0.163	0.038	0.010	27.65	0.030
Step 3								
~~D1 Ditch~~	~~1.704~~	~~0.093~~	~~0.055~~	~~0.131~~	~~0.030~~	~~0.008~~	~~6.861~~	~~0.008~~
~~D1 Stream~~	~~0.968~~	~~0.053~~	~~0.031~~	~~0.074~~	~~0.017~~	~~0.004~~	~~0.717~~	~~0.001~~
~~D2 Ditch~~	~~1.683~~	~~0.092~~	~~0.055~~	~~0.129~~	~~0.030~~	~~0.008~~	~~7.333~~	~~0.008~~
~~D2 Stream~~	~~1.463~~	~~0.080~~	~~0.048~~	~~0.113~~	~~0.026~~	~~0.007~~	~~6.131~~	~~0.007~~
D3 Ditch	1.273*/*1.114	0.069/0.061	0.041/0.036	0.09/0.086	0.022/0.020	0.005/0.005	1.477	0.002
D4 Pond	0.063	0.003	0.002	0.005	0.001	<0.001	0.421	<0.001
D4 Stream	1.096**/0.946	0.059/0.052	0.035/0.031	0.080/0.073	0.019/0.017	0.005/0.004	0.254	<0.001
D5 Pond	0.062	0.003	0.002	0.005	0.001	<0.001	0.431	<0.001
D5 Stream	1.183**/1.02	0.064/0.056	0.038/0.033	0.091/0.078	0.021/0.018	0.005/0.005	0.348	<0.001
~~D6 Ditch~~	~~1.202~~	~~0.066~~	~~0.039~~	~~0.092~~	~~0.021~~	~~0.006~~	~~3.575~~	~~0.004~~
R1 Pond	0.082	0.004	0.003	0.006	0.001	<0.001	0.56	0.001
R1 Stream	0.839**/0.723	0.045/0.040	0.027/0.023	0.064/0.056	0.015/0.013	0.0038//0.003	1.619	0.002
R3 Stream	1.182**/1.02	0.064/0.056	0.038/0.033	0.090/0.078	0.021/0.018	0.005/0.005	0.99	0.001
R4 Stream	0.838**/0.723	0.045/0.04	0.027/0.02	0.064/0.06	0.015/0.01	0.038/0.00	0.144	<0.001

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

*** Max PECsw between early (BBCH 27) and late (BBCH 69) applications,** single application**

Table 9.5-5: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prothioconazole metabolite prothioconazole-desthio (M04) for each organism group based on FOCUS Steps 1, 2 and 3 calculations for applications of 2 x 200 g a.s./ha in spring cereals (early application, BBCH 27)

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**		**Sediment dwelling inv.**
Test species		*Oncorhynchus mykiss*	*Oncorhynchus mykiss*	*Daphnia magna*	*Daphnia magna*	*Scenedesmus subspicatus*		*Chironomus riparius*
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)**	**PEC/RAC**					**PEC $_{sed}$ [µg/kg]**	**PEC/RAC**
Step 1	81.540	**1.230**	**244.132**	0.815	**8.154**	**1.483**	463.930	**2.320**
Step 2								
N-Europe	16.430	0.248	**49.192**	0.164	**1.643**	0.299	93.520	0.468
S-Europe	13.370	0.202	**40.030**	0.134	**1.337**	0.243	75.950	0.380
Step 3								
~~D1 ditch~~	~~0.033~~	~~<0.001~~	~~0.099~~	~~<0.001~~	~~0.003~~	~~0.001~~	~~1.012~~	~~0.005~~
~~D1 stream~~	~~0.021~~	~~<0.001~~	~~0.063~~	~~<0.001~~	~~0.002~~	~~<0.001~~	~~0.279~~	~~0.001~~
D3 ditch	0.069 ~~<0.001~~	0.0009 ~~<0.001~~	0.206 ~~0.003~~	0.00069 ~~<0.001~~	0.0069 ~~<0.001~~	0.00125 ~~<0.001~~	0.073 ~~0.043~~	0.00037 ~~<0.001~~
D4 pond	0.015 ~~0.004~~	0.00022 ~~<0.001~~	0.0449 ~~0.012~~	0.00015 ~~<0.001~~	0.0015 ~~<0.001~~	0.00027 ~~<0.001~~	0.125 ~~0.110~~	0.00062 ~~0.001~~
D4 stream	0.041 ~~0.011~~	0.00061 ~~<0.001~~	0.122 ~~0.033~~	0.41 ~~<0.001~~	0.0041 ~~0.001~~	0.00074 ~~<0.001~~	0.013	<0.001
D5 pond	0.015 ~~0.002~~	0.00022 ~~<0.001~~	0.045 ~~0.006~~	0.00015 ~~<0.001~~	0.0015 ~~<0.001~~	0.00027 ~~<0.001~~	0.130	0.001
D5 stream	0.044 ~~0.001~~	0.00066 ~~<0.001~~	0.13 ~~0.003~~	0.00044 ~~<0.001~~	0.0044 ~~<0.001~~	0.0008 ~~<0.001~~	0.002	<0.001
R4 stream	1.061 ~~0.943~~	0.016 ~~0.014~~	**3.17** ~~**2.823**~~	0.0106 ~~0.009~~	0.106 ~~0.094~~	0.019 ~~0.017~~	1.249 ~~1.182~~	0.0062 ~~0.006~~

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

Table 9.5-6: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prothioconazole metabolite prothioconazole-desthio (M04) for each organism group based on FOCUS Steps 1, 2 and 3 calculations for applications of 2 x 200 g a.s./ha in winter cereals (early application, BBCH 27)

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**		**Sediment dwelling inv.**
Test species		*Oncorhynchus mykiss*	*Oncorhynchus mykiss*	*Daphnia magna*	*Daphnia magna*	*Scenedesmus subspicatus*		*Chironomus riparius*
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)**	**PEC/RAC**					**PEC $_{sed}$ [µg/kg]**	
Step 1	81.540	**1.230**	**244.132**	0.815	**8.154**	**1.483**	463.930	**2.320**
Step 2								
N-Europe	16.430	0.248	**49.192**	0.164	**1.643**	0.299	93.520	0.468
S-Europe	13.370	0.202	**40.030**	0.134	**1.337**	0.243	75.950	0.380
Step 3								
D1 Ditch	0.014	<0.001	0.042	<0.001	0.001	<0.001	0.310	0.002
D1 Stream	0.009	<0.001	0.027	<0.001	0.001	<0.001	0.087	<0.001
D2 Ditch	0.102	0.002	0.305	0.001	0.010	0.002	0.358	0.002
D2 Stream	0.064	0.001	0.192	0.001	0.006	0.001	0.168	0.001
D3 Ditch	0.068 <0.001	0.0010 <0.001	0.20 0.003	0.00068 <0.001	0.0068 <0.001	0.0012 <0.001	0.072 0.041	0.00036 <0.001
D4 Pond	0.012 0.003	0.00018 <0.001	0.035 0.009	0.00012 <0.001	0.0012 <0.001	0.00021 <0.001	0.124 0.112	0.00062 0.001
D4 Stream	0.032 0.008	0.00048 <0.001	0.095 0.024	0.00032 <0.001	0.0032 0.001	0.00058 <0.001	0.008	<0.001
D5 Pond	0.015 0.002	0.00022 <0.001	0.050 0.006	0.00015 <0.001	0.0015 <0.001	0.00027 <0.001	0.132	0.001
D5 Stream	0.046 0.001	0.00069 <0.001	0.137 0.003	0.00046 <0.001	0.0046 <0.001	0.000084 <0.001	0.003	<0.001
D6 Ditch	<0.001	<0.001	0.003	<0.001	<0.001	<0.001	0.057	<0.001
R1 Pond	0.115 0.105	0.0017 0.002	0.34 0.314	0.0011 0.001	0.011 0.011	0.0020 0.002	1.066	0.005
R1 Stream	1.023 0.997	0.015 0.015	**3.06** **2.985**	0.010 0.010	0.10 0.100	0.018 0.018	1.055	0.005
R3 Stream	0.883 0.818	0.013 0.012	**2.64** **2.449**	0.0088 0.008	0.088 0.082	0.0176 0.015	1.075 1.034	0.005 0.005
R4 Stream	1.341	0.020	**4.015**	0.013	0.134	0.024	0.757	0.004

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 prothioconazole metabolite prothioconazole-desthio (M04) for each organism group based on FOCUS Steps 1, 2 and 3 calculations for applications of 2 x 200 g a.s./ha in spring cereals (late application, BBCH 69)

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**		**Sediment dwelling inv.**
Test species		*Oncorhynchus mykiss*	*Oncorhynchus mykiss*	*Daphnia magna*	*Daphnia magna*	*Scenedesmus subspicatus*		*Chironomus riparius*
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)**	**PEC/RAC**					**PEC $_{sed}$ [µg/kg]**	**PEC/RAC**
Step 1	81.540	**1.230**	**244.132**	0.815	**8.154**	**1.483**	463.930	**2.320**
Step 2								
N-Europe	2.980	0.045	**8.922**	0.030	0.298	0.299	16.180	0.081
S-Europe	3.900	0.059	**11.677**	0.039	0.390	0.243	21.450	0.107
Step 3								
~~D1 ditch~~	~~0.017~~	~~<0.001~~	~~0.051~~	~~<0.001~~	~~0.002~~	~~0.001~~	~~0.516~~	~~0.003~~
~~D1 stream~~	~~<0.001~~	~~<0.001~~	~~0.003~~	~~<0.001~~	~~<0.001~~	~~<0.001~~	~~0.022~~	~~<0.001~~
D3 ditch	0.116 ~~<0.001~~	0.0018 ~~<0.001~~	0.347 ~~0.003~~	0.0011 ~~<0.001~~	0.011 ~~<0.001~~	0.002 ~~<0.001~~	0.160 ~~0.062~~	<0.001
D4 pond	0.013	<0.001	0.039	<0.001	0.001	<0.001	0.166	0.001
D4 stream	0.053 ~~0.050~~	0.0008 ~~0.001~~	0.150	<0.001	0.005	<0.001	0.056	<0.001
D5 pond	0.016 ~~0.002~~	0.00024 ~~<0.001~~	0.048 ~~0.006~~	0.00016 ~~<0.001~~	0.0016 ~~<0.001~~	0.00029 ~~<0.001~~	0.129 ~~0.118~~	<0.001
D5 stream	0.067 ~~0.002~~	0.0010 ~~<0.001~~	0.20 ~~0.006~~	0.00067 ~~<0.001~~	0.0067 ~~<0.001~~	0.0012 ~~<0.001~~	0.028 ~~0.014~~	<0.001
R4 stream	0.640 ~~0.202~~	0.0096 ~~0.003~~	1.91 ~~0.605~~	0.0064 ~~0.002~~	0.064 ~~0.020~~	0.011 ~~0.017~~	0.373 ~~0.150~~	0.00185

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 9.5-8: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prothioconazole metabolite prothioconazole-desthio (M04) for each organism group based on FOCUS Steps 1, 2 and 3 calculations for applications of 2 x 200 g a.s./ha in winter cereals (late application, BBCH 69)

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**		**Sediment dwelling inv.**
Test species		*Oncorhynchus mykiss*	*Oncorhynchus mykiss*	*Daphnia magna*	*Daphnia magna*	*Scenedesmus subspicatus*		*Chironomus riparius*
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)**	**PEC/RAC**					**PEC $_{sed}$ [µg/kg]**	
Step 1	81.540	**1.230**	**244.132**	0.815	**8.154**	**1.483**	463.930	**2.320**
Step 2								
N-Europe	2.980	0.045	**8.922**	0.030	0.298	0.299	16.180	0.081
S-Europe	4.810	0.073	**14.401**	0.048	0.481	0.243	26.730	0.134
Step 3								
~~D1 Ditch~~	~~0.022~~	~~<0.001~~	~~0.066~~	~~<0.001~~	~~0.002~~	~~0.001~~	~~0.772~~	~~0.004~~
~~D1 Stream~~	~~0.014~~	~~<0.001~~	~~0.042~~	~~<0.001~~	~~0.001~~	~~<0.001~~	~~0.155~~	~~0.001~~
D2 Ditch	0.463	0.007	**1.386**	0.005	0.046	<0.001	1.239	0.006
D2 Stream	0.299	0.005	0.895	0.003	0.030	<0.001	0.697	0.003
D3 Ditch	0.128 ~~<0.001~~	0.0019 ~~<0.001~~	0.383 ~~0.003~~	0.00128 ~~<0.001~~	0.0128 ~~<0.001~~	0.0023 ~~<0.001~~	0.220 ~~0.066~~	0.0011 ~~<0.001~~
D4 Pond	0.016 ~~0.011~~	0.00024 ~~<0.001~~	0.047 ~~0.033~~	0.00016 ~~<0.001~~	0.0016 ~~0.001~~	0.00029 ~~<0.001~~	0.168 ~~0.154~~	0.00084 ~~0.001~~
D4 Stream	0.053 ~~0.043~~	0.0008 ~~0.001~~	0.158 ~~0.129~~	0.00053 ~~<0.001~~	0.0053 ~~0.004~~	0.00096 ~~<0.001~~	0.047	<0.001
D5 Pond	0.016 ~~0.002~~	0.00024 ~~<0.001~~	0.0479 ~~0.006~~	0.00016 ~~<0.001~~	0.0016 ~~<0.001~~	0.00029 ~~0.017~~	0.129 ~~0.119~~	0.000645 ~~0.001~~
D5 Stream	0.067 ~~0.002~~	0.0010 ~~<0.001~~	0.200 ~~0.006~~	0.00067 ~~<0.001~~	0.0067 ~~<0.001~~	0.0012 ~~<0.001~~	0.028 ~~0.014~~	<0.001
~~D6 Ditch~~	~~0.004~~	~~<0.001~~	~~0.012~~	~~<0.001~~	~~<0.001~~	~~<0.001~~	~~0.310~~	~~0.002~~
R1 Pond	0.130 ~~0.108~~	0.0019 ~~0.002~~	0.39 ~~0.323~~	0.0013 ~~0.001~~	0.013 ~~0.011~~	0.0023 ~~<0.001~~	0.978	0.005
R1 Stream	0.818 ~~0.771~~	0.012 ~~0.012~~	**2.45** ~~**2.308**~~	0.0081 ~~0.008~~	0.081 ~~0.077~~	0.0148 ~~<0.001~~	1.577	0.008
R3 Stream	0.198	0.003	0.593	0.002	0.020	<0.001	0.246	0.001
R4 Stream	0.165	0.002	0.494	0.002	0.017	<0.001	0.124	0.001

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 prothioconazole metabolite prothioconazole methyl (M01) for each organism group based on FOCUS Steps 1 and 2 calculations for applications of 2 x 200 g a.s./ha in spring and winter cereals (BBCH 27-69) (early and late applications)

Group		**Fish acute**	**Inverteb. acute**	**Algae**		**Sediment dwelling inv.**
Test species		*Oncorhynchus mykiss*	*Daphnia magna*	*Pseudokirchneriella subcapitata*		*Assumed parent toxicity/10*
Endpoint		LC_{50}	EC_{50}	ErC_{50}		NOEC
(µg/L)		1790	2800	47400		914
AF		100	100	10		10
RAC (µg/L)		17.9	28	4740		91.4
FOCUS Scenario	**PEC $_{gl\text{-}max*}$ (µg/L)**	**PEC/RAC**			**PEC $_{sed}$ [µg/kg]**	**PEC/RAC**
Spring cereals						
Step 1	9.16 ~~4.60~~	0.51 ~~0.257~~	0.33 0.164	0.00019 ~~0.001~~	221.72 ~~117.47~~	**2.42** ~~**1.285**~~
Step 2						
N-Europe	1.48	0.083	0.053	<0.001	37.88	0.414
S-Europe	1.19	0.066	0.043	<0.001	30.30	0.332
Winter cereals						
Step 1	9.16 ~~4.60~~	0.51 ~~0.257~~	0.33 0.164	0.00019 ~~0.001~~	221.72 ~~117.47~~	**2.42** ~~**1.285**~~
Step 2						
N-Europe	1.48	0.083	0.053	<0.001	37.88	0.414
S-Europe	1.19	0.066	0.043	<0.001	30.30	0.332

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* Max PECsw between early (BBCH27) and late (BBCH 69) applications

Table 9.5-10: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prothioconazole metabolite 1,2,4-triazole (M13) for each organism group based on FOCUS Steps 1 and 2 calculations for applications of 2 x 200 g a.s./ha in spring and winter cereals (BBCH 27-69) (early and late applications)

Group		**Fish acute**	**Inverteb. acute**	**Algae**		**Sediment dwelling inv.**
Test species		*Oncorhynchus mykiss*	*Daphnia magna*	*Pseudokirchneriella subcapitata*		*Assumed parent toxicity/10*
Endpoint		LC_{50}	EC_{50}	ErC_{50}		NOEC
(µg/L)		498000	900000	22500		914
AF		100	100	10		10
RAC (µg/L)		4980	9000	2250		91.4
FOCUS Scenario	**PEC $_{gl\text{-}max*}$ (µg/L)**	**PEC/RAC**			**PEC $_{sed}$ [µg/kg]**	**PEC/RAC**
Spring cereals						
Step 1	10.38 ~~9.65~~	0.002 ~~0.257~~	0.0011 ~~0.345~~	0.0046 ~~0.002~~	8.85 ~~8.56~~	0.096 ~~0.094~~
Step 2						
N-Europe	0.68	0.038	0.024	<0.001	0.60	0.007
S-Europe	0.59	0.033	0.021	<0.001	0.52	0.006
Winter cereals						
Step 1	10.38 ~~7.29~~	0.002 ~~0.407~~	0.0011 ~~0.260~~	0.0046 ~~0.002~~	8.85 ~~8.56~~	0.096 ~~0.094~~
Step 2						
N-Europe	0.68 ~~0.45~~	0.038 ~~0.025~~	0.024 ~~0.016~~	<0.001 ~~<0.001~~	0.60	0.007
S-Europe	0.59 ~~0.39~~	0.033 ~~0.022~~	0.021 ~~0.014~~	<0.001 ~~<0.001~~	0.52	0.006

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* Max PECsw between early (BBCH27) and late (BBCH 69) applications

For the intended uses in cereals, calculated PEC/RAC ratios for the active prothioconazole and its metabolites prothioconazole methyl (M01) and 1,2,4-triazole (M13), did indicate an acceptable risk for the most sensitive group of aquatic organisms in all FOCUS Steps 13 scenarios. Therefore, no further assessment is necessary.

For the intended uses of metabolite prothioconazole-desthio, calculated PEC/RAC ratios did not indicate an acceptable risk for the most sensitive group of aquatic organisms (chronic risk for fish as characterised by a NOEC for *Oncorhynchus mykiss* of 3.34 µg/L in connection with an assessment factor of 10) in FOCUS Steps 3 scenario R1 Stream for winter cereals. Therefore, further PEC/RAC ratios were calculated based on FOCUS Step 4 PEC_{SW} considering reduced exposure of surface water bodies.

Table 9.5-11: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for prothioconazole-desthio (M04) based on FOCUS Step 4 calculations and chronic toxicity data for fish with mitigation of spray drift and run-off for applications of 2 x 200 g a.s./ha in winter cereals (BBCH 27, early application).

Use	Nozzle reduction	Scenario	STEP 4 Max PECsw (µg/L)								
		Vegetative strip (m)	None	5	10	15	20	5	10	15	20
		No spray buffer (m)	None	5	10	15	20	5	10	15	20
Winter cereals BBCH 27	None	D1 Ditch	0.014	0.014	0.014	0.014	0.014	-	-	-	-
	None	D1 Stream	0.009	0.009	0.009	0.009	0.009	-	-	-	-
	None	D2 Ditch	0.102	0.102	0.102	0.102	0.102	-	-	-	-
	None	D2 Stream	0.064	0.064	0.064	0.064	0.064	-	-	-	-
	None	D3 Ditch	<0.001	<0.001	<0.001	<0.001	<0.001	-	-	-	-
	None	D4 Pond	0.003	0.003	0.003	0.002	0.002	-	-	-	-
	None	D4 Stream	0.008	0.008	0.008	0.008	0.008	-	-	-	-
	None	D5 Pond	0.002	0.002	0.001	0.001	0.001	-	-	-	-
	None	D5 Stream	0.001	0.001	0.001	0.001	0.001	-	-	-	-
	None	D6 Ditch	<0.001	<0.001	<0.001	<0.001	<0.001	-	-	-	-
	None	R1 Pond	0.115 ~~0.105~~	0.115 ~~0.105~~	0.115 ~~0.105~~	0.115 ~~0.105~~	0.115 ~~0.105~~	~~0.063~~	0.048 ~~0.042~~	~~0.032~~	0.021
	None	R1 Stream	**1.023** ~~0.997~~	**1.023** ~~0.997~~	**1.023** ~~0.997~~	**1.023** ~~0.997~~	**1.023** ~~0.997~~	~~0.650~~	**0.464** ~~0.453~~	~~0.347~~	0.244 ~~0.237~~
	None	R3 Stream	0.883 ~~0.818~~	0.883 ~~0.818~~	0.883 ~~0.818~~	0.883 ~~0.818~~	0.883 ~~0.818~~	~~0.535~~	**0.403** ~~0.373~~	~~0.287~~	0.212 ~~0.196~~
	None	R4 Stream	**1.341** ~~1.341~~	**1.341** ~~1.341~~	**1.341** ~~1.341~~	**1.341** ~~1.341~~	**1.341** ~~1.341~~	~~0.875~~	**0.610**	~~0.468~~	0.320
RAC (µg/L)	0.334										
PEC/RAC	None	D1 Ditch	0.042	0.042	0.042	0.042	0.0042				

	None	D1 Stream	0.027	0.027	0.027	0.027	0.027				
	None	D2 Ditch	0.305	0.305	0.305	0.305	0.305				
	None	D2 Stream	0.192	0.192	0.192	0.192	0.192				
	None	D3 Ditch	0.003	0.003	0.003	0.003	0.003				
	None	D4 Pond	0.009	0.009	0.009	0.006	0.006				
	None	D4 Stream	0.024	0.024	0.024	0.024	0.024				
	None	D5 Pond	0.006	0.006	0.003	0.003	0.003				
	None	D5 Stream	0.003	0.003	0.003	0.003	0.003				
	None	D6 Ditch	0.003	0.003	0.003	0.003	0.003				
	None	R1 Pond	0.344 ~~0.314~~	0.344 ~~0.314~~	0.344 ~~0.314~~	0.344 ~~0.314~~	0.344 ~~0.314~~	~~0.189~~	0.14 ~~0.126~~	~~0.096~~	0.063
	None	R1 Stream	**3.06** ~~2.985~~	**3.06** ~~2.985~~	**3.06** ~~2.985~~	**3.06** ~~2.985~~	**3.06** ~~2.985~~	~~1.946~~	**1.38** ~~1.356~~	~~1.039~~	0.73 ~~0.710~~
	None	R3 Stream	**2.64** ~~2.449~~	**2.64** ~~.449~~	**2.64** ~~2.449~~	**2.64** ~~2.449~~	**2.64** ~~2.449~~	~~1.602~~	**1.20** ~~1.117~~	~~0.859~~	0.63 ~~0.587~~
	None	R4 Stream	**4.01** ~~4.015~~	**4.01** ~~4.015~~	**4.01** ~~4.015~~	**4.01** ~~4.015~~	**4.01** ~~4.015~~	~~2.620~~	**1.826**	~~1.401~~	0.958

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: PEC calculation and acceptability of risk (PEC/RAC < 1) for prothioconazole-desthio (M04) based on FOCUS Step 4 calculations and chronic toxicity data for fish with mitigation of spray drift and run-off for applications of 2 x 200 g a.s./ha in winter cereals (BBCH 69, late application).

Use	**Nozzle reduction**	**Scenario**	**STEP 4 Max PECsw (µg/L)**								
		Vegetative strip (m)	None	5	10	15	20	5	10	15	20
		No spray buffer (m)	None	5	10	15	20	5	10	15	20
Winter cereals BBCH 69	None	D1 Ditch	0.022	0.022	0.022	0.022	0.022	-	-	-	-
	None	D1 Stream	0.014	0.014	0.014	0.014	0.014	-	-	-	-
	None	D2 Ditch	0.463	0.463	0.463	0.463	0.463	-	-	-	-
	None	D2 Stream	0.299	0.299	0.299	0.299	0.299	-	-	-	-
	None	D3 Ditch	<0.001	<0.001	<0.001	<0.001	<0.001	-	-	-	-
	None	D4 Pond	0.011	0.011	0.011	0.010	0.010	-	-	-	-
	None	D4 Stream	0.043	0.043	0.043	0.043	0.043	-	-	-	-
	None	D5 Pond	0.002	0.002	0.001	0.001	0.001	-	-	-	-
	None	D5 Stream	0.002	0.002	0.002	0.002	0.002	-	-	-	-

	None	D6 Ditch	0.004	0.001	<0.001	<0.001	<0.001	-	-	-	-
	None	R1 Pond	0.108	0.108	0.108	0.108	0.108	0.065	0.043	0.033	0.022
	None	R1 Stream	0.818 ~~0.771~~	0.818 ~~0.771~~	0.818 ~~0.771~~	0.818 ~~0.771~~	0.818 ~~0.771~~	~~0.502~~	0.372 ~~0.350~~	~~0.268~~	0.194 ~~0.183~~
	None	R3 Stream	0.198	0.198	0.198	0.198	0.198	0.129	0.090	0.069	0.047
	None	R4 Stream	0.165	0.165	0.165	0.165	0.165	0.108	0.075	0.058	0.039
RAC (µg/L)	0.334										
PEC/RAC	None	D1 Ditch	0.066	0.066	0.066	0.066	0.066				
	None	D1 Stream	0.042	0.042	0.042	0.042	0.042				
	None	D2 Ditch	**1.386**	**1.386**	**1.386**	**1.386**	**1.386**				
	None	D2 Stream	0.895	0.895	0.895	0.895	0.895				
	None	D3 Ditch	0.003	0.003	0.003	0.003	0.003				
	None	D4 Pond	0.033	0.033	0.033	0.030	0.030				
	None	D4 Stream	0.129	0.129	0.129	0.129	0.129				
	None	D5 Pond	0.006	0.006	0.003	0.003	0.003				
	None	D5 Stream	0.006	0.006	0.006	0.006	0.006				
	None	D6 Ditch	0.012	0.003	0.003	0.003	0.003				
	None	R1 Pond	0.323	0.323	0.323	0.323	0.323	0.195	0.129	0.099	0.066
	None	R1 Stream	**2.45** ~~**2.308**~~	**2.45** ~~**2.308**~~	**2.45** ~~**2.308**~~	**2.45** ~~**2.308**~~	**2.45** ~~**2.308**~~	~~**1.503**~~	**1.11** ~~**1.048**~~	~~0.802~~	0.58 ~~0.548~~
	None	R3 Stream	0.593	0.593	0.593	0.593	0.593	0.386	0.269	0.207	0.141
	None	R4 Stream	0.494	0.494	0.494	0.494	0.494	0.323	0.225	0.174	0.117

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 VFSmod calculations and chronic toxicity data for fish for applications of 2 x 200 g a.s./ha in winter cereals (BBCH 27-69, early and late application).**

Use	Vegetative filter strip (m)	5	10	15	20
	No spray buffer (m)	**5**	**10**	**15**	**20**
Winter Cereal BBCH 27					
VFSmod	R1 Pond	0.008	0.001	<0.001	<0.001
	R1 Stream	0.072	<0.001	<0.001	<0.001
	R3 Stream	0.368 **0.343**	0.231 0.216	0.149	<0.001
	R4 Stream	0.151	0.052	<0.001	<0.001
RAC (µg/L)	0.334				
PEC/RAC	R1 Pond	0.024	0.003	0.003	0.003
	R1 Stream	0.216	0.003	0.003	0.003
	R3 Stream	**1.10** 1.027	0.69 0.647	0.446	0.003
	R4 Stream	0.452	0.156	0.003	0.003
Winter Cereal BBCH 69					
VFSmod	R1 Pond	0.021	0.010	0.003	<0.001
	R1 Stream	0.124 0.119	0.059	0.016	<0.001
	R3 Stream	0.027	<0.001	<0.001	<0.001
	R4 Stream	0.011	0.001	0.001	<0.001
RAC (µg/L)	0.334				
PEC/RAC	R1 Pond	0.063	0.030	0.009	0.003
	R1 Stream	0.37 0.356	0.177	0.048	0.003
	R3 Stream	0.081	0.003	0.003	0.003
	R4 Stream	0.033	0.003	0.003	0.003

For chronic fish, acceptable risk for prothioconazole-desthio is demonstrated in spring cereals at FOCUS Step 3 and in winter cereals at FOCUS Step 4 with 20 m combined buffer zone and VFS. With VFSmod, mitigation in winter cereals can be reduced overall to 5 m combined buffer zone and VFS for Poland.

zRMS comments:

The aquatic risk assessment presented above has been amended accordingly with consideration of the surface water exposure agreed in the course of evaluation in area of Section 8.

Based on the performed calculations following conclusions may be derived:

1. Winter cereals at BBCH 27

- Prothioconazole: acceptable risk with no need for risk mitigation measures
- JU 6476-desthio:
  - D scenarios: risk acceptable with no need for risk mitigation measures
  - scenarios R1, R4: risk acceptable with 20 m VFS or 5 m VFS_{mod}
  - scenario R3: risk acceptable with 20 m VFS or 10 m VFS_{mod}

2. Winter cereals at BBCH 67

- Prothioconazole: acceptable risk with no need for risk mitigation measures
- JU 6476-desthio:
  - D scenarios and R3, R4 scenarios: risk acceptable with no need for risk mitigation measures
  - scenario R1: risk acceptable with 20 m VFS or 5 m VFS_{mod}

It should be noted that for chronic fish, acceptable risk for prothioconazole-desthio is demonstrated in spring cereals at FOCUS Step 3.
However, the risk from R scenarios not defined for spring cereals is covered by the risk assessment performed for these scenarios available for winter cereals.

Therefore finally, taking into account all relevant scenarios for Poland including D3, D4 and R1 for spring cereals and winter cereals - **a 5 m combined no-spray buffer zone and VFS is required**.

Concerned Member States must decide on applicability of indicated risk mitigation measures in their countries at the product authorisation.

Please note that additional aquatic risk assessment may be required by the concerned Member States that do not accept simulations performed according to FOCUS recommendations.

Sulphur

According to EFSA Scientific Report (2008) 221, the risk to aquatic organisms in the water column can be considered in general, as non-significant because the water solubility limit of the active substance is very low (0.063 mg a.s./L). In addition, no effect was observed at concentrations, which clearly exceeded the water solubility, by several orders of magnitude, please refer to Table 9.5-1.

Therefore, it was considered not necessary to assess the risk for aquatic organisms by calculating Toxicity Exposure Ratios (TER) and sulphur can be regarded of no concern for aquatic organisms.

However, since sulphur might adsorb to sediment after entering the surface water (EFSA Scientific Report (2008) 221), an assessment of exposure in the sediment compartment as well as an assessment of the risk for sediment-dwelling organisms is provided demonstrating an acceptable risk in consideration of the intended GAP uses of Patton Supra.
For completeness we reported the endpoints relating to benthic organisms as indicated in the conclusions of the RMS in sulphur revised Addendum DAR Vol.3 B9 2012: "A sediment-water toxicity test (sediment spiked) was conducted to evaluate the effects of sulphur dust on the sediment-dwelling midge *Chironomus riparius*, with a NOEC (28 d) = 949 mg/kg dw (nominal) = 608 mg/kg dw (initial measured sediment concentration). A sediment-water toxicity test (water spiked) was conducted to evaluate the effects of sodium sulphate on the sediment-dwelling midge *Chironomus riparius*. This study is not strictly required for

the confirmatory risk assessment, but was submitted as supportive information, with a NOEC (28 d) = 100 mg/L (nominal). The risk assessment for *Chironomus riparius* was therefore carried out by considering both the PEC_{SED} and PEC_{SW} values."
The entry routes spray drift, run-off and drainage were of potential relevance for loading of sediment with sulphur following spray application of the Patton Supra. For these routes of exposure, the predicted environmental concentrations in sediment were calculated based on simplified scenarios according to FOCUS recommendations based on very conservative assumptions as given in the model STEPS 1-2 in FOCUS (see Section B8, 8.9.1.2).

Table 9.5-14: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for sulphur for each organism group based on FOCUS Steps 1 and 2 calculations for the use of Patton Supra in spring and winter cereals.

Group		Sediment dwelling inv.
Test species		*Chironomus riparius*
Endpoint (mg/L)		NOEC 608000
AF		10
RAC (µg/L)		60800
FOCUS Scenario	**PEC sed [mg/kg]**	**PEC/RAC**
Step 1	12.845	0.0002
Step 2		
N-Europe	6.555	0.0001
S-Europe	5.305	0.0001

For the intended uses of sulphur in cereals, calculated TERs did indicate an acceptable risk for all groups of aquatic organisms in FOCUS Step 1-2. Therefore, no further assessment is necessary.

zRMS comments:

We agree with the calculations provided in the Table 9-5-14.
Due to no effects were observed at concentrations that exceeded the water solubility, by several orders of magnitude (table 9.5-1) it was not considered necessary to assess the risk for aquatic organisms by calculating RAC as sulphur can be considered of no concern for aquatic organisms.

However, since sulphur might adsorb to sediment after entering the surface water (EFSA Scientific Report (2008) 221), an assessment of exposure in the sediment compartment as well as an assessment of the risk for sediment-dwelling organisms is provided demonstrating an acceptable risk in consideration of the intended GAP uses of FHO04.

9.5.2.1 Formulation

The ratios between PEC_{SW} of the formulation due to drift (calculated based on the total amount of formulation that could be applied (4.0 L/ha)) and the aquatic organisms regulatory acceptable concentrations (RAC) for the formulation are reported in Table 9.5-15. PEC/RAC greater than 1 indicate acceptable risk.

Table 9.5-15: Acceptability of risk (PEC/RAC < 1) for formulation Patton Supra

Group		**Inverteb. acute**	**Algae**
Test species		*Daphnia magna*	*Pseudokirchneriella subcapitata*
Endpoint		EC_{50}	ErC_{50}
(µg/L)		37584	26363
AF		100	10
RAC (µg/L)		375.84	2636.3
Drift buffer distance	**PEC $_{gl-max}$ (µg/L)**	**PEC/RAC**	
1	61.653	0.164	0.023
3	21.035	0.056	0.008
5	12.693	0.034	0.0048
7.5	8.523	0.023	0.0032

For the intended use of formulation Patton Supra in cereals, calculated PEC/RAC did indicate an acceptable risk for all groups of aquatic organisms. Therefore, no further assessment is necessary.

zRMS comments:

We agree with the risk assessment for formulation provided in the Table 9.5-15. No risk mitigation measures are required for formulation FHO04.

9.5.3 Overall conclusions

An acceptable risk is demonstrated for prothioconazole, its relevant metabolites, sulphur, and the formation Patton Supra.

The active substance prothioconazole and two metabolites (prothioconazole S-methyl and 1,2,4-triazole) pass spring and winter applications at BBCH 27 and BBCH 69 at FOCUS Step 2, requiring no mitigations. In spring applications, the metabolite prothioconazole-desthio passes at FOCUS Step 3, for the relevant scenarios for Poland. However, to ensure an acceptable risk for prothioconazole-desthio in winter cereals for Poland, mitigations are required, with a 20 m combined no-spray buffer zone and VFS with VFSmod, the mitigation can be reduced overall to a 5 m combined no-spray buffer zone and VFS.
The risk from R scenarios not defined for spring cereals is covered by the risk assessment performed for these scenarios available for winter cereals.
Therefore finally, taking into account all relevant scenarios for Poland including D3, D4 and R1 for spring cereals and winter cereals - **a 5 m combined no-spray buffer zone and VFS is required**.
For the active substance sulphur, the sediment dwelling risk assessment was concluded as low risk. Finally, the formulation Patton Supra passes the aquatic risk assessment with no mitigations needed.

Overall, mitigations are required to ensure there is an acceptable risk to aquatic organisms, when using Patton Supra according to the GAP uses.

9.6 Effects on bees (KCP 10.3.1)

9.6.1 Toxicity data

Studies on the toxicity to bees have been carried out with prothioconazole and sulphur. Full details of these studies are provided in the respective EU DAR and related documents.
Effects on bees of formulation Patton Supra were not evaluated as part of the EU assessment of either prothioconazole or sulphur. New data submitted with this application are listed in and summarised in Appendix 2.
The selection of studies and endpoints for the risk assessments associated with the active substances is in line with the results of the EU review process. Justifications are provided below for the selection and studies for the formulation risk assessment.

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

Species	Substance	Exposure System	Results	Reference
Apis mellifera	Prothioconazole	Oral, acute	**LD_{50} > 71 µg/bee**	EFSA Conclusion 2007
Apis mellifera	Prothioconazole	Contact, acute	**LD_{50} > 200 µg/bee**	EFSA Conclusion 2007
Apis mellifera	Sulphur 80% WG	Oral, acute	**LD_{50} > 100 µg/bee**	EFSA Conclusion 2008
Apis mellifera	Sulphur 80% WG	Contact, acute	**LD_{50} > 100 µg/bee**	EFSA Conclusion 2008
Apis mellifera	Formulation FHO04	Oral, 24 h	**LD_{50} = >2232.57 µg/bee**	Ansaloni (2022a, KCP 3.1.1/01)
Apis mellifera	Formulation FHO04	Contact, 24 h	**LD_{50} = >2722.60 µg/bee**	Ansaloni (2022a, KCP 3.1.1/01)
Bombus terrestris	Formulation FHO04	Oral, 24 h	**LD_{50} = >6261 µg/bee**	Ripperger (2022, KCP 3.1.1/02)
Bombus terrestris	Formulation FHO04	Contact, 24 h	**LD_{50} = >2556 µg/bee**	Ripperger (2022, KCP 3.1.1/02)
Apis mellifera	Formulation FHO04	10 d feeding	**LDD_{50} = 523.03 µg/bee/day** LC_{50} = 26976.46 mg/kg diet	Ansaloni (2022b, KCP 3.1.2/01)
Apis mellifera	Formulation FHO04	22 d larval development	ED_{50} = 33.22 µg/larva/development period EC_{50} = 215.36 mg/kg diet **NOEC = 13.85 µg/larva**	Ansaloni (2022c, KCP 3.1.3/01)

zRMS comments:

Bees' toxicity data for prothioconazole, JAU 6476-desthio and Sulphur in Table 9.6-1 are in line with the EU agreed endpoints reported in EFSA Scientific Report (2007) 106, 1-98 and EFSA Scientific Report (2008) 221, 1-70, Confirmatory data 2012, respectively.
In addition, to bee studies, the acute studies on effects of FHO04 to bumblebees were performed by the Applicant. The laboratory studies on toxicity of FHO04 to bees and bumble bees were evaluated and considered acceptable by the zRMS.

For details of the evaluation please refer to Appendix 2.

9.6.1.1 Justification for new endpoints

Patton Supra was not the representative formulation assessed at EU-level as part of active substance approval. Effects on bees are therefore assessed using endpoints from the new formulation studies.

9.6.2 Risk assessment

The evaluation of the risk for bees was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002) and the recommendations of the EFSA bee Guidance Document (EFSA GD 2013) using the EFSA Bee calculator Tool (Bee-Tool v.3) for chronic risk assessment for adult bees and larva.

