Document Title

Summary of the ecotoxicological studies Prothioconazole FS 100 (100 g/L)

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Date	Data points containing amendments or additions[1] and brief description	Document identifier and version number

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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CP 10 ECOTOXICOLOGICAL STUDIES ON THE PLANT PROTECTION PRODUCT

Introduction

A dossier on prothioconazole (CAS No. 178928-70-6) was submitted February 2002 by Bayer CropScience to the EU RMS United Kingdom for agricultural use as a fungicide. Prothioconazole was included into Annex I of the Council Directive 91/414/EEC by the Commission Directive 2008/44/EC published 4 April 2008, with an entry into force by 1 August 2008.

This Supplemental Dossier contains only detailed summaries of studies, which were not part of the dossier during the first Annex I inclusion of prothioconazole and were, therefore, not evaluated during the first EU review of this compound. In order to facilitate discrimination between new and old information, the new information is written in black letters whereas *grey letters describe the old information.*

All studies, which have been already submitted by Bayer CropScience for the first Annex I inclusion, are contained in the Monograph and its Addenda and are included in the Baseline Dossier provided by Bayer CropScience. The summaries on the different endpoints were taken from the Monograph and its Addenda and supplemented with new information (new studies, references, further comments).

A synonymous name for prothioconazole used at several locations in this Supplemental Dossier is JAU 6476.

One of the representative formulations used for the submission for the renewal of approval of prothioconazole is the seed treatment formulation Prothioconazole FS 100. The summaries of those formulation studies, which had not been presented in the dossier during the first Annex I inclusion of prothioconazole as well as the risk assessment are presented in this dossier.

Ecotoxicological endpoints used in the following risk assessment were derived from studies with the formulated product Prothioconazole FS 100, the active substance prothioconazole and the metabolites listed in the residue definition for risk assessment.

In this dossier only endpoints used for the risk assessment are presented. For an overview of all available endpoints for prothioconazole and its metabolites please refer to the respective section of the MCA document. In order to facilitate discrimination between new and information submitted during the first Annex I inclusion process, *the previously evaluated information is written in grey letters*.

Use pattern considered in this risk assessment

Table CP 10- 1: Intended application pattern

Crop	Timing of application	Number of applications	Max. application rate, individual treatment [g a.s./ha] Prothioconazole*
Wheat (spring, winter), Barley (spring, winter), Oat, Spelt, Triticale	Seed treatment BBCH 00	1	18

* Maximum label rate: 0.180 L prod./ha; seeding rate: 180 kg seeds/ha; 0.100 L product/100 kg seeds (i.e. 10 g a.s./100 kg seeds)

Definition of the residue for risk assessment for prothioconazole

Due to changes in triggers for metabolites to be further assessed as well as due to new studies on the route of degradation in various environmental compartments, additional metabolites are proposed to be included in the residue definition for the risk assessment. Accordingly, studies have been prepared to describe the ecotoxicological profile of these metabolites in the relevant environmental compartment.

Table CP 10- 2: Definition of the residue for risk assessment*

Compartment	Residue definition for risk assessment
Soil	Prothioconazole, JAU 6476-S-methyl *(M01)* and JAU 6476-desthio *(M04)*
Groundwater	Prothioconazole, JAU 6476-S-methyl *(M01)* and JAU 6476-desthio *(M04)*
Surface water	Prothioconazole, JAU 6476-S-methyl *(M01)*, JAU 6476-desthio *(M04)*, JAU 6476-thiazocine *(M12)*, 1,2,4-triazole *(M13)* and JAU 6476-triazolylketone *(M42)*
Sediment	Prothioconazole, JAU 6476-S-methyl *(M01)*, JAU 6476-desthio *(M04)*, JAU 6476-thiazocine *(M12)*, 1,2,4-triazole *(M13)* and JAU 6476-triazolylketone *(M42)*
Air	Prothioconazole and JAU 6476-desthio *(M04)*

*Justification for the residue definition for risk assessment is provided in MCA Sec.7, Point 7.4.1

<u>Plant metabolites</u>

In addition to the active substance, its metabolite JAU 6476-desthio is assessed in the dietary exposure and risk assessment of terrestrial vertebrates (birds and mammals).

A list of metabolites, which contains the structures, the synonyms and code numbers attributed to the compound prothioconazole, is presented in Document N3 of this dossier.

CP 10.1 Effects on birds and other terrestrial vertebrates

The risk assessment has been performed according to "European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA" (EFSA Journal 2009; 7(12):1438), referred to in the following as "EFSA GD 2009".

CP 10.1.1 Effects on birds

Table CP 10.1.1- 1: Endpoints used in risk assessment

Test substance	Test species	Ecotoxicological endpoint		Reference
Prothioconazole	acute, oral *Colinus virginianus* (Bobwhite quail)	LD_{50}	**> 2000 mg a.s./kg bw**	[redacted] (1998) M-013030-01-1 KCA 8.1.1.1/01
	Reprod. 21 w dietary *Anas platyrhynchos* (Mallard duck)	NOEC NOEL	700 mg a.s./kg diet **78 mg a.s./kg bw/d**	[redacted] (2000) M-035123-01-1 KCA 8.1.1.3/02
JAU 6476-desthio	acute, oral *Colinus virginianus* (Bobwhite quail)	LD_{50}	**> 2000 mg p.m./kg bw**	[redacted] (1990) M-013915-01-1 KCA 8.1.1.1/02
	Reprod. 22 w dietary *Colinus virginianus* (Bobwhite quail)	NOEC NOEL	173 mg p.m./kg diet **14.8 mg p.m./kg bw/d**	[redacted] (2002), M-090509-01-1 KCA 8.1.1.3/03

Table CP 10.1.1- 2: Relevant avian indicator species feeding on seeds for risk assessment on Tier 1 level according to EFSA GD (2009)

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

Since prothioconazole and JAU 6476-desthio are non-systemic ([redacted] 2014a,b, M-486407-02-1, M-488935-01-3, KCP 10.1.1.2.18 & 19), no risk assessment for birds feeding on crop seedlings was performed.

Acute dietary risk assessment

Table CP 10.1.1- 3: Tier 1 acute TER calculation for birds feeding on seed treatment

Compound	Indicator species	LD_{50} [mg/kg bw]	Exposure		TER_A	Trigger
			FIR/bw	NAR [mg a.s./kg seeds][1)]		
Prothio-conazole	Small granivorous bird	> 2000	0.3	100	> 67	10
JAU 6476-desthio	Small granivorous bird	> 2000	0.3	100[2)]	> 67	10

[1)] Assuming a thousand grain weight of the seeds of 50 g [1]

[2)] The Tier 1 TER calculation for the metabolite JAU 6476-desthio was conducted with the application rate of the parent compound prothioconazole – representing a worst-case screening approach

The TER values of relevant scenarios, e.g. birds feeding on treated seeds meet the required trigger of 10 for acute exposure.

Risk assessment for birds drinking contaminated water

EFSA (2009, chapter 5.2.1) proposes to focus the risk assessment for birds and mammals on the dietary route of exposure. An assessment of the risk potentially posed by consumption of contaminated drinking water after the use of a pesticide as seed treatment is not required since this route seems unlikely to be a critical one or to lead to TER greater than direct dietary consumption.

Short-term dietary risk assessment

In the short-term dietary study with JAU 6476-desthio, mortalities occurred at the two top test levels after several days of reduced food consumption leading to severe body weight loss. The seven chicks dying around day 5 at 5000 ppm had a mean bodyweight of 16.3 g/bird; i.e less than 50% of the control bird weight of 35.2 g at day 5. All birds found dead were extremely emaciated. Since no other severe clinical symptoms were observed, it has to be assumed that they died on starvation.

During the post-exposure period the food consumption and bodyweight of the surviving birds started to recover.

The LC_{50} was determined at 4090 mg/kg feed. Based on the measured concentrations the 5-d lethal dietary dose (5-d LDD_{50}) of 603 mg/kg bw/day was calculated by [redacted] (2006; M-268832-02-1, KCA 8.1.1.2/04).

Effect profile and time course suggest that mortality occurred only after multiple dosing over several days, and is associated with increasing weight loss and starvation over the treatment duration.

Therefore the results of this study are not meaningful in the acute risk assessment which is intended to address a single day oral exposure event. The effects after a single day of exposure are appropriately addressed in the standard TER_A calculation with the single exposure LD_{50}.

[1] Faustzahlen für die Landwirtschaft (2005), published by Kuratorium für Technik und Bauwesen in der Landwirtschaft, Darmstadt, 13th edition, ISBN 3-7843-2194-1

Long-term reproductive risk assessment

Table CP 10.1.1- 4: Tier 1 reproductive risk assessment for birds feeding on seed treatment

Compound	Indicator species	NOEL [mg/kg bw/d]	Exposure			TER_{LT}	Trigger
			FIR/bw	NAR [mg a.s./kg seeds][1)]	f_{twa}		
Prothioconazole	Small granivorous bird	78	0.3	100	0.53	**4.9**	5
JAU 6476-desthio	Small granivorous bird	14.8	0.3	100[2)]	0.53	**0.9**	5

[1)] Assuming a thousand grain weight of the seeds of 50 g [2]

[2)] This value is taken from the parent compound and represents an unrealistic worst-case scenario.

Bold values do not meet the trigger

The TER values for birds feeding on treated seeds do not meet the trigger of 5 for long-term exposure. Accordingly, a refined risk assessment is needed.

Refinement parameters for long-term risk assessment for <u>small granivorous birds</u> exposed to <u>treated seeds</u>

Refinement of **focal bird species: skylark, chaffinch and yellowhammer**

PT and PD ([redacted]; 2006; M-279616-01-1, KCP 10.1.1.2/14)

Measured time-weighted average concentration for a 21-day window and formation fraction of JAU 6476-desthio ([redacted]P; 2001; M-088988-01-1, KCP 10.1.1.2/02, [redacted] 2014a,b, M-486407-02-1, M-488935-01-3, KCP 10.1.1.2/18 & 19), resulting in a **f_{TWA} of 0.085 for prothioconazole** and an **adjustment factor of 0.11 for JAU 6476**-desthio after kinetic evaluation ([redacted] & [redacted] 2015a, b, c, M-535724-01-1, M-534804-01-1, M-534805-01-1, KCP 10.1.1.2/15, 16 & 17).

Focal bird species and measured surface densities of cereal seeds on freshly drilled cereal fields

The relevant indicator species for the risk assessment is a 15 g granivorous bird, e.g. the linnet (*Carduelis cannabina*). Considering the linnet's feeding behaviour and habitat selection, it becomes obvious that linnets rely very much on small weed seeds throughout the year. To some extent, also crop seeds may be consumed during their milk ripe stages in summer or on stubble fields in autumn and winter. However, linnets are not considered to be the focal species for freshly drilled cereal fields, as cereal seeds tend to be slightly too big in size to fully match the linnets preference for smaller seeds (like weed seeds, see Cramp (1998)[3] and Eybert & Constant (1998)[4]. Moreover, the feeding activity of linnets is reported to be low on freshly drilled fields in cases where the number of seeds remaining on the soil surface is not very high. According to a study of Moorcroft *et al.* (2002)[5] linnets were rarely found on fields where densities of seeds important for their diets were lower than 250 seeds/m².

[2] Faustzahlen für die Landwirtschaft (2005), published by Kuratorium für Technik und Bauwesen in der Landwirtschaft, Darmstadt, 13th edition, ISBN 3-7843-2194-1

[3] [redacted] (1998): The complete birds of the western palearctic. Oxford University Press, UK. Database on CD-ROM (Printouts can be provided on request).

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

[5] [redacted] J D. (2002): The selection of stubble fields by wintering granivorous birds reflects vegetation cover and food abundance. Journal of Applied Ecology 39, 535-547. M-107939-01-1.

This conclusion was confirmed by the avian observations of Crocker & Irving (1999)[6]. Based on their observations and observations made by [redacted] (2006, M-279616-01-1, KCP 10.1.1.2/14), the **skylark** (*Alauda arvensis*), the **yellowhammer** (*Emberiza citrinella*) and the **chaffinch** (*Fringilla coelebs*) are considered to be the relevant focal bird species for the refined risk assessment due to their abundance and frequency of occurrence in freshly drilled cereal fields in combination with their dietary demands.

Portion of time (PT) birds

To obtain quantifiable information about the potential foraging time of the focal bird species in freshly drilled cereal fields, individuals of the three focal species (skylark, yellowhammer and chaffinch) were captured, radio-tagged and their presence in the field was followed for 24 hours ([redacted], 2006; KCP 10.1.1.2/14).

Based upon the field observations made, worst-case PT values were determined to be 100%, 35% and 22% (mean values) for skylark, yellowhammer and chaffinch, respectively. It should be mentioned that for the long-term risk, the 90th-%tile would overestimate the exposure situation, which is also underlined by the limited availability of cereal-seeds for a maximum of approx. 2-3 weeks (until seed emergence). Therefore, the use of mean PT-values measured in the field study could be considered in a further refinement if that should be necessary.
For the purpose of this document, the unrefined worst-case values will be used as a very conservative approach.