9.6.2.1 Hazard quotients for bees

Table 9.6-2: First-tier assessment of the risk for bees due to the use of formulation FHO04 in cereals (use 1)

Intended use	1 - Cereals		
Active substance	Prothioconazole		
Application rate (g/ha)	2 × 200 g/ha, 14 d interval		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	71	200	2.8
Contact toxicity	200		1.0
Intended use	1 - Cereals		
Active substance	Sulphur		
Application rate (g/ha)	2 × 2,500 g/ha, 14 d interval		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	100	2,500	25
Contact toxicity	100		25
Intended use	1 - Cereals		
Product	FHO04		
Application rate (g/ha)	2 × 4 L/ha, 14 d interval		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	2232.6	5,440*	2.4
Contact toxicity	2722.6		2.0

Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure. Q_H values shown in bold breach the relevant trigger.*applied dose considering density of the formulation of 1.36 kg/L

zRMS comments:

Acute risk assessment to bees

The acute risk assessment presented in Tables 9.6-2 is validated by the zRMS.
On the basis of calculated HQ values acceptable risk to bees may be concluded from all intended Central Zone uses of FHO04. Please note that the evaluation has been performed in line with SANCO/10329/2002 rev 2 final.

9.6.2.2 Chronic risk assessment for adult bees and larva (according to EFSA 2013)

The screening step risk assessment at the maximum single application rate of 4 L product/ha (= 5.440 kg product/ha) is presented in the following table.

Table 9.6-3: Screening assessment of the risk for bees due to the use of formulation FHO04 in cereals (use 1)

Intended use		1 - Cereals				
Product		Patton Supra (FHO04)				
Application rate (g/ha)		2 × 4 L/ha, 14 d interval				
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Calculation factor (Ef × SV)**	**Oral: ETR Contact: HQ**	**Trigger**	**Risk acceptable?**
Oral toxicity, chronic	525.03	5440*	7.6	**0.079**	0.03	**No**
Larval toxicity	13.85 µg/larva		4.4	**1.73**	0.2	**No**

*applied dose considering density of the formulation of 1.36 kg/L

From the screening assessment, potential chronic oral risk is indicated for adult honeybees and honeybee larvae, therefore, a first-tier assessment is required.

9.6.2.3 First-tier chronic assessment

The first-tier risk assessment for the chronic oral exposure of adult honeybee and honeybee larvae is presented in the following table.

Table 9.6-4: First-tier assessment of the risk for bees due to the use of FHO04 in cereals (use 1)

Intended use	1 - Cereals
Product	Patton Supra (FHO04)
Application rate (g/ha)	2 × 5,440 g/ha, 14 d interval

Toxicity category	**Scenario**	**BBCH**	**Honeybee**	
			ETR	**Trigger**
chronic	treated crop	10 - 29	0.01	0.03
chronic	treated crop	30 - 39	0.01	0.03
chronic	treated crop	40 - 69	0.01	0.03
chronic	weeds	10 - 29	0.02	0.03
chronic	weeds	30 - 39	0.01	0.03
chronic	weeds	40 - 69	0.01	0.03
chronic	field margin	10 - 29	0.00	0.03
chronic	field margin	30 - 39	0.00	0.03
chronic	field margin	40 - 69	0.00	0.03
chronic	adjacent crop	10 - 29	0.00	0.03
chronic	adjacent crop	30 - 39	0.00	0.03
chronic	adjacent crop	40 - 69	0.00	0.03
chronic	next crop	10 - 29	0.00	0.03
chronic	next crop	30 - 39	0.00	0.03
chronic	next crop	40 - 69	0.00	0.03
larva	treated crop	10 - 29	0.05	0.2
larva	treated crop	30 - 39	0.05	0.2
larva	treated crop	40 - 69	0.05	0.2
larva	weeds	10 - 29	**0.73**	0.2
larva	weeds	30 - 39	**0.37**	0.2
larva	weeds	40 - 69	**0.22**	0.2
larva	field margin	10 - 29	0.01	0.2
larva	field margin	30 - 39	0.01	0.2
larva	field margin	40 - 69	0.01	0.2
larva	adjacent crop	10 - 29	0.00	0.2
larva	adjacent crop	30 - 39	0.00	0.2

larva	adjacent crop	40 - 69	0.00	0.2
larva	next crop	10 - 29	0.13	0.2
larva	next crop	30 - 39	0.13	0.2
larva	next crop	40 - 69	0.13	0.2

Considering the proposed use, no unacceptable risks are expected for the chronic exposure of adult honeybees and honeybee larvae in the 'field margin', 'adjacent crop' and 'next crop' scenarios. However, 'weeds' did not pass in the first-tier risk assessment for honeybee larvae.

zRMS comments:

Chronic risk assessment to bees

It should be noted that according to conclusions of the Central Zone Harmonization Meeting in Warsaw in 2023, the chronic risk assessment according to recommendations of EFSA (2013) for adult and larvae bees should be presented for the zonal evaluations. In case of Poland, the chronic risk is not required until relevant EFSA Bee GD will implemented.
The chronic risk assessment presented in Tables 9.6-3 and 9.6-4 is validated by the zRMS.
Considering the proposed use, no unacceptable risks are expected for the chronic exposure of adult honeybees and honeybee larvae in the 'field margin', 'adjacent crop' and 'next crop' scenarios. However, 'weeds' did not pass in the first-tier risk assessment for honeybee larvae.

Therefore, further refinement for scenario; weeds'' is required at MSs level depend on their own requirements.

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

Not required.

9.6.3 Effects on bumble bees

A Tier 1 risk assessment using HQ acute oral and HQ acute contact is presented for bumblebees following both SANCO/10329/2002 and EFSA 2013. The SANCO approach is reported here for completeness only because SANCO/10329/2002 should not be applied to bumbles bees since no specific protection goals has been determined for this species and therefore there is no agreed trigger for HQ.

Table 9.6-5: First-tier assessment of the risk for bumblebees due to the use of formulation FHO04 in cereals (use 1)

Intended use	1 - Cereals					
Product	FHO04					
Application rate (g/ha)	2 × 4 L/ha, 14 d interval					
SANCO/10329/2002						
Test design	**LD_{50} (lab.) (µg/bee)**		**Single application rate (g/ha)**		**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**	
Oral toxicity	6261		5,440*		0.9	
Contact toxicity	2556				2.1	
EFSA 2013						
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Calculation factor (Ef × SV)**	**Oral: ETR Contact: HQ**	**Trigger**	**Risk acceptable?**
Oral toxicity, acute	6261	5440*	11.2	0.01	0.036	Yes
Contact toxicity	2556		1	2.1	7	Yes

*applied dose considering density of the formulation of 1.36 kg/L

zRMS comments:

The acute bumble bees risk assessment presented in Tables 9.6-5 is validated by the zRMS.

On the basis of calculated HQ values acceptable risk to bumble bees may be concluded from all intended Central Zone uses of FHO04.

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

Not relevant.

9.6.4 Effects on solitary bees

No information.

9.6.5 Overall conclusions

Acute oral and contact risk to bees following the proposed use of Patton Supra were performed in accordance with SANCO (2002) guidelines and show no risk.
Based on the EFSA guidance document 2013, the chronic risk to adult and larvae honeybees fails the screening assessment. However, both the chronic risk to adults and larvae pass the first-tier assessment for all scenarios, with the exception of the risk from larvae foraging on weeds in the treated field. This risk can be mitigated with the following label restriction: "*Do not apply when flowering weeds are present./Remove weeds before flowering.*" It is also noted that risks via weeds may be unrealistic in specific MS situations, since large amounts of flowering weeds are not compatible with profitable agriculture in many crops.
Overall, both the acute and chronic risks from the active substances prothioconazole and sulphur, and the formulation Patton Supra were acceptable at Tier 1 following the proposed use of Patton Supra.

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

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

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

Species	Substance	Exposure System	Results	Reference
Typholodromus pyri (protonymphs)	Prothioconazole EC 250	Laboratory test Coffin cells (2 D), 14 d	**LR_{50} = 18.7 g/ha**	EFSA Conclusion 2007
Typholodromus pyri (protonymphs)	Prothioconazole EC 250	Extended laboratory test Bean leaves (2 D), 14 d	LR_{50} = 445.5 g/ha	EFSA Conclusion 2007
Typholodromus pyri (protonymphs)	Prothioconazole EC 250	Extended laboratory test Aged residues, bean leaves (3 D), 14 d	Mortality 7 days after exposure, 1 day old residues: **14.5 %;** **15 day old residues: 6.4 % (corrected, 300 g/ha)** Reproduction 7 days	EFSA Conclusion 2007

Species	Substance	Exposure System	Results	Reference
			after exposure, 1 day old residues: 7.5 %; 15 day old residues: -28.8 % (300 g/ha)	
Aphidius rhodalosiphi (adults)	Prothioconazole EC 250	Laboratory test Glass plates (2 D), 14 d	**LR_{50} = 139.9 g/ha**	EFSA Conclusion 2007
Aphidius rhodalosiphi (adults)	Prothioconazole EC 250	Extended laboratory test Wheat leaves (3 D)	48 h mortality **<5 % in any test concentration up to 600 g/ha**	EFSA Conclusion 2007
Coccinella septumpunctata (larvae)	Prothioconazole EC 250	Laboratory test Glass plates (2 D), 46 d	LR_{50} = 229.8 g/ha	EFSA Conclusion 2007
Chrysoperla carnea (larvae)	Prothioconazole EC 250	Laboratory test Glass plates (2 D), 23 d d	Mortality: **15.2 % at 200 g/ha 28.3 % at 400 g/ha 41.3 % at 600 g/ha** No adverse effects on reproduction	EFSA Conclusion 2007
Poecilus cupreus (adults)	Prothioconazole EC 250	Quartz sand, 14 d	Mortality: 0.0 % at 400 g/ha 3.3 % at 600 g/ha No adverse effect on feeding rate	EFSA Conclusion 2007
Aleochara bilineata (adults / larvae)	Prothioconazole EC 250	Quartz sand, 87 d	Reproduction: 2.5 % at 42 g/ha 9.9 % at 200 g/ha 24.6 % at 600 g/ha	EFSA Conclusion 2007
Typhlodromus pyri	Sulphur dust	Laboratory test glass plates (2 D)	**LR_{50} =10,340 g/ha**	EFSA Conclusion 2008
Aphidius rhopalosiphi	Sulphur dust	Laboratory test glass plates (2 D)	**LR_{50} =486 g/ha**	EFSA Conclusion 2008
Typhlodromus pyri (protonymphs)	Formulation FHO04	Laboratory test glass plates (2 D)	**LR_{50} = 12,470.2 mL/ha** ER_{50} = 271.7 mL/ha	Leopold (2022a, KCP 10.3.2.1/01)
Aphidius rhopalosiphi (adults)	Formulation FHO04	Laboratory test glass plates (2 D)	**LR_{50} = 20,450 mL/ha** ER_{50} = >20,450 mL/ha	Leopold (2022b, KCP 10.3.2.1/02)
Typhlodromus pyri (protonymphs)	Formulation FHO04	Extended laboratory test Bean plants (3 D)	**LR_{50} = 13,558.7 mL/ha** ER_{50} = 892.6 mL/ha	Leopold (2022c, KCP 10.3.2.2/01)
Aphidius rhopalosiphi (adults)	Formulation FHO04	Extended laboratory test Barley plants (3 D)	**LR_{50} = 2,903.9 mL/ha** ER_{50} = >1 091 mL/ha	Leopold (2022d, KCP 10.3.2.2/02)
Chrysoperla carnea (larvae)	Formulation FHO04	Extended laboratory test Bean leaves (2D)	**LR_{50} = >20,450 mL/ha** ER_{50} = >20,450 mL/ha	Leopold (2022e, KCP 10.3.2.2/03)
Coccinella septumpuctata (adults)	Formulation FHO04	Extended laboratory test Bean leaves (2D)	**LR_{50} = >1,741.1 mL/ha** ER_{50} = >1091 mL/ha	Leopold (2022f, KCP 10.3.2.2/04)
Typhlodromus pyri (adults)	Formulation FHO04	Aged-residue test Bean leaves (3D)	Mortality at 2 × 4 L/ha: 14.5 % at 0 DALT 62.4 % at 7 DALT 29.0 % at 14 DALT (<50% for 35 – 70 DALT)	Fallowfield (2023, KCP 10.3.2.2/05)

Species	Substance	Exposure System	Results	Reference
			Reduction of reproduction at 2 × 4 L/ha: 94.7 % at 0 DALT 80.5 % at 14 DALT 53.2 % at 35 DALT 60.4 % at 42 DALT 68.3 % at 49 DALT **46.2% at 56 DALT** **18.7 % at 70 DALT**	
Aphidius rhopalosiphi (adults)	Formulation FHO04	Aged-residue test Bean leaves (2D)	Mortality at 2 × 4 L/ha: 82.5 % at 0 DALT 82.1 % at 7 DALT 65.8 % at 14 DALT **40.0 % at 28 DALT** **2.6 % at 42 DALT** Red. of reproduction at 2 × 4 L/ha: -2.1 % at 28 DALT -11.7 % at 42 DALT	Stevens (2022, KCP 10.3.2.2/06)
Coccinella septempunctata (larvae/adults)	Formulation FHO04	Aged-residue test Bean leaves (2D)	Mortality at 2 × 4 L/ha: **12.5 % at 0 DALT** **11.1 % at 7 DALT** No adverse effects on reproduction	White-Hall (2022, KCP 10.3.2.2/07)

zRMS comments:

Endpoints presented in Table 9.7-1 for NTA are in line with the EU agreed endpoints reported in EFSA Scientific Report (2007) 106, 1-98 and EFSA Scientific Report (2008) 221, 1-70.

In addition, the laboratory and higher tier 2 studies on toxicity of FHO04 to NTA were evaluated and validated by the zRMS. For details of the evaluation please refer to Appendix 2.

9.7.1.1 Justification for new endpoints

Patton Supra was not the representative formulation assessed at EU-level as part of active substance approval. Effects on non-target arthropods are therefore assessed using endpoints from the new formulation studies.

9.7.2 Risk assessment

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

9.7.2.1 Risk assessment for in-field exposure

~~**Table 9.7-2: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of prothioconazole following the use of formulation FHO04 in cereals (use 1)**~~

~~**Intended use**~~	~~Cereals~~
~~**Active substance**~~	~~Prothioconazole~~

Application rate (g/ha)	2 × 200 g/ha (14d)		
MAF	1.7		
Test species **Tier I**	**LR_{50} (lab.)** **(g/ha)**	**$PER_{in\text{-}field}$** **(g/ha)**	**$HQ_{in\text{-}field}$** **criterion: HQ ≤ 2**
Typhlodromus pyri	18.7	340	**18.2**
Aphidius rhopalosiphi	139.9		**2.4**
Test species **Higher-tier**	**Rate with ≤ 50 % effect** **(g/ha)**	**$PER_{in\text{-}field}$** **(g/ha)**	**$PER_{in\text{-}field}$ below rate with ≤ 50 % effect?**
Typhlodromus pyri	300	340	**No**
Aphidius rhopalosiphi	600	340	Yes

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

Table 9.7-3: First- and higher-tier assessment of the in-field risk for non-target arthropods due to sulphur following the use of formulation FHO04 in cereals (use 1)

Intended use	Cereals		
Active substance	Sulphur		
Application rate (g/ha)	2 × 2,500 g/ha (14d)		
MAF	1.7		
Test species **Tier I**	**LR_{50} (lab.)** **(g/ha)**	**$PER_{in\text{-}field}$** **(g/ha)**	**$HQ_{in\text{-}field}$** **criterion: HQ ≤ 2**
Typhlodromus pyri	10,340	4,250	0.4
Aphidius rhopalosiphi	486		**8.7**

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

Table 9.7-4: First- and higher-tier assessment of the in-field risk for non-target arthropods following the use of formulation FHO04 in cereals (use 1)

Intended use	Cereals		
Product	FHO04 (Prothioconazole: 53.14 g/L, Sulphur: 638.0 g/L)		
Application rate (L/ha)	2 × 4 L/ha (14d)		
MAF	1.7		
Test species **Tier I**	**LR_{50} (lab.)** **(mL/ha)**	**$PER_{in\text{-}field}$** **(mL/ha)**	**$HQ_{in\text{-}field}$** **criterion: HQ ≤ 2**
Typhlodromus pyri	12,470.2	6,800	0.545
Aphidius rhopalosiphi	20,450.0		0.333
Test species **Tier II**	**LR_{50} (lab.)/ER50** **(mL/ha)**	**$PER_{in\text{-}field}$** **(mL/ha)**	**PERin-field below rate with ≤ 50 % effect?** **HQin-field** **criterion: HQ ≤ 2**
Typhlodromus pyri	892.6 mL/ha 13,558.7	6,800	0.502 **(No)**
Aphidius rhopalosiphi	1091 mL/ha 2,903.9		2.342 **(No)**
Chrysoperla carnea	>20,450		0.333 (Yes)
Coccinella punctata	1091 mL/ha 1,741.1		3.906 **(No)**
Test species **Higher-tier**	**Rate with ≤ 50 % effect** **(L/ha) at xxx DALT**	**$PER_{in\text{-}field}$** **(L/ha)**	**$PER_{in\text{-}field}$ below rate with ≤ 50 % effect?**
Typhlodromus pyri	2 × 4 L/ha, 56 and 70 DALT	6.8	Yes

Aphidius rhopalosiphi	2 × 4 L/ha, 28 and 42 DALT		Yes
Coccinella septempunctata	2 × 4 L/ha, 0 and 7 DALT		Yes

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

zRMS comments:

The in-field risk assessment performed for the formulation FO04 is validated by the zRMS. The exposure calculated for particular active substances was not validated as not necessary since the risk assessment for this group of species is performed for formulation for which authorisation is sought.

The calculations of in-field risk assessment for FHO04 were performed according to relevant guidance.

At Tier-2 the PER in-field was below the ER_{50} for Chrysoperla carnea but above the ER_{50} for two indicator species: T.pyri and A. rhopalosiphi and one additional tested species-C. punctata requiring higher-tier studies.
Age residue studies for A.rhopalosiphi, C. septempuncta and T.pyri have been conducted and evaluated by zRMS to refine the risk in-field.
Based on the results obtained from these studies with two applications of product FO04 to French bean plants at a rate of 2 x 2400 mL product/ha, with a 14-day interval, foliar residues of FHO04 had no unacceptable effects on either survival or reproduction (i.e. < 50% effects, relative to the control).
Finally, an acceptable risk to NTA may be concluded from all intended Central Zone uses of FHO04.

9.7.2.2 Risk assessment for off-field exposure

Table 9.7-5: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of prothioconazole following the use of formulation FHO04 in cereals (use 1)

Intended use	Cereals				
Active substance	Prothioconazole				
Application rate (g/ha)	2 × 200 g/ha (14d)				
MAF	1.7				
vdf	10 (Tier I), 5 (Higher tier)				
Test species Tier I	**LR_{50} (lab.) (g/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g/ha)**	**CF**	**$HQ_{off\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	18.7	2.38 % (drift factor = 0.0238)	0.809 $0.081_{corr.}$	10	0.004
Aphidius rhopalosiphi	139.9				<0.001
Test species Higher-tier	**Rate with ≤ 50 % effect (g/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g/ha)**	**CF**	**corr. $PER_{off\text{-}field}$ below rate with ≤ 50 % effect?**
Typhlodromus pyri	300	2.38 % (drift factor = 0.0238)	1.618 $0.324_{corr.}$	5	Yes
Aphidius rhopalosiphi	600				Yes

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

Table 9.7-6: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of sulphur following the use of formulation FHO04 in cereals (use 1)

Intended use	Cereals
Active substance	Sulphur
Application rate (g/ha)	2 × 2,500 g/ha (14d)
MAF	1.7
vdf	10 (Tier I)

Test species Tier I	LR_{50} (lab.) (g/ha)	Drift rate	$PER_{off\text{-}field}$ (g/ha)	CF	$HQ_{off\text{-}field}$ criterion: HQ ≤ 2
Typhlodromus pyri	10,340	2.38 % (drift factor = 0.0238)	10.115 $1.012_{corr.}$	10	<0.001
Aphidius rhopalosiphi	486				0.002

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

Table 9.7-7: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of prothioconazole following the use of formulation FHO04 in cereals (use 1)

Intended use	Cereals
Product	FHO04 (Prothioconazole: 53.14 g/L, Sulphur: 638.0 g/L)
Application rate (L/ha)	2 × 4 L/ha (14d)
MAF	1.7
vdf	10 (Tier 1) / 5 (Higher-tier)

Test species **Tier I**	**LR_{50} (lab.)** **(mL/ha)**	**Drift rate**	**$PER_{off\text{-}field}$** **(mL/ha)**	**CF**	**$HQ_{off\text{-}field}$** **criterion: HQ ≤ 2**
Typhlodromus pyri	12,470.2	2.38 % (drift factor = 0.0238)	16.184 $1.618_{corr.}$	10	<0.001
Aphidius rhopalosiphi	20,450.0				<0.001
Test species **Tier II**	**LR_{50} (lab.)** **(mL/ha)**	**Drift rate**	**$PER_{off\text{-}field}$** **(mL/ha)**	**CF**	$HQ_{in\text{-}field}$ criterion: HQ ≤ 2 **corr. $PER_{off\text{-}field}$ below rate with ≤ 50 % effect?**
Typhlodromus pyri	892.6 13,558.7	2.38 % (drift factor = 0.0238)	80.92_{corr} 32.368 $6.474_{corr.}$	5	Yes <0.001
Aphidius rhopalosiphi	1091 2,903.9		809.2		Yes 0.002
Chrysoperla carnea	>20,450		80.92_{corr}		Yes <0.001
Coccinella punctata	1091 1,741.1		80.92_{Corr}		Yes 0.004
Test species Higher tier	Rate with ≤ 50 % effect (mL/ha)	Drift rate	$PER_{off\text{-}field}$ (mL/ha)	CF	corr. $PER_{off\text{-}field}$ below rate with ≤ 50 % effect?
Typhlodromus pyri	2 × 4 L/ha, 56 and 70 DALT	2.38 % (drift factor = 0.0238)	32.368 $6.474_{corr.}$	5	Yes
Aphidius rhopalosiphi	2 × 4 L/ha, 28 and 42 DALT				Yes
Coccinella septempunctata	2 × 4 L/ha, 0 and 7 DALT				Yes

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

zRMS comments:

The off-field risk assessment performed for the formulation FO04 included in Table 9.7-7 with consideration laboratory studies for 4 NTA species has benn amended by the zRMS according to recommendation given in ESCORT 2. Acceptable risk could be concluded with no need for risk mitigation measures.

Overall, an acceptable risk to non-target arthropods in in-field and off-field areas are expected following the application of the product FHO04 according to the proposed use pattern in cereals.

9.7.2.3 Additional higher-tier risk assessment

Not relevant.

9.7.2.4 Risk mitigation measures

No risk mitigation needed.

9.7.3 Overall conclusions

In-field and off-field HQ Higher tier and Tier I values based on laboratory studies with the formulation Patton Supra and the test organisms *Aphidius rhopalosiphi* and *Typhlodromus pyri* were below relevant trigger values indicating that the risk to in-field and off-field non-target arthropods is acceptable following the use of formulation Patton Supra according to the proposed use pattern.

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

9.8.1 Toxicity data

Studies on the toxicity to earthworms and other non-target soil organisms (meso- and macrofauna) have been carried out with prothioconazole and its relevant metabolites M01 (S-methyl) and M04 (desthio), and sulphur. Full details of these studies are provided in the respective EU DARs and related documents.

Effects on soil microorganisms of formulation FHO04 were not evaluated as part of the EU assessment of either active substance. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

According to the current guidance document SANCO/10239, EC 2002, endpoints (LC_{50}, NOEC or EC_{10}) considered in the risk assessment for soil macro- and mesofauna should be divided by a factor of 2, if the log P_{ow} is greater than 2, unless it can be demonstrated by soil sorption data or other evidence that the toxicity is independent of organic carbon content in the substrate.

As stated in the EFSA Scientific Report (2007) 106, the log P_{ow} for prothioconazole was determined > 2 and thus, a correction factor must be considered. The log P_{ow} values for the major metabolites of prothioconazole in soil were determined to be 4.19 (prothioconazole-methyl) and 3.04 (prothioconazole-desthio). As these values are above the relevant threshold of 2 as well, a correction factor of 2 was applied for the metabolite of concern for maximum conservatism.

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

Species	Substance	Exposure System	Results	Reference
~~*Eisenia fetida*~~	~~Prothioconazole~~	~~14 d, acute 10 % peat content~~	~~LC_{50} = >1000 mg/kg dw $LC_{50,corr}$ = 500 mg/kg dw*~~	~~EFSA Conclusion 2007~~
Eisenia fetida	M01 (S-methyl)	14 d, acute 10 % peat content	LC_{50} = >1000 mg/kg dw **$LC_{50,corr}$ = 500 mg/kg dw***	EFSA Conclusion 2007
Eisenia fetida	M04 (desthio)	14 d, acute 10 % peat content	LC_{50} = >1000 mg/kg dw	EFSA Conclusion 2007

Species	Substance	Exposure System	Results	Reference
			$LC_{50,corr}$ = 500 mg/kg dw*	
Eisenia fetida	Sulphur	Mixed into substrate 14 d, acute 10 % peat content	LC_{50} = 1576 mg/kg dw **$LC_{50,corr}$ = 788 mg/kg dw***	EFSA Conclusion 2008
Eisenia fetida	Prothioconazole	Mixed into substrate 56 d, chronic 10 % peat content	NOEC = 1.33 mg/kg dw **$NOEC_{corr}$ = 0.665 mg/kg dw***	EFSA Conclusion 2007
Eisenia fetida	M01 (S-methyl)	Mixed into substrate 56 d, chronic 10 % peat content	NOEC = 100 mg/kg dw **$NOEC_{corr}$ = 50 mg/kg dw***	EFSA Conclusion 2007
Eisenia fetida	M04 (desthio)	Mixed into substrate 56 d, chronic 10 % peat content	NOEC = 1 mg/kg dw **$NOEC_{corr}$ = 0.5 mg/kg dw***	EFSA Conclusion 2007
Folsomia candida	Prothioconazole	Mixed into substrate 28 d, chronic 10 % peat content	NOEC = 64 mg/kg dw **$NOEC_{corr}$ = 32 mg/kg dw***	EFSA Conclusion 2007
Hypoaspis aculeifer	Prothioconazole	Mixed into substrate 14 d, chronic	NOEC = 100 mg/kg dw **$NOEC_{corr}$ = 50 mg/kg dw***	EFSA Conclusion 2007
Folsomia candida	M01 (S-methyl)	Chronic 28 d, 10 % peat content	NOEC = 31.6 mg/kg dw **$NOEC_{corr}$ = 15.8 mg/kg dw***	EFSA Conclusion 2007
Folsomia candida	M04 (desthio)	Chronic 28 d, 10 % peat content	NOEC = 62.5 mg/kg dw **$NOEC_{corr}$ = 31.25 mg/kg dw***	EFSA Conclusion 2007
Eisenia fetida	Formulation FHO04	Mixed into substrate 56 d, chronic 10 % peat content	NOEC = 267.9 mg/kg dw **$NOEC_{corr}$ = 133.95 mg/kg dw*** **EC_{10}=258.82 mg/kg dw*** **EC_{10corr}=129.41 mg/kg dw***	Rana, 2022 UPL/2021/0569 KCP 10.4.1/01
Folsomia candida	Formulation FHO04	Mixed into substrate 28 d, chronic 5 % peat content	NOEC = 95.3 mg/kg dw **$NOEC_{corr}$ = 47.65 mg/kg dw*** **EC_{10} = 108.7 mg /kg dws**	Hübner, 2022 UPL/2021/0367 KCP 10.4.1/01
Hypoaspis aculeifer	Formulation FHO04	Mixed into substrate 14 d, chronic 5 % peat content	NOEC = ≥1000 mg/kg dw **$NOEC_{corr}$ = 500 mg/kg dw*** **EC_{10}=n.d.**	Hübner, 2022 UPL/2021/0368 KCP 10.4.1/02
Field studies				
Prothioconazole, formulation EC250 (EFSA conclusion, 2007)				
3 × 200 g a.s./ha on grassland				

Species	Substance	Exposure System	Results	Reference
5 different species identified and assessed (*Lumbricius terrestris, L. rubellus, L. castanea, Aporrectodea caliginosa, A. terrestris longa*). 46% reduction in the number of *A. caliginosa* juveniles 7 weeks after first application (2 weeks after final application). No adverse effect 5 month after first application. (Maximum measured soil PEC 0.052 mg prothioconazole/kg based on soil sampling depth of 10 cm which is equivalent to a soil PEC of 0.104 mg prothioconazole/kg over the standard 5 cm depth)				
Field study - Prothioconazole 250 g/L EC (Vollmer 2023, KCP 10.4.1.2/01				
Two spray applications of Prothioconazole 250 g/L EC, 14 days interval, with a maximum application rate covering the worst-case GAP for cereals of 2 x 200 g a.s./ha, with a safety factor of 4 (2 x 800 g prothioconazole/ha) during the main earthworm activity period in spring did not cause adverse effects (neither short-term, nor long-term up to 1 year after application) on earthworm field populations.				
Litter bag test – Prothioconazole, formulation FS100 then EC250 (EFSA conclusion, 2007)				
FS 100 (23.2 g a.s./ha) followed by JAU 6476 EC 250 (3 @ 200 g a.s./ha during 26-day period) after 34 days: test item: 51.7; control: 52.1 after 95 days: test item: 74.3; control: 78.4 after 126 days: test item: 92.0; control 91.2				

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

zRMS comments:

Endpoints presented in Table 9.8-1 are in line with the EU agreed endpoints reported in EFSA Scientific Report (2007) 106, 1-98 and EFSA Scientific Report (2008) 221, 1-70.

The laboratory studies for soli meso-and macrofauna and higher tier 2 study for earthworms by Volmer 2023 were evaluated and validated by the zRMS. For details of the evaluation please refer to Appendix 2.

9.8.1.1 Justification for new endpoints

Patton Supra was not the representative formulation assessed at EU-level as part of active substance approval. Effects on non-target soil meso- and macro-fauna are therefore assessed using endpoints from the new formulation studies.

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 worst-case PEC_{soil} for risk assessments covering the proposed GAP use are taken from Section 8 (Environmental Fate), Chapter 8.7.2. According to the assessment of environmental-fate data, multi-annual accumulation in soil does not need to be considered for either prothioconazole or sulphur.

Toxicity Exposure Ratios (TER) were calculated with the endpoints for chronic effects on earthworms and other soil organisms (*Hypoaspis aculeifer, Folsomia candida*) and the relevant PEC_{soil} values. The TER values are as follows:

Table 9.8-2: First-tier assessment of the acute and chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of formulation FHO04 in cereals (use 1)

Intended use	Cereals, Use 1
Acute effects on earthworms	

Active substance /metabolite	**LC_{50} (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_a (criterion TER ≥ 10)**
Prothioconazole	500	0.220	2273
M01 (S-methyl)	500	0.065	7692
M04 (desthio)	500	0.221	2262
Sulphur	788	6.667	118
Chronic effects on earthworms			
Active substance /metabo-lite/product	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
Prothioconazole	0.665	0.220	**3.023**
M01 (S-methyl)	50	0.065	769.231
M04 (desthio)	0.5	0.221	**2.262**
Formulation FHO04	129.41 ~~133.95~~	5.803	22.30 ~~23.083~~
Formulation FHO04 - prothioconazole	~~5.24~~ 5.05	0.22	~~23.818~~ 23.02
Formulation FHO04 - sulphur	~~62.93~~ 80.8	6.667	~~9.439~~ 12.12
Chronic effects on other soil macro- and mesofauna			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
Prothioconazole - Folsomia	32	0.220	145.455
Prothioconazole - Hypoaspis	50	0.22	227.273
M01 (S-methyl) - Folsomia	15.8	0.065	243.077
M04 (desthio) - Folsomia	31.25	0.221	141.403
FHO04 - Folsomia	47.65formulation is above triger of 5 indicatimg an acceptable risk	5.803	8.211
FHO04 - Hypoaspis	500	5.803	86.162

TER values shown in bold fall below the relevant trigger.

All TER_{LT} values for the formulated product and the metabolites of prothioconazole potentially relevant in soil are above the trigger value of 5, established for long-term exposure, indicating an overall acceptable risk for earthworms at Tier-1 level. Thus, no further considerations are required.

zRMS comments:

The soil exposure provided in Table 9.8-2 is confirmed to be in line with PEC_{SOIL} values agreed by the zRMS in area of Section 8. The risk assessment for both active substances and their metabolites is in general agreed by the zRMS.

TER_{LT} for soil meso-and macrofauna is above trigger of 5 indicating an acceptable risk. In case of metabolite of prothioconazole M04 an unacceptable risk is identified.

The field study carried by the Applicant (Vollmer, T. 2023) demonstrates that prothioconazole and its metabolite prothioconazole-desthio does not pose any risk to earthworm populations at the worst-case GAP application (2 x 200 g prothioconazole/ha) and up to 2 applications at 800 g prothioconazole/ha.

Overall, acceptable risk to soil macro- and meso-fauna may be concluded from the intended Central Zone uses of FHO04.

9.8.2.2 Higher-tier risk assessment

The field study carried by the Applicant (Vollmer, T. (2023) - Report No S21-03781) demonstrates that prothioconazole and its metabolite prothioconazole-desthio does not pose any risk to earthworm populations at the worst-case GAP application (2 x 200 g prothioconazole/ha) and up to 2 applications at 800 g prothioconazole/ha. Therefore, a safe use of formulation Patton Supra is demonstrated.

9.8.3 Overall conclusions

TER values for sulphur and formulation Patton Supra are above the trigger value of 5 indicating there is acceptable chronic risk to earthworms, meso-, and macrofauna at the proposed GAP. TER values for prothioconazole and its relevant metabolite are below the trigger value of 5 for earthworm, however a field study indicate that the chronic risk to earthworms is acceptable at the proposed GAP.

9.9 Effects on soil microbial activity (KCP 10.5)

9.9.1 Toxicity data

Studies on effects soil microorganisms have been carried out with prothioconazole and its relevant metabolites M01 (S-methyl) and M04 (desthio), and sulphur. Full details of these studies are provided in the respective EU DARs and related documents.
Effects on soil microorganisms of formulation Patton Supra were not evaluated as part of the EU assessment of either active substance. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	Prothioconazole	28 d, aerobic Silty sand	No influence at 2.0 kg a.s./ha (≡ **2.71 mg/kg**)	EFSA Conclusion 2007
N-mineralisation	M01 (S-methyl)	28 d, aerobic Silty sand	No influence at 2.0 kg p.m./ha (≡ **2.69 mg/kg**)	EFSA Conclusion 2007
N-mineralisation	M04 (desthio)	28 d, aerobic Silty sand	No influence at 1.0 kg p.m./ha (≡ **1.37 mg/kg**)	EFSA Conclusion 2007
N-mineralisation	Sulphur dust	28 d, aerobic	0 % effect at day 28 at 400 mg prod/kg d.w.soil (300 kg prod/ha)	EFSA Conclusion 2008
N-mineralisation	Sulphur 80 % WG	77 d, aerobic	**13.3 mg prod./kg d.w. soil** (10 kg prod/ha): - no effect during 77 days 133 mg prod/kg d.w.soil (100 kg prod/ha): - 14 % effect at day 77 - maximum effect of 86 % inhibition at day 21	EFSA Conclusion 2008
N-mineralisation	Formulation FHO04	28 d, aerobic Sandy-clay loam soil	For 40 L/ha (≡ **72.53 mg product/kg soil***): Nitrate formation rate	Bhosale, 2022 UPL/2021/0538 KCP 10.5/01

Endpoint	Substance	Exposure System	Results	Reference
			1.14 mg/kg soil dw/day Difference from control = 0.81 % (i.e. less than 25 %)	

*based on product density = 1.36 kg/L, and mass of 1 ha soil to 5 cm depth, assuming a bulk density of 1.5 g/cm^3 = 750,000 kg

zRMS comments:

Endpoints presented in Table 9.8-1 are in line with the EU agreed endpoints reported in EFSA Scientific Report (2007) 106, 1-98 and EFSA Scientific Report (2008) 221, 1-70.

The laboratory for micro-organism on toxicity of FHO04 were evaluated and validated by the zRMS.
For details of the evaluation please refer to Appendix 2.

9.9.1.1 Justification for new endpoints

Patton Supra was not the representative formulation assessed at EU-level as part of active substance approval. Effects on nitrogen transformation are therefore assessed using endpoints from the new formulation study.

9.9.2 Risk assessment

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

According to SANCO/10329/2002 rev 2 (final), the outcome of the soil microorganism test is directly assessed in terms of risk. Accordingly, effects within a range of ±25 % observed in the underlying tests are acceptable in a biological and ecological context provided that the concentrations/rates used in the tests covered the maximum PEC_{soil} / deposit rate.

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

Considering this maximum exposure level an acceptable risk for soil microorganisms with regard to C-/N transformation is indicated as outlined in the table below.

Table 9.9-2: Assessment of the risk for effects on soil micro-organisms due to the use of formulation FHO04 in cereals (use 1)

Intended use	1 – Cereals (BBCH 27, 20 % crop interception)		
N-mineralisation			
Active substance /metabolite/product	**Max. conc. with effects ≤ 25 % (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**Risk acceptable?**
Prothioconazole	2.71	0.220	Yes
M01 (S-methyl)	2.69	0.065	Yes
M04 (desthio)	1.37	0.221	Yes
Sulphur	13.3	6.667	Yes
Formulation FHO04	72.53	5.803	Yes

zRMS comments:

The risk assessment presented in Table 9.9-3 is in general agreed by the zRMS.

9.9.3 Overall conclusions

The risk to soil microorganisms following the proposed use of Patton Supra were performed in accordance with SANCO (2002) guidelines. Effects within a range of ± 25 % compared to the control were observed at exposure levels which clearly exceed the maximum PEC values in soil from the proposed use, thus an acceptable risk for soil microorganisms is indicated for all intended GAP uses of Patton Supra.

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

9.10.1 Toxicity data

Studies on the toxicity to non-target terrestrial plants have been carried out with prothioconazole and its relevant metabolites, and sulphur and its transformation product. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on non-target terrestrial plants of the formulation Patton Supra were not evaluated as part of the EU assessments of prothioconazole and sulphur. 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 because Patton Supra was not the representative formulation assessed for active substance approval.

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

Species	Substance	Exposure System	Results	Reference
Amaranthus retroflexus (d)	JAU 6476 a.s.	Pre-emergence	At 200 g a.s./ha, there was 5 % max. phytotoxic effect.	EFSA Conclusion 2007
Amaranthus retroflexus (d) *Beta vulgaris* (d)	JAU 6476 a.s.	Post emergence	At 250 g a.s./ha there was 10 % max. phytotoxic effect.	EFSA Conclusion 2007
Amaranthus retroflexus (d)	JAU 6476 EC 250	Pre-emergence	At 200 g a.s./ha, there was 5 % max. phytotoxic effect.	EFSA Conclusion 2007
Zea mays (m) *Avena sativa* (m) *Allium cepa* (m) *Brassica oleracea* (m) *Pisum sativum* (m) *Daucus carota* (m)	Sulphur 80% WG	Vegetative vigour	ER_{50} = > 25200 g/ha	EFSA Conclusion 2008
Dicotyledonous: Sugar beet (*Beta vulgaris*), oil seed rape (*Brassica napus*), soybean (*Glycine max*), tomato (*Solanum lycopersicum*)	FHO04: Prothioconazole: 53.14 g/L Sulphur: 638.0 g/L	21d Seedling Emergence and Seedling Growth	At 16 L product/ ha, no inhibition in seedling emergence, or post-emergence mortality occurred in any of the plant species.	Ripperger 2023 S21-05533(KCP 10.6.2/01)

Species	Substance	Exposure System	Results	Reference
Monocotyledonous: Onion (*Allium cepa*) and maize (*Zea mays*)				
Dicotyledonous: Sugar beet (*Beta vulgaris*), oil seed rape (*Brassica napus*), soybean (*Glycine max*), tomato (*Solanum lycopersicum*) Monocotyledonous: Onion (*Allium cepa*) and maize (*Zea mays*)	FHO04: Prothioconazole: 53.14 g/L Sulphur: 638.0 g/L	21 d Vegetative vigour	At 16 L product/ ha, no mortality nor symptoms of phytotoxicity occurred in any of the plant species..	Ripperger 2023 S21-05534KCP 10.6.2/02)

m: monocotyledonous; d: dicotyledonous

zRMS comments:

Endpoints presented in Table 9.10-1 are in line with the EU agreed endpoints reported in EFSA Scientific Report (2007) 106, 1-98 and EFSA Scientific Report (2008) 221, 1-70. The laboratory for NTTP on toxicity of FHO04 were evaluated and validated by the zRMS. For details of the evaluation please refer to Appendix 2.

9.10.1.1 Justification for new endpoints

Patton Supra was not the representative formulation assessed at EU-level as part of active substance approval. Effects on non-target terrestrial plants are therefore assessed using endpoints from the new formulation studies.

9.10.2 Risk assessment

9.10.2.1 Tier-1 risk assessment (based screening data)

Not relevant.

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

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

Table 9.10-2: Assessment of the risk for non-target plants due to the use of formulation Patton Supra, FHO04, in cereals (use 1)

Intended use	Cereals			
Product	FHO04 (Prothioconazole: 53.14 g/L, Sulphur: 638.0 g/L)			
Application rate (g/ha)	2 × 4 L/ha, 14 d interval			
MAF	1.7			
Test species	**ER_{50} (L/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (L/ha)**	**TER criterion: TER ≥ 5**

All species	> 16	2.38	0.16	100

MAF: Multiple application factor; PER: Predicted environmental rate; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

The calculated TER value for both vegetative vigour and seedling emergence is greater than the trigger, therefore a safe risk to non-target plants from the proposed use of formulation Patton Supra has been demonstrated.

zRMS comments:

Based on performed calculations acceptable risk to non-target terrestrial plants may be concluded for application of FHO04 at rate 2 x 2.4 L/ha without the needs of any specific risk mitigations to non - crop land.

9.10.2.3 Higher-tier risk assessment

Not relevant.

9.10.2.4 Risk mitigation measures

No risk mitigation needed.

9.10.3 Overall conclusions

The risk to non-target terrestrial plants following the proposed use of Patton Supra were performed in accordance with SANCO (2002) guidelines. The risk from the formulation was acceptable at Tier 2. No mitigation measures need to be applied.

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

N/A

9.12 Monitoring data (KCP 10.8)

N/A

9.13 Classification and Labelling

According to the criteria given in Regulation (EC) No 1272/2008, Patton Supra is classified as ‘very toxic to aquatic life’ (H400) and ‘toxic to aquatic life with long lasting effects’ (H411).

Table 9.13-1: Justified proposals for classification and labelling for Patton Supra IN002B1760 according to Regulation (EC) No 1272/2008

Hazard class(es), categories:	Acute Aquatic 1 Chronic Aquatic 2
Hazard pictograms or Code(s) for hazard pictogram(s):	GHS09
Signal word:	Warning
Hazard statement(s):	H410: Very toxic to aquatic life with long lasting effects
Precautionary statement(s):	P273: Avoid release to the environment. P391: Collect spillage P501: Dispose of contents and container to hazardous or special waste collection point, in accordance with local, regional, national and/or international regulation.
Additional labelling phrases:	EUH401: To avoid risks to man and the environment, comply with the instructions for use.

zRMS comments:

zRMS ~~doesn't~~ agrees with the classification for formulation FHO04 – H410 on the label proposed by the Applicant.

~~The zRMS's justification of H411 is provided below.~~

Acute aquatic hazard:

Valid test data for all the two trophic levels are available for the mixture as a whole, therefore there is a need to consider bridging principles or classification of individual components for acute hazard classification of the mixture. Test data showed that E_rC_{50} for primary producers is > 1 mg /L for Daphnia and algae but for fish summation method is required as no study for formulation for this organism is submitted.

Compound	Acute aquatic hazard	M	% in the product	H x M
Prothioconazole	Acute 1 (H400)	10	3.7	3.7 x 10 =37% >25%
Sulphur		-	46.12	-

Consequently, classification "Acute 1" (H400) for acute aquatic hazard is required.
The chronic toxicity studies with the product were available only for algae. In absence of chronic toxicity data for fish and aquatic invertebrates the classification for the chronic aquatic hazard should be thus based on summation method.

Long-term aquatic hazard:

In absence of chronic toxicity data for product the classification for the chronic aquatic hazard should be based on summation method.
Information on classification including associated M factors and the % of the components in the mixture are as follows:

Compound	Long-term aquatic hazard	M	C (%)
Prothioconazole	Chronic 1 (H410)	1	3.7
Sulphur	-	-	46.12

STEP 1: Classify as Chronic 1 if:

∑(Chronic 1 × M) ≥ 25 %

= (3.7× 1 + (24.0 × 100) < 25

STEP 2: Classify as Chronic 2 if:

(10 x Chronic 1 x M) ≥ 25 %

10 x 3.7× 1=37%

>25%

The classification “Acute 1” (H400) for acute aquatic hazard is required. According to the summation method, ∑(Chronic 1 × M) < 25 %, and the product should thus be classified as chronic 2 **(H411).**

Finaly the 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	Singh, P.D.	2023a	Acute immobilisation study of prothioconazole/sulphur (50+625) g/l sc to *Daphnia magna* Report NO. 502-3-07-29112 GLP Unpublished	N	UPL
KCP 10.2.1/02	Singh, P.D.	2023b	Alga (*Pseudokirchneriella subcaptitata*), growth inhibition test with prothioconazole/sulphur (50+625) g/l Report No. 502-3-07-29111 GLP Unpublished	N	UPL
KCP 10.3.1.1/01	Ansaloni, T.	2022a	Prothioconazole/Sulphur (50+625) g/L SC: Honey Bee (*Apis mellifera* L.) Acute Oral and Contact Toxicity Test under Laboratory Conditions Report No. S21-06043 GLP Unpublished	N	UPL
KCP 10.3.1.1/02	Ripperger, D.	2022a	Prothioconazole/Sulphur (50+625) g/L SC (FHO04): Acute Oral and Contact Toxicity to the Bumble Bee *Bombus terrestris* L. (Hymenoptera, Apidae) under Laboratory Conditions Report No. S21-06042 GLP Unpublished	N	UPL
KCP 10.3.1.2/01	Ansaloni, T.	2022b	Prothioconazole/Sulphur (50+625) g/L SC: Honey Bee (*Apis mellifera* L.) Chronic Oral Toxicity Test (10-Day Feeding) under Laboratory Conditions Report No. S21-06044 GLP Unpublished	N	UPL
KCP 10.3.1.3/01	Ansaloni, T.	2022c	Prothioconazole/Sulphur (50+625) g/L SC: Honey Bee (*Apis mellifera* L.) Larval Toxicity Test following Repeated Exposure under laboratory conditions Report No. S21-06046 GLP Unpublished	N	UPL
KCP 10.3.2.1/01	Leopold, J.	2022a	Prothioconazole/Sulphur (50+625) g/L SC: Effects on the Predatory Mite *Typhlodromus pyri* (Acari: Phytoseiidae) in the Laboratory. A Dose Response Test on Glass Plates	N	UPL

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. 163391063 GLP Unpublished		
KCP 10.3.2.1/02	Leopold, J.	2022b	Prothioconazole/Sulphur (50+625) g/L SC: Effects on the Parasitoid *Aphidius rhopalosiphi* (Hymenoptera: Braconidae) in the Laboratory. A Dose Response Test on Glass Plates Report No. 163391001 GLP Unpublished	N	UPL
KCP 10.3.2.2/01	Leopold, J.	2022c	Prothioconazole/Sulphur (50+625) g/L SC: Effects on the Predatory Mite *Typhlodromus pyri* (Acari: Phytoseiidae), Extended Laboratory Study - Dose Response Test Report No. 163391062 GLP Unpublished	N	UPL
KCP 10.3.2.2/02	Leopold, J.	2022d	Prothioconazole/Sulphur (50+625) g/L SC: Effects on the Parasitoid *Aphidius rhopalosiphi* (Hymenoptera, Braconidae), Extended Laboratory Study - Dose Response Testt Report No. 163391002 GLP Unpublished	N	UPL
KCP 10.3.2.2/03	Leopold, J.	2022e	Prothioconazole/Sulphur (50+625) g/L SC: Effects on the Lacewing *Chrysoperla carnea* (Neuroptera: Chrysopidae), , Extended Laboratory Study - Dose Response Test Report No. 163391047 GLP Unpublished	N	UPL
KCP 10.3.2.2/04	Leopold, J.	2022f	Prothioconazole/Sulphur (50+625) g/L SC: Effects on the Ladybird Beetle *Coccinella septempunctata* (Coleoptera, Coccinellidae), Extended Laboratory Study - Dose Response Test Report No. 163391012 GLP Unpublished	N	UPL
KCP 10.3.2.2/05	Fallowfield, L.	2022	Prothioconazole/Sulphur (50+625) SC – A Series of Aged-Residue Extended Laboratory Tests to Determine Effects on the Predatory Mite *Typhlodromus pyri* (Acari: Phytoseiidae) Report No. UPL-22-04 GLP Unpublished	N	UPL
KCP	Fallowfield, L.	2023	Prothioconazole/Sulphur (50+625) SC – A Series of Aged-Residue Extended Laboratory Tests to Determine	N	UPL

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
10.3.2.2/06			Effects on the Predatory Mite *Typhlodromus pyri* (Acari: Phytoseiidae) Report No. UPL-23-01 GLP Unpublished		
KCP 10.3.2.2/07	Stevens, J.	2022	Prothioconazole/Sulphur (50+625) SC – A Series of Aged-Residue Extended Laboratory Study on the Parasitic Wasp *Aphidius rhopalosiphi* (Hymenoptera, Brconidae) Report No. UPL-22-03 GLP Unpublished	N	UPL
KCP 10.3.2.2/08	White-Hall, C.	2022	Prothioconazole/Sulphur (50+625) SC – A Series of Aged-Residue Extended Laboratory Tests to Determine Effects on the Ladybird Beetle *Coccinella septempunctata* (Coleoptera: Coccinellidae) Report No. UPL-22-05 GLP Unpublished	N	UPL
KCP 10.4.1/01	Rana, J.R.	2022	Reproduction toxicity test of prothioconazole/sulphur (50+625) g/l sc to earthworm, *Eisenia fetida* 522-3-08-29113 GLP Unpublished	N	UPL
KCP 10.4.1.2/01	Vollmer, T.	2023	A Field Study to Evaluate the Effects of Metabolites of Prothioconazole on Earthworm Populations Report No S21-03781 GLP Unpublished	N	UPL
KCP 10.4.2/01	Hübner, S.	2022a	Effects on reproduction of collembola (*Folsomia candida*) in artificial soil 163391016 GLP Unpublished	N	UPL
KCP 10.4.2/02	Hübner, S.	2022b	Effects on reproduction of the predatory mite (*Hypoaspis aculeifer*) in artificial soil 163391089 GLP Upublished	N	UPL
KCP 10.5/01	Bhosale, J.D.	2022	Effect of prothioconazole/sulphur (50+625) g/l sc on soil microorganisms: nitrogen transformation test UPL report No.: 608-3-15-29110 GLP Unpublished	N	UPL

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 10.6.2/01	Ripperger, D	2022b	Prothioconazole/Sulphur (50+625) g/L SC (FHO04): Effects on the Seedling Emergence and Seedling Growth of Terrestrial Plant Species UPL report No.: S21-05533 GLP Unpublished	N	UPL
KCP 10.6.2/02	Ripperger, D	2023	Prothioconazole/Sulphur (50+625) g/L SC (FHO04): Effects on the Vegetative Vigour of Terrestrial Plant Species UPL report No.: S21-05534 GLP Unpublished	N	UPL

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

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

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

A 2.2.1.1 Study 1

Comments of zRMS	The study was conducted in line with OECD 202 with no deviations. Prothioconazole and sulphur in the test media was found to be stable up to 48 h (>80% of the nominal concentration). All the validity criteria were met and the study is considered acceptable with the following endpoint relevant for the risk assessment: 48 h EC_{50} =37.584 mg/L (based on nominal concentration)

Reference:	KCP 10.2.1/01
Report	ACUTE IMMOBILISATION STUDY OF PROTHIOCONAZOLE/SULPHUR (50+625) g/L SC TO *Daphnia magna I*, P. D. Singh, 2023a, Report No. 502-3-07-29112
Guideline(s):	Yes – OCSPP 850.1010 and OECD 202
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive summary

This study was performed to assess the immobilisation (EC_{50}) at 24 and 48 hours by FHO04 in *Daphnia magna*. For this purpose, daphnids were exposed to nominal concentrations of 7.5, 14.3, 27.1, 51.4 and 97.7 mg FHO04/L, each test treatment was replicated 4 times and contained 5 daphnids per replicate. The tested concentrations were selected by evaluating the results of the preliminary range finding study.
The test media was analysed for the test item stability and a.i. concentration. The concentrations selected were found to be stable within the guidelines limit (48 hours concentration).

Table 1: Endpoints for exposure of *Daphnia magna* to formulation FHO04

Exposure Time (h)	EC_{50} Value (mg/L)	95% Confidence Interval (mg/L)		Regression Equation (y = a + bx)	NOEC (mg/L)	LOEC (mg/L)
		Lower Limit	Upper Limit			
24	57.280	43.551	75.336	y = -0.064 + 2.880x	7.5	14.3
48	37.584	27.861	50.699	y = 0.322 + 2.970x		

Materials and methods

1. ***Test Item*:** FHO04
   - **Description:** Liquid
   - **Lot/Batch #:** 028421
   - **Purity:** Prothioconazole: 53.14 ± 0.64 g/L (39.13 ± 0.47 g/Kg)
     Sulphur: 638.0 ± 7.01 g/L (469.8 ± 5.16 g/Kg)
   - **CAS #:** Prothioconazole: 178928–70–6
     Sulphur: 7704-34-9
   - **Stability of test compound:** N/A - Stable
2. ***Vehicle and/or positive control*:** Vehicle: Reconstituted water
   Positive control: Potassium dichromate
3. ***Test system* -**
   - **Species:** *Daphnia magna*
   - **Age:** Less than 24 hours old
   - **Source:** Laboratory of Ecotoxicology, Jai Research Foundation
   - **Acclimation period:** 48 hr
   - **Water:** Reconstituted water
   - **Housing:** 120 mL in test vessel of 250 mL capacity
   - **Loading:** 20 daphnids per group, each consisting of 4 replicates of 5 daphnids per replicate
4. ***Environmental conditions* -**
   - **Temperature:** 20.1 to 20.7 °C
   - **pH:** 7.32 to 7.72
   - **Dissolved oxygen:** 96.7 to 191.8 %
   - **Hardness:** 213 mg/L as $CaCO_3$
   - **Photoperiod:** 16:8 light:dark hours, 1020 to 1050 Lux
   - **In life dates** February 12, 2022 to December 22, 2022

Study design

First instar daphnids (less than 24 h old neonates) were separated from the adults in a culture of daphnids derived from a single mother. This culture was previously acclimatised to the study conditions and was immediately transferred to labelled glass beakers (120 mL for each replicate). The mobility of the test organisms was verified immediately after the introduction by gentle swirling of the test container with visual inspection.

The test concentrations in the main study were based on the findings from a preliminary range finding study. The stock solution A (10.0 mg/mL) was prepared by mixing 200 mg test item in 20 mL of reconstituted water. Volumes of 450, 858, 1626, 3084 and 5867 µL were taken using micropipette from the stock solution A and made up to 598 mL with reconstituted water to obtain the nominal test concentrations of 7.5, 14.3, 27.1, 51.4 and 97.7 mg/L. The test solutions were prepared freshly prior to the exposure. For the control group reconstituted water was used.

All test daphnids were observed for immobility and abnormal behaviour at 0, 3, 24 and 48 hours of exposure.
Temperature, pH and dissolved oxygen were measured during the exposure while total hardness was measured prior to the treatment.
For determination of the stability and a.i. concentration of FHO04 in the test media sampling was carried out at 0 and 48 hours during the main study for all the test concentrations. Active ingredient concentration in water was determined using a validated analytical method.

Statistical analyses

The EC_{50} for FHO04 at 24 h intervals was calculated using the Probit of analysis method. [Statistical software package not stated]

Results and discussions

Analysis of test concentrations

Prothioconazole and sulphur in the test media was found to be stable up to 48 h (>80% of the nominal concentration). The test media was analysed for concentration and stability at 0 and 48 h during the main study and was within the acceptable limit (>80% of the nominal concentration).

Immobility

Percentage immobilisation observed at 48 hours was 0, 0, 5, 15, 30 and 20 at the test concentrations of 0 (control), 7.5, 14.3, 27.1, 51.4 and 97.7 mg FHO04 /L, respectively.

Table 2: Summary of the effects of FHO04 on *Daphnia magna* immobility at 24 and 48 h exposure period

Group	Test Conc. (mg/L)	N° of Replicate	N° of Daphnia /Group	Immobility N° and % at								Cumulative immobility at 48 h (%)
				0 h	%	3 h	%	24 h	%	48 h	%	
G1	0.0 (Control)	4	20	0	0	0	0	0	0	0	0	0
G2	7.5	4	20	0	0	0	0	0	0	0	0	0
G3	14.3	4	20	0	0	0	0	1	5	1	5	10
G4	27.1	4	20	0	0	0	0	4	20	3	15	35
G5	51.4	4	20	0	0	0	0	7	35	6	30	65
G6	97.7	4	20	0	0	0	0	16	80	4	20	100

Key: h = Hour, Conc. = Concentration

Other effects (behavioural observation)

Observations for signs of toxicity at 48 hours showed daphnids on the bottom at 14.3, 27.1, 51.4 and 97.7 mg/L. At concentration of 97.7 mg/L daphnids were observed complete immobilisation. No abnormal behaviour was observed in 0 (control) and 7.5 mg/L groups.

Table 3: Summary of the effects of FHO04 on *Daphnia magna* behaviour at 24 and 48 h exposure period

Group	Test Conc. (mg/L)	Behavioural Symptoms Observed at															
		0 h				3 h				24 h				48 h			
		R1	R2	R3	R4	R1	R2	R3	R4	R1	R2	R3	R4	R1	R2	R3	R4
G1	0.0 (Control)	1(5)	1(5)	1(5)	1(5)	1(5)	1(5)	1(5)	1(5)	1(5)	1(5)	1(5)	1(5)	1(5)	1(5)	1(5)	1(5)
G2	7.5	1(5)	1(5)	1(5)	1(5)	1(5)	1(5)	1(5)	1(5)	1(5)	1(5)	1(5)	1(5)	1(5)	1(5)	1(5)	1(5)
G3	14.3	1(5)	1(5)	1(5)	1(5)	1(5)	1(5)	1(5)	1(5)	1(5)	1(5)	1(5)	2(1), 1(4)	2(1), 1(4)	1(5)	1(5)	1(4)
G4	27.1	1(5)	1(5)	1(5)	1(5)	4(2), 1(3)	4(1), 1(4)	4(1), 1(4)	4(1), 1(4)	2(1), 1(4)	4(2), 1(3)	2(2), 1(3)	2(1), 1(4)	1(4)	2(2), 1(3)	1(3)	2(1), 1(3)
G5	51.4	1(5)	1(5)	1(5)	1(5)	4(2), 1(3)	4(2), 1(3)	4(3), 1(2)	4(2), 1(3)	4(2), 1(3)	2(2), 1(3)	2(4), 4(1)	2(1), 1(4)	2(2), 1(3)	2(1), 1(2)	2(1)	2(2), 1(2)
G6	97.7	1(5)	1(5)	1(5)	1(5)	4(4), 1(1)	4(3), 1(2)	4(3), 1(2)	4(4), 1(1)	2(5)	2(5)	2(3), 4(2)	2(3), 4(2)	*	*	2(2)	2(2)

Key: h = Hour, R = Replicate, Conc. = Concentration, * = Data not available, due to immobilisation

Behavioral symptom: 1 = Normal, 2 = Immobilisation, and 4 = Lethargy

Validity

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. The dissolved oxygen concentration was ≥8.35 mg/L in the control and test vessels, at the end of the test. Thus, validity criteria were met.

Conclusion

Results of this study indicate that Prothioconazole/Sulphur (50+625) g/L SC exposure caused concentration dependent toxicity to *Daphnia magna*.

Table 4: Endpoints for *Daphnia magna* exposed to FHO04

Exposure Time (h)	EC_{50} Value (mg/L)	95% Confidence Interval (mg/L)		Regression Equation (y = a + bx)	NOEC (mg/L)	LOEC (mg/L)
		Lower Limit	Upper Limit			
24	57.280	43.551	75.336	y = -0.064 + 2.880x	7.5	14.3
48	37.584	27.861	50.699	y = 0.322 + 2.970x		

A 2.2.1.2 Study 2

Comments of zRMS:	The study was conducted in line with OECD 202 with no deviations. Prothioconazole and sulphur in the test media was found to be stable up to 48 h (>80% of the nominal concentration). All the validity criteria were met and the study is considered acceptable with the following endpoint relevant for the risk assessment: 72 h E_rC_{50} = 26.363 mg/L (based on nominal concentration) 72 h NOE_rC= 0.6 mg/L (based on nominal concentration)

Reference:	KCP 10.2.1/02
Report	ALGA (*Pseudokirchneriella subcapitata*), GROWTH INHIBITION TEST WITH PROTHIOCONAZOLE/SULPHUR (50+625) g/L SC, P. D. Singh, 2023b, Report No. 501-3-07-29111
Guideline(s):	Yes – OCSPP 850.4500 and OECD 201
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive summary

This study was performed to assess the algae *Pseudokirchneriella subcaptata* growth inhibition caused by FHO04. For this purpose, an exponentially growing culture of algae (10556 cells/mL) was exposed to test concentrations of 0.0 (control), 0.2, 0.6, 1.7, 5.0, 15.0 and 45.0 mg FHO04/L to assess the algal growth at 24, 48, 72 and 96 hours.

Table 1: Formulation endpoints, 72 h exposure

Exposure Time (72 h)	I_bC_x Value for Biomass Inhibition	Concentr-ation (mg/L)	Concentr-ation (mg a.s./L)		NOEC (mg/L)	NOEC (mg a.s./L)		LOEC (mg/L)	LOEC (mg a.s./L)	
			Prothioc-onazole	Sulphur		Prothioc-onazole	Sulphur		Prothioc-onazole	Sulphur
	I_bC_{50}	8.395	0.328	3.944	0.6	0.023	0.282	1.7	0.067	0.799
	I_rC_{50}	26.363	1.032	12.385						
	I_yC_{50}	8.395	0.328	3.944						

Table 2: Formulation endpoints, 96 h exposure

Exposure Time (96 h)	I_bC_x Value for Biomass Inhibition	Concentr-ation (mg/L)	Concentr-ation (mg a.s./L)		NOEC (mg/L)	NOEC (mg a.s./L)		LOEC (mg/L)	LOEC (mg a.s./L)	
			Prothio-onazole	Sulphur		Prothio-onazole	Sulphur		Prothio-onazole	Sulphur
	I_bC_{50}	10.093	0.395	4.742	0.6	0.023	0.282	1.7	0.067	0.799
	I_rC_{50}	30.061	1.176	14.123						
	I_yC_{50}	12.134	0.475	5.701						

Materials and methods

1. ***Test Item*:** FHO04
   - **Description:** Liquid
   - **Lot/Batch #:** 028421
   - **Purity:** Prothioconazole: 53.14 ± 0.64 g/L (39.13 ± 0.47 g/kg)
     Sulphur: 638.0 ± 7.01 g/L (469.8 ± 5.16 g/kg
   - **CAS #:** Prothioconazole: 178928–70–6
     Sulphur: 7704-34-9
   - **Stability of test compound:** N/A - Stable
2. ***Vehicle and/or positive control*:** Vehicle: AAP medium (aqueous culture medium)
   Positive control: Potassium dichromate
3. ***Test system* -**
   - **Species:** A unicellular freshwater green alga *Pseudokirchneriella subcapitata* (Strain ATCC 22662)
   - **Age:** N/A

Source:	American Type Culture Collection, (ATCC), Manassas, USA.
Acclimation period:	The pre-culture was prepared 3 days prior to commencement of the study by transferring 3.65 mL from the latest sub-culture into a new culture vessel.
Water:	Distilled water
Housing:	In replicates, 250 mL capacity conical flasks
Loading:	0.75 mL, 10^4 cells/mL

4. ***Environmental conditions*** -

Temperature:	22.2 to 22.3 °C
pH:	7.25 to 7.97
Dissolved oxygen:	N/A
Hardness:	N/A
Photoperiod:	Continuous illumination, 4323 – 4347 (± 15% of the mean value) Lux
In life dates	February 12, 2022 to December 27, 2022

Study design

The cell concentration of the algal stock culture was 1900000 cells/mL. Each replicate was inoculated with 0.75 mL of algal culture to obtain the required cell concentration (approximately 1 x 10^4 cells/mL).

A quantity of 100 mg of the test item was mixed with 10 mL of algae culture medium to obtain the nominal concentration of 10 mg/mL (stock solution A). 1 mL of stock solution A was diluted with 10 mL of algae culture medium to obtain the nominal concentration of 1.0 mg/mL (stock solution B). A volume of 135 µL was taken from the test material stock solution (B**)** and transferred using a micropipette to each of the four replicates of each test concentration to obtain the nominal concentration of 0.2 mg FHO04. The volumes of 40.5, 114.8, 337.5, 1012.5 and 3037.5 µL were taken from the test material stock solution (A**)** and transferred using a micropipette to each of the four replicates of each test concentration to obtain the nominal concentrations of 0.6, 1.7, 5.0. 15.0 and 45 mg FHO04. The control group consisted of four replicates without the test item. Final volume was made up with sterilised culture medium to 135 mL in a 250 mL labelled conical flask.

The cells in the culture flasks were maintained in suspension by agitating the test flasks continuously at 100 rpm using an orbital shaker throughout the exposure period.

A volume of 10 mL of the test culture was collected from all replicate flasks at 24, 48, 72 and 96 hours. The cell concentration of each replicate was determined at regular intervals of 24 hours using a haemocytometer and microscope.

For determination of the stability and a.i. concentration of FHO04 in the test media, sampling was carried out at 0, 72 and 96 hours during the main study for all the test concentrations. Prothioconazole and sulphur concentrations in water were determined using a validated analytical method.

Statistical analyses

NOEC and LOEC were calculated with Brown-Forsythe test and ANOVA test. The IC_{50} (72 - 96 h) with its associated 95% confidence interval for FHO04 at 24 h intervals was calculated using the Probit analysis method. [Statistical software package not stated]

Results and discussions

Analysis of test concentrations

The stability test of FHO04 in the test media was performed during the method validation study and the test item in the test media was stable up to 96 h (>80 % of nominal concentration). The test media was analysed for active substance concentration and stability at 0, 72, and 96 h, during the main study and was within the acceptable limit (>80 % of nominal concentration).

Growth rate

In the untreated control the number of cells increased by a factor of 446.4 times over the 96 hour exposure period, therefore, the criterion of increase of biomass by at least a factor of 16 within three days was fulfilled.

In the treated groups increases in cell numbers decreased with increasing concentration of FHO04 during the 96 h exposure period.

The coefficient of variation of average specific growth rate during the whole test period in replicate control culture was 0.65 % (Coefficient of Variation of Average Growth between control replicates should not exceed 7 %). The mean coefficient of variation for days 0-1, 1-2, 2-3 and 3-4 in control culture was 4.42 %, respectively (Coefficient of Variation of Sectional (Daily) Growth Rate in control culture should not exceed 35 %).

Table 3: Mean Values of Algal Cell Count and Percent Inhibition of Yield

Nominal Concentration (mg FHO04)		Mean Number of Cells Counted/mL at					Percent Inhibition of Yield (%)	Percent Inhibition of growth rate (%)
	0 h	24 h	48 h	72 h	96 h	Biomass		
0 (Control)	10556	49375	2,33,750	10,53,750	47,12,500		-	
0.2		48750	234375	1055000	47,12,500	-0.03	0.00	-0.016
0.6		46875	233125	1061875	46,87,500	0.21	0.53	0.00
1.7		45625	205625	842500	39,56,250	16.99	16.08	2.83
5.0		36250	143750	68175	35,06,250	29.49	25.65	4.88
15.0		25625	114375	560000	29,06,250	42.12	38.41	8.03
45.0		12500	19375	28125	43,750	98.77	99.29	76.69

Based on the result of recovery phase, it can be concluded that Prothioconazole/Sulphur (50+625) g/L SC has algistatic effect on *Pseudokirchneriella subcapitata*.

Table 4: Algal cell count (recovery phase)

Group	Nominal Concentrations (mg/L)		Cell count at (cells/mL)			Increase of cell compare to 0 h
	Exposure Period	Recovery Phase	Day 0	Day 2	Day 4	
G1	Control (0.0)	Control (0.0)	78542	1730000	8225000	105
G7	45.0	0.75	729	15000	75000	103

Key: G = Group, h = Hour

Validity

The cell concentration in the control cultures increased exponentially by a factor of 99.8 within the test period of 72 h. The coefficient of variation of average specific growth rates during the test period of 72 h

in the replicate control cultures was 0.65%. The mean coefficients of variation for Day 0-1, 1-2, and 2-3 in control culture was 5.02%. Thus, validity criteria were met.

Conclusion

At 0.6 mg/L of FHO04 no significant effects on algal growth parameters were apparent. Therefore, this concentration was considered to be the NOEC in the present study. The lowest concentration exhibiting significant impact on algal growth parameters was 1.7 mg/L of FHO04 and was considered as a LOEC for the study.

Table 5: Results for *Pseudokirchneriella subcapitata* after 72 hr exposure to FHO04

Exposure Time (72 h)	I_bC_x Value for Biomass Inhibition	Concentration (mg/L)	95% Confidence Interval (mg/L)		Regression Equation (y = a + bx)	NOEC	LOEC
			Lower Limit	Upper Limit			
	I_bC_{05}	0.776	0.122	4.932	y = 3.530 + 1.591x	0.6	1.7
	I_bC_{10}	1.312	0.290	5.943			
	I_bC_{20}	2.483	0.793	7.780			
	I_bC_{50}	8.395	3.846	18.323			
Exposure Time (72 h)	I_rC_x Value for Growth Rate Inhibition	Concentration (mg/L)	95% Confidence Limits (mg/L)		Regression Equation (y = a + bx)		
			Lower Limit	Upper Limit			
	I_rC_{05}	3.451	1.045	11.402	y = 2.352 + 1.864x		
	I_rC_{10}	5.408	2.075	14.093			
	I_rC_{20}	9.311	4.375	19.815			
	I_rC_{50}	26.363	12.078	57.544			
Exposure Time (72 h)	I_yC_x Value for Yield Inhibition	Concentration (mg/L)	95% Confidence Limits (mg/L)		Regression Equation (y = a + bx)		
			Lower Limit	Upper Limit			
	I_yC_{05}	0.713	0.080	6.324	y = 3.581 + 1.535x		
	I_yC_{10}	1.230	0.208	7.278			
	I_yC_{20}	2.377	0.628	8.995			
	I_yC_{50}	8.395	3.404	20.701			

Key: h = Hour, y = Expected Response, x = Log concentration, a = Intercept, b =Slope

Table 6: Results for *Pseudokirchneriella subcapitata* after 96 hr exposure to FHO04

Exposure Time (96 h)	I_bC_x Value for Biomass Inhibition	Concentration (mg/L)	95% Confidence Interval (mg/L)		Regression Equation (y = a + bx)	NOEC	LOEC
			Lower Limit	Upper Limit			
	I_bC_{05}	0.998	0.134	7.430	y = 3.356 + 1.637x	0.6	1.7
	I_bC_{10}	1.663	0.325	8.511			
	I_bC_{20}	3.090	0.906	10.544			
	I_bC_{50}	10.093	3.963	25.704			
Exposure Time (96 h)	E_rC_x Value for Growth Rate Inhibition	Concentration (mg/L)	95% Confidence Limits (mg/L)		Regression Equation (y = a + bx)		
			Lower Limit	Upper Limit			
	I_rC_{05}	5.433	1.959	15.066	y = 1.727 + 2.214x		
	I_rC_{10}	7.925	3.451	18.197			
	I_rC_{20}	12.531	6.324	24.831			
	I_rC_{50}	30.061	14.322	63.096			
Exposure Time (96 h)	E_yC_x Value for Yield Inhibition	Concentration (mg/L)	95% Confidence Limits (mg/L)		Regression Equation (y = a + bx)		
			Lower Limit	Upper Limit			
	I_yC_{05}	1.127	0.151	8.395	y = 3.272 + 1.594x		
	I_yC_{10}	1.905	0.386	9.397			
	I_yC_{20}	3.597	1.089	11.885			
	I_yC_{50}	12.134	3.981	36.983			

Key: h = Hour, y = Expected Response, x = Log (concentration x 1000), x = Log concentration, a = Intercept, b = Slope

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 Study 1

Comments of zRMS:	The study was performed fully in line with OECD 213 and 214 with no deviations. All validity criteria were met and the study is considered acceptable with following end-points relevant for the risk assessment: 48h oral LD_{50} > 2232.57 µg product/bumble bee

	48h contact LD_{50} > 2722.60 µg product/bumble bee

Reference:	KCP 10.3.1.1/01 (covers KCP 10.3.1.1.1 and KCP 10.3.1.1.2)
Report	Prothioconazole/Sulphur (50+625) g/L SC: Honey Bee (*Apis mellifera* L.) Acute Oral and Contact Toxicity Test under Laboratory Conditions, T. Ansaloni, 2022a, Report No. S21-06043
Guideline(s):	Yes – OECD 213 and OECD 214
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive summary

Oral Toxicity

The study was conducted to determine the oral acute toxicity of FHO04 to honey bees. The study was conducted with the dose levels of 1484.98, 2078.97, 2910.56, 4074.78 and 5704.69 µg FHO04/bee. The validity of the test system was checked with a positive control (dimethoate) at the dose levels of 0.062, 0.093, 0.140 and 0.210 µg dimethoate/bee) and found to be valid. The control group (untreated control) was treated with 50% sucrose solution. Bees were observed for mortalities and abnormal behavioural symptoms at 4, 24 and 48 hours post dosing.

There was no mortality recorded in the control group (50% sucrose solution) or the group treated with formulation/test substance.

Table 1: Honey bee acute oral toxicity endpoints for FHO04

Endpoints	[µg t.i./bee]	[µg a.s.1/bee]*	[µg a.s.2/bee]*
24 h Oral LD_{50} [95% CI]	> 2232.57	> 86.10	> 1052.40
48 h Oral LD_{50} [95% CI]	> 2232.57	> 86.10	> 1052.40
24 h Oral NOED	≥ 2232.57	≥ 86.10	≥ 1052.40
48 h Oral NOED	≥ 2232.57	≥ 86.10	≥ 1052.40

*Endpoint equivalences based on the actual content of the active substances certified by the non-GLP CoA and the test item density. Active substance 1 = prothioconazole, active substance 2 = sulphur

Contact Toxicity

The study was conducted to determine the contact toxicity of FHO04 to honey bees. A positive control (dimethoate) at the dose levels of 0.08, 0.12, 0.18 and 0.27 µg/bee) was included to check the validity of the study. The untreated control group was treated with 0.1% Triton X solution. For each treatment group 2 µL of test solution was applied on the dorsal side of the thorax of each bee. Each treatment group was observed for mortalities and abnormal behavioural symptoms at 4, 24 and 48 hours post dosing.

No behavioural abnormalities were observed for any of the control bees or the bees exposed to the test item in the contact test throughout the Study.

Table 2: Honey bee acute contact toxicity endpoints for FHO04

Endpoints	[µg t.i./bee]	[µg a.s.1/bee]*	[µg a.s.2/bee]*
24 h Contact LD_{50}	> 2722.60	> 105.00	> 1283.40
48 h Contact LD_{50}	> 2722.60	> 105.00	> 1283.40
24 h Contact NOED	≥ 2722.60	≥ 105.00	> 1283.40
48 h Contact NOED	≥ 2722.60	≥ 105.00	≥ 1283.40

*Endpoint equivalences based on the actual content of the active substances certified by the non-GLP CoA and the test item density. Active substance 1 = prothioconazole, active substance 2 = sulphur

Materials and methods

Oral Toxicity

1. ***Test Material*:** FHO04
   Description: Liquid
   Lot/Batch #: 028421
   Purity: Prothioconazole: 53.14 g/L
   Sulphur: 638.0 g/L
   CAS #: Prothioconazole: 178928–70–6
   Sulphur: 7704-34-9
   Stability of test compound: Not provided
2. ***Vehicle and/or positive control*:** Vehicle: 50% sucrose solution containing 0.1% xanthan gum (used as thickener in order to keep the solutions homogenous over the feeding period of 24 hours)
   Positive control: Dimethoate
3. **Test animals -**
   Species: Honey bee, *Apis mellifera* L.
   Age: Worker bees from frames without brood
   Source: Commercial apiary near Eurofins Trialcamp S.L.U. facilities (Local Government Administration number 015-V-033)
   Acclimation period: Approximately 24 hours
   Feeding: The volume of ~ 2 mL test item feeding solution was offered to the test organisms in feeders (plastic syringes, approx. 2 mL). The syringes of the control groups and the reference item group contained ~ 2 mL feeding solution. The tip of each feeder was removed so that the bees had access to the feeding solution. Every morning, the syringes of all test cages were replaced by new syringes, filled with freshly prepared feeding solutions.
   Housing: Bees were kept in cages made of stainless steel (base: 8.5 cm x 4.5 cm; height: 6.0 cm, approximately). The front side of the cages was equipped with a transparent pane to enable observation. The bottom of the cages consisted of perforated steel, which guaranteed sufficient air supply. The cages were lined with filter paper.
   Loading: The test comprised 2 control groups, 5 test item treatment groups and 4 reference item treatment group. Each treatment

group consisted of 50 test organisms (divided into 5 replicates, containing 10 test organisms each).