Table CP 10.1.1- 5: Compilation of measured PT-values of focal bird species (portion of time birds spent potential foraging in freshly drilled cereal fields), determined via radio-tracking ([redacted], 2006; KCP 10.1.1.2/14)

	Focal bird species (no. of total tracking sessions)		
	Skylark (n = 11)	**Yellowhammer** (n = 14)	**Chaffinch** (n = 8)
90th %ile of potential foraging time (PT)	1.0 (100%)	0.35 (35%)	0.22 (22%)

Portion of Diet (PD)

In the field study of [redacted] (2006, M-279616-01-1, KCP 10.1.1.2/14), ingested food items of focal species have been determined by the analysis of stomach contents and faeces. The results are shown in the following table.
In all focal species (yellowhammer, chaffinch and skylark), food items from various sources have been identified, including cereal seeds. However, it must be taken into consideration that in and around the freshly drilled cereal fields under investigation, significant amounts of cereal and oil-seed rape (OSR) harvest remnants (comprising lots of un-treated seeds) were available from the previous crop.
These high-levels of seed-remnants (from the previous crop), easily accessible for birds on the soil surface, mostly result from the minimum soil cultivation practices followed in the study region (i.e. the abdication of mechanical soil-turning operations like ploughing between the harvest of the preceding crop and the drilling of (new) cereals, in combination with only a limited harrowing, see also above). That these un-treated cereal-seed-remnants (from the previous crop) posed at least for the skylark an

[6] Crocker, DR and Irving, PV (1999): Project PN0915, Improving estimates of wildlife exposure to pesticides in arable crops: milestone report 02/01, Variation of bird numbers on arable crops. M-290894-01-1.

important/easily exploitable food source, becomes obvious, when scrutinizing those skylarks in closer detail, which were captured on freshly drilled cereal fields and which were found to had cereal seeds in their gastro-intestinal tracts (GIT).

Table CP 10.1.1- 6: Content-list of the faeces and stomach flushes of scrutinized skylarks (n=10) and identification of the previous crop (from raw-data), [redacted] (2006, M-279616-01-1, KCP 10.1.1.2/14)

Food item	Sample ID									
	010	011	016	019	026	028	030	061	062	063
Animal matter										
Acari	1									
Coleoptera						1				
Diptera Tipulidae				1						
Diptera										
Hymenoptera Formicidae				1						
Rhynchota Cicadellidae				1						
Plant matter										
grass seed Poaceae				1						
plant fibre							2			
scale remains Brassicaceae									1	
Seed Poaceae [5mm]					6	1	1	1		
Other seed								2		
unknown plant material		1								
wheat grain [7mm]			1	1	1			1	1	1
Previous crop on the site on which the particular bird was captured	OSR	OSR	WW	WW	WW	Peas	WW	WW	WW	WW

OSR = oil-seed rape, WW = Winter Wheat

Only those skylarks had cereals seeds in their GIT, which were actually captured on fields that carried winter wheat as the previous crop. Thus, especially the skylark, as a bird species of the open landscape that avoids bush-and tree like structures at the field margins, can be expected to exploit the reservoir of un-incorporated and un-treated cereal-seed-remnants from the previous crop in the mid-field area. When calculating the portion of cereal-seeds in the diet of skylarks for the refined risk assessment, the calculated PD-value can therefore be considered to be a conservative estimate for this particular species. Moreover, unlike to yellowhammers and chaffinches, skylarks where never observed during the entire course of the study to feed on treated cereal seeds [redacted] (2006, M-279616-01-1, KCP 10.1.1.2/14).

To account for EU agreed input parameters the equations for the energy requirements as well as the values for energy and moisture content of the different feed items were calculated to comply with EFSA Guidance (2009), Appendix G. Full details of the mixed diets of each species are given in the following tables.

Table CP 10.1.1- 7: Daily Consumption and Energy Expenditure for 37.2 g Skylark

Species:	Passerine					
Body Weight (g):	37.2					
Proportion of diet based on	Dry					
Food	% in diet DRY wt	kJ/g Dry weight	Assimilation efficiency	Wt (g) dry food consumed	Wt (g) wet food consumed	**FIR/bw**
Dicot leaves	2	11.19	0.76	0.15	1.36	0.036
Grasses and cereal shoots		17.60	0.76			
non-grass herbs		17.80	0.76			
Browse		20.70	0.76			
Orchard topfruit		14.80	0.67			
Cereal seeds	**42**	**18.40**	**0.80**	**3.25**	**3.81**	**0.102**
Weed seeds	23	21.70	0.80	1.78	1.97	0.053
Small mammals		21.66	0.76			
Bird and mammal carrion		23.23	0.76			
Arthropods	33	22.70	0.76	2.55	8.18	0.220
Caterpillars		21.65	0.76			
Soil invertebrates		19.40	0.76			
Fish		21.00	0.76			
Aquatic invertebrates		20.90	0.76			
Aquatic vegetation		15.00	0.76			
Sum	100			7.74	15.32	
Daily Energy Expenditure	124.08	kJ/animal				

Table CP 10.1.1- 8: Daily Consumption and Energy Expenditure for 26.5 g Yellowhammer

Species:	Passerine					
Body Weight (g):	26.5					
Proportion of diet based on	Dry					
Food	% in diet DRY wt	kJ/g Dry weight	Assimilation efficiency	Wt (g) dry food consumed	Wt (g) wet food consumed	**FIR/bw**
Dicot leaves	2	11.19	0.76	0.13	1.11	0.042
Grasses and cereal shoots		17.60	0.76			
non-grass herbs		17.80	0.76			
Browse		20.70	0.76			
Orchard topfruit		14.80	0.63			
Cereal seeds	**58**	**18.40**	**0.80**	**3.66**	**4.29**	**0.162**
Weed seeds	12	21.70	0.80	0.76	0.84	0.032
Small mammals		21.66	0.76			
Bird and mammal carrion		23.23	0.76			
Arthropods	28	22.70	0.76	1.77	5.67	0.214
Caterpillars		21.65	0.76			
Soil invertebrates		19.40	0.76			
Fish		21.00	0.76			
Aquatic invertebrates		20.90	0.76			
Aquatic vegetation		15.00	0.76			
Sum	100			6.32	11.91	
Daily Energy Expenditure	98.65	kJ/animal				

Table CP 10.1.1- 9: Daily Consumption and Energy Expenditure for 20.9 g Chaffinch

Species:	Passerine					
Body Weight (g):	20.9					
Proportion of diet based on	Dry					
Food	% in diet DRY wt	kJ/g Dry weight	Assimilation efficiency	Wt (g) dry food consumed	Wt (g) wet food consumed	**FIR/bw**
Dicot leaves	3	11.19	0.76	0.16	1.37	0.065
Grasses and cereal shoots		17.60	0.76			
non-grass herbs		17.80	0.76			
Browse		20.70	0.76			
Orchard topfruit		14.80	0.63			
Cereal seeds	**32**	**18.40**	**0.80**	**1.66**	**1.95**	**0.093**
Weed seeds	4	21.70	0.80	0.21	0.23	0.011
Small mammals		21.66	0.76			
Bird and mammal carrion		23.23	0.76			
Arthropods	61	22.70	0.76	3.17	10.15	0.486
Caterpillars		21.65	0.76			
Soil invertebrates		19.40	0.76			
Fish		21.00	0.76			
Aquatic invertebrates		20.90	0.76			
Aquatic vegetation		15.00	0.76			
Sum	100			5.19	13.69	
Daily Energy Expenditure	84.03	kJ/animal				

Time-weighted-average (21-d f_{TWA}) and formation fraction

The dissipation of prothioconazole and JAU 6476-desthio from cereal seeds left on the soil surface after drilling was determined in several studies ([redacted]; 2001; M-088988-01-1, KCP 10.1.1.2/02, [redacted] 2014a,b, M-486407-02-1, M-488935-01-3, KCP 10.1.1.2/18 & 19).

DT_{50} values and formation fractions of these studies were re-evaluated using kinetic methods ([redacted] & [redacted] 2015a, b, c, M-535724-01-1, M-534804-01-1, M-534805-01-1, KCP 10.1.1.2/15, 16 & 17).

Combined evaluation of both parent and metabolite allows to better address the metabolite kinetics (the metabolite is at the same time formed and degraded).

In this kinetic evaluation, the formation of JAU 6476-desthio and its dissipation were investigated based on measured residue values from samples taken at different time intervals after sowing of prothioconazole treated cereal seeds. All eleven trials provided acceptable fits for kinetics of prothioconazole and eight of them provided acceptable fits for kinetics of JAU 6476-desthio.

Table CP 10.1.1- 10: SFO-DT_{50}s and time-weighted average factor for prothioconazole (cereal seeds)

Trial	PTZ DT_{50} (d)	PTZ f_{TWA} (21d)
EnSa-15-0606 LaacherHof early	1.47	0.101
EnSa-15-0606 LaacherHof late	0.45	0.031
EnSa-15-0606 Hoefchen early	0.21	0.014
EnSa-15-0607west	2.09	0.143
EnSa-15-0607 south	2.13	0.146
EnSa-15-0607 north	1.34	0.092
EnSa-15-0607 east	0.76	0.052
EnSa-15-0608 west	2.30	0.158
EnSa-15-0608 south	1.90	0.198
EnSa-15-0608 north	1.72	0.118
EnSa-15-0608 east	1.40	0.096
geomean	**1.23**	**0.085**

Based on these residue measurements of parent and metabolite in the same samples, both the maximum formation fraction "ff", and the SFO-DT_{50} for dissipation of the metabolite were determined for each trial and the 21-d f_{TWA} was calculated for each SFO-DT_{50}.
The resulting 21-d f_{TWA} for JAU 6476-desthio per trial was then multiplied with the corresponding formation fraction (ff) determined in the respective trial resulting in one "adjustment factor" per trial (see Table CP 10.1.1- 1). This adjustment factor takes into account formation as well as dissipation of JAU 6476-desthio.

Table CP 10.1.1- 11: SFO-DT_{50}s and time-weighted average factors for adjustment factor calculation for JAU 6476-desthio (cereal seeds)

Trial	DT_{50}	21-d f_{TWA}	ff	adjustment factor: 21-d f_{TWA} × ff
EnSa-15-0606 LaacherHof early	5.15	0.333	0.359	0.119
EnSa-15-0606 Hoefchen early	12.1	0.582	0.032	0.019
EnSa-15-0607west	2.62	0.179	1.000	0.179
EnSa-15-0607 south	2.34	0.160	0.950	0.152
EnSa-15-0608 west	4.81	0.314	0.540	0.170
EnSa-15-0608 south	2.14	0.145	0.660	0.096
EnSa-15-0608 north	3.68	0.248	0.540	0.134
EnSa-15-0608 east	7.81	0.453	0.370	0.168
			geomean	**0.110**

In the refined exposure assessment for JAU 6476-desthio, the adjustment factor is multiplied with the amount of parent applied (NAR, nominal application rate).

A total of 11 data points for prothioconazole were extracted, resulting in overall geometric mean **f_{TWA} values of 0.085 for prothioconazole.** For JAU 6476-desthio, a total of 8 DT_{50} values and 8 formation fraction values were obtained with an acceptable fit, resulting in a overall geometric mean **adjustment factor of 0.11.**

Refined long-term risk assessment

The refined long-term risk assessment for prothioconazole, and JAU 6476-desthio using the parameters described above is presented below.

Table CP 10.1.1- 12: Refined long-term TER calculation for birds feeding on cereal seeds treated with prothioconazole

Species	Skylark	Yellowhammer	Chaffinch
Treatment	Cereal seed treatment (100 mg prothioconazole/kg seed)		
Body weight (g)	37.2	26.5	20.9
f_{TWA}	0.085	0.085	0.085
FIR related to bw for cereal seeds [g bw/d]	0.102	0.162	0.093
PT (90th percentile)	1.0	0.35	0.22
DDD (mg a.s./kg bw /day)	0.867	0.482	0.174
Lowest long-term NOEL (mg a.s. /kg bw /day)	78		
Long-term TER	90	162	449

Table CP 10.1.1- 13: Refined long-term TER calculation for JAU 6476-desthio for birds feeding on cereal seeds treated with JAU 6476-desthio

Species	Skylark	Yellowhammer	Chaffinch
Treatment	Cereal seed treatment (100 mg of parent/kg seed)		
Body weight (g)	37.2	26.5	20.9
Adjustment factor	0.11	0.11	0.11
FIR related to bw for cereal seeds [g bw/d]	0.102	0.162	0.093
PT (90th percentile)	1.0	0.35	0.22
DDD (mg a.s./kg bw /day)	1.122	0.624	0.225
Lowest long-term NOEL (mg p.m. /kg bw /day)	14.8		
Long-term TER	13	24	347

Conclusion: The TER values meet the required trigger of 5 for long-term exposure. Hence, an acceptable risk can be concluded.

Amount of active ingredient in or on each item

The thousand gram weight (TGW) of cereals ranges from 30 to 55 g[7]. Therefore, the following calculations are based on a reasonable worst-case assumption of a TGW of 50 g.