4. ***Environmental conditions*** **-**

Temperature:	24.2 – 25.1°C
Humidity:	53.4 – 68.3 %
Photoperiod:	Dark
In life dates:	November 23, 2021 to November 25, 2021

Contact Toxicity

1. ***Test Material*****:**	FHO04
Description:	Liquid
Lot/Batch #:	028421
Purity:	Prothioconazole: 53.14 g/L Sulphur: 638.0 g/L
CAS #:	Prothioconazole: 178928–70–6 Sulphur: 7704-34-9
Stability of test compound:	Not provided
2. ***Vehicle and/or positive control*****:**	Vehicle: 0.1% Triton-X solution Positive control: Dimethoate
3. **Test animals -**	
Species:	Honey bee, *Apis mellifera* L.
Age:	Worker bees from frames without brood
Source:	Commercial apiary near Eurofins Trialcamp S.L.U. facilities (Local Government Administration number 015-V-033)
Acclimation period:	Approximately 24 hours
Feeding:	Provided *ad libitum* with 50 % (w/v) aqueous sucrose solution.
Housing:	Bees were kept in cages made of stainless steel (base: 8.5 cm × 4.5 cm; height: 6.0 cm, approximately). The front side of the cages was equipped with a transparent pane to enable observation. The bottom of the cages consisted of perforated steel, which guaranteed sufficient air supply. The cages were lined with filter paper.
Loading:	The test comprised 1 control group, 5 test item treatment groups and 4 reference item treatment group. Each treatment group consisted of 50 test organisms (divided into 5 replicates, containing 10 test organisms each).
4. ***Environmental conditions*** **-**	
Temperature:	24.2 – 25.1°C
Humidity:	53.4 – 68.3 %
Photoperiod:	Dark
In life dates:	November 23, 2021 to November 25, 2021

Study design

Oral Toxicity

FHO04 was weighed using a calibrated balance and diluted in 50 % sucrose solution containing 0.1 % xanthan. Young adult worker bees were exposed to a 50 % aqueous sucrose and 0.1 % xanthan solution containing 5 concentrations of FHO04 (1484.98, 2078.97, 2910.56, 4074.78 and 5704.69 µg FHO04/bee) by continuous and ad libitum feeding over a period of 2 days. The control groups were fed with 50 % untreated sucrose solution and 50 % sucrose solution containing 0.1 % xanthan.

Mortality and behavioural abnormalities were assessed 4 hours after application then at 24 and 48 hours. The effects of FHO04 were evaluated by comparing the results of the test item group to those of the solvent control group. Behavioural abnormalities were categorized as the following: moribund (bees cannot walk and show only very feeble movements and weak responses to stimulation); affected (bees still upright and attempting to walk, but with reduced coordination); cramps (bees contracting abdomen or entire body); apathy (bees show delayed reactions to stimulation and are sitting motionless in the unit or are able to walk but not correctly).

The consumption of feeding solution per bee per day was calculated by dividing the total daily consumption per replicate by the number of living bees at the beginning of the respective feeding interval. For each treatment group, the mean consumption of feeding solution per bee per day was calculated by averaging the replicate values.

Contact Toxicity

Test item application solutions were prepared at concentrations of 615.90, 893.06, 1294.93, 1877.66 and 2722.60 µg FHO04/bee. The reference item solution was prepared at a concentration of 0.080, 0.120, 0.180 and 0.270 µg/bee. The untreated control group (negative control) was treated with deionised water containing 0.1 % (v/v) Triton X-100.
The honey bees were anaesthetised with CO_2 prior to the exposure/application for ease of handling. The bees were individually treated and dosed with 2 µL of the test solution on the dorsal side of the thorax.

Mortality and behavioural abnormalities were assessed 4 hours after application then at 24 and 48 hours. The effects of FHO04 were evaluated by comparing the results of the test item group to those of the solvent control group. Behavioural abnormalities were categorized as the following: moribund (bees cannot walk and show only very feeble movements and weak responses to stimulation); affected (bees still upright and attempting to walk, but with reduced coordination); cramps (bees contracting abdomen or entire body); apathy (bees show delayed reactions to stimulation and are sitting motionless in the unit or are able to walk but not correctly).

Statistical analyses
For the statistical evaluation, the statistics program ToxRatPro® Version 3.3.0. was used.
In order to determine the LD_{50} (median Lethal Dietary Dose) contact values the Trimmed Spearman-Karber procedure was used. In order to determine the LD_{50} (median Lethal Dietary Dose) oral values the Probit analysis using linear max. likelihood regression was used.

For the test item groups in the oral and contact test, the 24-h and 48-h LD_{50} values could not be calculated since no test item dose caused > 50% mortality. Accordingly, these values were empirically estimated from the results. The 24-h and 48h NOED values for the oral and contact test were determined by a Multiple Sequentially-rejective Fisher Test After Bonferroni-Holm.

Results and discussions

Oral Toxicity
Mortality
No mortality was observed in the control group at the end of the observation period after 48 hours.

In the test item treatment groups, at the nominal doses of 1484.98, 2078.97, 2910.56, 4074.78, 5704.69 µg FHO04/bee, the actual consumed doses were 1385.15, 1698.12, 2049.62, 2232.57 and 1883.99 µg FHO04/bee, and the cumulative mean mortality was 0.00, 2.00, 0.00, 0.00 and 2.00%, respectively, 48 hours after start of exposure.

Table 3: Mean mortality and actual uptake (oral toxicity test)

Treatment group (Target dose)		Actual uptake	Mortality [%]		
			4 h	24 h	48 h
Control:					
C1	–	–	0.00	0.00	0.00
C2	–	–	0.00	0.00	0.00
Test item: Prothioconazole/Sulphur (50+625) g/L SC					
Treatment	**[µg t.i./bee]**	**[µg t.i./bee]**			
T1	1484.98	1385.15	0.00	0.00	0.00
T2	2078.97	1698.12	0.00	0.00	2.00
T3	2910.56	2049.62	0.00	0.00	0.00
T4	4074.78	2232.57	0.00	0.00	0.00
T5	5704.69	1883.99	0.00	2.00	2.00

Other effects (behavioural observation)
No behavioural abnormalities were observed for any of the control bees or the bees exposed to the test item in the contact test throughout the Study.

Contact Toxicity

Mortality
No mortality was observed in the control group at the end of the observation period after 48 hours.

In the test item nominal doses of 615.90, 893.06, 1294.93, 1877.66 and 2722.60 µg FHO04/bee, the cumulative mean mortality was 0.00, 0.00, 0.00, 0.00 and 4.00%, respectively, 48 hours after the start of exposure.

Table 4: Mean mortality (contact toxicity test)

Treatment group (Target dose)		Mortality [%]		
		4 h	24 h	48 h
Control:				
C	–	0.00	0.00	0.00
Test item: Prothioconazole/Sulphur (50+625) g/L SC				
Treatment	**[µg t.i./bee]**			
T1	615.90	0.00	0.00	0.00
T2	893.06	0.00	0.00	0.00
T3	1294.93	0.00	0.00	0.00
T4	1877.66	0.00	0.00	0.00
T5	2722.60	0.00	2.00	4.00

Other effects (behavioural observation)
No behavioural abnormalities were observed for any of the control bees or the bees exposed to the test item in the contact test throughout the Study.

Validity of the test

The study is valid since it fulfils the validity criteria: The average control mortality was ≤ 10% at the end of the test. Actual 0.00% mortality in both control groups of the oral toxicity test and in the control group of the contact toxicity tests. The 24-hour LD_{50} of the reference item met the specified range of: 0.10 to 0.35 µg dimethoate/bee (actual: 0.12 µg dimethoate/bee) in the oral toxicity test, as well as the specified range of: 0.10 to 0.30 µg dimethoate/bee (actual: 0.19 µg dimethoate/bee) in the contact toxicity test.

Conclusion

The 24-h oral median Lethal Dose (LD_{50}) value with 95% confidence limits for the reference item was 0.12 [0.11 – 0.13] µg dimethoate/bee. The 24-h contact Medial Lethal Dose (LD_{50}) value with 95% confidence limits for the reference item was 0.19 [0.17 – 0.21] µg dimethoate/bee.

A 2.3.1.1.2 Study 2

Comments of zRMS:	The study was performed fully in line with OECD 246 and 247 with no deviations. All validity criteria were met and the study is considered acceptable with following end-points relevant for the risk assessment: 48h oral LD_{50} > 6261 µg product/bumble bee 48h contact LD_{50} > 2556 µg product/bumble bee

Reference:	KCP 10.3.1.1/02 (covers KCP 10.3.1.1.1 and KCP 10.3.1.1.2)
Report	Prothioconazole/Sulphur (50+625) g/L SC (FHO04): Acute Oral and Contact Toxicity to the Bumble Bee *Bombus terrestris* L. (Hymenoptera, Apidae) under Laboratory Conditions, D., Ripperger, 2022a, Report No. S21-06042.
Guideline(s):	Yes – OECD 246 and OECD 247
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive summary

Oral Toxicity

The study was conducted to determine the contact toxicity of FHO04 to bumble bees. A positive control (dimethoate) at the dose level of 1.5 µg/bee was included to check the validity of the study. The untreated control groups were treated with 50 % sucrose solution. For each treatment group 40 µL of the test solutions were provided to each replicate in 1 mL feeders. Each treatment group was observed for mortalities and abnormal behavioural symptoms at 24 and 48 hours post dosing.

No mortality was observed for the test item at the dose levels of 15.6, 31.3, 62.5, 125 and 250 µg prothioconazole/bee at the end of the 48-hour test period. No mortality was observed in the negative control group. In the reference item group 55.9 % mortality was observed at the end of the 48-hour test period.

Table 1: Endpoints for mortality (oral toxicity test)

Endpoints	Oral toxicity test		
	[µg a.s. 1/bumble bee] [a b]	[µg a.s. 2/bumble bee] [c]	[µg product/bumble bee] [a d]
24 h LD_{50} (95 % lower / upper CL)	> 245 (n.d.)	> 2942 (n.d.)	> 6261 (n.d.)
48 h LD_{50} (95 % lower / upper CL)	> 245 (n.d.)	> 2942 (n.d.)	> 6261 (n.d.)
NOED	≥ 245	≥ 2942	≥ 6261

CL: confidence limit; n.d.: not determined
[a] based on actual food consumption
[b] based on analysed content of active substance (a.s.) 1, i.e. prothioconazole 3.913 % w/w
[c] based on analysed content of active substance (a.s.) 2, i.e. sulphur 46.98 % w/w
[d] calculated considering an analysed content of 3.913 % w/w of the active substance (prothioconazole)

Contact Toxicity

The study was conducted to determine the contact toxicity of FHO04 to bumble bees. A positive control (dimethoate) at the dose level of 13 µg/bee was included to check the validity of the study. The untreated control groups were treated with deionised water containing 0.1 % (v/v) Triton X-100. For each treatment group 5 µL of test solution was applied on the dorsal side of the thorax of each bee. Each treatment group was observed for mortalities and abnormal behavioural symptoms at 24 and 48 hours post dosing.

In the test item groups no mortality were observed at the doses of 6.25, 12.5, 25.0 and 100 µg prothioconazole/bumble bee and 3.3 % mortality was observed at the dose of 50.0 µg prothioconazole/bumble bee at the end of the 48 hour test period. In the negative control no mortality was observed at the end of the 48-hour test period. In the reference item group 86.7 % mortality was observed at the end of the 48-hour test period.

Table 2: Endpoints for mortality (contact toxicity test)

Endpoints	Contact toxicity test		
	[µg a.s. 1/bumble bee] [a]	[µg a.s. 2/bumble bee] [b]	[µg product/bumble bee] [c]
24 h LD_{50} (95 % lower / upper CL)	> 100 (n.d.)	> 1201 (n.d.)	> 2556 (n.d.)
48 h LD_{50} (95 % lower / upper CL)	> 100 (n.d.)	> 1201 (n.d.)	> 2556 (n.d.)
NOED	≥ 100	≥ 1201	≥ 2556

CL: confidence limit
n.d.: not determined
[a] based on the analysed content of active substance (a.s.) 1, i.e. prothioconazole 3.913 % w/w
[b] based on the analysed content of active substance (a.s.) 2, i.e. sulphur 46.98 % w/w
[c] calculated considering an analysed content of 3.913 % w/w of the active substance (prothioconazole)

Materials and methods

Oral Toxicity

1. ***Test Material*:** FHO04
   Description: Liquid
   Lot/Batch #: 028421
   Purity: Prothioconazole: 53.14 g/L
   Sulphur: 638.0 g/L
   CAS #: Prothioconazole: 178928–70–6
   Sulphur: 7704-34-9
   Stability of test compound: Not provided
2. ***Vehicle and/or positive control*:** Vehicle: Water
   Positive control: dimethoate
3. **Test animals -**
   Species: Bumble bee, *Bombus terrestris* L.

Age:	Adult worker bees from frames without brood
Source:	Biobest Belgium, Ilse Velden 18, 2260 Westerlo, Belgium
Acclimation period:	Approximately 24 hours
Feeding:	Provided *ad libitum* with 50 % (w/v) aqueous sucrose solution.
Housing:	In both test procedures, the bumble bees were kept individually (single housing) in Nicot cages (queen bee schooling cages: slightly conical perforated plastic cylinder; base: ~ 1 cm radius, height: 7 cm)
Loading:	The test comprised one control, five test item and one reference item groups. Each treatment group consisted of 35 test organisms (divided into 35 replicates, containing 1 test organisms each

4. ***Environmental conditions*** **-**

Temperature:	24.9 to 25.3 °C
Humidity:	54.7 to 60.6 %
Photoperiod:	Dark
In life dates:	March 09, 2022 to April 13, 2022

Contact Toxicity

1. ***Test Material*****:**	FHO04
Description:	Liquid
Lot/Batch #:	028421
Purity:	Prothioconazole: 53.14 g/L Sulphur: 638.0 g/L
CAS #:	Prothioconazole: 178928–70–6 Sulphur: 7704-34-9
Stability of test compound:	Not provided
2. ***Vehicle and/or positive control*****:**	Vehicle: Water Positive control: dimethoate

3. Test animals -

Species:	Bumble bee, *Bombus terrestris* L.
Age:	Adult worker bees from frames without brood
Source:	Biobest Belgium, Ilse Velden 18, 2260 Westerlo, Belgium
Acclimation period:	Approximately 24 hours
Feeding:	Provided *ad libitum* with 50 % (w/v) aqueous sucrose solution.
Housing:	In both test procedures, the bumble bees were kept individually (single housing) in Nicot cages (queen bee schooling cages: slightly conical perforated plastic cylinder; base: ~ 1 cm radius, height: 7 cm)
Loading:	The test comprised one control, five test item and one reference item groups. Each treatment group consisted of 30 test organisms (divided into 30 replicates, containing 1 test organisms each).

4. ***Environmental conditions*** **-**

Temperature:	24.9 to 25.3 °C
Humidity:	54.7 to 60.6 %
Photoperiod:	Dark
In life dates:	March 09, 2022 to April 13, 2022

Study design

Oral Toxicity

The stock solution was prepared by mixing 15.055 mL FHO04 in 40 mL deionised water. Test item application solutions were prepared at concentrations of 15.6, 31.3, 62.5, 125 and 250 µg prothioconazole/bee. The reference item solution was prepared at a concentration of 1.50 µg/bee. The untreated control group (negative control) was treated with 50 % (w/v) aqueous sucrose solution.

40 µL of the corresponding application solutions were provided to each replicate in 1 mL feeders. The bumble bees were starved for approx. 2 hours prior to start of exposure. Each replicate was provided with the application solution for up to 4 hours, to ensure a sufficient uptake. The feeders were then removed and the bumble bees were provided *ad libitum* with 50 % (w/v) aqueous sucrose solution. The actual amount of application solutions consumed was determined by weighing the feeders before and after the exposure period using calibrated equipment.

Contact Toxicity

The stock solution was prepared by mixing 15.055 mL FHO04 in 40 mL deionised water. Test item application solutions were prepared at concentrations of 6.25, 12.5, 25.0, 50.0 and 100.0 µg prothioconazole/bee. The reference item solution was prepared at a concentration of 13.0 µg/bee. The untreated control group (negative control) was treated with deionised water containing 0.1 % (v/v) Triton X-100.

The bumble bees were anaesthetised with CO_2 prior to the exposure/application for ease of handling. The bees were individually treated and dosed with 5 µL of the test solution on the dorsal side of the thorax.

At 4, 24 and 48 h post dosing, the bees were observed for mortality and any abnormal behavioural symptoms such as bumble bees still upright and attempting to walk but showing signs of reduced coordination and bumble bees cannot walk and show only very feeble movements of legs and antennae, only weak response to stimulation; e.g. light or blowing; bumble bees may recover but usually die.

Statistical analyses

NOED values were calculated using Fisher´s Exact Binomial Test with Bonferroni Correction.
The software used to perform the statistical analyses was ToxRat Professional 3.3.0.

Results and discussions

Oral Toxicity

Mortality

No mortality was observed for the test item at the dose levels of 15.6, 31.3, 62.5, 125 and 250 µg prothioconazole/bee at the end of the 48-hour test period.

No mortality was observed in the negative control group. In the reference item group 55.9 % mortality was observed at the end of the 48-hour test period.

Table 4: Mortality and actual uptake oral toxicity test in the control, test and reference item groups

Application rates [µg a.s. 1/bumble bee] [a]		Mortality [%]	
Target	Actual [b]	24 h	48 h
0 (C)	-	0.0	0.0
15.6	15.2	0.0	0.0
31.3	30.5	0.0	0.0
62.5	62.4	0.0	0.0
125	123	0.0	0.0
250	245	0.0	0.0
1.50 (R)	1.48	52.9	55.9

C: control; R: reference item [µg dimethoate/bumble bee]
[a] based on the analysed content of active substance (a.s.) 1, i.e. prothioconazole 3.913 % w/w
[b] based on actual food consumption

Other effects (behavioural observation)
One single moribund bumble bee was observed at the target dose of 62.5 µg prothioconazole/bumble bee 24 hours after start of exposure.

Validity of the test
The study is valid since it fulfils the validity criteria, i.e. the mortality in the control should be ≤ 10 % (actual value 0.0 %) and the mortality to the reference should be ≥ 50 % (actual value 55.9 %) at the end of the test.

Contact Toxicity

Mortality
In the test item groups no mortality were observed at the doses of 6.25, 12.5, 25.0 and 100 µg prothioconazole/bumble bee and 3.3 % mortality was observed at the dose of 50.0 µg prothioconazole/bumble bee at the end of the 48 hour test period.

In the negative control no mortality was observed at the end of the 48-hour test period. In the reference item group 86.7 % mortality was observed at the end of the 48-hour test period.

Table 4: Mortality in the contact toxicity test in the control, test and reference item groups

Application rates [µg a.s. 1/bumble bee] [a]	Mortality [%]	
Target	24 h	48 h
0 (C)	0.0	0.0
6.25	0.0	0.0
12.5	0.0	0.0
25.0	0.0	0.0
50.0	3.3	3.3
100	0.0	0.0
13.0 (R)	70.0	86.7

C: control; R: reference item [µg dimethoate/bumble bee]
[a] based on the analysed content of active substance (a.s.) 1, i.e. prothioconazole 3.913 % w/w

Other effects (behavioural observation)
One single moribund bee was observed at the dose of 50.0 µg prothioconazole/bumble bee 4 hours after start of exposure.

Validity of the test
The study is valid since it fulfils the validity criteria, i.e., the mortality in the control should be ≤ 10 % (actual value 0.0 %) and the mortality to the reference should be ≥ 50 % (actual value 86.7 %) at the end of the test.

Conclusion

Oral Toxicity
The acute oral 48-hour LD_{50} could not be calculated since no mortality occurred in the oral toxicity test. Therefore, the LD_{50} was considered to be > 245 µg prothioconazole/bumble bee, > 2942 µg sulphur/bumble bee or > 6261 µg product/bumble bee, respectively.

The LOED could not be determined, Therefore, the 48-hour NOED was considered to be > 245 µg prothioconazole/bumble bee, > 2942 µg sulphur/bumble bee or > 6261 µg product/bumble bee, respectively, the highest actual dose.
Contact Toxicity
The acute contact 48-hour LD_{50} could not be calculated since mortality was below 50 % in all the test item groups. Therefore, the LD_{50} was considered to be > 100 µg prothioconazole/bumble bee, > 1201 µg sulphur/bumble bee or > 2556 µg product/bumble bee, respectively.

The LOED could not be determined, Therefore, the 48-hour NOED was considered to be > 100 µg prothioconazole/bumble bee, > 1201 µg sulphur/bumble bee or > 2556 µg product/bumble bee, respectively, the highest dose tested.

Table 5: Endpoints for mortality (oral and contact toxicity)

Endpoints	Oral toxicity test [a]	Contact toxicity test	Oral toxicity test [a]	Contact toxicity test	Oral toxicity test [a]	Contact toxicity test
	[µg a.s. 1/bumble bee] [b]		[µg a.s. 2/bumble bee] [c]		[µg product/bumble bee] [d]	
24 h LD_{50} (95 % lower / upper CL)	> 245 (n.d.)	> 100 (n.d.)	> 2942 (n.d.)	> 1201 (n.d.)	> 6261 (n.d.)	> 2556 (n.d.)
48 h LD_{50} (95 % lower / upper CL)	> 245 (n.d.)	> 100 (n.d.)	> 2942 (n.d.)	> 1201 (n.d.)	> 6261 (n.d.)	> 2556 (n.d.)
NOED	≥ 245	≥ 100	≥ 2942	≥ 1201	≥ 6261	≥ 2556

CL: confidence limit; n.d. not determined
[a] based on actual food consumption
[b] based on analysed content of active substance (a.s.) 1, i.e. prothioconazole 3.913 % w/w
[c] based on analysed content of active substance (a.s.) 2, i.e. sulphur 46.98 % w/w
[d] calculated considering an analysed content of 3.913 % w/w of the active substance (a.s.) 1, prothioconazole

A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

A 2.3.1.2.1 Study 1

Comments of zRMS:	The study was performed fully in line with OECD 245 with no deviations. All validity criteria were met and the study is considered acceptable with following endpoints relevant for the risk assessment: LDD_{50} =523.03 µg product/bee/day NOEDD = 141.94 µg product/bee/day

Reference:	KCP 10.3.1.2/01
Report	Prothioconazole/Sulphur (50+625) g/L SC: Honey Bee (*Apis mellifera* L.) Chronic Oral Toxicity Test (10-Day Feeding) under Laboratory Conditions, T. Ansaloni, 2022b, Report No. S21-06044
Guideline(s):	Yes – OECD 245
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication	No

(if vertebrate study)

Executive summary

The objectives of this study were to determine the effects of FHO04 on the honey bee *Apis mellifera* L. in a 10-day chronic feeding test in the laboratory.

Young adult worker bees were exposed to a 50 % (w/v) aqueous sucrose solution 5 concentrations of FHO04 (8000.00, 12800.00, 20480.00, 32768.00 and 52428.80 mg product/kg food) by continuous and ad libitum feeding over a period of 10 days. The control groups were fed with 50 % untreated sucrose solution and 50% sucrose solution containing 0.1 % xanthan (used as a thickener in the treatment solutions).

Mortality and behavioural abnormalities were assessed daily. The chronic effects of FHO04 were evaluated by comparing the results of the test item group to those of the solvent control group.

Table 1: Chronic toxicity endpoints for honey bee exposed to FHO04

NOEC	8000.00 mg t.i./kg feeding solution
NOEDD	141.94 µg t.i./bee/day
LC_{10}	10053.14 mg t.i./kg feeding solution
LDD_{10}[1]	219.28 µg t.i./bee/day
LC_{20}	14107.82 mg t.i./kg feeding solution
LDD_{20}[1]	310.02 µg t.i./bee/day
LC_{50}	26976.46 mg t.i./kg feeding solution
LDD_{50}	523.03 µg t.i./bee/day

[1] Not reliable due to the very spread apart 95 % confidence intervals

Materials and methods

1. *Test Material*:	FHO04
Description:	Liquid
Lot/Batch #:	028421
Purity:	Prothioconazole: 53.14 g/L Sulphur: 638.0 g/L
CAS #:	Prothioconazole: 178928–70–6 Sulphur: 7704-34-9
Stability of test compound:	Not provided
2. *Vehicle and/or positive control*:	Vehicle: 50% sucrose solution containing 0.1% xanthan gum (used as thickener in order to keep the solutions homogenous over the feeding period of 24 hours) Positive control: Reference item (dimethoate)
3. Test animals -	
Species:	Honey bee, *Apis mellifera* L.
Age:	Young adult worker bees (newly hatched; 1 to 2 days old)
Source:	Commercial apiary near Eurofins Trialcamp S.L.U. facilities (Local Government Administration number 015-V-033)
Acclimation period:	Approximately 24 hours
Feeding:	The volume of ~ 1 mL test item feeding solution was offered to the test organisms in feeders (plastic syringes, approx. 5

mL). The syringes of the control groups and the reference item group contained ~ 1 mL feeding solution. The tip of each feeder was removed so that the bees had access to the feeding solution. Every morning, the syringes of all test cages were replaced by new syringes, filled with freshly prepared feeding solutions.

Housing: The bees were kept in cages made of stainless steel (base: 8.5 cm x 4.5 cm; height: 6.0 cm, all approximate). The front side of the cages was equipped with a transparent pane to enable observation. The bottom of the cages consisted of perforated steel, which guaranteed sufficient air supply. The cages were lined with filter paper.

Loading: The test comprised 2 control groups, 5 test item treatment groups and 1 reference item treatment group. Each treatment group consisted of 50 test organisms (divided into 5 replicates, containing 10 test organisms each).

4. ***Environmental conditions*** -

Temperature: 33.0 – 33.7 °C

Humidity: 45.7 – 59.6 %

Photoperiod: Dark

In life dates: November 17, 2021 to November 27, 2021

Study design

FHO04 was weighed using a calibrated balance and diluted in 50 % sucrose solution containing 0.1 % xanthan. Young adult worker bees were exposed to a 50 % aqueous sucrose solution containing 5 concentrations of FHO04 (8000.00, 12800.00, 20480.00, 32768.00 and 52428.80 mg product/kg food) by continuous and ad libitum feeding over a period of 10 days. The control groups were fed with 50 % untreated sucrose solution and 50 % sucrose solution containing 0.1 % xanthan.

Mortality and behavioural abnormalities were assessed daily during the 10-day exposure period. The chronic effects of FHO04 were evaluated by comparing the results of the test item group to those of the solvent control group. Behavioural abnormalities were categorized as the following: moribund (bees cannot walk and show only very feeble movements and weak responses to stimulation); affected (bees still upright and attempting to walk, but with reduced coordination); cramps (bees contracting abdomen or entire body); apathy (bees show delayed reactions to stimulation and are sitting motionless in the unit or are able to walk but not correctly); vomiting.

The consumption of feeding solution per bee per day was calculated by dividing the total daily consumption per replicate by the number of living bees at the beginning of the respective feeding interval. For each treatment group, the mean consumption of feeding solution per bee per day was calculated by averaging the replicate values.

Statistical analyses

For the statistical evaluation, the statistics program ToxRatPro® Version 3.3.0 was used. In order to determine the LC_{50} (median Lethal Concentration) values a Probit analysis using linear max. likelihood regression was used. In order to determine the LDD_{50} (median Lethal Dietary Dose) Weibull analysis using linear max. likelihood regression was used. Step-down Rao-Scott-Cochran-Armitage Test Procedure was used to evaluate whether there are significant differences between the mortality data of the control and the test item treatment group and to determine the NOEC (No Observed Effect Concentration) and NOEDD (No Observed Effect Dietary Dose) based on mortality.

Results and discussions

Mortality

In the control groups, -2.1 % cumulative mortality was observed after 10 days of continuous feeding. In the reference item group, the mortality continuously increased during the test period and reached 100 % after 10 days.

In the test item treatment groups, cumulative mortalities of 2.1, 14.6, 37.5, 43.8 and 87.5 % were observed after 10 days in the respective treatment groups of 8000.00, 12800.00, 20480.00, 32768.00 and 52428.80 mg product/kg food. Mortality in the three highest concentrated treatment groups were significantly different to the solvent control group.

Table 2: Cumulative and corrected cumulative mortality in the control, the test item and reference item treatment groups

Treatment	Concentration (mg t.i./kg feeding solution)	Total number of bees dosed	Final mortality (cumulative %)	SE	Corrected mortality[a] (cumulative %)
C1	--	50	2.0	2.0	-2.1
C2	--	50	4.0	2.5	--
T1	8000.00	50	6.0	4.0	2.1
T2	12800.00	50	18.0	9.2	14.6
T3	20480.00	50	40.0	12.3	37.5
T4	32768.00	50	46.0	5.1	43.8
T5	52428.80	50	88.0	4.9	87.5
R	0.90[b]	50	100.0	0.0	100.0

t.i. = test item; SE: Standard Error

[a] Corrected for the thickener control group according to the formula of Abbott (1925), modified by Schneider-Orelli (1947)

[b] For the reference item, the value indicates the amount of active substance (dimethoate)

Table 3: Cumulative, overall mean consumption of feeding solution, feeding dose (DD) and accumulated mean uptake of test item

Treatment	10 day cumulative mortality	Abbot's tranformed mortality [1]	Overall mean consumption of feeding solution	Feeding dose [2]	Accumulated mean uptake of test item
Control(s):					
	[%]	[%]	[mg/bee/day]	-	-
C1 (0)	2.0	-2.1	21.1	-	-
C2 (0)	4.0	-	19.4	-	-
Reference item: dimethoate [mg a.s./kg feeding solution]					
	[%]	[%]	[mg/bee/day]	[µg a.s./bee/day]	[µg a.s./bee]
R (0.90)	100.0	100.0	14.7	0.0133	0.1087
Test item: PROTHIOCONAZOLE/SULPHUR (50+625) G/L SC material [mg test item/kg feeding solution]					
	[%]	[%]	[mg/bee/day]	[µg t.i./bee/day]	[µg t.i./bee]
T1 (8000.00)	6.0	2.1	17.7	141.94	1419.41
T2 (12800.00)	18.0	14.6	18.5	236.30	2362.98
T3 (20480.00)	40.0	37.5	18.8	385.81	3858.13
T4 (32768.00)	46.0	43.8	17.7	580.21	5802.11
T5 (52428.80)	88.0	87.5	15.2	794.88	7630.87

[1] Corrected for the thickener control group C2

[2] Based on actual measured consumption of feeding solution

Other effects (behavioural observation)

The bees exposed to 8000.00 µg FHO04/bee and the control group exhibited normal behaviour during the testing period of 48 h post exposure.

Symptoms of intoxication (affected bees) were observed at the highest test item concentration (T5) starting on the sixth assessment day (D6), and at treatments T3 and T4 starting on the eighth assessment day (D8). By the end of the test (D10), 3.3 % and 3.7 % of the surviving bees were affected at the treatment levels T3 and T4, respectively. No other affected bees were observed at any of the other treatment levels at this same assessment. No symptoms of intoxication were observed for the control groups throughout the study.

Validity of the test

The study is valid since it fulfils the validity criteria, i.e. the average mortality across replicates for the untreated control and solvent control groups is ≤ 15 % at the end of the test (10 days following start of exposure); when a solvent control is included, the average mortality across replicates for the solvent control should also be ≤ 15 %. The average mortality in the reference substance treated group is ≥ 50 % at the end of the test (10 days following start of exposure).

Conclusion

The NOEC for mortality after 10 days of continuous exposure was determined to be 8000.00 mg product/kg food. The corresponding NOEDD, based on the actual consumption of the respective feeding solutions, was determined to be 141.94 µg product/bee/day.

The LC_{50} and LDD_{50} after 10 days of continuous exposure were determined to be 26976.46 mg product/kg food and 523.03 µg product/bee/day, respectively.

Table 4: Summary of chronic toxicity endpoints for honey bee exposed to FHO04

	µg/bee/day		
	test item	**prothioconazole[3]**	**sulphur[3]**
NOEDD [4]	141.94	5.55	66.68
LDD_{10} [95% CI] [5]	219.28 [48.17 – 329.26] [6]	8.58 [1.88 – 12.88] [6]	103.02 [22.63 – 154.69] [6]
LDD_{20} [95% CI] [5]	310.02 [114.25 – 420.36] [6]	12.13 [4.47 – 16.45] [6]	145.65 [53.68 – 197.49] [6]
LDD_{50} [95% CI] [5]	523.03 [365.21 – 700.91]	20.47 [14.29 – 27.43]	245.72 [171.58 – 329.29]
	mg/kg feeding solution		
	test item	**prothioconazole[3]**	**sulphur[3]**
NOEC[7]	8000.00	313.05	3758.47
LC_{10} (95% CI) [8]	10053.14 [7459.56 – 12306.41]	393.39 [291.90 – 481.56]	4723.05 [3504.57 – 5781.66]
LC_{20} (95% CI) [8]	14107.82 [11365.13 – 16537.09]	552.05 [444.73 – 647.11]	6627.97 [5339.44 – 7769.27]
LC_{50} (95% CI) [8]	26976.46 [23507.52 – 31488.66]	1055.62 [919.87 – 1232.19]	12673.77 [11044.03 – 14793.64]

CI: Confidence Interval

[3] Calculated on the basis of the content declared in the GLP Certificate of Analysis (prothioconazole 53.14 g/L, sulphur 638 g/L) and the test item density (1.358 g/cm³)

[4] Step-down Rao-Scott-Cochran-Armitage Test Procedure (α = 0.05, one sided greater)

[5] Weibull analysis using linear max. likelihood regression

[6] Not reliable due to the very spread apart 95 % confidence intervals

[7] Extrapolated form the NOEDD (concentration corresponding to the NOEDD)

[8] Probit analysis using linear max. likelihood regression

A 2.3.1.2 KCP 10.3.1.3 Effects on honey bee development and other honey bee life stages

A 2.3.1.2.1 Study 1

Comments of zRMS:	The study was performed fully in line with OECD 239 with no deviations. All validity criteria were met and the study is considered acceptable with following end-points relevant for the risk assessment: 22 d NOED =13.85 µg product/larva 22 d NOEC=89.94 mg product/kg diet 22 d ED_{50} =33.22 µg product /larva/developmental period 22 d EC_{50} =215.36 mg product/kg diet.

Reference:	KCP 10.3.1.3/01
Report	Prothioconazole/Sulphur (50+625) g/L SC: Honey Bee (*Apis mellifera* L.) Larval Toxicity Test following Repeated Exposure under laboratory conditions, T. Ansaloni, 2022c, Report No. S21-06046
Guideline(s):	Yes – OECD 239
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive summary

The objectives of this study were to determine the effects of Prothioconazole/Sulphur (50+625) g/L SC on honey bee (*Apis mellifera* L.) larvae, after a repeated exposure in a 21 days in vitro test.

The larvae were exposed to a diet with five different concentrations of 24.91, 47.34, 89.94, 170.88 and 324.67 mg FHO04/kg diet. Assessment of mortality was conducted on day D8 and D15 and assessment of emergence was conducted on D22.

The 22-Day NOED was determined to be 13.85 µg t.i./larva/developmental period. The 22-Day NOEC for adult emergence was determined to be 89.94 mg t.i./kg diet.

The 22-Day ED_{50} was estimated to be 33.22 µg t.i./larva/developmental period. The 22-Day EC_{50} was estimated to be 215.36 mg t.i./kg diet.

Table 1: Summary of endpoints at emergence on day 22 (D22)

Endpoints	Concentration			Dose		
	mg t.i./kg diet	mg a.s.1/ kg diet	mg a.s.2/kg diet	µg t.i./larva	µg a.s.1/larva	µg a.s.2/larva
NOEC/NOED[a]	89.94	3.52	42.25	13.85	0.54	6.51
LOEC/LOED[a]	170.88	6.69	80.28	26.32	1.03	12.36

EC_{50} / ED_{50} [95 % CI][b]	215.36 [185.22-250.41]	8.43 [7.25-9.80]	101.18 [87.02-117.64]	33.22 [28.62-38.59]	1.30 [1.12-1.51]	15.61 [13.45-18.13]

t.i.: test item (Prothioconazole/Sulphur (50+625) g/L SC)
a.s.1: active substance 1 (prothioconazole). a.s. 2: active substance 2 (sulphur)
[a] Fisher´s Exact Binomial Test with Bonferroni Correction (α = 0.05, one sided greater)
[b] Trimmed Spearman-Karber procedure
Active substances equivalences were determined based on the density of the test item and the prothioconazole and sulphur content declared in the GLP Certificate of Analysis.

Materials and methods

1. ***Test Material*:** FHO04
   - **Description:** Liquid
   - **Lot/Batch #:** 028421
   - **Purity:** Prothioconazole: 53.14 g/L
     Sulphur: 638.0 g/L
   - **CAS #:** Prothioconazole: 178928–70–6
     Sulphur: 7704-34-9
   - **Stability of test compound:** Not provided
2. ***Vehicle and/or positive control*:** Vehicle: artificial solution (see composition below)
   Positive control: Reference item (dimethoate)
3. **Test animals -**
   - **Species:** Honey bee, *Apis mellifera* L. synchronised first instar (L1) larvae
   - **Age:** Not older than 30 hours at grafting time.
   - **Source:** Commercial beehives from the in-house Test Facility stock
   - **Acclimation period:** Approximately 3 days
   - **Feeding:** On day 1-3, synchronised honey bee larvae were fed with a standardized amount of artificial diet consisting of 50 % weight of royal jelly + 50 % weight of an aqueous solution containing 2 % weight of yeast extract, 12 % weight of glucose and 12 % weight of fructose (20 µL/larva)
   - **Housing:** Crystal polystyrene grafting cells contained in a Plexiglass desiccator.
   - **Loading:** For each treatment group, 48 larvae from three different hives were tested. Each hive corresponded to one replicate; 16 larvae from each replicate were used.
4. ***Environmental conditions* -**
   - **Temperature:** 34.0 to 34.9°C
   - **Humidity:** 33.7 to 98.0 %
   - **Photoperiod:** Dark
   - **In life dates:** October 18, 2021 to November 08, 2021

Study design

The study was conducted as a dose response test with a duration of 21 days from grafting on day 1 (D1) to the final assessment on day 22 (D22). It comprised 1 control group (C), 5 test item groups (T1 – T5) with

five different concentrations of 24.91, 47.34, 89.94, 170.88 and 324.67 mg FHO04/kg diet, equivalent to 0.97, 1.85, 3.52, 6.69 and 12.70 mg prothioconazole/kg diet and 11.70, 22.24, 42.25, 80.28 and 152.53 mg sulphur/kg diet. Based on the cumulative application volume of 140 µL/larva, the corresponding doses were 3.84, 7.29, 13.85, 26.32 and 50.00 µg FHO04./larva, equivalent to 0.15, 0.29, 0.54, 1.03 and 1.96 µg prothioconazole/larva and 1.80, 3.42, 6.51, 12.36 and 23.49 µg sulphur/larva. One reference item group (R) with 48 mg dimethoate/kg diet, equivalent to a cumulative dose of 7.39 µg dimethoate/larva, was also included in the study. Just before feeding, from day 3 until day 6, the application solutions were added to the diet using a micropipette. The volume of application solution in the diet did not exceed 10 % of the final diet volume.

Assessment of larval mortality was conducted on D4, D5 and D6 before feeding and on D7 and D8. With the assistance of a stereo microscope, larvae were recorded as dead if no respiration (movement of spiracles) was observed. On D8, during the assessment of mortality, the presence of uneaten food was qualitatively recorded. Assessment of mortality during the pupation phase was conducted on day D15 and assessment of emergence was conducted on D22. At each assessment time, dead larvae and pupae were removed for sanitary reasons.

Statistical analyses

Statistical calculations were made with MS Excel 2016 and the statistical program ToxRatPro® Version 3.3.0.
To determine the NOEC and the LOEC values, a Fisher´s Exact Binomial Test with Bonferroni Correction ($\alpha = 0.05$, one sided greater) was used. The corresponding NOED and LOED values were extrapolated.
The ED_{50}/EC_{50} values and the respective 95 % confidence intervals were determined by means of Trimmed Spearman-Karber procedure.