[7] Faustzahlen für die Landwirtschaft (2005), published by Kuratorium für Technik und Bauwesen in der Landwirtschaft, Darmstadt, 13th edition, ISBN 3-7843-2194-1

Table CP 10.1.1- 14: Calculation of the maximum amount of active substance on one dressed seed

Crop	Max. dressing rate of the seed treatment product [A] [L/dt [B] seeds]	Content of active substances within the dressing product [g a.s./L product]	Nominal seed treatment rate [mg a.s./kg seeds]	Maximum amount of a.s. on one individual dressed seed [µg a.s./seed]
Prothioconazole				
Cereals	0.1	100	100	5

[A] assuming a thousand grain weight (TGW) of 50 g
[B] dt = deciton; 1 dt = 100 kg

Proportion of active ingredient LD_{50} per 100 items and per gram of items

Table CP 10.1.1- 15: Calculation of the proportion of the LD_{50} for the a.s. in 100 particles / gram particles

Crop	Maximum amount of a.s. on one individual dressed seed [A] [µg a.s./seed]	Content of active substance on 100 seeds [mg a.s.]	Amount of active substance on 100 seeds/LD_{50}	Content of active substance on 33 seeds = 1 g [mg a.s.]	Amount of active substance on 1 g seeds/LD_{50}
Prothioconazole					
Cereals	5	0.5	2.5×10^{-4}	0.1	$< 5 \times 10^{-5}$

[A] Assuming a thousand grain weight (TGW) of 50 g

Risk assessment of secondary poisoning

Substances with a high bioaccumulation potential could theoretically bear a risk of secondary poisoning for birds feeding on contaminated prey like fish or earthworms. For organic chemicals, a log $P_{OW} > 3$ is used to trigger an in-depth evaluation of the potential for bioaccumulation.

Prothioconazole has a log P_{OW} of 2.0 indicating no risk of bioaccumulation and, hence, secondary poisoning. However, the metabolites JAU 6476-desthio and JAU 6476-S-methyl have log P_{OW} values of 3.04 and 4.3, respectively, requiring an assessment of bioaccumulation and secondary poisoning potential. The following table provides an overview of the log P_{OW} values of the active substance and its metabolites.

Table CP 10.1.1- 16: Log P_{ow} values of prothioconazole and metabolites

Compounds	Log P_{ow}	Reference
Prothioconazole	2.0	[redacted] & [redacted], (2014) M-492539-01-1 KCA 2.7/02
JAU 6476-desthio	**3.04**	[redacted] (1992) M-010758-01-1 KCA 2.7/05
JAU 6476-S-methyl	**4.3**	[redacted] & [redacted], B (2008) M-297647-01-1 KCA 2.7/03
1,2,4-Triazole	-0.71	[redacted] (1985) M-045573-01-1 KCP 10.2.2/05
JAU 6476-triazolylketone	0.33	[redacted] & [redacted] (2015) M-528860-01-1 KCA 2.7/06
JAU 6476-thiazocine	1.9	[redacted] & [redacted] (2014) M-503411-01-1 KCA 2.7/04

Long-term DDD and TER calculation for earthworm-eating birds

Table CP 10.1.1- 17: Tier 1 long-term DDD and TER calculation for earthworm-eating birds

Compound	JAU 6476-desthio	JAU 6476-S-methyl	Origin of values
BCF_{worm} calculation:			
P_{OW}	1 096	19 953	See Table CP 10.1.1- 16
K_{OC} [mL/g]	575	2556.3	See MCA 7.1.3.1
f_{OC}	0.02	0.02	Default
BCF_{worm}	1.216	4.700	
PEC_{worm} calculation:			
PEC_{soil} (21 d-twa) [1] [mg/kg]	0.011	0.003	See MCP 9.1.3
PEC_{worm} [mg/kg]	0.013	0.014	
DDD calculation:			
FIR/bw	1.05	1.05	Default
DDD [mg/kg bw/d]	0.014	0.015	
TER_{LT} calculation:			
NO(A)EL [mg/kg bw/d]	14.8	7.8 [2]	See Table CP 10.1.1- 1
TER_{LT}	1 057	520	
Trigger	5	5	
Refined risk assessment required?	No	No	

[1] Worst case 21 d TWA_{soil} value based on 1 x 18 g/ha prothioconazole, 0% interception
[2] NOEL of the parent compound prothioconazole was divided by a factor of 10 (worst-case assumption)

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

Long-term DDD and TER calculation for fish-eating birds

Table CP 10.1.1- 18: Tier 1 long-term DDD and TER calculation for fish-eating birds

Compound	JAU 6476-desthio	JAU 6476-S-methyl	Origin of values
PEC_{fish} calculation			
BCF_{fish}	65	319.3[1)]	See MCA 8.2
PEC_{SW} (21d-twa) [2)] [mg/L]	0.000723	0.000082	See MCP 9.2.5
PEC_{fish} [mg/kg]	0.047	0.026	
DDD calculation:			
FIR/bw	0.159	0.159	Default
DDD [mg/kg bw/d]	0.007	0.004	
TER calculation:			
NO(A)EL [mg/kg bw/d]	14.8	7.8 [3)]	See Table CP 10.1.1- 1
TER_{LT}	2 114	1 950	
Trigger	5	5	
Refined risk assessment required?	No	No	

[1)] New BCF value resulting from a statement from [redacted] 2013 (M-459145-01-1; KCA 8.2.2.3/04)
[2)] Worst-case 21d-TWA_{SW} (winter cereals, 1 × 18 g a.s./ha, N-EU, Single)
[3)] NOEL of the parent compound prothioconazole was divided by a factor of 10 (worst-case assumption)

All TER values are well above the required trigger. Accordingly, the risk to fish-eating birds from the use of the product in cereals is considered acceptable.

CP 10.1.1.1 Acute oral toxicity

Toxicity of the formulated product

For animal welfare reasons, no acute oral toxicity study with the preparation was performed. Such a study is not deemed necessary, given the fact that the active substance is not acutely toxic to birds.

CP 10.1.1.2 Higher tier data on birds

The following studies are used for refining the risk assessment for birds.

Report:	KCP 10.1.1.2/14 [redacted]; 2006; M-279616-01-1
Title:	Generic field monitoring of birds in freshly drilled winter cereal fields in Autumn in Germany
Report No.:	BAR/FS-035
Document No.:	M-279616-01-1
Guideline(s):	The test was designed especially for the purpose of this study.
Guideline deviation(s):	none
GLP/GEP:	**yes**

Material and methods:

This generic study was performed to evaluate to which extent birds utilise freshly drilled treated cereal seeds as a food source.

Test locality: The study has been conducted in the area of the agrarian co-operative [redacted], which is located in the district of [redacted] in the natural preserve region "[redacted]"

(federal state of Sachsen-Anhalt, Germany). This region is a typical area for cereal cultivation in Europe and known to hold an essential population of the three pre-selected focal species Skylarks (*Alauda arvensis*). Chaffinch (*Fringilla coelobs*) and Yellowhammer (*Emberiza citrinella*) are also common.

Methods: From the literature ([redacted]. 2005, Crocker & Irving 1999) the three main focal species were deduced: the Skylark, the Chaffinch and the Yellowhammer. In order to obtain a reliable indicator for exposure of these species to treated seeds, the portion of time spent ‘potentially foraging’ on freshly drilled fields was acquired by radio tracking.
In total 13 Skylarks were trapped in winter cereal and adjacent habitats and tagged with radio transmitters; eight Skylarks were tracked for one (n = 5) or two (n = 3) daylight periods each. The respective number of trapped and radio tagged Chaffinches was 12. Telemetry sessions comprehended one (n = 6) or two (n = 1) daylight periods each. Eleven Yellowhammers were trapped and radio tagged. Eight individuals were tracked for one and three individuals for two daylight periods.
In order to assess the general relevance of winter cereal fields and other habitats as feeding locations for birds, 7 census counts each were carried out along five different transects, representing all main agrarian habitat types within the study area. These transects were walked once a week to acquire a full overview of bird life.
Additionally on six defined subareas of freshly drilled winter cereal fields (2 winter barley, 4 winter wheat) – including a small adjacent 'outside-area' - a scan sampling procedure was conducted. Here all bird activities were observed from dawn till dusk. This procedure was conducted once before drilling and two times after drilling to quantify any changes of bird activities possibly caused by the availability of treated cereal seeds. For each session the portion of scans a given species could be observed was calculated (frequency of occurrence = FO).
In order to gain information about food items selected by the focal species, 48 samples of faeces or stomach flushings were analysed quantitatively for their composition: taxonomic orders of plants, in particular components of winter cereals and arthropods or other identifiable items were recorded. Faeces and stomach flushing samples were taken during the handling of individual birds after mist netting and as well if defecating was observed during the telemetry session of tagged individuals.
To quantify the availability of winter cereal seeds to small and medium sized granivorous bird species, the initial exposure of the seeds was measured. Within 24 hours after the termination of drilling visible seeds were counted on the six different study plots when there was no scan sampling conducted.

Findings:

Table CP 10.1.1.2-1: Monitoring results of Yellowhammers, Skylarks and Chaffinches

PORTION OF TIME potentially foraging (PT) in cereal fields by radio tracked species				
Potential foraging time [1] radio tracked birds spent in freshly drilled winter cereal fields (wheat + barley)	species	mean [%]	90^{th} percentile [%]	tracking sessions (individuals)
	Yellowhammer	6.41	23.63	14 (11)
	Skylark	16.97	95.73	11 (8)
	Chaffinch	8.54	22.05	8 (7)
HABITAT PREFERENCE of species according to radio tracking				
Preference of winter cereals as a feeding habitat (D: Jacobs' Index, Range: -1 to +1; MCP [100%])	species	D		
	Yellowhammer	-0.79		
	Skylark	-0.29		
	Chaffinch	-0.89		
DIET of species in cereal fields				
Portion of samples [%] containing each food item after the analysis of faeces (19) and samples of stomach flushing (29) gathered in cereal fields (n: no. of faeces/flushings)	food items	Yellowhammer (n = 8/16)	Skylark (n = 6/3)	Chaffinch (n = 5/10)
	wheat seeds	75.00	60	80.00
	other cereal seeds	4.17	-	-
	other seeds	12.50	50	53.33
	other plant material	33.33	30	53.33
	Coleoptera	29.17	10	53.33
	Diptera	25.00	20	73.33
	Hymenoptera	8.33	10	13.33
	Dermaptera	4.17	-	20.00
	Rhynchota	12.50	10	26.67
	Araneae	12.50	-	26.67
	other animal material	4.17	10	20.00
	unidentified objects	16.67	-	-
BIRD ABUNDANCE in winter cereals according to transect counts (based on population) species				
Abundance of focal species and four other abundant species after seven transect counts covering 310.35 hectare	species	[Ind./ha]		
	Skylark	1.321		
	Starling	1.266		
	Chaffinch	1.260		
	Linnet	0.319		
	Yellowhammer	0.313		
	Wood pigeon	0.213		
	Mistle Trush	0.113		
BIRD FREQUENCY OF OCCURRENCE according to scan sampling				
Frequency of occurrence (mean of the results for each session; n = 12) of focal species and five other prevalent species on six fields	species	[%]		
	Yellowhammer	5.83		
	Black Redstart	5.54		
	Chaffinch	5.35		
	Jay	3.56		
	Blackbird	2.92		
	Brambling	2.90		
	Greenfinch	2.39		
	Skylark	0.16		

[1] Sum of behaviour categories 'foraging', 'active, maybe foraging' and 'unknown'

Conclusion:

Radio tracking Yellowhammers, Skylarks and Chaffinches in an agrarian landscape with a high number of freshly drilled winter cereal fields (wheat and barley) in the western part of Saxony-Anhalt showed that this field type was used as only a minor feeding habitat.

Eleven Yellowhammers (n = 14 sessions) were radio-tracked, of which three individuals did not use freshly drilled winter cereal fields as feeding habitat at all. Bird census and scan sampling data confirm that Yellowhammers evidently prefer non-crop habitats over freshly drilled winter cereal fields. However, when Yellowhammers visited freshly drilled cereal fields they could regularly be observed feeding on seeds. Additionally 75% of all diet samples contained wheat seeds. Whether these seeds were treated could not be ascertained in most instances. Four of 16 samples contained treated seeds deduced from the colour of the seed coating.
The radio tracking of 8 individual Skylarks (n = 11 sessions) showed that the majority of tagged Skylarks used plain fields and oilseed rape as feeding habitats. Only three individuals used freshly drilled cereal fields as a feeding habitat quite intensively. The results of the scan sampling approach seem to support the minor significance of freshly drilled cereal fields for Skylarks. However, the scan plots were situated close to the field edge so that the actual number of Skylarks in the whole field was possibly underestimated. According to the transect counts Skylarks reached their highest abundance on freshly drilled winter cereal fields (1.32 individuals per hectare). Additionally 55.6% of all diet samples contained wheat seeds, which proves the usage of this food source by Skylarks. Freshly drilled cereal fields offer a significant habitat for Skylarks, but individual Skylarks differ regarding their habitat choice and a variety of alternative habitats are also used extensively.

Radio tracking of 7 individual Chaffinches (n = 8 sessions) showed that freshly drilled winter cereal fields were used as a minor feeding habitat. Only four from these seven individuals were observed 'potentially foraging' in freshly drilled cereal fields. On average cereal fields were avoided (Jacobs' index [D]: -0.89). Bird census and scan sampling data confirm that Chaffinches evidently prefer non-crop habitats over freshly drilled winter cereal fields. However, direct observations demonstrate that Chaffinches feed on treated cereal seeds. Additionally 80% of all diet samples contained wheat seeds of unknown origin. Seed coating could not be found within the samples, which fits to the observation that Chaffinches mostly de-husk the seeds before swallowing.

The exposure assessment data show that cereal seeds were readily available for birds after drilling; the average number of seeds per m² was 11.8 on midfield areas and 36.7 on headlands. Yellowhammers and Chaffinches could be observed repeatedly feeding on coated seeds on freshly drilled winter cereal fields.

For risk assessment purposes a value for portion of diet obtained in treated area (PT – estimated from the time spent potentially foraging) can be derived for Yellowhammer, Skylark and Chaffinch from the study results: Yellowhammers spent on average 6.41% of their potentially foraging time in winter cereal fields (90th percentile = 23.63%); Skylarks spent 16.97% of their potentially foraging time in winter cereal fields (90th percentile = 95.73%) and Chaffinches spent 8.54% of their potentially foraging time in winter cereals (90th percentile = 22.05%).

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Report:	KCP 10.1.1.2/15 [redacted],;[redacted]; 2015; M-535724-01-1
Title:	Prothioconazole (PTZ) residue DT50 kinetics EUR - Residue dissipation of prothioconazole and its metabolite in or on wheat seeds: Kinetic evaluation
Report No.:	EnSa-15-0606
Document No.:	M-535724-01-1
Guideline(s):	not applicable
Guideline deviation(s):	not applicable
GLP/GEP:	**no**

This statement provides a kinetic evaluation of the residues of prothioconazole and its metabolite JAU 6476-desthio in wheat seeds in the field that may represent food items for seed-eating herbivorous birds or mammals. The residue decline data are available from a specifically performed residue study ([redacted],; 2001; M-088988-01-1, KCP 10.1.1.2/02).

The reliable single-first-order (SFO) half-lives of prothioconazole and JAU 6476-desthio derived in this evaluation are summarised in Table CP 10.1.1.2-1 below, along with the formation fraction of prothioconazole to desthio.

Table CP 10.1.1.2-2: Summary of DT_{50} values for prothioconazole and JAU 6476-desthio parent-to-metabolite kinetics

Trial code	Compound	Model	DT_{50} [d]	DT_{50} [d]	Formation fraction
			JAU 6476	Desthio	
[redacted] early drilling	JAU 6476	SFO	0.21		
	desthio	SFO		12.1	0.032
[redacted] early drilling	JAU 6476	SFO	1.47		
	desthio	SFO		5.15	0.359
[redacted] late drilling	JAU 6476	SFO	0.45		
	desthio	SFO		NR [1]	NR [1]

[1] Not reliable

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Report: KCP 10.1.1.2/16[redacted],; [redacted]; 2015; M-534804-01-1
Title: Prothioconazole (PTZ) Residue DT50 Kinetics EUR - Statement on residue dissipation of prothioconazole and its metabolite on wheat seeds in Germany: kinetic evaluation
Report No.: EnSa-15-0607
Document No.: M-534804-01-1
Guideline(s): not applicable
Guideline deviation(s): not applicable
GLP/GEP: **no**

This statement provides a kinetic evaluation of the residues of prothioconazole and its metabolite JAU 6476-desthio in wheat seeds in the field that may represent food items for seed-eating herbivorous birds or mammals. The residue decline data are available from a specifically performed residue study ([redacted]D; 2014; M-486407-02-1, KCP 10.1.1.2/18).

The reliable single-first-order (SFO) half-lives of prothioconazole and JAU 6476-desthio derived in this evaluation are summarised in Table CP 10.1.1.2-3 below, along with the formation fraction of prothioconazole to desthio.

Table CP 10.1.1.2-3: Summary of DT_{50} values for prothioconazole and JAU 6476-desthio parent-to-metabolite kinetics

Trial code	Compound	Model	DT_{50} [d]	DT_{50} [d]	Formation fraction
			JAU 6476	Desthio	
site west	JAU 6476	SFO	2.09		
	desthio	SFO		2.62	1
site south	JAU 6476	SFO	2.13		
	desthio	SFO		2.34	0.95
site north	JAU 6476	SFO	1.34		
	desthio	SFO		FNA[1]	FNA[1]
site east	JAU 6476	SFO	0.76		
	desthio	SFO		NR[2]	NR[2]

[1] Fit not acceptable
[2] Not reliable

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Report: KCP 10.1.1.2/17 [redacted]; [redacted]; 2015; M-534805-01-1
Title: Prothioconazole (PTZ) residue DT50 kinetics EUR- Statement on residue dissipation of prothioconazole and its metabolite on wheat seeds in Germany: kinetic evaluation
Report No.: EnSa-15-0608
Document No.: M-534805-01-1
Guideline(s): not applicable
Guideline deviation(s): not applicable
GLP/GEP: no

This statement provides a kinetic evaluation of the residues of prothioconazole and its metabolite JAU 6476-desthio in wheat seeds in the field that may represent food items for leaf-eating herbivorous birds or mammals. The residue decline data are available from a specifically performed residue study ([redacted]; 2014; M-488935-01-3; KCP 10.1.1.2/19).

The reliable single-first-order (SFO) half-lives of prothioconazole and JAU 6476-desthio derived in this evaluation are summarised in Table CP 10.1.1.2-4 below, along with the formation fraction of prothioconazole to desthio.

Table CP 10.1.1.2-4: DT_{50} values for prothioconazole and JAU 6476-desthio and results of the statistical analysis - scaled error (ε) and significance of the dissipation rate (t-test) for SFO model

Trial code	Compound	Model	DT_{50} [d]	DT_{50} [d]	formation fraction
			JAU 6476	Desthio	
west	JAU 6476	SFO	2.30		
	desthio	SFO		4.81	0.54
south	JAU 6476	SFO	2.90		
	desthio	SFO		2.11	0.66
north	JAU 6476	SFO	1.72		
	desthio	SFO		3.68	0.54
east	JAU 6476	SFO	1.40		
	desthio	SFO		7.81	0.37

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Report: KCP 10.1.1.2/18 [redacted],; 2014; M-486407-02-1
Title: Dissipation of triadimenol, prothioconazole and fluopyram on wheat seeds and seedlings in Germany
Report No.: B13017-1
Document No.: M-486407-02-1
Guideline(s): For the present study type no official test guideline is available. The study was conducted
under consideration of the recommendations in the current guidance document on risk
assessment for birds & mammals (EFSA 2009)
Guideline deviation(s): none
GLP/GEP: **yes**

Objective:

The purpose of the study was to quantify residue amounts of triadimenol, prothioconazole, JAU 6476-desthio and fluopyram residues after seed treatment with the Baytan 3 (fluopyram + prothioconazole + triadimenol FS 217.5) on spring wheat under field conditions:
1) on wheat seeds remaining on the soil surface if not buried after drilling,
2) in seedlings emerging from drilled wheat seeds,
following the recommendations of the Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. (EFSA Journal 2009; 7(12):1438)

Study Materials and Sites:

Study sites: The study was conducted on plain field stripes (headland) at four different locations distributed over Germany, (1) near [redacted], Rhineland (west), (2) near [redacted], Saxony (east), (3) near [redacted] Hesse-Palatine (south) and (4) [redacted], Lower Saxony (north). An area of approximately 150 m2 of fallow land was demarcated per study site. Three plots, serving as replicates, were installed at each location.

Test item and application: fluopyram + prothioconazole + triadimenol FS 217.5 (Baytan 3) was applied with nominal 200 mL product/100 kg seeds on spring wheat.

Sampling: Treated seeds scattered on the soil surface were collected on DAT +1, +2, +3,+ 5, +7, +10, +15, +21 and +28. On the day of sowing (DAT 0), seed samples were taken directly from the package. Seedlings emerging from drilled seeds were sampled starting at BBCH 10/11 every two days until day

21 after first sampling. Seedlings were cut with scissors close to the soil surface with a targeted minimum seedling biomass per sampling day and replicate of approximately 10 - 12 g wet weight.
Residue analysis: All samples (seeds and seedlings) were analysed for their content of triadimenol (analytical method 01072/M001), fluopyram (analytical method 00984/M002), prothioconazole and JAU 6476-desthio (analytical method 01013) via HPLC-MS/MS. Residues are reported in terms of mg active substance/kg or pure metabolite (mg a.s.(p.m.)/kg). The Limit of Quantification (LOQ) value was 0.01 mg/kg.
Calculations: Mean and time-weighted-average concentrations were calculated with MS Office Excel 2010. The residue decline (DT50) of fluopyram, triadimenol and prothioconazole on wheat seeds and seedlings was determined, assuming a first-order kinetic using KinGUI2.1.

Results:
For the purpose of refining the risk assessment for the product presented in this dossier only the results pertaining to prothioconazole and its desthio-metabolite are presented in this summary.
Mean measured initial concentrations on seeds, 21d-TWA concentrations and resulting f_{twa} values relevant for risk assessments for terrestrial vertebrates summarised in the report are only used after a kinetic evaluation. Therefore they are not presented here in detail to avoid confusion.

Neither prothioconazole nor its metabolite JAU 6476-desthio were detected in seedlings in significant quantities (<LOQ and 0.014 mg p.m./kg f.w., respectively). Therefore, prothioconazole can be considered as „not systemic“ when used as a seed treatment.

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Report:	KCP 10.1.1.2/19 [redacted], 2014; M-488935-01-3
Title:	Dissipation of fludioxonil, penflufen and prothioconazole on wheat seeds and seedlings in Germany
Report No.:	M-488935-01-3
Document No.:	M-488935-01-3
Guideline(s):	For the present study type no official test guideline is available. The study was conducted under consideration of the recommendations in the current guidance document on risk assessment for birds & mammals (EFSA 2009)
Guideline deviation(s):	not applicable
GLP/GEP:	**yes**

Objective:
The purpose of the study was to quantify residue amounts of fludioxonil, penflufen, prothioconazole, and metabolite JAU 6476-desthio after seed treatment with the product (fludioxonil + penflufen + prothioconazole FS 150) on spring wheat under field conditions:
1) on wheat seeds remaining on the soil surface if not buried after drilling,
2) in seedlings emerging from drilled wheat seeds,
following the recommendations of the Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. (EFSA Journal 2009; 7(12):1438)

Study Materials and Sites
Study sites: The study was conducted on plain field stripes (headland) at four different locations distributed over Germany, (1) [redacted], Rhineland (west), (2) [redacted], Saxony (east), (3) near [redacted], Hesse-Palatine (south) and (4) [redacted], Lower Saxony (north). An area of approximately 150 m^2 of fallow land was demarcated per study site. Three plots, serving as replicates, were installed at each location.

Test item and application: fludioxonil + penflufen + prothioconazole FS 150 was applied with nominal 100 mL product/100 kg seeds on spring wheat.

Sampling: Treated seeds scattered on the soil surface were collected on DAT +1, +2, +3,+ 5, +7, +10, +15, +21 and +28. On the day of sowing (DAT 0), seed samples were taken directly from the package. Seedlings emerging from drilled seeds were sampled starting at BBCH 10-11 every two days until day 21 after first sampling. Seedlings were cut with scissors close to the soil surface with a targeted minimum seedling biomass per sampling day and replicate of approximately 10 – 12 wet weight.

Residue analysis: All samples (seeds and seedlings) were analysed for their content of fludioxonil, penflufen, prothioconazole and JAU 6476-desthio (analytical method 01013) via HPLC-MS/MS. Residues are reported in terms of mg active substance/kg (mg a.s./kg). The Limit of Quantification (LOQ) value was 0.01 mg/kg.

Calculations: Mean and time-weighted-average concentrations were calculated with MS Office Excel 2010. The residue decline (DT_{50}) of fludioxonil, penflufen and prothioconazole on wheat seeds and seedlings was determined, assuming a first-order kinetic using KinGUI2.

Results

For the purpose of refining the risk assessment for the product presented in this dossier only the results pertaining to prothioconazole and its desthio-metabolite are presented in this summary.
Mean measured initial concentrations on seeds, 21d-TWA concentrations and resulting f_{TWA} values relevant for risk assessments for terrestrial vertebrates summarised in the report are only used after a kinetic evaluation. Therefore they are not presented here in detail to avoid confusion.

None of the compounds was detected in seedlings in significant quantities. Prothioconazole can be considered as "not systemic" when used as a seed treatment.