Results and discussions

Mortality

Mean cumulative larval mortality on day 8 (D8) of the test item treatment groups of 24.91, 47.34, 89.94, 170.88 and 324.67 mg FHO04/kg diet was 6.3, 8.3, 4.2, 25.0 and 52.1 %, respectively, compared to 4.2 % in the control group.
Mean cumulative larval mortality on day 15 (D15) of the test item treatment groups of 24.91, 47.34, 89.94, 170.88 and 324.67 mg FHO04/kg diet was 18.8, 12.5, 18.8, 35.4 and 64.6 %, respectively, compared to 10.4 % in the control group.
Mean cumulative larval mortality on day 22 (D22) of the test item treatment groups of 24.91, 47.34, 89.94, 170.88 and 324.67 mg FHO04/kg diet was 18.8, 12.5, 20.8, 37.5 and 64.6 %, respectively, compared to 10.4 % in the control group.

Table 2:The effects of prothioconazole/sulphur (50+625) g/l sc on honey bee (*Apis mellifera* L.) larvae from repeated exposure

Treatment Group	Cumulative Mortality [%]						
[mg t.i./kg diet]	D4	D5	D6	D7	D8	D15	D22
C [0]	0.0	4.2	4.2	4.2	4.2	10.4	10.4
T1 [24.91]	2.1	4.2	4.2	4.2	6.3	18.8	18.8
T2 [47.34]	2.1	6.3	6.3	6.3	8.3	12.5	12.5
T3 [89.94]	2.1	4.2	4.2	4.2	4.2	18.8	20.8
T4 [170.88]	6.3	12.5	16.7	25.0	25.0	35.4	37.5
T5 [324.67]	6.3	18.8	41.7	52.1	52.1	64.6	64.6
R [48.00] [a]	33.3	62.5	87.5	95.8	97.9	97.9	97.9

[a] For the reference item, the values indicate the amount of active substance (dimethoate)
t.i.: test item (Prothioconazole/Sulphur (50+625) g/L SC)

Table 3:The effects of prothioconazole/sulphur (50+625) g/L SC on honey bee (*Apis mellifera* L.) larvae from repeated exposure (corrected mortality)

Treatment Group [mg t.i./kg diet]	Corrected Mortality [%][a]						
	D4	D5	D6	D7	D8	D15	D22
T1 [24.91]	2.1	0.0	0.0	0.0	2.2	9.3	9.3
T2 [47.34]	2.1	2.2	2.2	2.2	4.3	2.3	2.3
T3 [89.94]	2.1	0.0	0.0	0.0	0.0	9.3	11.6
T4 [170.88]	6.3	8.7	13.0	21.7	21.7	27.9	30.2
T5 [324.67]	6.3	15.2	39.1	50.0	50.0	60.5	60.5

[a] Corrected for control mortality according to Abbott modified by Schneider-Orelli. Negative values represent lower mortality compared to the control group.
t.i.: test item (Prothioconazole/Sulphur (50+625) g/L SC)

Emergence rate on day 22 was 81.3, 87.5, 79.2, 62.5 and 35.4 %, respectively, compared to 89.6 % in the control group.

Table 4:Mortality during pupation phase (D8 – D22) and emergence rate (D22)

Treatment Group [mg t.i./kg diet]	Mortality D8-D15 [%]	Mortality D15-D22 [%]	Mortality D8-D22 [%]	Emergence D22 [%]
C [0]	6.5	0.0	6.5	89.6
T1 [24.91]	13.3	0.0	13.3	81.3
T2 [47.34]	4.5	0.0	4.5	87.5
T3 [89.94]	15.2	2.6	17.4	79.2
T4 [170.88]	13.9	3.2	16.7	62.5
T5 [324.67]	26.1	0.0	26.1	35.4

t.i.: test item (Prothioconazole/Sulphur (50+625) g/L SC)

On day 8, no individuals with presence of uneaten food were observed. At the end of the test, in the final assessment of the emergence on day 22, no emerged bees were recorded as being affected (i.e. malformation).

Validity of the test

The study is valid since it fulfils the validity criteria, i.e. for the control, the cumulative larval mortality from day 3 (D3) until day 8 (D8) was ≤ 15 % across all replicates (actual value 4.2 %) and on day 22 (D22) the adult emergence rate was ≥ 70 % across all replicates (actual value 89.6 %). For the reference, the cumulative larval mortality was ≥ 50 % across all replicates on day 8 (D8) (actual value 97.9 %).

Conclusion

The 22-Day NOED was determined to be 13.85 µg t.i./larva/developmental period. The 22-Day NOEC for adult emergence was determined to be 89.94 mg t.i./kg diet.

The 22-Day ED_{50} was estimated to be 33.22 µg t.i./larva/developmental period. The 22-Day EC_{50} was estimated to be 215.36 mg t.i./kg diet.

Table 5: Endpoints at emergence on day 22 (D22)

Endpoints	Concentration			Dose		
	mg t.i./kg diet	mg a.s.1/ kg diet	mg a.s.2/kg diet	µg t.i./larva	µg a.s.1/larva	µg a.s.2/larva
NOEC/NOED[a]	89.94	3.52	42.25	13.85	0.54	6.51
LOEC/LOED[a]	170.88	6.69	80.28	26.32	1.03	12.36
EC_{10} / ED_{10}	> 47.34	> 1.85	> 22.24	> 7.29	> 0.29	> 3.42
EC_{20} / ED_{20}	> 89.94	> 3.52	> 42.25	> 13.85	> 0.54	> 6.51
EC_{50} / ED_{50} [95 % CI][b]	215.36 [185.22-250.41]	8.43 [7.25-9.80]	101.18 [87.02-117.64]	33.22 [28.62-38.59]	1.30 [1.12-1.51]	15.61 [13.45-18.13]

t.i.: test item (Prothioconazole/Sulphur (50+625) g/L SC)
a.s.1: active substance 1 (prothioconazole). a.s. 2: active substance 2 (sulphur)
[a] Fisher´s Exact Binomial Test with Bonferroni Correction (α = 0.05, one sided greater)
[b] Trimmed Spearman-Karber procedure
Active substances equivalences were determined based on the density of the test item and the prothioconazole and sulphur content declared in the GLP Certificate of Analysis.

A 2.3.1.3 KCP 10.3.2 Effects on arthropods other than bees

A 2.3.2.1 Study 1

Comments of zRMS:	The study was performed in line with the respective guideline with no major deviations. All validity criteria were met and the study is considered acceptable with following endpoints relevant for the risk assessment: LR_{50} = 12470.2 mL product/ha ER_{50} = 271.7 mL product/ha

Reference:	KCP 10.3.2.1/01
Report	Prothioconazole/Sulphur (50+625) g/L SC: Effects on the Predatory Mite *Typhlodromus pyri* (Acari: Phytoseiidae) in the Laboratory. A Dose Response Test on Glass Plates., J. Leopold, 2022a, Report No. 163391063
Guideline(s):	Yes - Blümel *et al.* 2000
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive summary

The effect of FHO04 on the mortality and fecundity of the predatory mite *Typhlodromus pyri* was determined by exposing mites to FHO04 on treated glass surfaces for 2 days. Test organisms were exposed to freshly dried residues at rates of 253, 758-, 2273-, 6817- and 20450-mL product/ha). Dimethoate was used as a toxic standard and water was used as a control. Each test group was replicated 3 times (20 mites per replicate). Treatment groups were assessed for mortality on day 7. A reproduction test was performed on day 7.

On day 7, the corrected mortality was between 7.0 and 61.4 % for different test rates. Statistically significant difference was observed at the treatment rates of 2273, 6817- and 20450-mL product/ha. The NOER and LOER based on lethal effects were 758- and 2273-mL product/ha, respectively. The LR_{50} of FHO04 was 12470.2 mL product/ha.

The effects on reproduction were calculated between 35.0 % and 98.6 %. Reproduction was statistically significantly reduced compared to the control at all test item application rates. The NOER and LOER based on the offspring production were considered to be < 253 and < 253 mL product/ha.

Materials and methods

1. *Test Item*:	FHO04
Description:	Liquid
Lot/Batch #:	028421
Purity:	Prothioconazole: 53.14 g/L Sulphur: 638.0 g/L
CAS #:	Prothioconazole: 178928–70–6 Sulphur: 7704-34-9
Stability of test compound:	N/A
2. *Vehicle and/or positive control*:	Vehicle: water Positive Control: dimethoate
3. *Test organism*:	
Species:	Predatory Mite *Typhlodromus pyri*
Age:	No older than 1 day old
Source:	Katz Biotech AG, An der Birkenpfuhlheide 10, D-15837 Baruth
Acclimation period:	N/A
Feeding:	A mixture of pine (*Pinus* sp.) and birch (*Betula* sp.) pollen (3:1) *ad libitum* on the day of the test start and on each assessment day except for the last one resp. at least every four days.
Test unit:	Formed by 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.
Loading:	20 mites per replicate
4. *Environmental conditions* -	
Temperature:	23 - 26 °C
Humidity	64 - 72 %
Photoperiod:	16 hours light: 8 hours dark (310 - 410 lux)
In-life dates:	October 2021 to January 2022

Study design

There were 7 test groups along with the control and the reference item group. The tested concentrations were 253, 758-, 2273-, 6817- and 20450-mL product/ha A toxic reference item (dimethoate) applied at a rate of 9 mL product/ha was included, as an indicator of the relative sensitivity of the test organisms and the test system. Each group was replicated 3 times with 20 mites per replicate.

A stock solution was prepared by mixing a defined amount of test item with a defined amount of de-ionized water to get tested concentrations. All solutions were prepared with deionised water and were sprayed on

the surface to be treated and let them dry.

Healthy and undamaged predatory mite *Typhlodromus pyri* were impartially selected and introduced into each test unit at the start of the assessment.

Assessment of adult mortality was carried out after 7 days after the application. For the reproduction assessment, surviving mites from the control and from all test item groups where the corrected mortality was < 50 % were sexed and the number of eggs per female was recorded at 3 assessment days within one week.

During the exposure, temperature, relative humidity, and photoperiod were recorded.

Statistical analyses
Mortality: Step-down Cochran-Armitage Test, Fisher's Exact Binomial Test (both one-sided greater, α = 0.05), LR50 calculation by Weibull Analysis.
Reproduction: Williams t-Test, (one-sided smaller, α = 0.05); ER50 calculation by 3-parametric normal CDF.
The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ® ToxRat Solutions GmbH.

Results and discussions

Mortality data
The corrected mortality of the mites was 7.0, 5.3, 14.0, 42.1 and 61.4 at the test concentrations of control, 253, 758-, 2273-, 6817- and 20450-mL product/ha, respectively. The % corrected mortality for the reference item was 80.7 %.

Table 1: Effect of FHO04 on the mortality of predatory mite *Typhlodromus pyri* at the end of the test

Treatments	Concentrations (mL product/ha)	% Mean Mortality	% Corrected Mortality
Control	0.0	5.0 ± 5.0	-
Test Item	253	11.7 ± 2.9	7.0
	758	10.0 ± 5.0	5.3
	2273	18.3 ± 10.4	14.0
	6817	45.0 ± 5.0	42.1
	20450	63.3 ± 10.4	61.4
Reference item	9	81.7 ± 2.9	80.7

Reproduction performance
The percent reduction observed was 67.6, 35.0, 70.9 and 98.7 at the test concentrations of control, 253-, 758-, 2273- and 6817-mL product/ha, respectively.

Table 2: Effect of FHO04 on the reproduction performance of predatory mite *Typhlodromus pyri* after 7 days of exposure

Treatments	Concentrations (mL product/ha)	Reproduction (eggs/female)	Effect on reproduction (%)
Control	0.0	4.9	-
Test Item	253	1.6	67.6
	758	3.2	35.0
	2273	1.4	70.9
	6817	0.1	98.6
	20450	-	-

Validity Criteria

The study is valid since it fulfils the validity criteria. The mortality in the control should not exceed 13 % (actual value 0.0 %). The mortality in the reference should result in at least 50 % corrected mortality (actual value 80.7 %). The number of eggs per female in the control should be ≥ 4 eggs for the second week (actual value 4.9).

Conclusion

Statistically significant difference in mortality was observed at the treatment rates of 2273, 6817- and 20450-mL product/ha. The LR_{50} of FHO04 was 12470.2 mL product/ha. Reproduction was statistically significantly reduced compared to the control at all test item application rates. The ER_{50} of FHO04 was 271.7 mL product/ha.

Table 3: Endpoints for predatory mite *Typhlodromus pyri* exposed to FHO04

Endpoint for Mortality [mL product/ha]	
LR_{50}	12470.2
NOECR	758
LOER	2273
Endpoint for Reproduction [mL product/ha]	
ER_{50}	271.7
NOER	< 253
LOER	253

A 2.3.2.1

Study 2

Comments of zRMS:	The study was performed in line with the respective guideline with no major deviations. All validity criteria were met and the study is considered acceptable with following end-points relevant for the risk assessment: LR_{50} > 20450 mL product/ha ER_{50} >20450 mL product/ha

Reference:	KCP 10.3.2.1/02
Report	Prothioconazole/Sulphur (50+625) g/L SC: Effects on the Parasitoid *Aphidius rhopalosiphi* (Hymenoptera: Braconidae) in the Laboratory. A Dose Response Test on Glass Plates, J. Leopold, 2022b, Report No. 163391001
Guideline(s):	Yes – Mead-Briggs *et al*. 2000 and Mead-Briggs *et al*. 2010.
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive summary

The effect of FHO04 on the mortality and fecundity of the parasitoid wasp *Aphidius rhopalosiphi* was determined by exposing parasitoid wasps to FHO04 on treated glass surfaces for 2 days. Test organisms were exposed to freshly dried residues at rates of 253, 758-, 2273-, 6817- and 20450-mL product/ha. Dimethoate was used as a toxic standard and water was used as a control. Each test group was replicated 4 times (10 parasitoid wasps per replicate). Treatment groups were assessed for mortality after 2, 24 and 48 hours after test initiation. A reproduction test was performed on day 11-12.

On day 2, the corrected mortality was between 2.5 and 10.0 % for different test rates. The mortality of *Aphidius rhopalosiphi* was not statistically significantly increased compared to the control up to and including the highest application rate of 20450 mL product/ha. The NOER and LOER based on lethal effects was 20450 mL product/ha. The LR_{50} of FHO04 was 20450 mL product/ha.

Reproduction was not statistically significantly reduced compared to the control up to and including the application rate of 2273 mL product/ha. The NOER and LOER based on the offspring production were considered to be 2273- and 6817-mL product/ha, respectively. The ER_{50} of FHO04 was ≥ 20450 mL product/ha.

Materials and methods

1. *Test Item*:	FHO04
Description:	Liquid
Lot/Batch #:	028421
Purity:	Prothioconazole: 53.14 g/L Sulphur: 638.0 g/L
CAS #:	Prothioconazole: 178928–70–6 Sulphur: 7704-34-9
Stability of test compound:	N/A
2. *Vehicle and/or positive control*:	Vehicle: water Positive Control: dimethoate
3. *Test organism*:	
Species:	Parasitoid *Aphidius rhopalosiphi*
Age:	Not older than 2 days old
Source:	Katz Biotech AG, An der Birkenpfuhlheide 10, D-15837 Baruth
Acclimation period:	1-2 days
Feeding:	10 % fructose solution
Test unit:	Comprising 2 treated glass plates (13 cm x 13 cm) which were held apart by an untreated aluminium 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 (approximately 1 cm in diameter) covered with a cloth. The 4th side of the frame had 1 small hole (approximately 1 cm in diameter) for inserting and feeding the test organisms
Loading:	10 parasitoids per replicate
4. *Environmental conditions* -	
Temperature:	18 - 21 °C
Humidity	72 - 79 %
Photoperiod:	16 hours light:8 hours dark (770 - 1720 lux)
In-life dates:	October 2021 to January 2022

Study design

There were 5 test groups along with the control and the reference item group. The tested concentrations were 253, 758-, 2273-, 6817- and 20450-mL product/ha. A toxic reference item (dimethoate) applied at a rate of 0.3 mL product/ha was included, as an indicator of the relative sensitivity of the test organisms and the test system. Each group was replicated 4 times with 10 per replicate.

A stock solution was prepared by mixing a defined amount of test item with a defined amount of de-ionized water to get tested concentrations. All solutions were prepared with deionised water and were sprayed on the surface to be treated and let them dry.

Healthy and undamaged parasitoid *Aphidius rhopalosiphi* were impartially selected and introduced into each test unit at the start of the assessment.
Assessment of adult mortality and offspring production were carried out 2, 24 and 48 hours after the application. The number of parasitoids alive, affected, moribund and dead was recorded. Moribund parasitoids were counted as dead.
During the exposure, temperature, relative humidity, and photoperiod were recorded.

Statistical analyses
Data for mortality were analysed for significance using the Chi^2 2x2 Table Test with Bonferroni Correction and the two-sample comparison between the reference item and control was analysed using the Fisher´s Exact Binomial Test. Data for fecundity were analysed using 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 a linear trend was revealed, the Williams 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.

Results and discussions

Mortality data
The corrected mortality of the parasitoids was 5.0, 2.5, 2.5, 7.5 and 10 & at the test concentrations of control, 253, 758-, 2273-, 6817- and 20450-mL product/ha, respectively. The % corrected mortality for the reference item was 100.0 %.

Table 1: Effect of FHO4 on the mortality of parasitoid *Aphidius rhopalosiphi* at the end of the test

Treatments	Concentrations (mL product/ha)	% Mean Mortality	% Corrected Mortality
Control	0.0	0.0 ± 0.0	-
Test Item	253	5.0 ± 5.8	5.0
	758	2.5 ± 5.0	2.5
	2273	2.5 ± 5.0	2.5
	6817	7.5 ± 9.6	7.5
	20450	10.0 ± 8.2	10.0
Reference item	0.3	100.0 ± 0.0	100.0

Reproduction performance

The percent reduction observed was -8.5, 19.1, 7.0, 22.7 and 34.2 at the test concentrations of control, 253, 758-, 2273-, 6817- and 20450-mL product/ha, respectively.
Reproduction was not affected up to and including the test item application rate of 2273 mL product/ha.

Table 2: Effect of FHO04 on the reproduction performance of parasitoid *Aphidius rhopalosiphi* after 2 days of exposure

Treatments	Concentrations (mL product/ha)	Parasitisation rate (mummies per female)	Reduction of parasitisation efficiency (%)
Control	0.0	33.5 ± 16.8	-

Test Item	253	36.3 ± 11.4	-8.5
	758	27.1 ± 11.1	19.1
	2273	31.1 ± 13.1	7.0
	6817	25.8 ± 13.1	22.7
	20450	22.0 ± 9.9	34.2

Validity Criteria

The study is valid since it fulfils the validity criteria. The mortality in the control should not exceed 13 % (actual value 0.0 %). The mortality in the reference should result in at least 50 % corrected mortality (actual value 100 %). The reproduction rate in the control should be ≥ 5 mummies per female (actual value 33.5) and there should be no more than 2 parasitoids producing zero values (actual value – no parasitoids produced zero values).

Conclusion

The mortality of *Aphidius rhopalosiphi* was not statistically significantly increased compared to the control up to and including the highest application rate of 20450 mL product/ha. The LR_{50} of FHO04 was > 20450 mL product/ha.

Reproduction was not statistically significantly reduced compared to the control up to and including the application rate of 2273 mL product/ha. The ER_{50} of FHO04 was > 20450 mL product/ha.

Table 3: Endpoints for parasitoid *Aphidius rhopalosiphi* exposed to FHO04

Endpoint for Mortality [mL product/ha]	
LR_{50}	20450
NOER	20450
LOER	20450
Endpoint for Reproduction [mL product/ha]	
ER_{50}	> 20450
NOER	2273
LOER	6817

A 2.3.2.2 Study 1

Comments of zRMS:	The study was performed in line with the respective guideline with no major deviations. All validity criteria were met and the study is considered acceptable with following endpoints relevant for the risk assessment: LR_{50} = 13558.7 mL product/ha ER_{50} = 892.6 mL product/ha

Reference:	KCP 10.3.2.2/01
Report	Prothioconazole/Sulphur (50+625) g/L SC: Effects on the Predatory Mite *Typhlodromus pyri* (Acari: Phytoseiidae), Extended Laboratory Study - Dose Response Test -, J. Leopold, 2022c, Report No.163391062.
Guideline(s):	Yes - Blümel *et al.* 2000 and Oomen 1988
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Duplication (if vertebrate study)	No

Executive summary

The effect of FHO04 on the mortality and fecundity of the predatory mite *Typhlodromus pyri* was determined by exposing mites to FHO04 on bean plants (*Phaseolus vulgaris* L. `Maxi´, 18 days old) for 7 days. Test organisms were exposed to freshly dried residues at rates of 436, 1091-, 2727-, 6817- and 17041-mL product/ha. Dimethoate was used as a toxic standard and water was used as a control. Each test group was replicated 6 times (10 mites per replicate). Treatment groups were assessed for mortality on day 7. A reproduction test was performed on day 7.

On day 7, the corrected mortality was between 10.5 and 56.1 % for different test rates. At all test item rates, mortality was statistically significantly increased compared to the control. The NOER and LOER based on lethal effects were < 436- and 436-mL product/ha, respectively. The LR_{50} of FHO04 was 13558.7 mL product/ha.

The effects on reproduction were calculated between 28.0 % and 95.3 %. Reproduction was statistically significantly reduced compared to the control up to and including the application rate of 6817 mL product/ha. The NOER and LOER based on the offspring production were considered to be < 436 and 436 product/ha.

Materials and methods

1. *Test Item*:	FHO04
Description:	Liquid
Lot/Batch #:	028421
Purity:	Prothioconazole: 53.14 g/L Sulphur: 638.0 g/L
CAS #:	Prothioconazole: 178928–70–6 Sulphur: 7704-34-9
Stability of test compound:	N/A
2. *Vehicle and/or positive control*:	Vehicle: water Positive Control: dimethoate
3. *Test organism*:	
Species:	Predatory Mite *Typhlodromus pyri*
Age:	No older than 1 day old
Source:	Katz Biotech AG, An der Birkenpfuhlheide 10, D-15837 Baruth
Acclimation period:	N/A
Feeding:	A mixture of pine (*Pinus sp*.) and birch (*Betula sp*.) pollen (3:1) *ad libitum* on the day of the test start and on each assessment day except for the last one respectively at least every four days.
Test unit:	The test unit was placed with its treated side upward on a wet cotton wool pad in a petri dish. The petri dish was constantly filled with tap water during the trial. A glue barrier was added to prevent the escaping of the mites. A small strip of filter paper was partly laid onto the leaf and extended onto the cotton wool pad. This provided a water source for the mites.
Loading:	10 mites per replicate

4. ***Environmental conditions*** -

Temperature:	23 - 26 °C
Humidity	64 - 69 %
Photoperiod:	16 hours light: 8hours dark (280 - 410 lux)
In-life dates:	November 2021 to December 2021

Study design

There were 5 test groups along with the control and the reference item group. The tested concentrations were 436, 1091-, 2727-, 6817- and 17041-mL product/ha. A toxic reference item (dimethoate) applied at a rate of 40 mL product/ha was included, as an indicator of the relative sensitivity of the test organisms and the test system. Each group was replicated 6 times with 10 organisms per replicate.

A stock solution was prepared by mixing a defined amount of test item with a defined amount of de-ionized water to get tested concentrations. All solutions were prepared with deionised water and were sprayed on the surface to be treated and let them dry.

Healthy and undamaged predatory mite *Typhlodromus pyri* were impartially selected and introduced into each test unit at the start of the assessment.

Assessment of adult mortality and offspring production were carried out 7 days after the application.

During the exposure, temperature, relative humidity and photoperiod were recorded.

Statistical analyses
Standard procedures, mortality: Step-down Cochran-Armitage Test, Fisher‘s Exact Binomial Test (both one-sided greater, $\alpha = 0.05$), LR_{50} calculation by Weibull Analysis.
Reproduction: Williams t-Test (one-sided smaller, $\alpha = 0.05$); ER_{50} calculation by Probit Analysis.
The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ® ToxRat Solutions GmbH

Results and discussions

Mortality data
The corrected mortality of the mites was 10.5, 15.8, 33.3, 31.6 and 56.1 at the test concentrations of control, 436, 1091, 2727, 6817 and 17041 mL product/ha, respectively. The % corrected mortality for the reference item was 100.0 %.

Table 1: Effect of FHO04 on the mortality of predatory mite *Typhlodromus pyri* at the end of the test

Treatments	Concentrations (mL product/ha)	% Mean Mortality	% Corrected Mortality
Control	0.0	5.0 ± 8.4	-
Test Item	436	15.0 ± 17.6	10.5
	1091	20.0 ± 17.9	15.8
	2727	36.7 ± 20.7	33.3
	6817	35.0 ± 12.2	31.6
	17041	58.3 ± 7.5	56.1
Reference item	40	100.0 ± 0.0	100.0

Reproduction performance

The percent reduction observed was 28.0, 55.9, 82.9 and 95.3 at the test concentrations of control, 436, 1091, 2727 and 6817 mL product/ha, respectively.

Reproduction was statistically significantly reduced compared to the control up to and including the application rate of 6817 mL product/ha.

Table 2: Effect of FHO04 on the reproduction performance of predatory mite *Typhlodromus pyri* after 7 days of exposure

Treatments	Concentrations (mL product/ha)	Reproduction (eggs/female)	Effect on reproduction (%)
Control	0.0	5.0 ± 1.6	-
Test Item	436	3.6 ± 0.7	28.0
	1091	2.2 ± 1.7	55.9
	2727	0.9 ± 0.8	82.9
	6817	0.2 ± 0.3	95.3

Validity Criteria
The study is valid since it fulfils the validity criteria. The mortality in the control should not exceed 20 % (actual value 5.0 %). The mortality in the reference should result in at least 50 % corrected mortality (actual value 100.0 %). The number of eggs per female in the control should be ≥ 4 eggs for the second week (actual value 5.0).

Conclusion

Statistically significant difference in mortality was observed at all test item application rates. The LR_{50} of FHO04 was 13558.7 mL product/ha.
Reproduction was statistically significantly reduced compared to the control up to and including the application rate of 6817 mL product/ha. The ER_{50} of FHO04 was 892.6 mL product/ha.

Table 3: Endpoints for predatory mite *Typhlodromus pyri* exposed to FHO04

Endpoint for Mortality [mL product/ha]	
LR_{50}	13558.7
NOER	< 436 mL
LOER	436
Endpoint for Reproduction [mL product/ha]	
ER_{50}	892.6
NOER	< 436
LOER	436

A 2.3.2.2 Study 2

Comments of zRMS:	The study was performed in line with the respective guideline with no major deviations. All validity criteria were met and the study is considered acceptable with following endpoints relevant for the risk assessment: LR_{50} = 2903.6 mL product/ha ER_{50} > 1091 mL product/ha

Reference:	KCP 10.3.2.2/02
Report	Prothioconazole/Sulphur (50+625) g/L SC: Effects on the Parasitoid *Aphidius rhopalosiphi* (Hymenoptera, Braconidae), Extended Laboratory Study - Dose Response Test -, J. Leopold, 2022d, Report No.163391002

Guideline(s):	Yes - Mead-Briggs *et al.* 2010
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive summary

The effect of FHO04 on the mortality and fecundity of the parasitoid *Aphidius rhopalosiphi* was determined by exposing adults to FHO04 on barley seedlings for 2 days. Test organisms were exposed to freshly dried residues at rates of 436, 1091, 2727, 6817 and 17041 mL product/ha. Dimethoate was used as a toxic standard and water was used as a control. Each test group was replicated 6 times with 5 females per replicate (exposure). Treatment groups were assessed for mortality on day 2. A reproduction test was performed on day 15.

On day 2, the corrected mortality was between 3.3 and 93.3 % for different test rates. Statistically significant difference was observed at the treatment rates of 2727 mL/ha. The NOER and LOER based on lethal effects was 1091 and 2727 mL product/ha, respectively. The LR_{50} of FHO04 was 2903.9 mL product/ha.

Reproduction was statistically significantly reduced compared to the control up to and including the application rate of 1091 mL product/ha. The NOER and LOER based on the offspring production were considered to be < 436- and 436-mL product/ha, respectively. The ER_{50} of FHO04 was > 1091 mL product/ha.

Materials and methods

1. *Test Item*:	FHO04
Description:	Liquid
Lot/Batch #:	028421
Purity:	Prothioconazole: 53.14 g/L Sulphur: 638.0 g/L
CAS #:	Prothioconazole: 178928–70–6 Sulphur: 7704-34-9
Stability of test compound:	N/A
2. *Vehicle and/or positive control*:	Vehicle: water Positive Control: dimethoate
3. *Test organism*:	
Species:	parasitoid *Aphidius rhopalosiphi*
Age:	No older than 2 days
Source:	Katz Biotech AG, An der Birkenpfuhlheide 10, D-15837 Baruth
Acclimation period:	1 – 2 days
Feeding:	10 % (w/w) fructose solution
Test unit:	Treated pots (13 cm in diameter) with 8 - 10 barley seedlings (*Hordeum vulgare* 'Sunshine') per pot. The plants were used for the bioassay, when at the 2nd leaf growth stage, i.e. BBCH Growth Stage 12. The plants were trimmed to a uniform height of 12 cm tall prior to the test commencing. The plants were enclosed within a clear polyacrylic cylinder (20 cm high and 10 cm in diameter) with a hole (approximately 2 cm in diameter) for the introduction

of the parasitoids. After introduction the hole was closed by a stopper with a hole where the ventilation tube was inserted. The opening of ventilation tube was closed with a fine mesh gauze. The top of the cylinder was closed with a fine mesh gauze. The soil surface was covered with a thin layer of quartz sand before treatment.

Loading: 5 females per replicate (exposure), 1 female per replicate (post-exposure)

4. ***Environmental conditions*** -

Temperature: 18 - 21 °C
Humidity 71 - 78 %
Photoperiod: 16 hours light:8 hours dark (880 - 1140 lux)
In-life dates: October 2021 to January 2022

Study design

There were 5 test groups along with the control and the reference item group. The tested concentrations were 436, 1091, 2727, 6817 and 17041 mL product/ha. A toxic reference item (dimethoate) applied at a rate of 10 mL product/ha was included, as an indicator of the relative sensitivity of the test organisms and the test system. Each group was replicated 6 times with 5 females per replicate (exposure).

A stock solution was prepared by mixing a defined amount of test item with a defined amount of de-ionized water to get tested concentrations. All solutions were prepared with deionised water and were sprayed on the surface to be treated and let them dry.

Healthy and undamaged parasitoids were impartially selected and introduced into each test unit at the start of the assessment.

Assessment of adult mortality was carried out 2, 24 and 48 hours after test initiation. The number of parasitoids alive, affected, moribund and dead were recorded. Moribund parasitoids were counted as dead. Assessment of reproduction involved counting the number of aphid mummies 12 days after the 24 hours parasitisation period in all replicates where the females were alive after the 24 hour parasitisation period (n = 10 - 18).
During the exposure, temperature, relative humidity and photoperiod were recorded.

Statistical analyses
Mortality: Step-down Rao-Scott-Cochran-Armitage Test, Fisher's Exact Binomial Test (both one-sided greater, $\alpha = 0.05$), LR50 calculation by Logit Analysis. Settling of parasitoids: Williams t-Test, Student t-Test (both one-sided smaller, $\alpha = 0.05$)
Reproduction: Williams t-Test (one-sided smaller, $\alpha = 0.05$).
The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ® ToxRat Solutions GmbH

Results and discussions

Mortality data
The corrected mortality of the parasitoid wasps was 3.3, 6.7, 60.0, 80.0 and 93.3 % at the test concentrations of control, 436, 1091, 2727, 6817 and 17041 mL product/ha, respectively. The % corrected mortality for the reference item was 100 %.

Table 1: Effect of FHO04 on the mortality of parasitoid *Aphidius rhopalosiphi* at the end of the test

Treatments	Concentrations (mL product/ha)	% Mean Mortality	% Corrected Mortality
Control	0.0	0.0 ± 0.0	-
Test Item	436	3.3 ± 8.2	3.3

	1091	6.7 ± 10.3	6.7
	2727	60.0 ± 40.0	60.0
	6817	80.0 ± 17.9	80.0
	17041	93.3 ± 10.3	93.3
Reference item	10	100.0 ± 0.0	100.0

Reproduction performance

The effect on parasitisation efficiency observed was 25.7 and 31.6 % at the test concentrations of control, 436 and 1091 mL product/ha, respectively.

There was no statistically significant difference observed at any treatment rate when compared to the control.

Table 2: Effect of FHO04 on the reproduction performance of parasitoid *Aphidius rhopalosiphi* after 2 days of exposure

Treatments	Concentrations (mL product/ha)	Parasitisation rate (mummies per female)	Effect on parasitisation efficiency (%)
Control	0.0	74.2 ± 27.3	-
Test Item	436	55.1 ± 20.7	25.7
	1091	50.7 ± 10.9	31.6

Validity Criteria

The study is valid since it fulfils the validity criteria. The mortality in the control should not exceed 13 % (actual value 0.0 %). The mortality in the reference should result in at least 50 % corrected mortality (actual value 100 %). The reproduction rate in the control should be ≥ 5 mummies per female (actual value 74.2) and there should be no more than 2 parasitoids producing zero values (actual value – no parasitoids produced zero values).

Conclusion

The mortality of *Aphidius rhopalosiphi* was not statistically significantly increased compared to the control up to and including the application rate of 1091 mL product/ha. The LC_{50} of FHO04 was 2903.9 mL product/ha.

Reproduction was statistically significantly reduced compared to the control up to and including the application rate of 1091 mL product/ha. The ER_{50} of FHO04 was > 1091 mL product/ha.

Table 3: Endpoints for parasitoid *Aphidius rhopalosiphi* exposed to FHO04

Endpoint for Mortality [mL product/ha]	
LR_{50}	2903.9
NOER	1091
LOER	2727
Endpoint for Reproduction [mL product/ha]	
ER_{50}	> 1091
NOER	< 436
LOER	436

A.2.3.2.2 Study 3

Comments of zRMS:	The study was performed in line with the respective guideline with no major deviations. All validity criteria were met and the study is considered acceptable with following endpoints relevant for the risk assessment: LR_{50} = 20450 mL product/ha

	ER_{50} >20450 mL product/ha

Reference:	KCP 10.3.2.2/03
Report	Prothioconazole/Sulphur (50+625) g/L SC: Effects on the Lacewing *Chrysoperla carnea* (Neuroptera: Chrysopidae), Extended Laboratory Study - Dose Response Test, J. Leopold, 2022e, Report No. 163391047
Guideline(s):	Yes - Vogt *et al.* 2000
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive summary

The effect of FHO04 on the mortality and fecundity of the lacewing *Chrysoperla carnea* was determined by exposing larvae to FHO04 on leaves of bean plants (*Phaseolus vulgaris* L. `Maxi´, 22 days old) for 27 days. Test organisms were exposed to freshly dried residues at rates of 253, 758, 2273, 6817 and 20450 mL product/ha. Dimethoate was used as a toxic standard and water was used as a control. Each test group was replicated 40 times (1 larva per replicate). Treatment groups were assessed for mortality on day 27. A reproduction test was performed by counting the number of eggs after 24 hour egg-laying periods (checks) and 2 checks were done within one week. The number of larvae (from the gauze) was determined after hatching of all larvae and the hatching rate was calculated.

On day 17, the corrected mortality was between -3.1 and 18.8 % for different test rates. It was not statistically significantly increased compared to the control up to and including the application rate of 20450 mL product/ha. The NOER and LOER based on lethal effects were > 20450 mL product/ha. The LR_{50} of FHO04 was > 20450 mL product/ha.

The effects on reproduction were calculated between -4.4 and 20.5 %. There was no statistically significant difference observed at any treatment rate when compared to the control.

Materials and methods

1. ***Test Item*:** FHO04
   - **Description:** Liquid
   - **Lot/Batch #:** 028421
   - **Purity:** Prothioconazole: 53.14 g/L
     Sulphur: 638.0 g/L
   - **CAS #:** Prothioconazole: 178928–70–6
     Sulphur: 7704-34-9
   - **Stability of test compound:** N/A
2. ***Vehicle and/or positive control*:** Vehicle: water
   Positive Control: dimethoate
3. ***Test organism*:**
   - **Species:** Lacewing *Chrysoperla carnea*
   - **Age:** 2-3 days old
   - **Source:** Katz Biotech AG, An der Birkenpfuhlheide 10, D-15837 Baruth

Acclimation period:	2-3 days
Feeding:	Larvae: UV-sterilised *Sitotroga cerealella* Oliv. eggs, *ad libitum* Adults: artificial diet: 1 egg, 1 egg yolk, 15 mL condensed milk, 20 g fructose, 30 g honey, 30 g brewer's yeast, 50 g wheat germ and deionised water (approximately 45 mL) mixed homogeneously, *ad libitum*
Test unit:	Detached primary leaves of bean plants (*Phaseolus vulgaris* L. `Maxi´, 22 days old) were cut to discs of about 55 mm in diameter. These leaf cuts were treated on their upper surface. The leaf discs were placed with their treated side upwards on a wet cotton wool pad in a petri dish (60 mm in diameter). The petri dish had a hole for a wick. A Fluon treated cylinder was fixed on each leaf by two elastic bands to guarantee a close position on the leaves. The height of the cylinder was 30 mm and the diameter was 46 mm. The lower part of the cylinder was not treated with Fluon to avoid contamination of the larvae. Escaping of the larvae was prevented by the close position of the cylinder to the leaf and the Fluon on the walls of the cylinder. The exposure units of one treatment group were placed in a bowl. A wick was connected with the cotton wool pad in the exposure units and was wetted regularly.
Loading:	1 larva per replicate

4. ***Environmental conditions*** -

Temperature:	23 - 27 °C
Humidity	60 - 74 %
Photoperiod:	16 hours light:8 hours dark (1030 - 1150 lux)
In-life dates:	October 2021 to April 2022

Study design
There were 5 test groups along with the control and the reference item group. The tested concentrations were 253, 758, 2273, 6817 and 20450 mL product/ha. A toxic reference item (dimethoate) applied at a rate of 170 mL product/ha was included, as an indicator of the relative sensitivity of the test organisms and the test system. Each group was replicated 40 times with 1 larva per replicate.

A stock solution was prepared by mixing a defined amount of test item with a defined amount of de-ionized water to get tested concentrations. All solutions were prepared with deionised water and were sprayed on the surface to be treated and let them dry.

Healthy and undamaged lacewing *Chrysoperla carnea* were impartially selected and introduced into each test unit at the start of the assessment.

Assessment of adult mortality and offspring production were carried out on 27 days after the application.

During the exposure, temperature, relative humidity and photoperiod were recorded.

Statistical analyses
Mortality: Chi^2 2x2 Table Test with Bonferroni Correction, Fisher's Exact Binomial Test (both one-sided greater, $\alpha = 0.05$).
Reproduction: In agreement with the guideline, no statistical evaluation was performed.
The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ® ToxRat Solutions GmbH.

Results and discussions

Mortality data

The corrected mortality of the lacewings was 9.4, 3.1, -3.1, 9.4 and 18.8 % at the test concentrations of control, 253, 758, 2273, 6817 and 20450 mL product/ha, respectively. The % corrected mortality for the reference item was 90.6 %.