CP 10.1.2 Effects on terrestrial vertebrates other than birds

Table CP 10.1.2- 1: Endpoints used in risk assessment

Test substance	Test species	Ecotoxicological endpoint		Reference
Prothioconazole	acute, oral Rat	LD_{50}	**>6200 mg a.s./kg bw**	[redacted] (1998) M-012312-01-1 KCA 5.2.1/01
	Long-term (2-gen.-repro study) Rat	NO(A)EL	**95.6 mg a.s./kg bw/d**	[redacted] (2001) M-036206-01-1 KCA 5.6.1/02
JAU 6476-desthio	Acute, oral Mouse	$LD_{50\ (male)}$ $LD_{50\ (female)}$	**2235 mg p.m./kg bw** 3459 mg p.m./kg bw	[redacted] (1991) M-008521-01-1 KCA 5.8.1/34
	Long-term (2-gen.-repro study) Rat	NOEL	**10 mg p.m./kg bw/d**	[redacted] & [redacted] (2001) M-036130-01-1 KCA 5.8.1/23

a.s.: active substance; p.m.: pure metabolite; bw = body weight

Table CP 10.1.2- 2: Relevant generic focal species feeding on seeds for Tier 1 risk assessment

Type of seeds	Generic focal species	FIR/bw
'Large seeds' (maize, beans or peas)	Small omnivorous mammal	0.24
'Small seeds' (not maize, beans or peas)	Small omnivorous mammal	0.24

Since prothioconazole and JAU 6476-desthio are non-systemic ([redacted] 2014a, b, M-486407-01-1, M-488935-01-3, KCP 10.1.1.2/18 & 19), no risk assessment for birds feeding on crop seedlings was performed.

Acute dietary risk assessment

Table CP 10.1.2- 3: Tier 1 acute TER calculation for mammals feeding on seed treatment

Compound	Generic focal species	LD_{50} [mg/kg bw]	Exposure		TER_A	Trigger
			FIR/bw	NAR [mg a.s./kg seeds][1)]		
Prothio-conazole	Small omnivorous mammal	6200	0.24	100	258	10
JAU 6476-desthio	Small omnivorous mammal	2235	0.24	100[2)]	93	10

[1)] Assuming a thousand grain weight of the seeds of 50 g [8]

[2)] This value is taken from the parent compound and represents an unrealistic worst-case scenario

The TER values for mammals feeding on treated seeds or crop seedlings meet the required trigger of 10 for acute exposure, indicating no unacceptable risk to mammals for the use of the product.

Tier 1 risk assessment for mammals drinking contaminated water

EFSA (2009, chapter 5.2.1) proposes to focus the risk assessment for birds and mammals on the dietary route of exposure. An assessment of the risk potentially posed by consumption of contaminated drinking water after the use of a pesticide as seed treatment is not required since this route seems unlikely to be a critical one or to lead to TER greater than direct dietary consumption.

[8] Faustzahlen für die Landwirtschaft (2005), published by Kuratorium für Technik und Bauwesen in der Landwirtschaft, Darmstadt, 13th edition, ISBN 3-7843-2194-1

Long-term reproductive risk assessment

Table CP 10.1.2- 4: Tier 1 long-term TER calculation for mammals feeding on seed treatment

Compound	Generic focal species	NOEL [mg/kg bw/d]	Exposure			TER_{LT}	Trigger
			FIR/bw	NAR [mg a.s./kg seeds][1)]	f_{TWA}		
Prothioconazole	Small omnivorous mammal	95.6	0.24	100	0.53	5	5
JAU 6476-desthio	Small omnivorous mammal	10	0.24	100[2)]	0.53	**0.8**	5

[1)] Assuming a thousand grain weight of the seeds of 50 g [9]

[2)] The application rate is taken from the parent compound and represents an unrealistic worst-case scenario

Bold values do not meet the trigger

The TER values for mammals feeding on treated seeds do not meet the required trigger of 5 for long-term exposure to JAU 6476-desthio. Accordingly, a refined risk assessment is needed .

Refined risk assessment for long-term exposure for small omnivorous mammals feeding on seeds treated with JAU 6476-desthio

Focal species

Freshly drilled cereal fields are best represented by the bare field scenarios, numbers 1-3 in the "Mammal Tier 1 tables of the EFSA Guidance (2009). In the case of seed treatments, the treated seeds are the only feed items containing residues of the plant protection product. Accordingly only the granivorous mammal with a single diet of seeds has to be considered in the risk assessment.

Scenario #2 lists the Wood mouse (*Apodemus sylvaticus*) as the generic focal species for Tier 1 risk assessment. Relevant ecological parameters are a PD of 1 (single diet of seeds) and a FIR/bw of 0.17. These figures will be used in the refined risk assessment for granivorous mammals.

Residue on feed

As outlined in chapter 10.1.2 (birds) an adjustment factor of 0.11 taking into account formation and dissipation of JAU 6476-desthio can be used for the long-term risk assessment of granivorous birds. The same refinement will be used here for the long-term risk assessment for granivorous mammals.

[9] Faustzahlen für die Landwirtschaft (2005), published by Kuratorium für Technik und Bauwesen in der Landwirtschaft, Darmstadt, 13th edition, ISBN 3-7843-2194-1

Table CP 10.1.2- 5: TER calculation based on long-term toxicity and exposure to JAU 6476-desthio

Application	Seed dressing (JAU 6476-desthio)
Nominal seed treatment rate of the parent compound [mg a.s./kg seeds]	100
Generic focal species	Wood mouse
Feed	Treated seeds
FIR/bw (Wood mouse)	0.17
Adjustment factor	0.11
DDD [mg p.m./kg bw]	1.87
NOEL [mg p.m./kg bw]	10.0
TER_{LT}	5.3

The refined long-term risk assessment for the exposure of birds to residues of the metabolite JAU 6476-desthio after the application of the product indicates that no unacceptable adverse effects on mammals are to be expected.

Long-term risk assessment for mammals drinking contaminated water

EFSA (2009, chapter 5.2.1) proposes to focus the risk assessment for birds and mammals on the dietary route of exposure. An assessment of the risk potentially posed by consumption of contaminated drinking water after the use of a pesticide as seed treatment is not required since this route seems unlikely to be a critical one or to lead to TER greater than direct dietary consumption.

Risk assessment of secondary poisoning

Substances with a high bioaccumulation potential could theoretically bear a risk of secondary poisoning for mammals if feeding on contaminated prey like fish or earthworms. For organic chemicals, a log P_{OW} > 3 is used to trigger an in-depth evaluation of the potential for bioaccumulation.
Prothioconazole, however, has a log P_{OW} of 2.0, indicating a very low risk of bioaccumulation and, hence, secondary poisoning.

Prothioconazole metabolites JAU 6476-desthio (log Pow 3.04) and JAU 6476-S-methyl (log Pow 4.19) will be evaluated for potential effects of secondary poisoning of mammals.

Long-term DDD and TER calculation for earthworm-eating mammals

Table CP 10.1.2- 6: Tier 1 long-term DDD and TER calculation for earthworm-eating mammals

Compound	JAU 6476-desthio	JAU 6476-S-methyl	Origin of values
PEC_{worm} [mg/kg]	0.013	0.014	see Table CP 10.1.1- 17
DDD calculation:			
FIR/bw	1.28	1.28	Default
DDD [mg/kg bw/d]	0.017	0.018	
TER calculation:			
NO(A)EL [mg/kg bw/d]	10	9.56 [1]	See Table CP 10.1.2- 1
TER_{LT}	588	531	
Trigger	5	5	
Refined risk assessment required?	No	No	

[1)] NOEL of the parent compound prothioconazole was divided by a factor of 10 (worst-case assumption)

Both TER values are above the trigger of 5. Accordingly, the risk to earthworm-eating mammals following the use of the product in cereals is acceptable.

Long-term DDD and TER calculation for fish-eating mammals

Table CP 10.1.2- 7: Tier 1 long-term DDD and TER calculation for fish-eating mammals

Compound	JAU 6476-desthio	JAU 6476-S-methyl	Origin of values
PEC_{fish} [mg/kg]	0.047	0.026	See Table CP 10.1.1- 18
DDD calculation:			
FIR/bw	0.142	0.142	Default
DDD [mg/kg bw/d]	0.007	0.004	
TER calculation:			
NO(A)EL [mg/kg bw/d]	10	9.56 [1]	See Table CP 10.1.2- 1
TER_{LT}	1 429	2 390	
Trigger	5	5	
Refined risk assessment required?	No	No	

[1] NOEL of the parent compound prothioconazole was divided by a factor of 10 (worst-case assumption)

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

CP 10.1.2.1 Acute oral toxicity to mammals

An acute study on rats was conducted with Prothioconazole FS 100 ([redacted]F; 2001; M-137432-01-1, KCP 7.1.1/01). According to OECD guideline 423, the result corresponds with LD_{50} >2000 mg prod./kg bw.

CP 10.1.2.2 Higher tier data on mammals

Additional data is presented to support the short half-life of prothioconazole and JAU 6476-desthio on seeds. This data is provided in chapter CP 10.1.1.2 and employed in the refined risk assessment for omnivorous mammals.

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

No additional studies are available or required under the data requirements of EC 1107/2009.

CP 10.2 Effects on aquatic organisms

The risk assessment has been performed according to the Regulation (EC) No 1107/2009 and following the EFSA Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (2013; cited in the following paragraphs as "EFSA AGD").

Ecotoxicological endpoints used in risk assessment

The relevant endpoint from each aquatic study was defined according to the current data requirements from the EU Regulation 283/2013 and the EFSA AGD (2013), and based on recommendations from the relevant standard test guideline e.g. Growth rate (r) is the most suitable endpoint from algae inhibition tests for use in risk assessment, as stated by OECD Guideline 201 and the EFSA AGD (2013). TER and RAC calculations presented in this dossier are thus based on the E_rC_{50} values. Indeed, processes in ecosystems are dominantly rate driven and therefore, the unit development per time (growth rate) appears more suitable to measure effects in algae. Also, growth rates and their inhibition can easily be compared between species, test durations and test conditions, which is not the case for biomass. Moreover, the current test guidelines OECD TG 201, the EU-Method C3, the EC regulation for Classification and Labelling (EC regulation 1272/2008) and the PPR Opinion (EFSA Journal 461, 1-44; 2007) list growth rate as the most suitable endpoint of the algae inhibition test.

In accordance with Regulation (EC) No 1107/2009 and with the EFSA AGD (2013), studies resulting in lower endpoints were used for the risk assessment. Although Regulation (EC) No 1107/2009 place no data requirement on marine species, marine studies resulting in lower endpoints compared to freshwater studies were considered for risk assessment as a conservative approach.

For the aquatic risk assessment an envelope approach was performed. Therebye, the highest FOCUS Step 2 PEC values were used to calculate the risk to aquatic organisms. This clearly represents the worst-case situation covering all other FOCUS STEP 2 scenarios.
If the trigger was not met using this calculation, worst-case FOCUS STEP 3 PEC values were used as refinement until a safe use of each intended application can be considered.

Risk assessment for aquatic organisms

Table CP 10.2- 1: Endpoints relevant for risk assessment

Test substance	Test species	Endpoint		Reference
Prothio-conazole	Fish, acute *Oncorhynchus mykiss* (Rainbow trout)	LC_{50}	**1.83 mg a.s./L**	[redacted] (1999) M-015215-01-1 KCA 8.2.1/01
	Fish, early life stage *Oncorhynchus mykiss* (Rainbow trout)	NOEC	**0.49 mg a.s./L**	[redacted] & [redacted] (2007) M-291414-01-1 KCA 8.2.2.1/03
	Invertebrate, acute *Daphnia magna* (Cladoceran)	EC_{50}	**1.3 mg a.s./L**	[redacted] (1999) M-013690-01-1 KCA 8.2.4.1/01
	Invertebrate, acute *Americamysis bahia* (Mysid shrimp)	LC_{50}	**2.4 mg a.s./L**	[redacted] et al. (2002) M-083057-01-1 KCA 8.2.4.2/02
	Invertebrate, chronic *Daphnia magna* (Cladoceran)	NOEC	**0.56 mg a.s./L**	[redacted] & [redacted] (2001) M-055997-01-1 KCA 8.2.5.1/01
	Sediment dweller, chronic *Chironomus riparius* (Chironomid)	NOEC	**9.14 mg a.s./L**	[redacted] (2000) M-047356-01-1 KCA 8.2.5.4/01
	Skeletonema costatum (Marine diatom)	E_rC_{50}	**0.046 mg a.s./L** [5]	[redacted] & [redacted] (2004) M-000954-01-1 KCA 8.2.6.2/01
	Lemna gibba (Duckweed)	E_rC_{50}	**> 0.404 mg a.s./L**	[redacted] et al. (2004) M-000532-01-1 KCA 8.2.7/01
JAU 6476-desthio	Fish, acute *Oncorhynchus mykiss* (Rainbow trout)	LC_{50}	**6.63 mg p.m./L**	[redacted] (1990) M-013303-01-1 KCA 8.2.1/04
	Fish, early life stage *Oncorhynchus mykiss* (Rainbow trout)	NOEC	**0.00334 mg p.m./L**	[redacted] (2002) M-038386-01-1 KCA 8.2.2.1/02
	Invertebrate, acute *Daphnia magna* (Cladoceran)	EC_{50}	**> 10 mg p.m./L**	[redacted] (1990) M-013308-01-1 KCA 8.2.4.1/02
	Invertebrate, acute *Americamysis bahia* (Mysid shrimp)	LC_{50}	**> 1.009 mg p.m./L**	[redacted] et al. (2003) M-104620-01-1 KCA 8.2.5.2/02
	Invertebrate, chronic *Daphnia magna* (Cladoceran)	NOEC	**0.10 mg p.m./L**	[redacted] & [redacted] (2001) M-073861-01-1 KCA 8.2.5.1/02
	Invertebrate, chronic *Americamysis bahia* (Mysid shrimp)	NOEC	**0.064 mg p.m./L**	[redacted] et al. (2003) M-104620-01-1 KCA 8.2.5.2/02
	Sediment dweller, chronic *Chironomus riparius* (Chironomid)	NOEC	**2.0 mg p.m./L**[1)]	[redacted] (2000) M-023234-01-1 KCA 8.2.5.4/02
	Scenedesmus subspicatus (Green alga)	E_rC_{50}	**0.55 mg p.m./L**	[redacted] (1990) M-013305-01-1 KCA 8.2.6.1/02

Test substance	Test species	Endpoint		Reference
	Lemna gibba (Duckweed)	E_rC_{50}	**0.0809 mg p.m./L**	[redacted] et al. (2003) M-104599-01-1 KCA 8.2.7/02
JAU 6476-S-methyl	Fish, acute *Oncorhynchus mykiss* (Rainbow trout)	LC_{50}	**1.79 mg p.m./L**	[redacted] & [redacted] (2001) M-074388-01-1 KCA 8.2.1/05
	Invertebrate, acute *Daphnia magna* (Cladoceran)	EC_{50}	**2.8 mg p.m./L**	[redacted] [redacted] (2001) M-071853-01-1 KCA 8.2.4.1/03
	Peudokirchneriella subcapitata (Green alga)	E_rC_{50}	**47.4 mg p.m./L**	[redacted] & [redacted] (2001) M-061047-01-1 KCA 8.2.6.1/02
	Sediment dweller, chronic *Chironomus riparius* (Chironomid)	NOEC	**0.1 mg p.m./L**	[redacted] (2006) M-266605-01-1 KCA 8.2.5.4/04
1,2,4-Triazole	Fish, acute *Oncorhynchus mykiss* (Rainbow trout)	LC_{50}	**498 mg p.m./L**	[redacted] (1983) M-046022-01-1 KCA 8.2.1/06
	Fish, juvenile growth test *Oncorhynchus mykiss* (Rainbow trout)	NOEC	**3.2 mg p.m./L**	[redacted] & [redacted] (2002) M-030491-01-1 KCA 8.2.2/01
	Invertebrate, acute *Daphnia magna* (Cladoceran)	EC_{50}	**> 100 mg p.m./L** 2)	[redacted] (1995) M-088901-01-1 KCA 8.2.4.1/06
	Peudokirchneriella subcapitata (Green alga)	E_rC_{50}	**> 31 mg p.m./L** 3)	[redacted] et al. (2001) M-077067-01-1 KCA 8.2.6.1/04
JAU 6476 - triazolyl-ketone	Fish, acute *Oncorhynchus mykiss* (Rainbow trout)	LC_{50}	**> 100 mg p.m./L**	[redacted] (2006) M-266572-01-1 KCA 8.2.1/11
	Invertebrate, acute *Daphnia magna* (Cladoceran)	EC_{50}	**> 100 mg p.m./L**	[redacted] (2006) M-266597-01-1 KCA 8.2.4.1/07
	Peudokirchneriella subcapitata (Green alga)	E_rC_{50}	**> 100 mg p.m./L**	[redacted] (2006) M-266567-01-1 KCA 8.2.6.1/05
JAU 6476 - thiazocine	Fish, acute *Oncorhynchus mykiss* (Rainbow trout)	LC_{50}	**1.83 mg a.s./L** 4)	[redacted] (1999) M-015215-01-1 KCA 8.2.1/01
	Fish, early life stage *Oncorhynchus mykiss* (Rainbow trout)	NOEC	**0.49 mg a.s./L** 4)	[redacted] & [redacted] (2007) M-291414-01-1 KCA 8.2.2.1/03
	Invertebrate, acute *Daphnia magna* (Cladoceran)	EC_{50}	**1.3 mg a.s./L** 4)	[redacted] (1999) M-013690-01-1 KCA 8.2.4.1/01

Test substance	Test species	Endpoint		Reference
	Invertebrate, chronic *Daphnia magna* (Cladoceran)	NOEC	**0.56 mg a.s./L** [4]	[redacted] & [redacted] (2001) M-055997-01-1 KCA 8.2.4.1/01
	Pseudokirchneriella subcapitata (Green alga)	E_rC_{50}	**2.18 mg a.s./L** [4]	[redacted] (2005) M-027625-01-1 KCA 8.2.6.1/01
PTZ FS 100	Fish, acute *Oncorhynchus mykiss* (Rainbow trout)	LC_{50}	**16.4 mg prod./L**	[redacted] 2015 M-525350-01-1 KCP 10.2.1/01
	Invertebrate, acute *Daphnia magna* (cladoceran)	EC_{50}	**9.8 mg prod./L**	[redacted] 2015 M-525311-01-1 KCP 10.2.1/02
	Pseudokirchneriella subcapitata (Green alga)	E_rC_{50}	**18.6 mg prod./L**	[redacted] 2015 M-525311-01-1 KCP 10.2.1/03

a.s.: active substance; p.m.: pure metabolite; prod. = formulated product

Bold values: Endpoints considered relevant for risk assessment

[1] NOEC according to the list of endpoints given in the EFSA conclusion on prothioconazole (2007). The original study endpoint is the EC_{15} = 4.4 mg/L; the cited NOEC was not statistically derived, as was explained in the DAR by the RMS but proposed as a conservative endpoint.

[2] EU agreed endpoint for 1,2,4-triazole derived from the PRAPeR expert meeting on triazole metabolites (PRAPeR 13, 2007).

[3] EU agreed endpoint is derived from the EFSA Scientific Report (2014) 12(1):3485, Conclusion on the peer review of tebuconazole.

[4] JAU 6476-thiazocine has lost the toxophore and shows no pesticidal activity, as explained in detail in a statement by [redacted] 2015, (M-536612-01-1; KCA 8.2/01). For metabolites with such properties, the 'EFSA Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge of field surface waters (2013)' prescribes to assume "that the acute and chronic toxicity of the metabolite is equal to the toxicity of the a.s. (parent compound) for all first tier taxonomic groups". Therefore, the endpoints of the parent compound prothioconazole from studies on first tier species were used for the acute and chronic risk assessment of JAU 6476-thiazocine.

[5] Although Regulation (EC) No 1107/2009 place no data requirement on marine species, the endpoint from a study on the marine diatom *Skeletonema costatum* is used for algae risk assessment for prothioconazole as a conservative approach. Indeed this endpoint is lower than the one from the standard species (green algae, *P. subcapitata,* E_rC_{50} = 2.18 mg a.s./L).

Predicted environmental concentrations used in risk assessment

Full details of the predicted environmental concentrations are given in MCP 9 (KCP 9.2.5/08).

Table CP 10.2- 2: Initial max. PEC_{sw} values for prothioconazole and its metabolites – Use in winter and spring cereals (FOCUS Step 1 & 2)

	Compound	Prothio-conazole	JAU 6476-desthio		JAU 6476-S-methyl		1,2,4-triazole	Thiazocine	Triazolyl-ketone
Use pattern	Scenario	PEC_{sw} [µg/L]	PEC_{sw} [µg/L]	21d-TWA [µg/L]	PEC_{sw} [µg/L]	21d-TWA [µg/L]	PEC_{sw} [µg/L]	PEC_{sw} [µg/L]	PEC_{sw} [µg/L]
Winter cereals 1×18 g a.s./ha	Step 1	1.789	3.441	3.028	0.385	0.352	0.453	0.668	0.294
	Step 2								
	N-EU Si	**0.124**	**0.937**	0.723*	**0.110**	0.082*	**0.031**	**0.046**	**0.020**
	S-EU Si	0.099	0.749	0.578	0.088	0.066	0.025	0.037	0.016
Spring cereals 1×18 g a.s./ha	Step 1	1.789	3.441	3.028	0.385	0.352	0.453	0.668	0.294
	Step 2								
	N-EU Si	0.049	0.375	0.289	0.044	0.033	0.013	0.018	0.008
	S-EU Si	0.099	0.749	0.578	0.088	0.066	0.025	0.037	0.016

Bold values were considered in risk assessment
* Values used for secondary poisoning (see 10.1.1, Table CP 10.1.1-18)

Table CP 10.2- 3: Initial max. PEC_{sw} values for the prothioconazole metabolite JAU 6476-desthio – Use in winter and spring cereals (FOCUS Step 3)

	Use pattern	1 × 18 g a.s./ha	
Compound	FOCUS Scenario	Winter cereals $PEC_{sw, max}$ [µg/L]	Spring cereals $PEC_{sw, max}$ [µg/L]
JAU 6476-desthio	D1 (ditch, 1st)	<0.001	<0.001
	D1 (stream, 1st)	<0.001	<0.001
	D2 (ditch, 1st)	<0.001	-
	D2 (stream, 1st)	<0.001	-
	D3 (ditch, 1st)	<0.001	<0.001
	D4 (pond, 1st)	<0.001	<0.001
	D4 (stream, 1st)	<0.001	<0.001
	D5 (pond, 1st)	<0.001	<0.001
	D5 (stream, 1st)	<0.001	<0.001
	D6 (ditch, 1st)	<0.001	-
	R1 (pond, 1st)	<0.001	-
	R1 (stream, 1st)	<0.001	-
	R3 (stream, 1st)	<0.001	-
	R4 (stream, 1st)	<0.001	<0.001

Acute Risk Assessment For Aquatic Organisms

Based on the risk envelope approach, the highest PEC values were used to calculate the acute risk to aquatic organisms. This clearly represents the worst-case situation covering all other intended uses of the product.

If the trigger was not met using this calculation, worst-case FOCUS STEP 3 PEC values were used as refinement until a safe use of each intended application could be assumed.

Table CP 10.2- 4: TER$_A$ calculations based on FOCUS Step 2

Compound	Test species	Endpoint [µg/L]		PEC$_{sw,max}$ [µg/L]	TER$_A$	Trigger
Cereals (winter/spring)						
Prothioconazole	Fish, acute *Oncorhynchus mykiss*	LC$_{50}$	1830	0.124	14758	100
	Invertebrate, acute *Daphnia magna*	EC$_{50}$	1300	0.124	10484	100
	Invertebrate, acute *Americamysis bahia*	EC$_{50}$	2400	0.124	19355	100
JAU 6476-desthio	Fish, acute *Oncorhynchus mykiss*	LC$_{50}$	6630	0.937	7076	100
	Invertebrate, acute *Daphnia magna*	EC$_{50}$	>10000	0.937	>10672	100
	Invertebrate, acute *Americamysis bahia*	LC$_{50}$	>1009	0.937	>1077	100
JAU 6476-S-methyl	Fish, acute *Oncorhynchus mykiss*	LC$_{50}$	1790	0.110	16273	100
	Invertebrate, acute *Daphnia magna*	EC$_{50}$	2800	0.110	25455	100
1,2,4-Triazole	Fish, acute *Oncorhynchus mykiss*	LC$_{50}$	498000	0.031	16064516	100
	Invertebrate, acute *Daphnia magna*	EC$_{50}$	>100000	0.031	>3225806	100
JAU 6476-thiazocine	Fish, acute *Oncorhynchus mykiss*	LC$_{50}$	1830*	0.046	39783	100
	Invertebrate, acute *Daphnia magna*	EC$_{50}$	1300*	0.046	28261	100
JAU 6476-triazolylketone	Fish, acute *Oncorhynchus mykiss*	LC$_{50}$	>100000	0.020	>5000000	100
	Invertebrate, acute *Daphnia magna*	EC$_{50}$	>100000	0.020	>5000000	100

* Endpoints from parent prothioconazole from studies on first tier species were used for risk assessment of M12 (see Table CP 10.2-1 and MCA, point 8.2 for more details)

Table CP 10.2- 5: $RAC_{sw;\ ac}$ calculations based on FOCUS Step 2 (acceptability of risk: PEC/RAC < 1)

Compound	Test species	Endpoint [µg/L]	$RAC_{sw;\ ac}$ (LC_{50}/100)	$PEC_{sw,max}$ [µg/L]	PEC/RAC
Cereals (winter/spring)					
Prothioconazole	Fish, acute *Oncorhynchus mykiss*	LC_{50} 1830	18.3	0.124	0.01
	Invertebrate, acute *Daphnia magna*	EC_{50} 1300	13.0	0.124	0.01
	Invertebrate, acute *Americamysis bahia*	EC_{50} 2400	24.0	0.124	0.01
JAU 6476-desthio	Fish, acute *Oncorhynchus mykiss*	LC_{50} 6630	66.3	0.937	0.01
	Invertebrate, acute *Daphnia magna*	EC_{50} >10000	>100	0.937	< 0.01
	Invertebrate, acute *Americamysis bahia*	LC_{50} 1009	10.09	0.937	< 0.09
JAU 6476-S-methyl	Fish, acute *Oncorhynchus mykiss*	LC_{50} 1790	17.9	0.110	0.01
	Invertebrate, acute *Daphnia magna*	EC_{50} 2800	28.0	0.110	0.004
1,2,4-Triazole	Fish, acute *Oncorhynchus mykiss*	LC_{50} 498000	4980	0.031	0.00001
	Invertebrate, acute *Daphnia magna*	EC_{50} >100000	>1000	0.031	< 0.00003
JAU 6476-thiazocine	Fish, acute *Oncorhynchus mykiss*	LC_{50} 1830*	18.3	0.046	0.003
	Invertebrate, acute *Daphnia magna*	EC_{50} 1300*	13.0	0.046	0.004
JAU 6476-triazolylketone	Fish, acute *Oncorhynchus mykiss*	LC_{50} >100000	>1000	0.020	< 0.00002
	Invertebrate, acute *Daphnia magna*	EC_{50} >100000	>1000	0.020	< 0.00002

* Endpoints from parent prothioconazole from studies on first tier species were used for risk assessment of M12 (see Table CP 10.2-1 and MCA, point 8.2 for more details)

The TER trigger was exceeded for all organisms for prothioconazole and all its metabolites.