Table 1: Effect of FHO04 on the mortality of lacewing *Chrysoperla carnea* at the end of the test

Treatments	Concentrations (mL product/ha)	% Mean Mortality	% Corrected Mortality
Control	0.0	20.0	-
Test Item	253	27.5	9.4
	758	22.5	3.1
	2273	17.5	-3.1
	6817	27.5	9.4
	20450	35.0	18.8
Reference item	170	92.5	90.6

Reproduction performance

The percent effect on reproduction observed was 20.5, 0.2, 10.1, -4.4 and 4.6 % at the test concentrations of control, 253, 758, 2273, 6817 and 20450 mL product/ha, respectively.

Table 2: Effect of FHO04 on the reproduction performance of lacewing *Chrysoperla carnea* 27 days of exposure

Treatments	Concentrations (mL product/ha)	Reproduction (eggs/female/day)	Effect on reproduction (%)
Control	0.0	30.8 ± 4.3	-
Test Item	253	24.5 ± 4.0	20.5
	758	30.8 ± 2.9	0.2
	2273	27.7 ± 2.4	10.1
	6817	32.2 ± 3.3	-4.4
	20450	29.4 ± 2.8	4.6

Validity Criteria

The study is valid since it fulfils the validity criteria. The mortality in the control should not exceed 20 % (actual value 20.0 %). The mortality in the reference should result in at least 50 % corrected mortality (actual value 90.6 %). The mean fecundity in the control group should be at least 15 eggs per female per day (actual value 30.8). The mean larval hatching rate of the control group should be at least 70 % (actual value 96.2 %).

Conclusion

Statistically significant difference in mortality was not observed at the treatment rates up to and including the application rate of 20450 mL The LR_{50} of FHO04 was > 20450 mL product/ha.

The effects on reproduction were calculated between -4.4 to 20.5 %. No clear dose-response relationship was recognizable. ER_{50}, based on the offspring production was > 20450 mL product/ha

Table 3: Endpoints for lacewing *Chrysoperla carnea* exposed to FHO04

Endpoint for Mortality [mL product/ha]	
LR_{50}	> 20450
NOER	> 20450
LOER	> 20450

Endpoint for Reproduction [mL product/ha]	
ER_{50}	> 20450
NOEC	-
LOEC	-

A.2.3.2.2 Study 4

Comments of zRMS:	The study was performed in line with the respective guideline with no major deviations. All validity criteria were met and the study is considered acceptable with following end-points relevant for the risk assessment: LR_{50} =1741.1 mL product/ha ER_{50}, > 1091 mL product/ha

Reference:	KCP 10.3.2.2/04
Report	Prothioconazole/Sulphur (50+625) g/L SC: Effects on the Ladybird Beetle *Coccinella septempunctata* (Coleoptera, Coccinellidae), Extended Laboratory Study - Dose Response Test -, J. Leopold, 2022f, Report No. 163391012
Guideline(s):	Yes - Schmuck *et al*. 2000
Deviations:	Yes, guideline was modified for exposure of *C. septempunctata* on natural substrate. Deviation does not have a deleterious impact on study results.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive summary

The effect of FHO04 on the mortality and fecundity of the ladybird beetle *Coccinella septempunctata* was determined by exposing adults to FHO04 on leaves of bean plants (*Phaseolus vulgaris* L. `Maxi´, 22 days old) for 19 days. Test organisms were exposed to freshly dried residues at rates of 174, 436, 1091, 2727 and 6817 mL product/ha. Dimethoate was used as a toxic standard and water was used as a control. Each test group was replicated 40 times (1 beetle per replicate). Treatment groups were assessed for mortality on day 19. A reproduction test was carried out by counting the number of eggs every day within the subsequent two weeks of oviposition. The number of larvae hatched was counted daily.

On day 19, the corrected mortality was between -5.7 and 77.1 % for different test rates. The mortality of *Coccinella septempunctata* was not statistically significantly increased compared to the control up to and including the application rate of 436 mL product/ha. The NOER and LOER based on lethal effects were 436 and 1091 mL product/ha, respectively. The LR_{50} of FHO04 was 1741.1 mL product/ha.

Reproduction was assessed in the control and at 174, 436 and 1091 mL product/ha. The mean number of fertile eggs per female and day was 6.2 in the control treatment. In the test item treatments, it ranged from 7.7 eggs per female and day (174 mL product/ha) to 15.2 eggs per female and day (1091 mL product/ha) and was > 2 fertile eggs per female and day at all test item rates. Reproduction performance was observed to be improved compared to the control, but the reproductive output is within the historical data base for control beetles and therefore this parameter is considered not to have been impacted by the treatment (Schmuck et al. 2000) up to and including 1091 mL product/ha. The ER_{50} of FHO04 was > 1091 mL product/ha.

Materials and methods

1. *Test Item*:	FHO04
Description:	Liquid
Lot/Batch #:	028421
Purity:	Prothioconazole: 53.14 g/L Sulphur: 638.0 g/L
CAS #:	Prothioconazole: 178928–70–6 Sulphur: 7704-34-9
Stability of test compound:	N/A
2. *Vehicle and/or positive control*:	Vehicle: water Positive Control: dimethoate
3. *Test organism*:	
Species:	Ladybird beetle *Coccinella septempunctata*
Age:	3-4 days old
Source:	Katz Biotech AG, An der Birkenpfuhlheide 10, D-15837 Baruth
Acclimation period:	3-4 days
Feeding:	Larvae: Live aphids (*Acyrthosiphon pisum*) *ad libitum.* Aphids were replaced or added each day until larvae had entered the pupal stage. Adults: Broad bean plants (*Vicia faba*) infested with aphids, *Acyrthosiphon pisum*; the infested plants were replaced once a week by fresh ones. If necessary additional aphids were added. Pollen and honey were given *ad libitum*.
Test unit:	Detached primary leaves of bean plants (*Phaseolus vulgaris* L. `Maxi´, 22 days old) were cut into discs of about 55 mm in diameter. These leaf discs were treated on their upper surface. The leaf discs were placed with their treated side upward on a wet cotton wool pad (approximately 55 mm in diameter) in a petri dish (60 mm in diameter). The petri dish had a hole for a wick to pass through. A Fluon treated cylinder of 30 mm height and 40 mm in diameter (approximately the lower 3 mm were not treated with Fluon to avoid contamination of the larvae) was fixed on each leaf by two elastic bands to guarantee a close position on the leaves. Escaping of the larvae and the aphids (food, see 6.5) was prevented by the direct contact of the cylinder with the leaf and the Fluon on the walls of the cylinder. The exposure units of one treatment group were placed in a bowl. A wick was connected with the cotton wool pad in the exposure units and was wetted regularly. At the end of exposure the cylinders were covered on the top with a plastic lid to prevent emerging beetles from escape.
Loading:	1 larva per replicate
4. *Environmental conditions* -	
Temperature:	23 - 27 °C
Humidity	60 - 74 %
Photoperiod:	16 hours light:8 hours dark (1010 - 1280 lux)
In-life dates:	October 2021 to February 2022

Study design

There were 5 test groups along with the control and the reference item group. The tested concentrations were 174, 436, 1091, 2727 and 6817 mL product/ha. A toxic reference item (dimethoate) applied at a rate of 50 mL product/ha was included, as an indicator of the relative sensitivity of the test organisms and the test system. Each group was replicated 40 times with 1 organism per replicate.

A stock solution was prepared by mixing a defined amount of test item with a defined amount of de-ionized water to get tested concentrations. All solutions were prepared with deionised water and were sprayed on the surface to be treated and let them dry.

Healthy and undamaged ladybird beetle *Coccinella septempunctata* were impartially selected and introduced into each test unit at the start of the assessment.

Assessment of adult mortality was carried out on 19 days after the application. Assessment of offspring production was carried out by counting the number of eggs every day within the subsequent two weeks of oviposition. The number of larvae hatched was counted daily.

During the exposure, temperature, relative humidity and photoperiod were recorded.

Statistical analyses
Mortality: Step-down-Cochran-Armitage Test, Fisher's Exact Binomial Test (both one-sided greater, $\alpha = 0.05$); LR50 calculation by Probit Analysis.
Reproduction data were not analysed statistically in accordance with the guideline. Instead, recorded mean values were compared qualitatively with the lower limit given by the historical data base of Schmuck *et al*. 2000.

Results and discussions

Mortality data
The corrected mortality of the beetles was -5.7, 5.7, 51.4, 71.4 and 77.1 % at the test concentrations of control, 174, 436, 1091, 2727 and 6817 mL product/ha, respectively. The % corrected mortality for the reference item was 100.0 %.

Table 1: Effect of FHO04 on the mortality of ladybird beetle *Coccinella* septempunctata at the end of the test

Treatments	Concentrations (mL product/ha)	% Mean Mortality	% Corrected Mortality
Control	0.0	12.5	-
Test Item	174	7.5	-5.7
	436	17.5	5.7
	1091	57.5	51.4
	2727	75.0	71.4
	6817	80.0	77.1
Reference item	50	100.0	100.0

Reproduction performance
The larval hatching rate observed was 93.4, 93.9 and 72.8 % at the test concentrations of control, 174, 436 and 1091 mL product/ha, respectively.

Table 2: Effect of FHO04 on the reproduction performance of ladybird beetle *Coccinella* septempunctata after 14 days of oviposition.

Treatments	Concentrations (mL product/ha)	Fertile eggs per female per day	Larval hatching rate (%)
Control	0.0	6.2 ± 3.8	94.4
Test Item	174	7.7 ± 5.5	93.4

	436	14.7 ± 8.7	93.9
	1091	15.2 ± 7.7	72.8

Validity Criteria
The study is valid since it fulfils the validity criteria. The pre-imaginal mortality in the control should not exceed 30 % (actual value 12.5 %). The pre-imaginal mortality in the reference should result in at least 40 % corrected mortality (actual value 100.0 %). The number of eggs per female per day in the control should be ≥ 2 fertile eggs per viable female per day (actual value 6.2).

Conclusion

The mortality of *Coccinella septempunctata* was not statistically significantly increased compared to the control up to and including the application rate of 436 mL product/ha. The LR_{50} of FHO04 was 1741.1 mL product/ha. The effects on reproduction were calculated between 72.8 % and 93.9 %. EC_{50}, based on the offspring production was > 1091 mL product/ha.

Table 3: Endpoints for ladybird beetle *Coccinella* septempunctata exposed to FHO04

Endpoint for Mortality [mL product/ha]	
LR_{50}	1741.1
NOEC	436
LOEC	1091
Endpoint for Reproduction [mL product/ha]	
ER_{50}	> 1091
NOEC	-
LOEC	-

A.2.3.2.2 Study 5

Comments of zRMS:	The study was performed in line with the respective guideline. Results indicated that, when applied to dwarf French bean plants at a rate equivalent to 4 L test item/ha, on two occasions with 14-day interval in-between, fresh-dried residues, 7-, 14-, 28- and 42-day field-aged residues resulted in no unacceptable effects on the survival of the mites (i.e. < 50% corrected mortality relative to the respective control). For reproduction, fresh-dried residues, 7- and 28-day field-aged residues resulted in a > 50% reduction in reproduction relative to the respective control (i.e. unacceptable effects on the reproductive capacity), whereas the 14- and 42-day field aged residues resulted in a < 50% reduction in reproduction relative to the respective control (i.e. no unacceptable effects on the reproductive capacity). Unfortunately, because of the unacceptable effects on reproduction with 28-day field aged residues, which may just be an anomaly, there were no two consecutive bioassays with < 50% reduction in re-production. A repeat study by L. Fallowfield, 2023, with field aged residues covering a longer period of time than 42 days, was therefore performed and evaluated by zRMS.

Reference:	KCP 10.3.2.2/05
Report	Prothioconazole/Sulphur (50+625) SC – A Series of Aged-Residue Extended Laboratory Tests to Determine Effects on the Predatory Mite *Typhlodromus pyri* (Acari: Phytoseiidae), L. Fallowfield, 2022, Report No. UPL-22-04
Guideline(s):	Yes - Blümel *et al*. (2000)
Deviations:	No

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

Executive summary

The test item in this study was prothioconazole/sulphur (50+625) g/L SC, a suspension concentrate formulation containing the active substances prothioconazole (nominally 50 g/L) and sulphur (nominally 625 g/L). The aim of this study was to determine the effects of both freshly-dried and field-aged residues this test item on the predatory mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae), in a series of extended laboratory tests.

Prothioconazole/sulphur (50+625) g/L SC was evaluated at a single application rate, equivalent to 4 L test item/ha. The treatment was applied to the test plants on two occasions (times TI and T2) with a 14-day interval in-between. This treatment was compared to a water control. A toxic reference treatment of dimethoate (an EC formulation containing nominally 400 g a.s./L, applied at a rate of 60 mL product/ha) was also included in the study. All treatments were applied to dwarf French bean plants (Phaseolus vulgaris), using a laboratory track-sprayer, at a volume rate equivalent to 400 L spray solution/ha. After both T1 and T2 applications, the treated plants were placed under UV permeable rain protection and extended laboratory bioassays were carried out using leaves collected from the plants at 0, 7, 14, 28 and 42 DAT (days after treatment), commencing after the second spray applications (time T2).

For each bioassay, 5-cm leaf discs were cut from the treated leaves (n = 5 per treatment). These were each laid, with the treated upper (adaxial) surface exposed, onto a layer of water-saturated cotton wool lining a Petri dish. A line of a non-drying sticky gel was drawn around the edge of each leaf disc, to serve as a barrier to mite dispersal. Twenty protonymphal mites were placed at the centre of each arena and untreated pollen and water were provided for nourishment. The survival of the mites was assessed after 7 days, by which time the mites in the control treatment were adult. The sex of the surviving mites was determined and they were then left in situ so that their reproduction could be assessed over a further 7 days. The mean number of eggs produced per female between 7 and 14 days after initiation (DAI) of the test was calculated. These reproduction assessments were made for the control and for the test-item treatment only.

The testing programme was to be continued until residues no longer resulted in unacceptable effects (i.e. where corrected mortality was < 50% and any reduction in reproduction was < 50% when compared to the respective control), in two consecutive bioassays. However, this criterion was not achieved for reproduction due to there being no more usable bean leaves after the 42 DAT bioassay to set up further bioassays.

Results indicated that, when applied to dwarf French bean plants at a rate equivalent to 4 L test item/ha, on two occasions with a 14-day interval in-between, fresh-dried residues, 7-, 14-, 28- and 42-day field-aged residues resulted in no unacceptable effects on the survival of the mites (i.e. < 50% corrected mortality relative to the respective control). For reproduction, fresh-dried residues, 7- and 28-day field-aged residues resulted in a > 50% reduction in reproduction relative to the respective control (i.e. unacceptable effects on the reproductive capacity), whereas the 14- and 42-day field aged residues resulted in a < 50% reduction in reproduction relative to the respective control (i.e. no unacceptable effects on the reproductive capacity).

Unfortunately, because of the unacceptable effects on reproduction with 28-day field aged residues, which may just be an anomaly, there were no two consecutive bioassays with < 50% reduction in reproduction.

A repeat study, with field aged residues covering a longer period of time than 42 days, was therefore performed.

A.2.3.2.2 Study 6

Comments of zRMS:	The study was performed in line with the respective guideline with no major deviations.

	All validity criteria were met and the study is considered acceptable with following end-points relevant for the risk assessment: The effects of freshly-dried and field-aged foliar residues of FHO04 on the predatory mite *Typhlodromus pyri* were evaluated in a series of extended laboratory tests. When applied to dwarf French bean plants at a rate equivalent to 4 L test item/ha, applied on two occasions with a 14-day interval in-between, 56-day and 70-day field-aged residues resulted in no unacceptable effects on either the survival or the subsequent reproductive capacity of the mites, (i.e. < 50% corrected mortality and < 50% reduction in reproduction, relative to the respective control).

Reference:	KCP 10.3.2.2/06
Report	Prothioconazole/Sulphur (50+625) SC – A Series of Aged-Residue Extended Laboratory Tests to Determine Effects on the Predatory Mite *Typhlodromus pyri* (Acari: Phytoseiidae), L. Fallowfield, 2023, Report No. UPL-23-01
Guideline(s):	Yes - Blümel *et al*. (2000)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive summary

The effect of FHO04 on the mortality and fecundity of the predatory mite *Typhlodromus pyri* was determined by exposing mites to FHO04 on leaves of the dwarf French bean, *Phaseolus vulgaris* L. (var. The Prince). The treatment variants evaluated were a single application rate of the test item and a water control, both applied twice with a 14-day interval (T1 and T2).

Test organisms were exposed to fresh and aged foliar residues at a rate of 4 L product/ha. Dimethoate was used as a toxic standard and water was used as a control. Each test group was replicated 5 times (20 mites per replicate).

An extended laboratory bioassay commenced within 1 h of the second treatment application (i.e. 0 days after treatment = 0 DAT) and then additional bioassays were initiated at 7, 14, 35, 42, 49, 56 and 70 DAT. The endpoints for the bioassays were an assessment of mite mortality 7 days after initiation of the test (7 DAI) and an assessment of the reproductive capacity of the mites (as mean number of eggs produced per female) over the subsequent 7 days, i.e. 7-14 DAI, where corrected mortality in the test item treatment was ≤ 50% at 7 DAI.

The effects of freshly dried and field-aged foliar residues of FHO04 on the predatory mite *Typhlodromus pyri* were evaluated in a series of extended laboratory tests. When applied to dwarf French bean plants at a rate equivalent to 4 L test item/ha, applied on two occasions with a 14-day interval in-between, 56-day and 70-day field-aged residues resulted in no unacceptable effects on either the survival or the subsequent reproductive capacity of the mites, (i.e. < 50% corrected mortality and < 50% reduction in reproduction, relative to the respective control).

Materials and methods

1. ***Test Item*:** FHO04
   Description: Liquid
   Lot/Batch #: 028421

Purity:	Prothioconazole: 53.14 g/L Sulphur: 638.0 g/L
CAS #:	Prothioconazole: 178928–70–6 Sulphur: 7704-34-9
Stability of test compound:	N/A
2. *Vehicle and/or positive control*:	Vehicle: water Positive Control: Dimethoate
3. *Test organism*:	
Species:	Predatory mite *Typhlodromus pyri*
Age:	1-2s day old
Source:	P.K. Nützlingszuchten, Welzheim, Germany
Acclimation period:	N/A
Feeding:	1:1 v/v mixture of almond (*Prunus* sp. var. a mix of Aldrich, Nonpareil and Wood Colony) and apple (*Malus* sp. var. Red Delicious) pollen
Test unit:	The test arenas comprised plastic Petri dish bases (9 cm in diameter) lined with water-saturated cotton wool.
Loading:	20 mites per replicate
4. *Environmental conditions* -	
Temperature:	23 to 27 °C
Humidity	60 to 90 %
Photoperiod:	16 hours light:8 hours dark (400-1500 lux)
In-life dates:	18 April 2023 to 25 July 2023

Study design
FHO04 was evaluated at a single application rate, equivalent to 4 L test item/ha. The treatment was applied to the test plants on two occasions (times TI and T2) with a 14-day interval in-between. This treatment was compared to a water control. A toxic reference treatment of dimethoate (60 mL product/ha) was also included in the study, applied at T2 only. Each group was replicated 5 times with 20 mites per replicate.

A stock solution was prepared by mixing a defined amount of test item with a defined amount of de-ionized water to get tested concentrations. All solutions were prepared with deionised water and were sprayed on the surface to be treated and let them dry.

Healthy and undamaged predatory mites were impartially selected and introduced into each test unit at the start of the assessment.

Assessment of adult mortality and offspring production were carried out 7, 14, 35, 42, 49, 56 and 70 days after treatments (DAT).

During the exposure, temperature, relative humidity and photoperiod were recorded.

Statistical analyses
Treatment mortalities were compared to the respective control using the chi^2 2x2 table test ($\alpha = 0.05$, one-sided, > respective control). Mortality was corrected for respective control treatment deaths using Abbott's formula.
Treatment reproduction effects were compared to the respective control by Student's t-test for homogenous variances ($\alpha = 0.05$, one-sided, < respective control).
ToxRat Professional, ToxRat® Solutions GmbH, 2018, version 3.3.0) was used for the analysis.

Results and discussions

Mortality data

The corrected mortality of the mites was 62.4, 29.0, 19.8, 8.9, 0.0, 3.2 and 1.1 % after 7, 14, 35, 42, 49, 56 and 70 days after treatments (DAT). The % corrected mortality for the reference item was 100 %.

Table 1: Effect of FHO04 on the mortality of Predatory mite *Typhlodromus pyri*

Number of days after treatment	Treatment	Test item rate (L/ha)	% Mean Mortality	% Corrected Mortality
0	Control	-	17	-
	FHO04	4	29	14.5
	Dimethoate	-	100	100
7	Control	-	7	-
	FHO04	4	65	62.4
14	Control	-	7	-
	FHO04	4	34	29.0
35	Control	-	9	-
	FHO04	4	27	19.8
42	Control	-	10	-
	FHO04	4	18	8.9
49	Control	-	2	-
	FHO04	4	2	0.0
56	Control	-	6	-
	FHO04	4	9	3.2
70	Control	-	9	-
	FHO04	4	10	1.1

Reproduction performance

The percent reduction in reproduction observed was 94.7, 80.5, 53.2, 60.4. 68.3, 46.2 and 18.7 after 14, 35, 42, 49, 56 and 70 days after treatments (DAT).

Table 2: Effect of FHO04 on the reproduction performance of Predatory mite *Typhlodromus pyri*

Number of days after treatment	Treatment	Test item rate (L/ha)	Mean number of eggs per female (7-14 DAI)	% reduction in reproduction, relative to the respective control
0	Control	-	6.6	-
	FHO04	4	0.4	94.7
14	Control	-	8.8	-
	FHO04	4	1.7	80.5
35	Control	-	7.9	-
	FHO04	4	3.7	53.2
42	Control	-	9.6	-
	FHO04	4	3.8	60.4
49	Control	-	10.0	-
	FHO04	4	3.2	68.3
56	Control	-	8.2	-
	FHO04	4	4.4	46.2
70	Control	-	10.1	-
	FHO04	4	8.2	18.7

Validity Criteria

The study is valid since it fulfils the validity criteria. The mortality in the control treatment over the initial 7 days of a bioassay should not exceed 20 % (actual value 17 %). The corrected mortality in the toxic reference treatment should be 50-100 % (actual value 100 %). The mean cumulative number of eggs produced between 7 and 14 days should be equal to or exceed 4.0 per female in any control treatment (actual

value 7).

Conclusion

The effects of freshly-dried and field-aged foliar residues of FHO04 on the predatory mite *Typhlodromus pyri* were evaluated in a series of extended laboratory tests. When applied to dwarf French bean plants at a rate equivalent to 4 L test item/ha, applied on two occasions with a 14-day interval in-between, 56-day and 70-day field-aged residues resulted in no unacceptable effects on either the survival or the subsequent reproductive capacity of the mites, (i.e. < 50% corrected mortality and < 50% reduction in reproduction, relative to the respective control).

A.2.3.2.2 Study 7

Comments of zRMS:	The study was performed in line with the respective guideline with no major deviations. All validity criteria were met and the study is considered acceptable with following endpoints relevant for the risk assessment: The effects of both fresh and aged foliar residues of FHO04 on the parasitic wasp, *Aphidius rhopalosiphi*, were evaluated under extended laboratory conditions. Following two applications to French bean plants at a rate of 4 L test item/ha, with a 14-day interval, both 28 and 42-day-old foliar residues had no unacceptable effects on either wasp survival or reproduction (i.e. < 50% effects, relative to the control).

Reference:	KCP 10.3.2.2/07
Report	Prothioconazole/Sulphur (50+625) g/L SC – An aged-residue extended laboratory study on the parasitic wasp *Aphidius rhopalosiphi* (Hymenoptera, Braconidae), J. Stevens, 2022, UPL-22-03
Guideline(s):	Yes - Mead-Briggs *et al.* (2000)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive summary

The effect of FHO04 on the mortality and fecundity of the parasitic wasp *Aphidius rhopalosiphi* was determined by exposing wasps to FHO04 on leaves of the dwarf French bean, *Phaseolus vulgaris* L. (var. The Prince). The treatment variants evaluated were a single application rate of the test item and a water control, both applied twice with a 14-day interval (T1 and T2).

Test organisms were exposed to fresh and ages foliar residues at a rate of 4 L product/ha. Dimethoate was used as a toxic standard and water was used as a control. Each test group was replicated 4 times (10 wasps per replicate).

Treatments were applied to potted French bean plants which were then maintained outdoors until foliage was required for bioassays being carried out under extended laboratory test conditions. For each bioassay, adult wasps were confined on excised leaves once residues had dried after the second application (at time T2), i.e. at 0 days after treatment (DAT), and then at 7, 14, 28 and 42 DAT. The endpoints of the individual bioassays were an assessment of wasp mortality after 48 h and an assessment of the subsequent reproductive capacity of individually-confined females. The fecundity assessments were made for the control and test-item treatment in the 28 and 48 DAT bioassays as they resulted in ≤ 50% corrected mortality.

The effects of both fresh and aged foliar residues of FHO04 on the parasitic wasp, *Aphidius rhopalosiphi*, were evaluated under extended laboratory conditions. Following two applications to French bean plants at a rate of 4 L test item/ha, with a 14-day interval, both 28 and 42-day-old foliar residues had no unacceptable effects on either wasp survival or reproduction (i.e. < 50% effects, relative to the control).

Materials and methods

1. *Test Item*:	FHO04
Description:	Liquid
Lot/Batch #:	028421
Purity:	Prothioconazole: 53.14 g/L Sulphur: 638.0 g/L
CAS #:	Prothioconazole: 178928–70–6 Sulphur: 7704-34-9
Stability of test compound:	N/A
2. *Vehicle and/or positive control*:	Vehicle: water Positive Control: Dimethoate
3. *Test organism*:	
Species:	Parasitic wasp *Aphidius rhopalosiphi*
Age:	2 days after emergence
Source:	Katz Biotech AG, Baruth, Germany
Acclimation period:	N/A
Feeding:	10% fructose solution
Test unit:	Treated bean leaves were used to form the floor and ceiling of shallow arenas, with their upper surfaces facing inwards. The arenas comprised circular frames made from clear acrylic tubing (these were of approx. 5.1 cm internal diameter and 15 mm deep) [Fig. 1] and held in place with 3 elastic bands. Holes (four in number and ca. 8 mm in diameter) had been drilled through the side wall of the frame to provide ventilation and 3 of these were covered with nylon netting (0.5 mm x 0.5 mm mesh). The fourth hole was left uncovered as an access hole for the introduction of the parasitoids and was then sealed with a cotton wool bung.
Loading:	10 wasps per replicate
4. *Environmental conditions* -	
Temperature:	20.4 to 21.9 °C
Humidity	66 to 78 %
Photoperiod:	16 hours light:8 hours dark (901-1304 lux)
In-life dates:	26 July 2022 to 03 October 2022

Study design

A single treatment rate (4 product/ha) of the test item, applied on two occasions with a 14-day interval, was evaluated and compared to a water-treated control and, for the initial bioassay, a toxic reference treatment (dimethoate, 60 mL product/ha). Treatments were applied to potted French bean plants which were then maintained outdoors until foliage was required for bioassays being carried out under extended laboratory test conditions. For each bioassay, adult wasps were confined on excised leaves once residues had dried after the second application (at time T2), i.e. at 0 days after treatment (DAT), and then at 7, 14, 28 and 42 DAT. The endpoints of the individual bioassays were an assessment of wasp mortality after 48 h and an assessment of the subsequent reproductive capacity of individually-confined females. The fecundity assessments were made for the control and test-item treatment in the 28 and 48 DAT bioassays as they resulted in ≤ 50% corrected mortality.

During the exposure, temperature, relative humidity and photoperiod were recorded.

Statistical analyses

Treatment mortalities were compared to the respective control using Fisher's exact binomial test (one-sided, > control, $\alpha = 0.05$). Mortality was corrected for respective control treatment deaths using Abbott's formula.

Treatment reproduction effects were compared to the respective control by Student's t-test for homogenous variances ($\alpha = 0.05$, one-sided, < respective control).

ToxRat Professional, ToxRat® Solutions GmbH, 2018, version 3.3.0) was used for the analysis.

Results and discussions

Mortality data

The corrected mortality of the wasps was 82.5, 82.1, 65.8, 40.0 and 2.6 % after 7, 14, 28 and 42 days after treatments (DAT). The % corrected mortality for the reference item was 100 %.

Table 1: Effect of FHO04 on the mortality of parasitic wasp *Aphidius rhopalosiphi*

Number of days after treatment	Treatment	Test item rate (L/ha)	% Mean Mortality (48 hr)	% Corrected Mortality (48 hr)
0	Control	-	0.0	-
	FHO04	2 x 4	82.5	82.5
	Dimethoate	-	100	100
7	Control	-	2.5	-
	FHO04	2 x 4	82.5	82.1
14	Control	-	5.0	-
	FHO04	2 x 4	67.5	65.8
28	Control	-	0.0	-
	FHO04	2 x 4	40.0	40.0
42	Control	-	2.5	-
	FHO04	2 x 4	5.0	2.6

Reproduction performance

The percent reduction in reproduction observed was -2.1 and -11.7 after 28 and 42 days after treatments (DAT).

Table 2: Effect of FHO04 on the reproduction performance of parasitic wasp *Aphidius rhopalosiphi*

Number of days after treatment	Treatment	Test item rate (L/ha)	Number of wasps evaluated	Mean number mummies per surviving female	% reduction in reproduction
0	Control	-	-	-	-
	FHO04	2 x 4	-	-	-
7	Control	-	-	-	-
	FHO04	2 x 4	-	-	-
14	Control	-	-	-	-
	FHO04	2 x 4	-	-	-
28	Control	-	14	29.7	-
	FHO04	2 x 4	15	30.3	-2.1
42	Control	-	14	42.6	-
	FHO04	2 x 4	13	47.6	-11.7

Validity Criteria
The study is valid since it fulfils the validity criteria. The mortality in the control treatment should not exceed 13 % (highest actual value 5.0 %) at 48 hrs. The corrected mortality in the toxic reference treatment should be 50-100 % (actual value 100 %) at 48 hrs. The mean number of mummies in the control treatment should be ≥ 5.0 per female and there should not be more than two zero values in the control treatment (lowest actual value 29.7).

Conclusion
The effects of both fresh and aged foliar residues of FHO04 on the parasitic wasp, *Aphidius rhopalosiphi*, were evaluated under extended laboratory conditions. Following two applications to French bean plants at a rate of 4 L test item/ha, with a 14-day interval, both 28 and 42-day-old foliar residues had no unacceptable effects on either wasp survival or reproduction (i.e. < 50% effects, relative to the control).

A.2.3.2.2 Study 8

Comments of zRMS:	The study was performed in line with the respective guideline with no major deviations. All validity criteria were met and the study is considered acceptable with following end-points relevant for the risk assessment: When applied at a rate equivalent to 4.0 L product/ha, on two occasions with a 14-day interval, fresh residues (0-day-old) of FHO04 and the subsequent bioassay evaluating 7- day-old foliar residues of FHO04, showed no unacceptable effects on either the mortality, or the subsequent reproductive capacity of the ladybird.

Reference:	KCP 10.3.2.2/08
Report	Prothioconazole/Sulphur (50+625) g/L SC – A series of aged-residue extended laboratory tests to determine effects on the ladybird beetle, *Coccinella septempunctata* (Coleoptera: Coccinellidae), C. White-Hall, 2022, Report No. UPL-22-05.
Guideline(s):	Yes - Schmuck *et al*. (2000)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive summary

The effect of FHO04 on the mortality and fecundity of the ladybird beetle *Coccinella septempunctata* was determined by exposing beetles to FHO04 on leaves of the dwarf French bean, *Phaseolus vulgaris* L. (var. The Prince). The treatment variants evaluated were a single application rate of the test item and a water control, both applied twice with a 14-day interval (T1 and T2).

Test organisms were exposed to fresh and aged foliar residues at a rate of 4 L product/ha. Dimethoate was used as a toxic standard and water was used as a control. Each test group was replicated 40 times (1 beetle per replicate).

For each bioassay, larvae were confined on excised leaves once residues had dried after the second application (at time T2), i.e. at 0 days after treatment (DAT), and then at 7 DAT. The endpoints of the individual bioassays were an assessment of pre-imaginal mortality and the subsequent assessment of the reproductive capacity of adult ladybirds that survived their initial exposure. The fecundity assessments were made for

the control and test-item treatment in both bioassays since the latter had resulted in ≤ 50% corrected mortality.

When applied at a rate equivalent to 4.0 L test item/ha, on two occasions with a 14-day interval, fresh residues (0-day-old) of FHO04 and the subsequent bioassay evaluating 7- day-old foliar residues of FHO04, showed no unacceptable effects on either the mortality, or the subsequent reproductive capacity of the ladybird.

Materials and methods

1. *Test Item*:	FHO04
Description:	Liquid
Lot/Batch #:	028421
Purity:	Prothioconazole: 53.14 g/L Sulphur: 638.0 g/L
CAS #:	Prothioconazole: 178928–70–6 Sulphur: 7704-34-9
Stability of test compound:	N/A
2. *Vehicle and/or positive control*:	Vehicle: water Positive Control : dimethoate
3. *Test organism*:	
Species:	Ladybird beetle *C. septempunctata*
Age:	2-5 day old
Source:	Katz Biotech AG, Baruth, Germany
Acclimation period:	N/A
Feeding:	Pea aphids (*Acyrthosiphon pisum* (Harris)) were provided *ad libitum*
Test unit:	Comprising of a square glass plate (7.5 cm x 7.5 cm), a Perspex® supporting plate of similar size, with a 5-cm-diameter hole cut through it, and an acrylic cylinder (5 cm outer diameter, 4.4 cm inner diameter, 2.5 cm tall).
Loading:	40 per replicate
4. *Environmental conditions* -	
Temperature:	24.6 to 25.6°C
Humidity	69 to 77 %
Photoperiod:	16 hours light:8 hours dark (4000-5800)
In-life dates:	02 August 2022 to 10 October 2022

Study design

FHO04 was evaluated at a single application rate, equivalent to 4 L test item/ha. The treatment was applied to the test plants on two occasions (times TI and T2) with a 14-day interval in-between. This treatment was compared to a water control. A toxic reference treatment of dimethoate (60 mL product/ha) was also included in the study, applied at T2 only. Each group was replicated 5 times with 20 beetles per replicate.

Treatments were applied to potted French bean plants which were then maintained outdoors until foliage was required for bioassays being carried out under extended laboratory test conditions. For each bioassay, larvae were confined on excised leaves once residues had dried after the second application (at time T2), i.e. at 0 days after treatment (DAT), and then at 7 DAT.

A stock solution was prepared by mixing a defined amount of test item with a defined amount of de-ionized water to get tested concentrations. All solutions were prepared with deionised water and were sprayed on the surface to be treated and let them dry.

Healthy and undamaged ladybird beetles were impartially selected and introduced into each test unit at the start of the assessment.

Assessment of adult mortality and offspring production were carried out 0 and 7 days after treatments (DAT).

Statistical analyses
Treatment mortalities were compared to the respective control using Fisher's exact binomial test (one-sided, > control, $\alpha = 0.05$). Mortality was corrected for respective control treatment deaths using Abbott's formula.
ToxRat Professional, ToxRat® Solutions GmbH, 2018, version 3.3.0) was used for the analysis.

Results and discussions

Mortality data
The corrected mortality of the beetles was 12.5 and 11.1 % after 0 and 7 days after treatments (DAT). The % corrected mortality for the reference item was 97.5 %.

Table 1: Effect of FHO04 on the mortality of ladybird beetle *Coccinella septempunctata*

Number of days after treatment	Treatment	Test item rate (L/ha)	% Pre-imaginal Mortality	% Corrected Pre-imaginal Mortality
0	Control	-	0.0	-
	FHO04	4	12.5	12.5
	Dimethoate	-	97.5	97.5
7	Control	-	10.0	-
	FHO04	4	20.0	11.1

Reproduction performance

In the 0 DAT bioassay, the mean number of viable eggs per female per day was 6.8 in the control, compared with 8.3 in the 4 L test item/ha treatment rate of FHO04. In the bioassay initiated at 7 DAT, the mean number of viable eggs per female per day was 11.4 in the control, compared with 7.5 in the 4 L test item/ha treatment rate of FHO04.

Table 2: Effect of FHO04 on the reproduction performance of ladybird beetle *Coccinella septempunctata*

Number of days after treatment	Treatment	Test item rate (L/ha)	Mean no. eggs/female/day	Mean % egg viability	Mean no. viable eggs/female/day
0	Control	-	14.0	48.7	6.8
	FHO04	4	14.9	55.4	8.3
7	Control	-	19.8	57.3	11.4
	FHO04	4	16.0	46.8	7.5

Validity Criteria
The study is valid since it fulfils the validity criteria. The pre-imaginal mortality in the control treatment should not exceed 30 % (actual value 10 %). The level of mortality in the toxic reference treatment should be ≥ 50% (actual value 97.5 %). Mean egg production should be > 2 viable eggs/female/day in the control treatment (actual value 7.5).

Conclusion
When applied at a rate equivalent to 4.0 L test item/ha, on two occasions with a 14-day interval, fresh residues (0-day-old) of FHO04 and the subsequent bioassay evaluating 7- day-old foliar residues of FHO04, showed no unacceptable effects on either the mortality, or the subsequent reproductive capacity of the ladybird.

A 2.4 KCP 10.4 Effects on non-target soil meso- and macrofauna

A 2.4.1 KCP 10.4.1 Earthworms

Comments of zRMS:	The study was performed in line with OECD 222 with no deviations. All validity criteria were met. The test design was relevant to derive both, NOEC and ECx values (8 concentrations, 8 replicates for control, 4 replicates per treatment group). Reliability of the EC_{10} value has been evaluated in line with recommendations of EFSA Supporting publication 2019:EN-1673: • NW (normalised width) of 0.49 was calculated, which results with rating "good" in line with Table E9 in EFSA Supporting publication 2019:EN-1673, • median EC_{10} is lower than $EC_{20,low}$, • the dose-response curve is shallow with steepness of 0.28 Based on NW of 0.49 and dose-response curve it is considered that EC_{10} to be not sufficiently reliable and should be used in the risk assessment. Overall, the study is considered acceptable with following endpoints relevant for the risk assessment: EC_{10} = 258.82 mg product/kg dws EC_{20} =399.95 mg product/kg dws EC_{50} = 920.45 mg product/kg dws NOEC = 267.9 mg product/kg dws

Reference:	KCP 10.4.1/01
Report	Reproduction toxicity test of prothioconazole/sulphur (50+625) g/l sc to earthworm, *Eisenia fetida*, Rana, J.R., 2022, report No 522-3-08-29113, sponsor study No. UPL/2021/0569, Authority registration No GLP/C-139/2019
Guideline(s):	Yes – OECD 222 - Earthworm Reproduction Test (*Eisenia fetida / Eisenia andrei*)
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No – invertebrate study

Executive summary

This study was performed to assess the reproductive toxicity (EC_{50}) of formulation FHO04 (prothioconazole/sulphur (50+625) g/L SC) to earthworm (*Eisenia foetida*). The earthworms were exposed to the test concentration of 0 (untreated control), 99.7, 138.6, 192.7, 267.9, 372.4, 517.6, 719.4, and 1000.0 mg test item/kg dry weight of the artificial soil for a period of 56 days. Each concentration was replicated 4 times with 10 earthworms per replicate. The moisture content of the artificial soil during the exposure period was 27.15 % (59.01 % of water-holding capacity) and 25.31 % (55.03 % of water-holding capacity on the day of treatment and at the end of the experiment, respectively.