Chronic risk assessment for aquatic organisms

For all metabolites where a complete chronic data package is available (e.g. JAU 6476-desthio), TER_{LT} and $RAC_{sw,ch}$ calculations are presented below. For those metabolites where chronic data are not available for every first tier taxonomic group relevant to fungicide risk assessment (as defined in EFSA AGD (2013)[10]), TER_{LT} and $RAC_{sw,ch}$ calculations are presented with the available studies. In addition, a complementary chronic risk assessment following the stepwise approach as recommended by EFSA AGD (see point 10.2.4 'Risk assessment scheme for metabolites', page 143) is performed in a stand-alone document ([redacted]; 2015; M-536697-01-1, KCP 10.2/02). This EFSA stepwise

[10] First tier taxonomic groups relevant to fungicide risk assessment as defined in EFSA AGD (2013) are fish, invertebrates and algae. Sediment dwellers should also be considered, when metabolites accumulate in sediment (> 10% of the metabolite found in sediment at the end of the water/sediment study) and when toxicity to daphnids is expected (daphnid endpoint < 0.1 mg/L).

approach was placed in a stand-alone document because, as this approach is new, there is currently no agreed template how to formally include it in the Section 10 of the MCP Document. Further information about this approach and its results is presented after the TER / RAC tables below.

Based on the risk envelope approach, the highest PEC values were used to calculate the chronic risk to aquatic organisms. This clearly represents the worst-case situation covering all other intended uses of the product. If the trigger was not met using this calculation, worst-case FOCUS STEP 3 PEC values were used as refinement until a safe use of each intended application could be assumed.

Table CP 10.2- 6: TER_{LT} calculations based on FOCUS Step 2

Compound	Test species	Endpoint [µg/L]	$PEC_{sw, max}$ [µg/L]	TER_{LT}	Trigger
Cereals (winter/spring)					
Prothioconazole	Fish, early life stage *Oncorhynchus mykiss*	NOEC 490	0.124	3952	10
	Invertebrate, chronic *Daphnia magna*	NOEC 560	0.124	4516	10
	Sediment dweller, chronic *Chironomus riparius*	NOEC 9140	0.124	73710	10
	Marine diatom, chronic *Skeletonema costatum*	E_rC_{50} 46	0.124	371	10
	Aquatic plant, chronic *Lemna gibba*	E_rC_{50} > 404	0.124	> 3258	10
JAU 6476-desthio	Fish, early life stage *Oncorhynchus mykiss*	NOEC 3.34	0.937	**3.6**	10
	Invertebrate, chronic *Daphnia magna*	NOEC 100	0.937	107	10
	Invertebrate, chronic *Americamysis bahia*	NOEC 64	0.937	68	10
	Sediment dweller, chronic *Chironomus riparius*	NOEC 2000	0.937	2134	10
	Green alga, chronic *Scenedesmus subspicatus*	E_rC_{50} 550	0.937	587	10
	Aquatic plant, chronic *Lemna gibba*	E_rC_{50} 80.9	0.937	86	10
JAU 6476-S-methyl	Sediment dweller, chronic *Chironomus riparius*	NOEC 100	0.110	909	10
	Green algae, chronic *Pseudokirchneriella subcapitata*	E_rC_{50} 47400	0.110	430909	10
1,2,4-Triazole	Fish, juvenile growth *Oncorhynchus mykiss*	NOEC 3200	0.031	103226	10
	Green alga, chronic *Pseudokirchneriella subcapitata*	E_rC_{50} > 31000	0.031	>1000000	10

Compound	Test species	Endpoint [µg/L]	$PEC_{sw, max}$ [µg/L]	TER_{LT}	Trigger
Cereals (winter/spring)					
JAU 6476-thiazocine	Fish, early life stage *Oncorhynchus mykiss*	NOEC 490*	0.046	10652	10
	Invertebrate, chronic *Daphnia magna*	NOEC 560*	0.046	12174	10
	Green alga, chronic *Pseudokirchneriella subcapitata*	E_rC_{50} 2180*	0.046	47391	10
JAU 6476-triazolylketone	Green algae, chronic *Pseudokirchneriella subcapitata*	E_rC_{50} > 100000	0.020	>5000000	10

* Endpoints from parent prothioconazole from studies on first tier species were used for risk assessment of M12 (see Table CP 10.2-1 and MCA, point 8.2 for more details)
Bold values do not meet the trigger

Table CP 10.2- 7: $RAC_{sw, ch}$ calculations based on FOCUS Step 2 (acceptability of risk: PEC/RAC < 1)

Compound	Test species	Endpoint [µg/L]	$RAC_{sw, ch}$ (NOEC/10) (E_rC_{50}/10)	$PEC_{sw,max}$ [µg/L]	PEC/RAC
Cereals (winter/spring)					
Prothioconazole	Fish, early life stage *Oncorhynchus mykiss*	NOEC 490	49.0	0.124	0.003
	Invertebrate, chronic *Daphnia magna*	NOEC 560	56.0	0.124	0.002
	Sediment dweller, chronic *Chironomus riparius*	NOEC 9140	914	0.124	0.0001
	Marine diatom, chronic *Skeletonema costatum*	E_rC_{50} 46	4.6	0.124	0.03
	Aquatic plant, chronic *Lemna gibba*	E_rC_{50} > 404	> 40.4	0.124	< 0.003
JAU 6476-desthio	Fish, early life stage *Oncorhynchus mykiss*	NOEC 3.34	0.334	0.937	**2.81**
	Invertebrate, chronic *Daphnia magna*	NOEC 100	10.0	0.937	0.09
	Invertebrate, chronic *Americamysis bahia*	NOEC 64	6.4	0.937	0.15
	Sediment dweller, chronic *Chironomus riparius*	NOEC 2000	200	0.937	0.005
	Green alga, chronic *Scenedesmus subspicatus*	E_rC_{50} 550	55.0	0.937	0.02
	Aquatic plant, chronic *Lemna gibba*	E_rC_{50} 80.9	8.09	0.937	0.12
JAU 6476-S-methyl	Sediment dweller, chronic *Chironomus riparius*	NOEC 100	10.0	0.110	0.01
	Green alga, chronic *Pseudokirchneriella subcapitata*	E_rC_{50} 47400	4740	0.110	0.00002
1,2,4-Triazole	Fish, juvenile growth *Oncorhynchus mykiss*	NOEC 3200	320	0.031	0.0001
	Green algae, chronic *P. subcapitata*	E_rC_{50} > 31000	> 3100	0.031	< 0.00001
JAU 6476-thiazocine	Fish, early life stage *Oncorhynchus mykiss*	NOEC 490*	49.0	0.046	0.001
	Invertebrate, chronic *Daphnia magna*	NOEC 560*	56.0	0.046	0.001
	Green alga, chronic *Pseudokirchneriella subcapitata*	E_rC_{50} 2180*	218	0.046	0.0002
JAU 6476-triazolylketone	Green alga, chronic *Pseudokirchneriella subcapitata*	E_rC_{50} > 100000	> 10000	0.020	< 0.000002

* Endpoints from parent prothioconazole from studies on first tier species were used for risk assessment of M12 (see Table CP 10.2-1 and MCA, point 8.2 for more details)

For JAU 6476-desthio the chronic trigger was not met for fish. Therefore, a refined risk assessment is required. The consideration of the more realistic FOCUS STEP 3 surface water concentrations is presented below.

Table CP 10.2- 8: TER_{LT} calculations for winter and spring cereals based on FOCUS Step 3

Test species	Endpoint [µg/L]	$PEC_{sw, max}$ [µg/L]	FOCUS scenario	TER_{LT}	Trigger
JAU 6476-desthio, winter & spring cereals, 1 x 18 g a.s./ha					
Fish, early life stage *Oncorhynchus mykiss*	NOEC 3.34	<0.001	All (D1 to R4)	> 3340	10

Table CP 10.2- 9: $RAC_{sw; ch}$ calculations for winter and spring cereals based on FOCUS Step 3 acceptability of risk: PEC/RAC < 1)

Test species	Endpoint [µg/L]	$RAC_{sw; ch}$ (NOEC/10) (E_rC_{50}/10)	$PEC_{sw, max}$ [µg/L]	FOCUS scenario	PEC/RAC
JAU 6476-desthio, winter & spring cereals, 1 x 18 g a.s./ha					
Fish, early life stage *Oncorhynchus mykiss*	NOEC 3.34	0.334	<0.001	All (D1 to R4)	< 0.003

The TER trigger is exceeded based on FOCUS STEP 3 values for both winter and spring cereals.

Stepwise approach (EFSA AGD 2013)

Report:	KCP 10.2/02[redacted]Q;[redacted]; 2015; M-536697-01-1
Title:	Stepwise approach for the risk assessment of major aquatic metabolites of prothioconazole (formulated as prothioconazole FS 100 g/L) following the EFSA guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (2013)
Report No.:	M-536697-01-1
Document No.:	M-536697-01-1
Guideline(s):	none
Guideline deviation(s):	none
GLP/GEP:	**no**

The EFSA AGD (2013) stepwise approach was used for all metabolites where chronic data is not available for each first tier taxonomic group relevant to fungicide risk assessment (i.e. JAU 6476-S-methyl (M01), JAU 6476-thiazocine (M12), 1,2,4-Triazole (M13), and JAU 6476-triazolylketone (M42)). The EFSA AGD (2013) "risk assessment scheme for metabolites" (point 10.2.4, page 143 of the EFSA AGD) was followed, and the rationale for decision at each step of the scheme was explained in detail.

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Overall conclusion

In conclusion, a chronic risk assessment of all major aquatic metabolites of prothioconazole was provided addressing risks to all first tier taxonomic groups (including sediment dwellers, where relevant). The 'classical' approach based on TER- and RAC calculations as presented above was combined with the new stepwise approach from the EFSA AGD (2013) (see [redacted] and [redacted], 2015). Based on the results from this combined approach, a low chronic risk is concluded for all aquatic metabolites of prothioconazole. For each of the assessed metabolites, the chronic trigger is met for all evaluated scenarios. Consequently, a safe use can be assumed according to the proposed GAP.

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

Report:	KCP 10.2.1/01[redacted]2; 2015; M-525350-01-1
Title:	Acute toxicity of prothioconazole FS 100 G to fish (Oncorhynchus mykiss) under static conditions
Report No.:	E 203 4796- 1
Document No.:	M-525350-01-1
Guideline(s):	US EPA OCSPP 850.1075; EPA-FIFRA § 72-1/SEP-EPA-540/9-85-006 (1982/1985); OCSPP 850.1075 (Public Draft, 1996); Council Regulation (EC) No 440/2008, C.1 (2008); OECD No. 203 (rev.1992); JMAFF, 12 Nousan No. 8147 (2000)
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:

The aim of the study was to determine the acute toxicity of Prothioconazole FS 100 to the Rainbow trout (*Oncorhynchus mykiss*), expressed as 96 hours LC_{50}.

Material and methods:
Test item: Prothioconazole FS 100 G, analyzed content a.s. content: 97.86 g/L (8.47 % w/w); Batch No. 2015-001031, Specification No. 102000006421, TOX10850-00.