Prothioconazole/ Sulphur (50+625) g/L SC (mg/kg a.soil)	**Control**	**99.7**	**138.6**	**192.7**	**267.9**	**372.4**	**517.6**	**719.4**	**1000.0**
Mortality (day 28) (%)	0	0	0	0	0	0	0	0	0
Statistical Significance	-	-	-	-	-	-	-	-	-
Body weight change (day 0-28) (%)	14.30	16.70	10.30	12.60	6.30	13.20	12.48	17.90	12.03
Statistical Significance	-	ns	ns	ns	ns	ns	ns	ns	ns
Mean No. of Juveniles (day 56)	165.25	166.75	169.25	166.75	152.00	128.00	119.75	101.50	76.75
Statistical Significance	-	ns	ns	ns	ns	*	*	*	*
% reduction in reproductive output (day 56)	-	-0.91	-2.42	-0.91	8.02	22.54	27.53	38.58	53.56
Food consumption	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

Key : a.soil = artificial soil, - = Not Applicable, ns = not significantly different compared to control, * = Significantly different compared to control (1% level, p≤0.01), ≠ = End points are calculated from the prothioconazole (39.13 g/kg) and sulphur concentration (469.8 g/kg) with purity given in the CoA.

	Endpoints		
	mg test item/kg a.soil	mg prothioconazole/kg a. soil≠	mg sulphur/kg a. soil≠
NOEC (day 28 mortality)	≥1000.0	≥39.13	≥469.8
LOEC (day 28 mortality)	≥1000.0	≥39.13	≥469.8
LC_{50}	≥1000.0	≥39.13	≥469.8
NOEC (day 56 reproduction)	267.9	10.48	125.86
LOEC (day 56 reproduction)	372.4	14.57	174.95
EC_{10} (reproduction) and 95% confidence levels	258.82 202.77 – 330.37	10.13 7.93 – 12.93	121.59 95.26 – 155.21
EC_{20} (reproduction) and 95% confidence levels	399.95 343.56 – 465.59	15.65 13.44 – 18.22	187.90 161.40 – 218.73
EC_{50} (reproduction) and 95% confidence levels	920.45 770.90 – 1099.01	36.02 30.17 – 43.00	432.43 362.17 – 516.31

Materials and methods

1. ***Test Item*:** Formulation FHO04

Description: Slightly yellow opaque homogeneous liquid

Lot/Batch #: 028421

Purity: Prothioconazole: 53.14 ± 0.64 g/L (39.13 ± 0.47 g/kg)
Sulphur: 638.0 ± 7.01 g/L (469.8 ± 5.16 g/kg)

CAS #: Prothioconazole: 178928–70–6
Sulphur: 7704-34-9

Stability of test compound: Manufactured April 14, 2021, expiry April 14 2023

2. *Vehicle and/or positive control*:	Vehicle: distilled water Positive Control: carbendazim technical
3. *Test organism*:	
Species:	Earthworm, *Eisenia foetida*
Age:	~8 months old mature earthworms with clitella
Body weight:	315.4 - 598.4 mg
Source:	Original source: Annapurna Bio Farm, West Bengal, India Test organisms from in-house organisms sub-cultured and maintained on farmyard manure.
Acclimation period:	24 hours
Housing:	Glass beakers of 2 L capacity, each containing 600 g of artificial soil
Loading:	10 earthworms per 600 g artificial soil
4. *Environmental conditions* -	
Temperature:	19 - 21 °C
Photoperiod:	16 h light and 8 h darkness (474 - 586 Lux)
pH:	6.40 (before start of the experiment) 6.28 - 6.40 (at the start of experiment) 6.40 - 6.49 (at the end of the experiment)
Moisture content (%):	On day of treatment - 27.15 % (59.01 % of WHC) At the end of the experiment - 25.31 % (55.03 % of WHC) Change in the moisture content between the start of the test and on day 56 was 6.75 % (within 10 % of initial)
In-life dates:	September 29, 2021 to January 13, 2022

Study design
The artificial soil (10 % peat) was freshly prepared one day prior to the conduct of the test.

The earthworms were conditioned for 24 hours in artificial soil prior to treatment.

Based on the result of the preliminary range-finding study a quantity of 10,000 mg formulation was dissolved in 100 mL of distilled water and thoroughly mixed. Aliquots of stock solution were removed, made up to 180 mL with distilled water and added to each 600 g replicate of artificial soil to obtain the test concentrations of 99.7, 138.6, 192.7, 267.9, 372.4, 517.6, 719.4 and 1000.0, mg test item/kg artificial soil. A volume of 180 mL distilled water was mixed with each 600 g replicate of artificial soil for the control group. There were four replicates for the treatment group and eight replicates for the control group.

On the day of treatment, the earthworms were separated, washed with distilled water and dried with blotting paper. Earthworms were weighed individually. For each treatment and untreated control 10 earthworms per replicate were used.

Earthworms were observed for signs of toxicity and mortality on day 28. Body weight of individual earthworms was recorded on days 0 and 28. The moisture content of the test medium was measured on days 0 and 56.

Statistical analyses
Validated software developed in-house was used for all statistical analyses.

Data for 0, 28-day body weight and 56-day juvenile count were subjected to Shapiro-Wilk's test for normal distribution of data followed by Bartlett's test to meet the homogeneity of variance before conducting an analysis of variance (ANOVA) and Dunnett's "t" test.
Data for percent change in body weight between day 0-28 were subjected to Shapiro-Wilk's test for normal distribution of data followed by Bartlett's test to meet the homogeneity of variance before conducting an

analysis of variance (ANOVA).

Results and discussions

Mortality data
No sign of toxicity or adult mortality was observed on day 28, at the test concentration levels of 0.0 (control) 99.7, 138.6, 192.7, 267.9, 372.4, 517.6, 719.4, and 1000.0 mg prothioconazole/sulphur (50+625) g/L SC/kg dry weight of the artificial soil.

Body weight changes
The average percent body weight change between days 0 – 28 was 14.30, 16.70, 10.30, 12.60, 6.30, 13.20, 12.48, 17.90, and 12.03 at the test concentration levels of 0.0 (control), 99.7, 138.6, 192.7, 267.9, 372.4, 517.6, 719.4, and 1000 mg test item/kg dry weight of the artificial soil, respectively. No statistically significant differences in percent change in mean body weight were observed at the test concentration levels of 99.7, 138.6, 192.7, 267.9, 372.4, 517.6, 719.4, and 1000 mg Prothioconazole/Sulphur (50+625) g/L SC/kg dry weight of the artificial soil, when compared with that of the control group between days 0-28.

Juveniles
The mean number of juveniles observed on day 56 at test concentration levels of 0.0 (control) 99.7, 138.6, 192.7, 267.9, 372.4, 517.6, 719.4, and 1000.0 mg prothioconazole/sulphur (50+625) g/L SC/kg dry weight of the artificial soil was 165.25, 166.75, 169.25, 166.75, 152.00, 128.00, 119.75, 101.50, and 76.75, respectively. Statistically, a significant decrease was observed in the mean number of juveniles at test concentration levels of 372.4, 517.6, 719.4, and 1000.0 mg prothioconazole/sulphur (50+625) g/L SC/kg dry weight of the artificial soil. In contrast, statistically, no significant difference was observed in the mean number of juveniles at test concentration levels of 99.7, 138.6, 192.7, and 267.9 mg prothioconazole/sulphur (50+625) g/L SC/kg dry weight of the artificial soil when compared with that of the control group.

Deficiencies
None recorded.

Validity
The test was considered valid as per the below mentioned criteria:
No adult mortality was observed over the initial 4 weeks of the test in the control group. Each replicate (containing 10 adult earthworms) by the end of the test in the control group produced 150 to 186 juveniles (>30 juveniles).
The coefficient of variation of the reproduction in the control group was 6.96% (<30%).

Conclusion

The LC_{50}, NOEC and LOEC values for adult worm (28 days) based on mortality were greater than 1000 mg test item/kg dry weight of the artificial soil (based on nominal concentrations).

The NOEC and LOEC based on change in body weight were greater than 1000.0 mg test item/kg dry weight of the artificial soil.

The EC_{10}, EC_{20}, and EC_{50} values for juvenile production were determined to be 258.82, 399.95 and 920.45 mg test item/kg dry weight of the artificial soil, respectively.
The NOEC, based on juvenile production, was 267.9 mg test item/kg dry weight of the artificial soil and the LOEC were 372.4 mg test item/kg dry weight of the artificial soil (based on nominal concentrations).
The regression equation was $y = -1.886 + 2.323x$.

A 2.4.1.1 KCP 10.4.1.1 Earthworms - sub-lethal effects

A 2.4.1.2 KCP 10.4.1.2 Earthworms - field studies

Comments of zRMS:	The study was conducted to investigate potential effects of the two metabolites, desthio prothioconazole, and s-methyl-prothioconazole on natural earthworm populations. The earthworms were treated with a control (tap water), 2 x 50, 2 x 100, 2 x 200, 2 x 400 and 2 x 800 g prothioconazole/ha with 6 replicates per treatment group. Daily surface-density counts of alive, moribund, and dead earthworms within the first 2 days after each application were performed in the control and test item treated plots. The earthworm population was assessed for its abundance and biomass prior to the application, and approximately 1, 6, 10, and 12 months after the first application. The design covered the worst-case GAP for cereals and oil seed rape of 2 x 200 g a.s./ha (equivalent to treatment T3) with a safety factor of 4 (highest tested rate T5 = 2 x 800 g a.s./ha). The site was located in southern Germany in the federal state of Baden-Wuerttemberg between Stuttgart and Pforzheim in an area with mostly small fields (0.5–5 ha) of mixed land use. The site was cropped with cereals (triticale), but the crop was cut and removed before the first application and cut again before the second application to ensure maximum exposure of the soil surface to the applied items. Grass-clover was sown by hand on 27 May 2021 and the grass-clover crop was maintained during the study period. Maximum mean total residue levels (sum of parent and the analysed metabolites, expressed in equivalents of prothioconazole) ranged from 0.022 ± 0.001 mg/kg soil (T1, 2 x 50 g a.s./ha) to 0.532 ± 0.144 mg/kg soil (T5, 2 x 800 g a.s./ha) after the second application of the test item. The maximum percentage of s-methyl-prothioconazole of total residues was 11.3 %. The parent dissipated quickly as expected. Desthio-prothioconazole was the main metabolite at all sampling timings. Earthworm abundance in the plots treated with Prothioconazole 250 g/L EC was not affected by the treatment throughout the study period of approximately 1 year after application A1 (360 DAA1). In the plots treated with Prothioconazole 250 g/L EC, there were no statistically significant reductions ($p \leq 0.05$) in the abundance of total earthworms, or in the abundance of any of the evaluated species or species groups at any of the four post application samplings with the exception of one statistically significant reduction of L. *terrestris* adults observed at 28 DAA1 (58 %) in treatment group T2 (Table 1). A reduction of this magnitude was not seen in the other tested rates (up to 8-fold amount of applied a.s.). The maximum observed reduction in the other treatment groups for L. *terrestris* adults after application of the test item was 19 % (300 DAA1, T3, 2 x 200 g a.s./ha and 28 DAA1, T5, 2 x 800 g a.s./ha). The observation is therefore not considered treatment-related, but rather attributable to a random deviation in treatment group T2. Even in this treatment group, the observed reduction decreased to 29 % at the following sampling timing and further afterwards (1 % increase at 300 DAA1, 14 % reduction at 360 DAA1). The general discriminatory power of the test system to detect treatment-related effects as indicated by the calculation of MDDs was satisfactory. 48 % of the evaluated endpoints fell in the best two MDD classes IV and III, which means, “small to medium-sized effects can be determined statistically”. It can be concluded that two spray applications of Prothioconazole 250 g/L EC at an interval of 14 days with a maximum application rate equivalent to 2 x 800 g prothioconazole/ha during the main earth-worm activity period in spring did not cause adverse effects (neither short-term, nor long-term up to 1 year after application) on earthworm field populations.

Reference:	KCP 10.4.1.2/01
Report	A Field Study to Evaluate the Effects of Metabolites of Prothioconazole on Earthworm Populations, Vollmer, T., 2023, report No S21-03781.

Guideline(s):	Yes – ISO 11268-3 (2014) and ISO Guideline 23611-1 (2018)
Deviations:	Yes – Part of the field site was accidentally reseeded with a grass/clover mix by the farmer during work on the surrounding field. In total, this affected 5 plots: plots 38 (T3d) and 39 (Cd) completely and the plots 1 (Ra), 10 (T4b) and 19 (Ce) with approximately 60 % of the plot area (see separate scheme for details). A shallow tillage operation (rotary harrow to 5 cm depth) in combination with drilling was performed on the affected parts of the plots listed above on 06 Sep 2021. Impact on the earthworm population was assessed in detail by comparing the population in neighbouring affected and unaffected areas outside the plots as described in study plan amendment 4. The conclusion of the evaluation was given in study plan amendment 6. It was concluded that the earthworm population in the accidentally reseeded plots were significantly impacted. Plots Cd and T3d were thus excluded from statistical evaluations after sampling SEW 2. The general outcome of the study was not influenced by the deviation (5 replicates remaining in control and treatment group T3, which exceeds requirements of ISO guideline 11268-3).
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Yes

Executive summary

This study was performed to assess the potential effects of two metabolites of prothioconazole (prothioconazole-desthio (M01) and prothioconazole-s-methyl (M04)) to natural earthworm populations. The earthworms were treated with a control (tap water), 2 x 50, 2 x 100, 2 x 200, 2 x 400 and 2 x 800 g prothioconazole/ha with 6 replicates per treatment group. The reference item (Bendazol) was applied at 10000 g/ha. The test item was applied on two occasions with a 14-day interval between sprays.

Daily surface-density counts of alive, moribund, and dead earthworms within the first 2 days after each application were performed in the control and test item treated plots. The earthworm population was assessed for its abundance and biomass prior to the application, and approximately 1, 6, 10, and 12 months after the first application.

It can be concluded that two spray applications of Prothioconazole 250 g/L EC at an interval of 14 days with a maximum application rate equivalent to 2 x 800 g prothioconazole/ha during the main earthworm activity period in spring did not cause adverse effects (neither short-term, nor long-term up to 1 year after application) on earthworm field populations.

Materials and methods

1. *Test Item*: **Description:** **Lot/Batch #:** **Purity:** **CAS #:** **Stability of test compound:**	Prothioconazole 250 g/L (batch: 20191219001, a.s.: prothioconazole) Content of a.s. nominal: 250 g/L Content of a.s. analysed: 252.8 g/L Yellow liquid 20191219001 250 g/L 178928-70-6 N/A
2. *Vehicle and/or positive control*:	Vehicle: tap water Positive Control: Bendazol
3. *Test organism*:	Naturally occurring field populations of earthworms in all stages (juveniles and adults)
4. *Application rates*:	C: Tap water

	T1: 2 x 50 g prothioconazole/ha T2: 2 x 100 g prothioconazole/ha T3: 2 x 200 g prothioconazole/ha T4: 2 x 400 g prothioconazole/ha T5: 2 x 800 g prothioconazole/ha R: 1 x 10000 g carbendazim/ha

Study design

The study was conducted to investigate potential effects of the two metabolites, desthio prothioconazole, and s-methyl-prothioconazole on natural earthworm populations. The test item was applied as a commercially formulated product containing prothioconazole as the active ingredient. Metabolites were formed and dissipated in the soil within the treated plots at realistic levels and over a relevant time frame throughout the study.

The study had an extended design based on ISO 11268-3 including a control, five test item groups, and a toxic reference, following a combined NOEC/ECx design with increased replication (6 replicates per treatment group) of the control (C) and of one test item treatment (T3), plus two additional lower- (T1, T2) and higher- (T4, T5) test item treatment rates with reduced replication (three replicates per treatment group). The design covered the worst-case GAP for cereals and oil seed rape of 2 x 200 g a.s./ha (equivalent to treatment T3) with a safety factor of 4 (highest tested rate T5 = 2 x 800 g a.s./ha).

The site was located in southern Germany in the federal state of Baden-Wuerttemberg between Stuttgart and Pforzheim in an area with mostly small fields (0.5–5 ha) of mixed land use. The site was cropped with cereals (triticale), but the crop was cut and removed before the first application and cut again before the second application to ensure maximum exposure of the soil surface to the applied items. Grass-clover was sown by hand on 27 May 2021 and the grass-clover crop was maintained during the study period.

The test item was applied to field plots with very low vegetation coverage as spray application on two occasions in spring 2021, with a 14-day interval at five different rates. The control plots were treated with tap water and the reference item plots were treated once with the standard reference item carbendazim. Daily surface-density counts of alive, moribund, and dead earthworms within the first 2 days after each application were performed in the control and test item treated plots. The earthworm population was assessed for its abundance and biomass prior to the application, and approximately 1, 6, 10, and 12 months after the first application. All earthworm samples were stored in a preserving solution for species identification counting and biomass determination. The potential effect(s) of the test item treatments were assessed by comparing the data from the plots treated with the test item to the water treated control.

For application rate verification, the difference between prepared and remaining spray solution was measured for each plot using calibrated equipment and the difference between prepared and remaining spray solution was calculated. Additionally, spray solution samples (at applications A1 and A2), soil samples (immediately after applications A1 and A2), and Petri dishes (at applications A1 and A2) were collected and analysed for prothioconazole (spray solution samples and Petri dishes) and for prothioconazole and its metabolites desthio-prothioconazole and s-methyl prothioconazole (soil samples only). Further soil samples were taken at 3 and 12 days after application A1 and at 3, 7, 13, 27, and 56 days after application A2. The soil samples taken at 12 days after application A1 and at 56 days after application A2 were not analysed (analysis was optional and not requested by the study sponsors). The samples taken at 3 days after application A1 and at 3 days and 7 days after application A2 were analysed for residues of prothioconazole and its metabolites desthio-prothioconazole and s-methyl-prothioconazole, while the samples taken at the other timings were analysed for residues of the two metabolites only. Residues were analysed within the analytical phase of the study (S21-03781-L1) and the results of the analysis are summarized in an analytical phase report.

Eighteen different earthworm taxa (including thirteen different species and two morphological groups) were observed and identified in samples from the study site during the study period. Daily surface-density counts of alive, moribund, and dead earthworms within the first 2 days after each application were performed in the control and test item treated plots. The earthworm population was assessed for its abundance and biomass prior to the application, and approximately 1, 6, 10, and 12 months after the first application.

Endpoints
Abundance and biomass of the taxa "total earthworms", "total adult earthworms", "endogeic earthworms", "*Allolobophora chlorotica* adults", "*Aporrectodea caliginosa* adults", "*A. rosea* adults", "anecic earthworms", "*A. longa* total", "*A. longa* adults", "A. *longa* juveniles", "*Lumbricus terrestris* total", "*L. terrestris* adults", "*L. terrestris* juveniles", "total juveniles", "epilobous juveniles", "tanylobous juveniles"
Application rates
C: Tap water
T1: 2 x 50 g prothioconazole/ha
T2: 2 x 100 g prothioconazole/ha
T3: 2 x 200 g prothioconazole/ha
T4: 2 x 400 g prothioconazole/ha
T5: 2 x 800 g prothioconazole/ha
R: 1 x 10000 g carbendazim/ha

Test conditions
Exposure of the earthworm community to the test item was enhanced through additional irrigation of the field site directly after the applications. The combined natural rainfall and irrigation resulted in soil moisture levels that ensured constant earthworm activity and thus exposure to the treatments.

Validity criteria
Initial earthworm population at the study site (size and presence of key species) was in conformity with the requirements laid out in the guideline ISO 11268-3.
Negative impact of the toxic reference treatment on earthworm abundance and / or biomass exceeded 50% and was statistically significant.

Statistical analyses
All statistical evaluations were done using the statistical software program SAS® for Windows, release 9.4 (SAS Inc., Cary, NC, USA).

Results and discussions
The results of the soil residue analysis confirm correct application of the test item and characterises the exposure regime established in the field study. For the ecotoxicologically most relevant metabolite desthio-prothioconazole, the maximum residue levels detected in the soil samples after the second application (A2) were close to those that could be expected using worst-case assumptions for the degradation kinetics (using the highest documented DT50 values from field (parent, desthio-prothioconazole) or lab (s-methyl-prothioconazole) studies as given in EFSA, 2007). Expressed in prothioconazole equivalents, they ranged from 0.015 mg/kg dry soil in the lowest treatment rate T1 (52 % of expected) to 0.359 mg/kg dry soil in the highest treatment rate T5 (77 % of expected). Percentages for the parent prothioconazole (22–51 % of expected) and for the metabolite s-methyl-prothioconazole (0–31 % of expected) were lower.

Maximum mean total residue levels (sum of parent and the analysed metabolites, expressed in equivalents of prothioconazole) ranged from 0.022 ± 0.001 mg/kg soil (T1, 2 x 50 g a.s./ha) to 0.532 ± 0.144 mg/kg soil (T5, 2 x 800 g a.s./ha) after the second application of the test item. The maximum percentage of s-methyl-prothioconazole of total residues was 11.3 %. The parent dissipated quickly as expected. Desthio-prothioconazole was the main metabolite at all sampling timings.

Earthworm abundance in the plots treated with Prothioconazole 250 g/L EC was not affected by the treatment throughout the study period of approximately 1 year after application A1 (360 DAA1). In the plots treated with Prothioconazole 250 g/L EC, there were no statistically significant reductions ($p \leq 0.05$) in the abundance of total earthworms, or in the abundance of any of the evaluated species or species groups at any of the four post application samplings with the exception of one statistically significant reduction of L. *terrestris* adults observed at 28 DAA1 (58 %) in treatment group T2 (Table 1). A reduction of this magnitude was not seen in the other tested rates (up to 8-fold amount of applied a.s.). The maximum observed reduction in the other treatment groups for L. *terrestris* adults after application of the test item was 19 % (300 DAA1, T3, 2 x 200 g a.s./ha and 28 DAA1, T5, 2 x 800 g a.s./ha). The observation is therefore not

considered treatment-related, but rather attributable to a random deviation in treatment group T2. Even in this treatment group, the observed reduction decreased to 29 % at the following sampling timing and further afterwards (1 % increase at 300 DAA1, 14 % reduction at 360 DAA1).

The general discriminatory power of the test system to detect treatment-related effects as indicated by the calculation of MDDs was satisfactory. 48 % of the evaluated endpoints fell in the best two MDD classes IV and III, which means, “small to medium-sized effects can be determined statistically”.

Overall, no dose-response relationship was visible in the dataset, neither based on statistically significant reductions, nor based on relative changes compared to the control. In the following, the results will be discussed across all treatment groups with a focus on deviations from the control exceeding 35 %. Deviations below 35 % are considered to be “negligible” or “small” and are “deemed to allow for internal recovery of earthworm populations so that biodiversity levels and the provision of the ecosystem-services in agricultural field soils is assured in relevant time frames” according to EFSA PPR PANEL (2017). Such fluctuations are within the range of normal sampling variability. It should be noted that the threshold of 35 % is an arbitrary one, used here solely as a worst-case criterion to cover all potentially ecologically relevant deviations seen in the study. Even larger deviations may be well within the typical range of variability within a field study.

Table 1: Summary of the statistically significant ($p \leq 0.05$) reductions (⇩) of earthworm abundance compared to the control in test item treatments T1–T5 and in the reference treatment R.

Sampling (days after application A1)	-11	28	181	300	360	-11	28	181	300	360	-11	28	181	300	360
Treatment	T1 (2 x 50 g prothioconazole/ha)					T2 (2x 100 g prothioconazole/ha)					T3 (2 x 200 g prothioconazole/ha)				
Taxon															
Total															
Total Adults															
Endogeic															
A. chlorotica adults															
A. caliginosa adults															
A. rosea adults															
Anecic															
A. longa total															
A. longa adults															
A. longa juveniles															
L. terrestris total															
L. terrestris adults							⇩								
L. terrestris juveniles															
Total Juveniles															
Epilobous juveniles															
Tanylobous juveniles															

Sampling (days after application A1)	-11	28	181	300	360	-11	28	181	300	360	-11	28	181	300	360
Treatment	T4 (2 x 400 g prothioconazole/ha)					T5 (2x 800 g prothioconazole/ha)					R (10 000 g carbendazim/ha)				
Taxon															
Total											⇩	⇩	⇩	⇩	
Total Adults											⇩	⇩	⇩		
Endogeic											⇩				
A. chlorotica adults											⇩				
A. caliginosa adults											⇩				
A. rosea adults											⇩	⇩	⇩		
Anecic											⇩	⇩	⇩		
A. longa total											⇩	⇩	⇩		
A. longa adults											⇩	⇩			
A. longa juveniles											⇩		⇩	⇩	
L. terrestris total											⇩	⇩	⇩	⇩	
L. terrestris adults											⇩	⇩	⇩		
L. terrestris juveniles											⇩			⇩	
Total Juveniles											⇩	⇩	⇩	⇩	
Epilobous juveniles											⇩	⇩	⇩	⇩	
Tanylobous juveniles											⇩	⇩	⇩	⇩	

In the plots treated with Prothioconazole 250 g/L EC, there were no statistically significant reductions ($p \leq 0.05$) in the biomass of total earthworms, or in the biomass of any of the evaluated species or species groups at any of the four post application samplings with the exception of one statistically significant reduction of L. *terrestris* adults observed at 28 DAA1 (63 %) in treatment group T2 (Table 2). A reduction of this magnitude is not seen in the other tested rates (up to 8-fold amount of applied a.s.). The maximum observed reduction in the other treatment groups for L. *terrestris* adults after application of the test item is 21 % (300 DAA1, T3, 2 x 200 g a.s./ha). The observation is therefore not considered treatment-related, but rather attributable to a random deviation in treatment group T2. Even in this treatment group, the observed reduction decreased to 28 % at the following sampling timing and further afterwards (7 % reduction at 300 DAA1, 16 % reduction at 360 DAA1).

The general discriminatory power of the test system to detect treatment-related effects as indicated by the calculation of MDDs was satisfactory. 53 % of the evaluated endpoints fell in the best two MDD classes IV and III, which means, “small to medium-sized effects can be determined statistically”.

Overall, no dose-response relationship was visible in the dataset, neither based on statistically significant reductions, nor based on relative changes compared to the control. In the following, the results will be

discussed across all treatment groups with a focus on deviations from the control exceeding 35 %. Deviations below 35 % are considered to be “negligible” or “small” and are “deemed to allow for internal recovery of earthworm populations so that biodiversity levels and the provision of the ecosystem-services in agricultural field soils is assured in relevant time frames” according to EFSA PPR PANEL (2017). Such fluctuations are within the range of normal sampling variability. It should be noted that the threshold of 35 % is an arbitrary one, used here solely as a worst-case criterion to cover all potentially ecologically relevant deviations seen in the study. Even larger deviations may be well within the typical range of variability within a field study.

Table 2: Summary of statistically significant (p≤0.05) reductions (⇩) of earthworm biomass compared to the control in test item treatments T1–T5 and in the reference treatment R/

Sampling (days after application A1)	-11	28	181	300	360	-11	28	181	300	360	-11	28	181	300	360
Treatment / Taxon	**T1 (2 x 50 g prothioconazole/ha)**					**T2 (2x 100 g prothioconazole/ha)**					**T3 (2 x 200 g prothioconazole/ha)**				
Total															
Total Adults															
Endogeic															
A. chlorotica adults															
A. caliginosa adults															
A. rosea adults															
Anecic															
A. longa total															
A. longa adults															
A. longa juveniles															
L. terrestris total															
L. terrestris adults							⇩								
L. terrestris juveniles															
Total Juveniles															
Epilobous juveniles															
Tanylobous juveniles															
Sampling (days after application A1)	-11	28	181	300	360	-11	28	181	300	360	-11	28	181	300	360
Treatment / Taxon	**T4 (2 x 400 g prothioconazole/ha)**					**T5 (2x 800 g prothioconazole/ha)**					**R (10 000 g carbendazim/ha)**				
Total												⇩	⇩	⇩	⇩
Total Adults												⇩	⇩	⇩	
Endogeic												⇩			
A. chlorotica adults												⇩			
A. caliginosa adults												⇩			
A. rosea adults												⇩	⇩	⇩	
Anecic												⇩	⇩	⇩	
A. longa total												⇩	⇩	⇩	
A. longa adults												⇩	⇩		
A. longa juveniles												⇩	⇩	⇩	
L. terrestris total												⇩	⇩	⇩	⇩
L. terrestris adults												⇩	⇩	⇩	
L. terrestris juveniles												⇩			
Total Juveniles												⇩	⇩	⇩	⇩
Epilobous juveniles												⇩	⇩	⇩	⇩
Tanylobous juveniles												⇩	⇩		

Deficiencies
None recorded.

Conclusion

A field study was carried out on an arable field site in Southern Germany to assess potential effects of two spray applications of Prothioconazole 250 g/L EC at an interval of 14 days with a maximum application rate equivalent to 2 x g 800 prothioconazole/ha on earthworm field populations. The test item was applied at five different rates ranging from 2 x 50 g a.s./ha to 2 x 800 g a.s./ha. The field site had an initial earthworm population that met the requirements described in the guideline ISO 11268-3 (ISO, 2014). The observed strong and statistically significant reduction of earthworm abundance and biomass after application of the toxic reference item Bendazol (a.s. carbendazim, 10000 g/ha) indicated appropriate sensitivity of the test system and exposure of the earthworm population to the applied items.

Verification of the field application rates by residue analysis of soil cores taken at different timings after each application, by analysis of test item concentrations in Petri dishes exposed at the soil surface during

both applications, and by analysis of test item concentrations in spray solution samples taken at both applications confirmed that the target test item rates had actually been applied and that no contamination of the control (water-treated) plots occurred. The maximum mean residue level for the sum of the active substance prothioconazole and its main metabolites desthio-prothioconazole and s-methyl-prothioconazole in the 0-10 cm soil layer (expressed in equivalents of the parent prothioconazole) was observed immediately after the second application with 0.532 mg a.s./kg dry soil (treatment T5, 2x 800 g a.s./ha). Maximum residue levels of the main metabolite desthio-prothioconazole were reached 7 days after the second application with 0.359 mg/kg dry soil (treatment T5, 2x 800 g a.s./ha). The maximum percentage of s-methyl-prothioconazole of total residues was 11.3 %. The parent dissipated quickly as expected. Desthio-prothioconazole was the main metabolite at all sampling timings.

No adverse effects of the test item applications on the earthworm field population were observed in this study up to and including the highest applied rate. A single, statistically significant ($p \leq 0.05$) deviation from control levels was detected in the test item treatments during the study period: at 28 DAA1, both, abundance, and biomass of adults of the anecic species L. *terrestris* were lower in the test item treatment T2 (2 x 100 g a.s./ha) than in the control, with associated reductions of 58 % and 63 %, respectively. No such reductions were seen in the other treatments that received up to eightfold rates of the test item and this observation was therefore not considered treatment related.

No other statistically significant reductions of total earthworm abundance or biomass nor of abundance and biomass of any earthworm species, taxonomical or ecological group or earthworms occurred at any of the four post treatment samplings performed at 28, 181, 300 and 360 days after first application of the test item.

Overall, no dose-response relationship was visible in the dataset, neither based on statistically significant reductions, nor based on relative changes of abundance or biomass in the test item treatments compared to the control.

It can be concluded that two spray applications of Prothioconazole 250 g/L EC at an interval of 14 days with a maximum application rate equivalent to 2 x 800 g prothioconazole/ha during the main earthworm activity period in spring did not cause adverse effects (neither short-term, nor long-term up to 1 year after application) on earthworm field populations.

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

Study 1

Comments of zRMS:	The study was performed in line with the respective guideline with no major deviations. All validity criteria were met and the study is considered acceptable with following endpoints relevant for the risk assessment: EC_{10} = 108.7 mg product /kg dws EC_{20} = 195.1 mg product /kg dws EC_{50} = 472.2 mg product/kg dws $NOEC_{rep}$ = 95.3 mg product/kg dws

Reference:	KCP 10.4.2/01
Report	Effects on reproduction of collembola (*Folsomia candida*) in artificial soil, Hübner, S, 2022a, report No 163391016, UPL/2021/0367
Guideline(s):	Yes OECD 232, 2016 and ISO 11267, 2014
Deviations:	None

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

Executive summary

This study was performed to assess the acute toxicity (LC_{50}) of formulation FHO04 (prothioconazole/sulphur (50+625) g/L SC) to collembola (*Folsomia candida*). The collembola were exposed to the test concentration of 0 (untreated control), 16.3, 29.4, 52.9, 95.3, 171, 309, 556 and 1000 mg FHO04/kg soil dry weight equivalent to 0.639 + 7.67, 1.15 + 13.8, 2.07 + 24.9, 3.73 + 44.8, 6.71 + 80.6, 12.1 + 145, 21.7 + 261 and 39.1 + 470 mg prothioconazole + mg sulphur/kg soil dry weight based on the content of 53.14 g/L prothioconazole and 638.0 g/L sulphur and a density of 1.358 g/mL) in artificial soil for a period of 28 days. Each concentration was replicated 4 times with 10 individuals per replicate. The moisture content of the artificial soil during the exposure period was water content at experimental start 17.5 % to 18.0 % (51.6 % to 52.9 % of the maximum water holding capacity); water content at experimental end 15.3 % to 17.1 % (44.9 % to 50.2 % of the maximum water holding capacity).

Table 1. Summary of the Effects of FHO04 on Collembola (*Folsomia candida*) in a 28-day Reproduction Study

FHO04 [mg/kg soil dry weight]	**Control**	**16.3**	**29.4**	**52.9**	**95.3**	**171**	**309**	**556**	**1000**
mg Prothioconazole + mg Sulphur/kg soil dry weight		**0.639 + 7.67**	**1.15 + 13.8**	**2.07 + 24.9**	**3.73 + 44.8**	**6.71 + 80.6**	**12.1 + 145**	**21.7 + 261**	**39.1 + 470**
Mean mortality (day 28) [%]	4	5	5	5	0	3	5	10	58
Significance [1)]	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*
Mean no. of juveniles (day 28)	1546	1521	1663 [a)]	1468	1406	1279	997	904 [a)]	100
Reproduction in [%] of control (day 28)	-	98	108 [a)]	95	91	83	64	58 [a)]	6
Statistical significance [2)]	-	n.s.	n.s.	n.s.	n.s.	*	*	*	*

Endpoints [mg test item/kg soil dry weight]			
NOEC (mortality)	556		
LOEC (mortality)	1000		
LC_{50} (mortality) [3)]	902.3 (95% confidence limits of 807.3 – 1008.6)		
NOEC (reproduction)	95.3		
LOEC (reproduction)	171		
EC Values (reproduction) [4)]	EC_{10}: 108.7	EC_{20}: 195.1	EC_{50}: 472.2
95% confidence limits	47.8 – 166.5	113.3 – 263.9	373.3 – 591.5

Endpoints [mg Prothioconazole + mg Sulphur/kg soil dry weight]			
NOEC (mortality)	21.7 + 261		
LOEC (mortality)	39.1 + 470		
LC_{50} (mortality) [3)]	35.3 + 423.9 (95% confidence limits of 31.6 – 39.5+ 379.3 – 473.8)		
NOEC (reproduction)	3.73 + 44.8		
LOEC (reproduction)	6.71 + 80.6		
EC Values (reproduction) [4)]	EC_{10}: 4.25 + 51.1	EC_{20}: 7.63 + 91.7	EC_{50}: 18.5 + 221.8
95% confidence limits	1.87 – 6.52 + 22.5 – 78.2	4.43 – 10.3 + 53.2 – 124.0	14.6 – 23.1 + 175.4 – 277.9

n.s. = not significantly different compared to the control
* = significantly different compared to the control
[1)] Step-Down Cochran-Armitage Test, $\alpha = 0.05$, one-sided greater
[2)] Williams t-test, $\alpha = 0.05$, one-sided smaller
[3)] Trimmed Spearman Karber procedure
[4)] Weibull Analysis
- not applicable
[a)] = one replicate of this treatment group was found to be an outlier, the replicate was therefore excluded from further statistical evaluation (Outlier-test after Hampel, $\alpha = 0.05$)

Materials and methods

1. ***Test Item*:** Formulation FHO04

Description: Slightly yellow liquid

Lot/Batch #: 028421

Purity: Prothioconazole: 53.14 ± 0.64 g/L (39.13 ± 0.47 g/kg)

	Sulphur: 638.0 ± 7.01 g/L (469.8 ± 5.16 g/kg), according to GLP-CoA (based on analysis)
CAS #:	Prothioconazole: 178928–70–6 Sulphur: 7704-34-9
Stability of test compound:	Expiry date: November 05, 2023
2. *Vehicle and/or positive control*:	Vehicle: distilled water Positive Control: Boric acid
3. *Test organism*:	
Species:	Collembola; Isotomidae (commonly known as springtails) *Folsomia candida* (Willem 1902)
Age:	Synchronised juveniles 10 – 12 days (at experimental start), adults
Source:	In-house stock bred and maintained at Ibacon test facility.
Acclimation period:	None
Housing:	Glass containers (volume: 100 mL; diameter: 5 cm), closed tightly to avoid water evaporation, filled with 30 g ± 1.0 g artificial soil dry weight.
Loading:	10 individuals per 30 g artificial soil
4. *Environmental conditions* -	
Temperature:	18°C to 22 °C
Photoperiod:	16 h light, 8 h dark (400 lux to 800 lux)
pH:	Determined according to ISO 11465 and ISO 10390 ($CaCl_2$) at test start and test end. At test start: 6.2 to 6.4; at test end: 5.6 to 6.4
Moisture content (%):	At test start: 17.5 % to 18.0 % (51.6 % to 52.9 % of max. WHC, i.e. within the recommended range 40-60 % of total WHC) At test end: 15.3 % to 17.1 % (44.9 % to 50.2 % of max. WHC)
In-life dates:	Experiment start date: November 10, 2021 Completion date: December 28, 2021

Study design

The artificial soil was moistened to approximately half of the final water content 3 days before the application. The additional water required to achieve the final water content was added when applying the test item.

A stock solution was prepared by adding deionized water to 1030.0 mg of FHO04 to obtain a final net weight of 112.1 g, equivalent to a concentration of 9.1882 mg test item/g. A dilution series was prepared from this stock solution. 38.1 g of the stock solution and of the corresponding dilutions were added to artificial soil equivalent to 350 g dry weight.

Target concentration [mg test item /kg soil dry weight]	dilution series	Concentration of the added dilution [mg test item /g]	Achieved nominal concentration [mg test item /kg soil dry weight]
16.3	60.0 g dilution 6 + 48.2 g water	0.1497	16.29
29.4	60.0 g dilution 5 + 48.0 g water (= dilution 6)	0.2699	29.38
52.9	60.0 g dilution 4 + 48.1 g water (= dilution 5)	0.4858	52.88
95.3	60.0 g dilution 3 + 47.7 g water (= dilution 4)	0.8753	95.28
171	60.0 g dilution 2 + 48.4 g water (= dilution 3)	1.5711	171.0
309	60.0 g dilution 1 + 48.0 g water (= dilution 2)	2.8385	309.0
556	60.0 g stock solution + 47.9 g water (= dilution 1)	5.1093	556.2
1000	stock solution	9.1882	1000.2

On the day of treatment, 10 individual collembola were collected with an aspirator and put into a small glass tube, before placement onto the surface of the treated artificial soil.

After 28 days exposure, collembola were extracted from artificial soil by flotation. Adult animals were counted visually, juveniles were counted using the digital phot evaluation software 'FolsomiaCounter' Version 1.24 2021; © Visionalytics).

Twenty eight days after application the numbers of living adult collembola (Mortality) and juvenile collembola (Reproduction) were recorded. Missing adult collembola were recorded as dead (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 (Behaviour).

Statistical analyses

Mortality data were statistically analysed using Step-down Cochran-Armitage Test ($\alpha = 0.05$, one-sided greater). An LC_{50} value and its 95 % confidence limits at day 28 was calculated by applying Trimmed Spearman-Karber procedure. Values were compensated for control mortality using Abbott's formula. The reproduction data were tested for outliers (Outlier-test after Hampel, $\alpha = 0.05$). The replicates that were found to be outliers were excluded from further statistical evaluation. Reproduction data were tested for normal distribution and homogeneity of variance using Shapiro-Wilk's test and Cochran'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, $\alpha = 0.05$, 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 for reproduction were calculated by Weibull Analysis. The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ToxRat® Solutions GmbH.

Results and discussions

Mortality data

Mortality of Folsomia candida was not statistically significantly different compared with the control up to and including the test concentration of 556 mg test item/kg soil dry weight (Step-down Cochran-Armitage Test, $\alpha = 0.05$, one-sided greater). At the test concentration of 1000 mg test item/kg soil dry weight a statistically significant increased mortality was observed.

Table 3. Mortality of Adult Collembola after 28 days

Treatment Group	Number of Surviving Adults per Replicate								Mean Mortality [%]	Standard Deviation	Signifi-cance[1]
	1	2	3	4	5	6	7	8			
Control	9	10	10	9	10	10	9	10	4	± 5%	-
16.3	8	10	10	10	-	-	-	-	5	± 10%	n.s.
29.4	10	10	10	8	-	-	-	-	5	± 10%	n.s.
52.9	10	8	10	10	-	-	-	-	5	± 10%	n.s.
95.3	10	10	10	10	-	-	-	-	0	± 0%	n.s.
171	10	9	10	10	-	-	-	-	3	± 5%	n.s.
309	10	10	8	10	-	-	-	-	5	± 10%	n.s.
556	10	8	9	9	-	-	-	-	10	± 8%	n.s.
1000	5	3	4	5	-	-	-	-	58	± 10%	*

The results represent rounded values calculated from the exact raw data
Test item dosages are given as mg test item/kg artificial soil dry weight
[1] Step-down Cochran-Armitage Test, one-sided greater, α = 0.05
- Not applicable
n.s. = Not statistically significantly different compared to the control
* = Statistically significantly different compared to the control

Reproduction

One replicate each in the treatments of 29.4 mg test item/kg soil dry weight and 556 mg test item/kg soil dry weight was found to be an outlier and was therefore excluded from further evaluation (Outlier test after Hampel, α = 0.05). There were no statistically significant effects on reproduction of *Folsomia candida* up to and including the concentration of 95.3 mg test item/kg soil dry weight (Williams t-test, α = 0.05, one-sided smaller). At the concentration of 171 mg test item/kg soil dry weight and above reproduction was statistically significant reduced compared to the control.

Table 4. Reproduction of Collembola after 28 days

Treatment Group	Number of Juveniles per Replicate								Mean	Standard Deviation	% of Control	Signifi-cance[1]
	1	2	3	4	5	6	7	8				
Control	1484	1566	1677	1396	1617	1447	1589	1590	1546	± 95	-	-
16.3	1713	1563	1412	1396	-	-	-	-	1521	± 148	98	n.s.
29.4	1668	1644	1527[a)]	1677	-	-	-	-	1663	± 17	108	n.s.
52.9	1439	1474	1486	1471	-	-	-	-	1468	± 20	95	n.s.
95.3	999	1651	1823	1151	-	-	-	-	1406	± 394	91	n.s.
171	971	1323	1619	1202	-	-	-	-	1279	± 270	83	*
309	1274	546	665	1501	-	-	-	-	997	± 463	64	*
556	945	836	571[a)]	931	-	-	-	-	904	± 59	58	*
1000	115	72	48	165	-	-	-	-	100	± 51	6	*

The results represent rounded values calculated from the exact raw data
Test item dosages are given as mg test item/kg artificial soil dry weight
[1] Williams t-test, α = 0.05, one-sided smaller
[a)] Outlier after Hampel, α = 0.05
- Not applicable
n.s. = Not significantly different compared to the control
* = Significantly different compared to the control

Behavioural abnormalities

No abnormal behaviour was observed with the surviving collembola.

Deficiencies

None recorded.

Validity

Mean mortality was 4 %, validity criterion was met.
The number of juvenile collembola per replicate was 1396 to 1677, validity criterion was met.
The coefficient of variation of the control reproduction was 6.1 %, validity criterion was met:
Conclusion

The NOEC for reproduction was determined to be 95.3 mg test item/kg soil dry weight and the LOEC for reproduction was determined to be 171 mg test item/kg soil dry weight.

The EC_{10} for *Folsomia candida* in artificial soil was determined to be 108.7 mg test item/kg soil dry weight (95 % confidence limits of 47.8 to 166.5 mg test item/kg soil dry weight, Weibull Analysis). The EC_{20} was determined to be 195.1 mg test item/kg soil dry weight (95 % confidence limits of 113.3 to 263.9 mg test item/kg soil dry weight). The EC_{50} was determined to be 472.2 mg test item/kg soil dry weight (95 % confidence limits of 373.3 to 591.5 mg test item/kg soil dry weight).

Study 2

Comments of zRMS:	The study was performed in line with the respective guideline with no major deviations. All validity criteria were met and the study is considered acceptable with following end-points relevant for the risk assessment: EC_{10} = n.d EC_{20} = n.d EC_{50} >1000.0 mg product/kg dws NOECrep ≥ 1000.0 mg product/kg dws

Reference:	KCP 10.4.2/02
Report	Effects on reproduction of the predatory mite (*Hypoaspis aculeifer*) in artificial soil, Hübner, S, 2022b, report No 163391089, UPL/2021/0368
Guideline(s):	Yes - OECD 226, 2016
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No – invertebrate study

Executive summary
This study was performed to assess the acute toxicity (LC_{50}) of formulation FHO04 (prothioconazole/sulphur (50+625) g/L SC) to predatory mite (*Hypoaspis aculeifer*). The mites were exposed to the test concentration of 0 (untreated control), 16.3, 29.4, 52.9, 95.3, 171, 309, 556 and 1000 mg FHO04/kg soil dry weight equivalent to 0.639 + 7.67, 1.15 + 13.8, 2.07 + 24.9, 3.73 + 44.8, 6.71 + 80.6, 12.1 + 145, 21.7 + 261 and 39.1 + 470 mg prothioconazole + mg sulphur/kg soil dry weight based on the content of 53.14 g/L prothioconazole and 638.0 g/L sulphur and a density of 1.358 g/mL) in artificial soil for a period of 14 days. Each concentration was replicated 4 times with 10 individuals per replicate. The moisture content of the artificial soil during the exposure period was water content at experimental start 17.5 % to 18.0 % (51.6 % to 52.9 % of the maximum water holding capacity); water content at experimental end 16.5 % to 17.9 % (48.5 % to 52.7 % of the maximum water holding capacity).

Table 1. Summary of the Effects of FHO04 on the Predatory Mite *Hypoaspis aculeifer* in a 14-day Reproduction Study

FHO04 [mg/kg soil dry weight]	**Control**	**16.3**	**29.4**	**52.9**	**95.3**	**171**	**309**	**556**	**1000**
mg Prothioconazole + mg Sulphur/kg soil dry weight		**0.639 + 7.67**	**1.15 + 13.8**	**2.07 + 24.9**	**3.73 + 44.8**	**6.71 + 80.6**	**12.1 + 145**	**21.7 + 261**	**39.1 + 470**
Mortality (day 14) [%]	6	0	5	8	10	5	0	8	0
Statistical significance[1)]	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
No. of juveniles (day 14)	175	163	200	209	204	194	117	160	172
Reproduction in [%] of control (day 14)	-	93	114	119	117	111	67	91	98
Statistical significance [2)]	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Endpoints [mg test item/kg soil dry weight]									
NOEC (mortality)	≥1000								
LOEC (mortality)	>1000								
LC_{50} (mortality) [3)]	>1000								
NOEC (reproduction)	≥1000								
LOEC (reproduction)	>1000								
EC_{50} (reproduction) [3)]	>1000								
Endpoints [mg Prothioconazole + mg Sulphur/kg soil dry weight]									
NOEC (mortality)	≥39.1 + ≥470								
LOEC (mortality)	>39.1 + >470								
LC_{50} (mortality) [3)]	>39.1 + >470								
NOEC (reproduction)	≥39.1 + ≥470								
LOEC (reproduction)	>39.1 + >470								
EC_{50} (reproduction) [3)]	>39.1 + >470								

n.s. = not 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
[3)] estimated value
- not applicable

Materials and methods

1. ***Test Item*:** Formulation FHO04

Description: Slightly yellow liquid

Lot/Batch #: 028421

Purity: Prothioconazole: 53.14 ± 0.64 g/L (39.13 ± 0.47 g/kg)
Sulphur: 638.0 ± 7.01 g/L (469.8 ± 5.16 g/kg), according to GLP-CoA (based on analysis)

CAS #: Prothioconazole: 178928–70–6
Sulphur: 7704-34-9

Stability of test compound: Expiry date: November 05, 2023

2. ***Vehicle and/or positive control*:** Vehicle: distilled water
Positive Control: Dimethoate

3. ***Test organism*:**

Species: Predatory mites (Acari: Laelapidae)
Hypoaspis aculeifer (Canestrini 1883)

Age: Adults, approximately 12 days after reaching the adult stage (33 days after placing adult females in clean rearing vessels over a period of 2 days)

Source: Katz Biotech AG, An der Birkenpfuhlheide 10, 15837 Baruth, Germany

Acclimation period: None

Housing: Glass containers (volume: 100 mL; diameter: 5 cm), closed tightly to avoid water evaporation, filled with 20 g ± 1.0 g artificial soil dry weight.

Loading: 10 individuals per 30 g artificial soil

4. ***Environmental conditions* -**

Temperature: 18°C to 22 °C

Photoperiod: 16 h light, 8 h dark (400 lux to 800 lux)

pH: Determined according to ISO 11465 and ISO 10390 ($CaCl_2$) at test start and test end.
At test start: 6.2 to 6.4; at test end: 5.9 to 6.1

Moisture content (%): At test start: 17.5 % to 18.0 % (51.6 % to 52.9 % of max. WHC, i.e. within the recommended range 40-60 % of total WHC)
At test end: 16.5 % to 17.9 % (48.5 % to 52.7 % of max. WHC)

In-life dates: Experiment start date: November 10, 2021
Completion date: December 15, 2021

Study design

The artificial soil (10 % peat) was moistened to approximately half of the final water content 3 days before the application. The additional water required to achieve the final water content was added when applying the test item.

A stock solution was prepared by adding deionized water to 1030.0 mg of FHO04 to obtain a final net weight of 112.1 g, equivalent to a concentration of 9.1882 mg test item/g. A dilution series was prepared from this stock solution. 38.1 g of the stock solution and of the corresponding dilutions were added to artificial soil equivalent to 350 g dry weight.

Target concentration [mg test item /kg soil dry weight]	dilution series	Concentration of the added dilution [mg test item /g]	Achieved nominal concentration [mg test item /kg soil dry weight]
16.3	60.0 g dilution 6 + 48.2 g water	0.1497	16.29
29.4	60.0 g dilution 5 + 48.0 g water (= dilution 6)	0.2699	29.38
52.9	60.0 g dilution 4 + 48.1 g water (= dilution 5)	0.4858	52.88
95.3	60.0 g dilution 3 + 47.7 g water (= dilution 4)	0.8753	95.28
171	60.0 g dilution 2 + 48.4 g water (= dilution 3)	1.5711	171.0
309	60.0 g dilution 1 + 48.0 g water (= dilution 2)	2.8385	309.0
556	60.0 g stock solution + 47.9 g water (= dilution 1)	5.1093	556.2
1000	stock solution	9.1882	1000.2

On the day of treatment, 10 adult females were collected with paint brush and put into a small glass tube,

before placement onto the surface of the treated artificial soil 2 hours after addition of the test substance.

After 14 days' exposure, mites were extracted from artificial soil by exposing the soil to increasing heat (from ~25 to 30 °C during a ~2 day period). Displaced animals were collected in fixation solution (glycol + detergent). Adult animals were counted visually, juveniles were counted twice using a binocular microscope. Nine of the replicates were counted three times because the first two counts deviated more than 10 % from their mean value.

Fourteen days after application the numbers of extracted adult female predatory mites (Mortality) and juvenile mites (Reproduction) were recorded. Missing adult predatory mites were recorded as dead (it was assumed that missing adult mites had died and degraded during the test period). Extracted mites were observed for any abnormal behaviour or conditions (Behaviour).

Statistical analyses

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 Since the reproduction data were normally distributed and homogeneous and did follow a monotonicity trend (contrast trend) the Williams t-test (multiple comparison, $\alpha = 0.05$, 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_{50} was not determined by statistical analysis as no reduction of reproduction above 50 % was observed. The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ToxRat® Solutions GmbH.

Results and discussions

Mortality data

Mortality of *Hypoaspis aculeifer* in the test item treated group ranged from 0 % to 10 %. The values were not statistically significantly different compared to the control, where 6 % of the adult mites died (Fisher's Exact Test, $\alpha = 0.05$, one-sided greater). Therefore, the NOEC for 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.

Table 3. Mortality of adult *Hypoaspis aculeifer* after 14 days

Treatment Group	Number of Surviving Females per Replicate								Mean Mortality [%]	Standard Deviation [%]	Significance[1]
	1	2	3	4	5	6	7	8			
Control	10	10	9	8	10	9	9	10	6	± 7	-
16.3	10	10	10	10	-	-	-	-	0	± 0	n.s.
29.4	9	10	9	10	-	-	-	-	5	± 6	n.s.
52.9	9	8	10	10	-	-	-	-	8	± 10	n.s.
95.3	9	9	9	9	-	-	-	-	10	± 0	n.s.
171	9	10	9	10	-	-	-	-	5	± 6	n.s.
309	10	10	10	10	-	-	-	-	0	± 0	n.s.
556	10	9	9	9	-	-	-	-	8	± 5	n.s.
1000	10	10	10	10	-	-	-	-	0	± 0	n.s.

The results represent rounded values calculated from the exact raw data
Test item dosages are given as mg test item/kg artificial soil dry weight
[1] Fisher´s Exact Test , one-sided greater, $\alpha = 0.05$
- Not applicable
n.s. = Not statistically significantly different compared to the control

Reproduction

There were no statistically significant effects on reproduction of *Hypoaspis aculeifer* up to and including the highest test concentration of 1000 mg test item/kg soil dry weight (Williams t-test, $\alpha = 0.05$, one-sided

smaller).

Table 4. Reproduction of *Hypoaspis aculeifer* after 14 days

Treatment Group	Number of Juveniles per Replicate[1]								Mean	Standard Deviation	% of Control	Signifi-cance[2]
	1	2	3	4	5	6	7	8				
Control	188	153	174	213	157	175	176	168	175	± 19	-	-
16.3	104	211	197	139	-	-	-	-	163	± 50	93	n.s.
29.4	208	152	170	271	-	-	-	-	200	± 53	114	n.s.
52.9	238	190	218	192	-	-	-	-	209	± 22	119	n.s.
95.3	205	192	176	246	-	-	-	-	204	± 30	117	n.s.
171	191	172	204	209	-	-	-	-	194	± 17	111	n.s.
309	67	122	123	157	-	-	-	-	117	± 37	67	n.s.
556	188	168	156	128	-	-	-	-	160	± 25	91	n.s.
1000	176	176	155	181	-	-	-	-	172	± 12	98	n.s.

The results represent rounded values calculated from the exact raw data
Test item dosages are given as mg test item/kg artificial soil dry weight
[1] Mean of two counts; numbers underlined are mean of three counts
[2] Williams t-test, α = 0.05, one-sided smaller
- Not applicable
n.s. = Not significantly different compared to the control

Behavioural abnormalities
No behavioural abnormalities were observed in any of the treatment groups.

Deficiencies
None recorded.

Validity
Mean mortality was 6 %, validity criterion was met.
The number of juvenile mites per replicate was 153 to 213, validity criterion was met.
The coefficient of variation of the control reproduction was 10.8 %, validity criterion was met:

Conclusion
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.
LC_{50} and EC_{50} values were estimated to be >1000 mg test item/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

Comments of zRMS:	The study was performed in line with the respective guideline with no major deviations. All validity criteria were met and the study is considered acceptable with following end-points relevant for the risk assessment: It can be concluded that product FH004 does not have any long-term influence on nitrogen transformation in soil, at its maximum application rate per season (1T), i.e., 8.00 L/ha or 10.67 µL/kg dry weight soil and at its 5 times of the maximum application rate per season (5T), i.e., 40.00 L/ha or 53.33 µL/kg of soil.

Reference: KCP 10.5/01

Report	Effect of prothioconazole/sulphur (50+625) g/l sc on soil microorganisms: nitrogen transformation test, Bhosale, J.D., 2022, report No 608-3-15-29110, sponsor's study No. UPL/2021/0538, Authority registration No GLP/C-139/2019
Guideline(s):	Yes – OECD 216, Soil microorganisms: nitrogen transformation test.
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No – soil microorganism study

Executive summary

The purpose of this study was to assess the effects of formulation FHO04 (prothioconazole/sulphur (50+625) g/L SC) on the nitrogen transformation activity of soil microflora under laboratory conditions through comparison of test item treated soil with a non-treated soil. EFM-59 (C/N) soil (sandy-clay loam texture, USDA) collected from pasture adjacent to the Par River and amended with nitrogen-rich dried lucerne was used. Distilled water without test item was added to non-treated soil and the test item was applied to treated soil at dose level 8.00 L/ha or 10.67 µL/kg dry weight soil (maximum application rate per season, 1T), and at dose level 40.00 L/ha or 53.33 µL/kg dry weight soil (5 times of the maximum application rate per season, 5T). Treatments were replicated in triplicate and incubated at 20 ± 2 °C in the dark. Nitrate content was measured on the day 0, 7, 14, and 28 of the test item application.

As the difference in the mean nitrate content between the lower treatment (1T) and the control was -0.27 % (less than 25 %) on day 28, the experiment was terminated on day 28, and further analysis was not performed.

Day	Mean Nitrate Content (mg/kg dry weight soil)			Percent Deviation of Nitrate Content	
	Control	Treatment (1T)	Treatment (5T)	Treatment (1T)	Treatment (5T)
0	61.47	64.27	68.13	-4.56	-10.83
7	71.20	74.13	74.27	-4.12	-4.31
14	92.27	95.07	96.53	-3.03	-4.62
28	99.20	99.47	100.00	-0.27	-0.81

Note: (-) sign shows promoted nitrogen transformation activity of the soil microorganisms, compared to the control.

Sample Details	Nitrate Formation Rate (mg nitrate/ kg dry weight soil/day)		
	0 to 7	0 to 14	0 to 28
Control	1.39	2.20	1.35
Treatment (1T)	1.41	2.20	1.26
Treatment (5T)	0.88	2.03	1.14

The results of this study revealed that the test item does not have a long-term influence on the nitrogen transformation in the soil at its maximum application rate per season (1T), i.e., 8.00 L/ha or 10.67 µL/kg dry weight soil, or at its 5 times of maximum application rate per season (5T), i.e., 40.00 L/ha or 53.33 µL/kg of soil.

Materials and methods

1. *Test Item*:	Formulation FHO04
Description:	Slightly yellow opaque homogeneous liquid
Lot/Batch #:	028421
Purity:	Prothioconazole: 53.14 ± 0.64 g/L (39.13 ± 0.47 g/kg) Sulphur: 638.0 ± 7.01 g/L (469.8 ± 5.16 g/kg)
CAS #:	Prothioconazole: 178928–70–6 Sulphur: 7704-34-9
Stability of test compound:	Manufactured April 14, 2021, expiry April 14, 2023
2. *Control* -	Distilled water without test item
3. *Soil* -	
Name:	EFM-59 (C/N)
Collection site:	Latitude – 20.5373042 and Longitude – 73.1705381 of Dharampur, India
pH:	7.46 (water)
Organic carbon:	0.64 %
Sand:	70.48 %
Silt:	6.24 %
Clay:	23.28 %
Texture:	sandy-clay loam (USDA)
Water holding capacity:	57.87 %
Organic substrate:	5 g Lucerne grass green meal powder [C: N ratio (13.59:1)] added to one kg soil (dry weight)
4. *Environmental conditions* -	
Temperature:	20 ± 2 °C
Moisture content:	50 ± 5% of maximum water holding capacity
In life dates	September 14, 2021 to October 11, 2021

Study design

The formulation FHO04 (Prothioconazole/Sulphur (50+625) g/L SC) was applied to sandy-clay loam textured soil (EFM-59 (C/N)) collected from pasture adjacent to the Par River, at dose level 8.00 L/ha or 10.67 µL/kg dry weight soil (maximum application rate per season, 1T), and at dose level 40.00 L/ha or 53.33 µL/kg dry weight soil (5 times of the maximum application rate per season, 5T) (three treatments). Untreated EFM-59 (C/N) soil was tested as the control. There were three replicates per treatment. Nitrate content was measured on the day 0, 7, 14, and 28 of the test item application. As the difference in the mean nitrate content between the lower treatment (1T) and the control was -0.27% (less than 25%) on day 28, the experiment was terminated on day 28, and further analysis was not performed.

Nitrate analysis

Soil (32 g of soil = 25 g dry weight equivalent) from control and the treated soil containers was extracted with 125 mL 0.1 M potassium chloride solution by shaking at 150 ± 5 rpm for 60 minutes at 20 ± 2 °C. After centrifugation (5000 rpm, 10 min.) and filtration (Whatman No. 1) 2 mL of 2 M ammonium sulphate were added to 100 mL of filtrate and nitrate (NO_3^-) concentrations determined using a pre-calibrated ISE meter using nitrate (NO_3^-) electrode.

Statistical analyses

Statistical analysis of mean nitrate content data was performed by using in-house developed validated statistical computer software for the data homogeneity (F test for homogeneity of variance).

Results and discussions

Nitrogen transformation – Nitrate content

TABLE 1: Nitrate (NO_3^-) Content at Different Time Points

(Refer: APPENDIX 1)

Nitrate Content (mg/kg dry weight soil)					
Sample		Day			
		0	7	14	28
Control	CR_1	60.40	71.20	92.00	98.00
	CR_2	61.60	72.40	94.40	99.60
	CR_3	62.40	70.00	90.40	100.00
	Mean	**61.47**	**71.20**	**92.27**	**99.20**
Treatment (1T)	$1TR_1$	63.20	73.60	94.00	98.40
	$1TR_2$	64.00	73.60	95.60	99.60
	$1TR_3$	65.60	75.20	95.60	100.40
	Mean	**64.27**	**74.13**	**95.07**	**99.47**
Treatment (5T)	$5TR_1$	68.00	72.40	96.40	99.60
	$5TR_2$	67.20	74.00	98.00	100.00
	$5TR_3$	69.20	76.40	95.20	100.40
	Mean	**68.13**	**74.27**	**96.53**	**100.00**

Statistically, no significant difference in the mean nitrate content of treatment (1T), and (5T) was observed on day 0, 7, 14, and 28 when compared with that of the control.

Nitrogen transformation – Mineral nitrogen content

Since the difference in the mean nitrate (NO_3^-) content between the lower treatment (1T) and the control, was less than 25% on day 28, this study was terminated on day 28, and further analysis was not performed.

Nitrogen transformation – Nitrate formation rates

Mean nitrogen formation rates are summarised in Table 2, below.

TABLE 2: Nitrate (NO_3^-) Formation Rate at Different Time Intervals

(Refer: APPENDIX 2)

Sample Details	Nitrate Formation Rate (mg nitrate/kg dry weight soil/day)		
	0 to 7	0 to 14	0 to 28
Control	1.39	2.20	1.35
Treatment (1T)	1.41	2.20	1.26
Treatment (5T)	0.88	2.03	1.14

TABLE 4: Percent Deviation of Mean Nitrate Content on Different Days

(Refer: APPENDIX 1)

Days	Percent Deviation of Mean Nitrate Content	
	Treatment (1T)	Treatment (5T)
0	-4.56	-10.83
7	-4.12	-4.31
14	-3.03	-4.62
28	-0.27	-0.81

Key:

- = Sign shows promoted nitrogen transformation activity of soil microorganisms, compared to the control

Validity criteria

The variation between the replicate control samples was less than 15 % which fulfilled the validity criterion for the nitrogen transformation test throughout the study period.

Conclusion

Results of this study revealed that test item, does not have any long-term influence on nitrogen transformation in soil, at its maximum application rate per season (1T), i.e., 8.00 L/ha or 10.67 µL/kg dry weight soil and at its 5 times of the maximum application rate per season (5T), i.e., 40.00 L/ha or 53.33 µL/kg of 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 Study 1: seedling emergence test

Comments of zRMS:	The study was performed in line with OECD 208 with no major deviations. All validity criteria were met and the study is considered acceptable. Overall, the study is considered acceptable with following endpoints relevant for the risk assessment: ER_{50} > 16 L FH004 /ha. Visual phytotoxicity: There was no phytotoxic symptom observed in any of the species tested.

Reference:	KCP 10.6.2/01
Report	Prothioconazole/Sulphur (50+625) g/L SC (FHO04): Effects on the Seedling Emergence and Seedling Growth of Terrestrial Plant Species, D. Ripperger, 2022b, Report No. S21-05533
Guideline(s):	Yes – OECD 208
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive summary

The effects on plants following deposition of the test item FHO04 on seedling emergence and early growth of higher plants were tested in a greenhouse study. The dicotyledon species tested were sugar beet (*Beta vulgaris*), oil seed rape (*Brassica napus*), soybean (*Glycine max*) and tomato (*Solanum lycopersicum*). The monocotyledon species tested were onion (*Allium cepa*) and maize (*Zea mays*).

For each species, 5 treatment groups were used with application rates 0.0 (control), 1.0, 2.0, 4.0, 8.0, and 16.0 L product/ha. The pots were placed in saucers on cultivation tables under the greenhouse condition.

The seedling emergence and the conditions of the plants, with regard to mortality and visual phytotoxicity were recorded on days 7, 14 and 21 after 50% of the control plants had emerged. At the end of the test, all plants surviving were cut at soil level and weighed.

FHO04 did not significantly decrease the emergence or the fresh weight of any of the species tested.

Table 1: Effects on terrestrial plants following soil application

Species	ER_{50} (L FHO04/ha)	NOER (L FHO04/ha)
	Rate	
Beta vulgaris	> 16.0	> 16.0

Brassica napus	> 16.0	> 16.0
Glycine max	> 16.0	> 16.0
Solanum lycopersicum	> 16.0	> 16.0
Allium cepa	> 16.0	> 16.0
Zea mays	> 16.0	> 16.0

Materials and methods

1. ***Test Item*:** FHO04
   - **Description:** Liquid
   - **Lot/Batch #:** 028421
   - **Purity:** Prothioconazole: 53.14 g/L
     Sulphur: 638.0 g/L
   - **CAS #:** Prothioconazole: 178928–70–6
     Sulphur: 7704-34-9
   - **Stability of test compound:** N/A - Stable
2. ***Vehicle*:** Tap water
3. ***Test system* -**
   - **Species:** Sugar beet (*Beta vulgaris*), oil seed rape (*Brassica napus*), soybean (*Glycine max*), tomato (*Solanum lycopersicum*), onion (*Allium cepa*) and maize (*Zea mays*)
   - **Growth stage:** -
   - **Substrate:** Soil, sandy loam texture, 1.26 % organic C, pH 7.26
4. ***Environmental conditions* -**
   - **Temperature:** 12.5 - 27.5 °C
   - **Humidity:** 52.0 - 100.0 %
   - **Light intensity:** 331.3 – 679.4 Lux
   - **Photoperiod:** 16 h light 8 hours dark
   - **In life dates** November 25, 2021 to May 05, 2022

Study design

All the species were germinated and grown in a greenhouse located in E-46900 Torrent, Spain. The seeds were not coated with insecticide/fungicide and no plant protection products were used. Two seeds for all dicotyledonous species and *Zea mays* and 4 seeds for *Allium cepa* per pot were sown into pots at a depth of 0.5-2 cm making 10 replicates per treatment for each plant species.

Table 2: Details of replicate numbers and plant species

Species	Common name	Family	No of repli-cates	No of seeds/replicate
Beta vulgaris	sugar beet	Amaranthaceae	10	2
Brassica napus	oil seed rape	Brassicaceae	10	2
Glycine max	soybean	Fabaceae	10	2
Solanum lycopersicum	tomato	Solanaceae	10	2
Allium cepa	onion	Amaryllidaceae	5	4
Zea mays	maize	Poaceae	10	2

There were 5 test groups together with the control (water only) for each species. The highest concentration of FHO04 solution (16 L product/ha) was prepared by weighing 163.3560 g of FHO04 in 1380.00 g of tap water. Lower dose rates (1.0, 2.0, 4.0 and 8.0 mL/ha) were prepared by diluting the16 L product/ha solution with tap water.

Table 3: Application rates

Group	FHO04 (L/ha)
Control	0 (tap water)
Treatment 1	1.0
Treatment 2	2.0
Treatment 3	4.0
Treatment 4	8.0
Treatment 5	16.0

Applications were made using a Laboratory Track-Sprayer (Schachtner) at 60 cm above the soil surface. The Laboratory Track-Sprayer (Schachtner) was calibrated to deliver output of 200 L/ha ± 5 %.
The pots were placed in saucers containing water and kept under greenhouse conditions.

The determination of FHO04 in the treatment solution was performed by HPLC and the test item was found stable with the mean recovery of the highest application rate (16.0 L product/ha) being within the range of 80 to 120 % of the nominal concentrations.

The plants were assessed for visual phytotoxicity, emergence and mortality on days 1, 14 and 21 after 50% of the control group had emerged.

All plants surviving were cut at soil level and weighed in each replicate.

Statistical analyses
For data evaluation the statistical programme ToxRat Professional 3.3.0 was used.
Seedling emergence: Multiple Fisher's exact test with Bonferroni-Holm adjustment
Post-emergence mortality: Multiple Fisher's exact test with Bonferroni-Holm adjustment
Shoot dry weight: Williams' test, Dunnett's t-test, Jonckheere-Terpstra test. ER_{50} with 3-parameter normal cumulative distribution function.

Results and discussions

Mortality
Cumulative post-emergence mortality occurred only in two species tested (*Glycine max* and *Allium cepa*) with a mortality of 5.0 and 5.6 %, respectively. Both mortalities occurred with an application rate of 1.0 L product/ha.

Seedling emergence
There was no significant decrease observed in any of the species tested.

Final biomass
A statistically significant reduction in shoot dry weight occurred in Brassica napus and Allium cepa. The statistically significant reduction in Brassica napus occurred at 16.0 L product/ha. Thus, the respective LOER and NOER were determined as 16.0 and 8.00 L product/ha. A statistically significant reduction in Allium cepa occurred at 8.00 and 16.0 L product/ha. Thus, the respective LOER and NOER were determined as 8.00 and 4.00 L product/ha**.**

Visual phytotoxicity

There was no phytotoxic symptom observed in any of the species tested.

Deficiencies

None.

Validity criteria

Validity criteria

Parameters	Required	Actual
Control Seedling Emergence [%] (for each particular species)	≥ 70	70 to 100
Control Phytotoxicity The control seedlings of each species did not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis, wilting, leaf and stem deformations) and control plants exhibit only normal variation in growth and morphology for that particular species	none	none
Control Survival [%] Mean survival of emerged control seedlings	≥ 90	100
Cultivation Conditions The environmental conditions for each particular species were identical and growing media contained the same amount of soil matrix, support media, or substrate from the same source	identical for each species	identical for each species

All required validity criteria were met. Accordingly, the study is valid.

Conclusion

The ER_{50} for seedling emergence, post-emergence mortality and shoot dry weight, could not be calculated due to a lack of inhibition equal or above 50 %. Therefore, the ER_{50} is considered > 16.0 L product/ha for all species tested.

A 2.6.2.2 Study 2: vegetative vigour test

Comments of zRMS:	The study was performed in line with OECD 227 with no major deviations. All validity criteria were met and the study is considered acceptable. Overall, the study is considered acceptable with following endpoints relevant for the risk assessment: ER_{50} > 16 L FH004 /ha. <u>Visual phytotoxicity:</u> No symptoms of phytotoxicity occurred in *Brassica napus* and the monocotyledonous species. Slight phytotoxicity symptoms were observed for *Beta vulgaris* at 16.0 L product/ha (10 % necrosis), for *Glycine max* at 4.00, 8.00 and 16.0 L product/ha (10 to 20 % leaf deformation and necrosis), and for *Solanum lycopersicum* at 8.00 and 16.0 L product/ha (10 to 20 % leaf deformation and necrosis).

Reference:	KCP 10.6.2/02
Report	Prothioconazole/Sulphur (50+625) g/L SC (FHO04): Effects on the Vegetative Vigour of Terrestrial Plant Species, D. Ripperger, 2023, Report No. S21-

	05534
Guideline(s):	Yes – OECD 227
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive summary

The effects on plants following deposition of the test item FHO04 on seedling emergence and early growth of higher plants were tested in a greenhouse study. The dicotyledon species tested were sugar beet (*Beta vulgaris*), oil seed rape (*Brassica napus*), soybean (*Glycine max*) and tomato (*Solanum lycopersicum*). The monocotyledon species tested were onion (*Allium cepa*) and maize (*Zea mays*).

For each species, 5 treatment groups were used with application rates 0.0 (control), 1.0, 2.0, 4.0, 8.0, and 16.0 L product/ha. The pots were placed in saucers on cultivation tables under the greenhouse condition.

Evaluations of the plant for mortality and visual phytotoxicity were conducted on days 7, 14 and 21. At the end of the test, all plants surviving were cut at soil level and weighed.

FHO04 did not significantly decrease the emergence or the fresh weight of any of the species tested.

Table 1: Effects on terrestrial plants following foliar application

Species	ER_{50} (L FHO04/ha)	NOER (L FHO04/ha)
	Rate	
Beta vulgaris	> 16.0	> 16.0
Brassica napus	> 16.0	> 16.0
Glycine max	> 16.0	> 16.0
Solanum lycopersicum	> 16.0	> 16.0
Allium cepa	> 16.0	> 16.0
Zea mays	> 16.0	> 16.0

Materials and methods

1. ***Test Item*:** FHO04
   - **Description:** Liquid
   - **Lot/Batch #:** 028421
   - **Purity:** Prothioconazole: 53.14 g/L
     Sulphur: 638.0 g/L
   - **CAS #:** Prothioconazole: 178928–70–6
     Sulphur: 7704-34-9
   - **Stability of test compound:** N/A - Stable
2. ***Vehicle*:** Tap water
3. ***Test system* -**

Species:	Sugar beet (*Beta vulgaris*), oil seed rape (*Brassica napus*), soybean (*Glycine max*), tomato (*Solanum lycopersicum*), onion (*Allium cepa*) and maize (*Zea mays*)
Growth stage:	2 – 4 leaves (BBCH 12) at the time of application and grown on for further 21 days
Substrate:	Soil, sandy loam texture, 1.26 % organic C, pH 7.26

4. ***Environmental conditions*** **-**

Temperature:	13.0 to 27.5 °C
Humidity:	52.0 to 100.0 %
Light intensity:	395.4 to 547.7 Lux
Photoperiod:	Minimum 16 hours light
In life dates	December 03, 2021 to February 09. dd, 2022

Study design

All the species were germinated and grown in a greenhouse. The seeds were not coated with insecticide/fungicide and no plant protection products were used.

All the species were grown in non-porous plastic pots. No plant protection products were used on the plants. There were 5 test groups together with the control (water only) for each species. Each treatment and the control were replicated 10 times and each replicate consisted of 2 plants for all dicotyledonous species and *Zea mays*. Each treatment and the control were replicated 5 times and each replicate consisted of 4 plants for all dicotyledonous species and *Allium cepa*.

Table 2: Details of replicate numbers and plant species

Species	Common name	Family	No of replicates	No of seeds/replicate
Beta vulgaris	sugar beet	Amaranthaceae	10	2
Brassica napus	oil seed rape	Brassicaceae	10	2
Glycine max	soybean	Fabaceae	10	2
Solanum lycopersicum	tomato	Solanaceae	10	2
Allium cepa	onion	Amaryllidaceae	5	4
Zea mays	maize	Poaceae	10	2

The highest concentration of FHO04 solution (16 L product/ha) was prepared by weighing 163.3560 g of FHO04 in 1380.00 g of tap water. Lower dose rates (1.0, 2.0, 4.0 and 8.0 mL/ha) were prepared by diluting the16 L product/ha solution with tap water.

Table 3: Application rates

Group	FHO04 (L/ha)
Control	0 (tap water)
Treatment 1	1.0
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Treatment 3	4.0
Treatment 4	8.0
Treatment 5	16.0

Applications were made using a Laboratory Track-Sprayer (Schachtner) at 60 cm above the soil surface. The Laboratory Track-Sprayer (Schachtner) was calibrated to deliver output of 200 L/ha ± 5 %.
The pots were placed in saucers containing water under greenhouse conditions.
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The determination of FHO04 in the treatment solution was performed by HPLC and the test item was found stable with the mean recovery of the highest application rate (16.0 L product/ha) being within the range of 80 to 120 % of the nominal concentrations.

The plants were assessed for visual phytotoxicity and mortality on days 7, 14 and 21 after application.

All living plants were cut at soil level and weighed at the end of the experiment to determine effect on vegetative weight.

Statistical analyses
Shoot dry weight: Wiliams' test, Dunnett's t-test, both tests one-sided smaller, $\alpha = 0.05$. ER_{50} with 3-parameter normal cumulative distribution function. the statistical programme ToxRat Professional 3.3.0 was used.

Results and discussions

Mortality
At the end of the test no significant mortality recorded with any species tested.

Final biomass
Statistically significant differences regarding shoot dry weight occurred in *Glycine max, Solanum lycopersicum* and *Zea mays* at 16.0 L product/ha (inhibitions compared to control 20.8, 24.6 and 18.3 %), but these were not considered to be adverse effects (i.e. greater than 50 %).

Visual phytotoxicity
No symptoms of phytotoxicity occurred in *Brassica napus* and the monocotyledonous species. Slight phytotoxicity symptoms were observed for *Beta vulgaris* at 16.0 L product/ha (10 % necrosis), for *Glycine max* at 4.00, 8.00 and 16.0 L product/ha (10 to 20 % leaf deformation and necrosis), and for *Solanum lycopersicum* at 8.00 and 16.0 L product/ha (10 to 20 % leaf deformation and necrosis).

Deficiencies
None.

Validity criteria

Validity criteria

Parameters	Required	Actual
Seedling Emergence [%] (for each particular species)	≥ 70	97.4 to 99.3
Control Phytotoxicity The control seedlings of each species did not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis, wilting, leaf and stem deformations) and control plants exhibit only normal variation in growth and morphology for that particular species	none	none
Control Survival [%] Mean survival of emerged control seedlings	≥ 90	100
Cultivation Conditions The environmental conditions for each particular species were identical and growing media contained the same amount of soil matrix, support media, or substrate from the same source	identical for each species	identical for each species

All required validity criteria were met. Accordingly, the study is valid.

Conclusion

The ER_{50} for mortality and shoot dry weight, could not be calculated due to a lack of inhibition equal or above 50 %. Therefore, the ER_{50} is considered to be > 16.0 L product/ha for all species tested.

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