Rainbow trout (*Oncorhynchus mykiss*), mean body length 5.6 cm, mean body weight 2.6 g. The used fish batch (Lot F 4/15) was delivered on February 12, 2015. The biomass loading for this test was 0.65 g fish / L test medium.
The test was conducted according to FIFRA Guideline 72-1, OCSPP 850.1075, OECD TG 203 and JMAFF, 12 Nousan No. 8147. Ten fish in each test level were exposed for 96 hours under static conditions to nominal concentrations of 1.28, 2.82, 6.20, 13.6 and 30.0 mg prod./L. A control with further 10 fish was tested in parallel. Dissolved oxygen concentrations ranged from 70 % to 104 % oxygen saturation, the pH values ranged from 6.6 to 7.3 and the water temperature ranged from 12.8 °C to 13.6 °C in all aquaria over the whole testing period. Prothioconazole was analyzed in all test levels after 0 hours, on day 2 and on day 4 of the exposure period to confirm nominal concentrations. In case 100% mortality occurred in a concentration prior to test termination, the analytical determinations in this concentration were conducted at the respective assessment date.
During the test, fish were examined after four hours and then daily for mortalities and signs of poisoning.

Findings:
Validity criteria:
All validity criteria were met as presented below.

Table CP 10.2.1- 1: Validity criteria

Validity criteria	Recommended	Obtained
Mortality within the 48-hour settling-in period	≤ 5 %	< 5 %
Mortality in the control	≤10 %	0 %
Dissolved oxygen saturation throughout the test	≥60 %	70 %– 104 %
pH variation	≤ 1.0	0.7

Analytical results:
The chemical analysis of prothioconazole (in water by HPLC - UV) revealed that measured recoveries at experimental start ranged from 109 to 115% of nominal values, confirming the correct application of the test item. On day 2 the recoveries were between 70 and 94% and were between 47 and 90% at test termination. The mean recovery over the whole testing period of 96 hours was 47% to 115% of nominal. Due to the dissipation of prothioconazole in aqueous solution (mean measured concentration were below 80% of nominal values), the biological results are based on geometric mean measured concentrations of prothioconazole. The geometric mean measured concentrations of prothioconazole were recalculated to the following formulation concentrations: 0 (control), 0.926, 2.23, 5.08, 10.6 and 29.2 mg prod./L.

Biological results:
In the controls no mortalities or sub-lethal findings were observed.
In all test levels ≥ 5.08 mg prod./L behavioral changes were observed during the entire exposure period. After 96 hours of exposure to the nominal concentration of 5.08 mg prod./L ten fish showed the following behavioural symptoms:
• remaining for unusually long periods on the bottom of the aquarium

• showed labored respiration
• strongly extended gills
• reduced activity; apathy

Table CP 10.2.1- 2: Effect of Prothioconazole FS 100 G on *Oncorhynchus mykiss*

Test item	Prothioconazole FS 100 G
Test species	*Oncorhynchus mykiss*
Exposure	96 hours static design

Exposure time		**4 hours**		**24 hours**		**48 hours**		**72 hours**		**96 hours**	
Test conc. nominal [mg prod. / L]	Geometric mean [mg prod. / L]	no. of dead	% dead	no. of dead	% dead	no. of dead	% dead	no. of dead	% dead	no. of dead	% dead
control	control	0	0	0	0	0	0	0	0	0	0
1.28	0.926	0	0	0	0	0	0	0	0	0	0
2.82	2.23	0	0	0	0	0	0	0	0	0	0
6.20	5.08	0	0	0	0	0	0	0	0	0	0
13.6	10.6	0	0	0	0	0	0	0	0	0	0
30.0	29.2	0	0	7	70	8	80	8	80	10	100

LC_{50} 96 hours (95% C.I.):	**16.4 mg prod./L (C.I. 95%: n.d.)**
LOEC:	5.08 mg prod./L
NOEC:	2.23 mg prod./L
LC0: (no mortality)	10.6 mg prod./L
LC_{100} (100 % mortality):	29.2 mg prod./L

C.I.: Confidence interval
n.d. not determined due to mathematical reasons or inappropriate data

Conclusion:
Based on recalculated geometric mean concentrations, the LC_{50} after 96 h of static exposure to Prothioconaole FS 100 G was 16.4 mg prod./L.

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Report: KCP 10.2.1/02 [redacted].; 2015; M-525311-01-1
Title: Acute toxicity of prothioconazole FS 100 G to the waterflea Daphnia magna in a static laboratory test system
Report No: E 202-4719-5
Document No.: M-525311-01-1
Guideline(s): US EPA, OCSPP 850.1010; OECD guideline 202,(2004); EC Council Regulation No 440/2008, Method C.2 (2008); U.S. EPA P.A.G., Subdivision E, § 72-2 (1982); OPPTS Guideline 850.1010 public draft 1996 (modified); JMAFF 12 Nousan No. 8147 (2000)
Guideline deviation(s): none
GLP/GEP: yes

Objective:
The study was performed to detect possible effects of Prothioconazole FS 100 on mobility of *Daphnia magna* caused by 48 hours of exposure in a static laboratory test system, expressed as EC_{50} for immobilisation.

Material and methods:
Test item: Prothioconazole FS 100 G, analyzed a.s. content: 8.47 % w/w, Batch No. 2015-001031, TOX10850-00, Specification No. 102000006421 (V.5).

The test was conducted according to OECD Guideline 202, FIFRA Guideline 72-2, OPPTS Guideline 850.1010 and JMAFF 12 Nousan No. 8147. *Daphnia magna* (1st instars ≤ 24 h old, 6 × 5 animals per concentration) were exposed in a static test system for 48 hours to nominal concentrations of 0, 0.5, 1.0, 2.0, 4.0, 8.0 and 16 mg prod./L without feeding.
The content of prothioconazole in exposure media was measured for verification of the test item concentrations.
The test vessels consisted of 100 mL glass beakers, individually labelled and filled with 50 mL of the test solution (10 mL test solution per daphnid). Groups of five animals were randomly assigned to individual test vessels (replicates). The test solutions were not artificially aerated during exposure.
After 24 and 48 hours, behaviour of the water fleas was visually evaluated by counting mobile daphnids, defined as animals with swimming movements (slight movements of antennae were not interpreted as swimming movement) within approximately 15 seconds after gentle agitation of the test vessel.
Water temperatures within the test system were recorded at start and end of exposure from one vessel of the untreated control group and of the highest treatment group, using a hand-held measuring device.
PH-value and of dissolved oxygen were determined for all freshly prepared solutions (batch sample) and again in the aged solutions (composite replicates) at the end of exposure periode.

Findings:
Validity criteria:
All validity criteria were met as presented below:

Table CP 10.2.1- 3: Validity criteria

Validity criteria	Recommended	Obtained
Control mortality	≤ 10 %	0.0%

Analytical results:
The chemical analysis of prothioconazole in the freshly prepared test solutions at test initiation revealed measured concentrations between 111% and 120% (mean: 116%) of the aspired nominal concentrations. Concentrations in the aged test solutions at the end of the 48 hours exposure period ranged between 37% and 71% (mean: 49%) of nominal. No contaminations of prothioconazole were detected in samples from untreated water control. Due to the dissipation of prothioconazole in aqueous solution (mean measured concentration were below 80% of nominal values), EC_{50} calculations were performed for recalculated test concentrations, based on the geometric mean values of the measured a.s. concentrations.

Biological results:
No immobilities or other effects on behaviour occurred in untreated control within 48 hours of exposure. Effects on the mobility of daphnids in treated vessels are presented below. Observations on sublethal effects revealed no abnormal behaviour of the exposed daphnids over the entire exposure period of 48 hours.
EC_{50} alues for immobilization after 24h and 48h of exposure were calculating using probit analysis.

Table CP 10.2.1- 4: Effect of Prothioconazole FS 100 G on *Daphnia magna*

Test item		Prothioconazole FS 100 G				
Test species		*Daphnia magna*				
Exposure		acute, static laboratory				
Test concentration [mg prod./L]		**Exposed daphnids**	**24 h**		**48 h**	
Nominally	**Recalculated**	**(=100%)**	**n**	**%**	**N**	**%**
control	0	30	0	0.0	0	0.0
0.5	0.3	30	0	0.0	0	0.0
1.0	0.8	30	0	0.0	0	0.0
2.0	1.3	30	0	0.0	0	0.0
4.0	2.8	30	0	0.0	0	0.0
8.0	7.1	30	0	0.0	9	30.0
16.0	12.1	30	7	23.3	19	63.3

Probit analysis for data obtained after	EC_{50}		lower 95% cl mg form./L		upper 95% cl mg form./L	
	Nominally	Re-calculated by measured a.s. content	nominally	recalculated by measured a.s. content	nominally	recalculated by measured a.s. content
24 hours	30.0	23.1	n.d.	n.d.	n.d.	n.d.
48 h	12.2	9.8	9.1	7.7	18.3	13.7

Conclusion: The EC_{50} value for immobilization after 48 h of static exposure to prothioconazole FS 100 G was 9.8 mg prod./L based on mean measured concentrations.

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Report: KCP 10.2.1/03 [redacted], 2015, M-525317-01-1
Title: Pseudokirchneriella subcapitata growth inhibition test with prothioconazole FS 100 G
Report No.: E 201 4788-0
Document No.: M-525317-01-1
Guideline(s): OECD Guideline 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test (July 28, 2011)
Guideline deviation(s): none
GLP/GEP: yes

Objective:
The aim of the study was to determine the influence of Prothioconazole FS 100 on exponentially growing populations of *Pseudokirchneriella subcapitata* expressed as NOEC, LOEC and EC_x for growth rate of algal biomass (cells per volume).

Material and methods:
Test item: Prothioconazole FS 100 G analysed content: 8.47 % w/w prothioconazole, Batch No. 2015-001031, TOX10850-00, Specification No. 102000006421.

The test was conducted according to OECD Guideline 201. *Pseudokirchneriella subcapitata* (freshwater microalgae, formerly known as *Selenastrum capricornutum*) were exposed in a chronic multigeneration test for 72 hours under static exposure conditions to nominal concentrations of 0.954, 3.05, 9.77, 31.3

and 100 mg prod./L in comparison to control. Concentrations of prothioconazole were measured in all treatment groups and in the controls at test start and test end (72 hours).
The pH values were 8.0 in the control replicates and the incubation temperature ranged from 22.3 °C to 23.5 °C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 4.67 klux (mean value).

Findings:

Validity criteria:

All validity criteria were met as presented below:

Table CP 10.2.1- 5: Validity criteria

Validity criteria	Recommended	Obtained
Control biomass increase	Factor of ≥16 within 72 h	57.6
Mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3) in the control cultures	≤35 %	29.6 %
coefficient of variation of average specific growth rates during the whole test period between the replicate control cultures	≤7 %	1.7 %

Analytical results:

The analytical findings showed that prothioconazole concentrations on day 0 in the treated vessels ranged between 86.1% and 103 % of nominal (average 93.4%). After 72 hours, concentrations ranged between 55.3% and 91.3% of nominal (average 65.7%). Due to the dissipation of the prothioconazole in aqueous solution (mean measured concentration were below 80% of nominal values), endpoint values were based on geometric mean measured test concentrations of the formulation. Geometric mean measured concentrations were 0.659, 2.24, 7.36, 25.7 and 97.4 mg prod./L, respectively.

Biological results:

The static algae growth inhibition test provided the following tabulated effects after 72 hours. No effect occurred in untreated control within 72 hours of exposure.

Table CP 10.2.1- 6: Effect of Prothioconazole FS 100 G on *Pseudokirchneriella subcapitata*

Test item	Prothioconazole FS 100 G		
Test species	*Pseudokirchneriella subcapitata*		
Exposure	Growth inhibtion test, 72 h		
geo. mean meas. concentration [mg form./L]	**cell number after 72 h (means) per mL**	**(0-72h)-average specific growth rates [days-1]**	**inhibition of average specific growth rate [%]**
control	576 000	1.351	0.0
0.659	510 000	1.304	3.5
2.24	526 000	1.320	2.3
7.36	254 000	1.077	20.3●
25.7	55 000	0.547	59.5●
97.4	6 000	-0.173	112.8●

test initiation with 10,000 cells/mL
● significantly (α=0.05, one-sided smaller) reduced, based on Williams multiple sequential t-test procedure

Based on these results, the E_rC_{50} were estimated to be 18.6 mg prod./L and the NOErC was 2.24 mg prod./L.

Conclusion:
After 72 hours of exposure, the E_rC_{50} for Prothioconazole FS 100 G for the green alga *Pseudokirchneriella subcapitata* was 18.6 mg prod./L (95% CI: 16.2 – 21.3 mg form./L).

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

No further testing of the Prothioconazole FS 100 formulation has been performed, nor is it required.

CP 10.2.3 Further testing on aquatic organisms

No further testing of the Prothioconazole FS 100 formulation has been performed, nor is it required.

CP 10.3 Effects on arthropods

CP 10.3.1 Effects on bees

The risk assessment has been performed according to the existing guidance in force at the time of the preparation and submission of this dossier namely the EU Guidance Document on Terrestrial Ecotoxicology (SANCO/ 10329/2002 rev 2) and EPPO Standard PP 3/10 (3) Environmental Risk Assessment Scheme for Plant Protection Products - Chapter 10: honey bees.

Commission Regulations (EU) 283/2013 and 284/2013 require where bees are likely to be exposed, testing by both acute (oral and contact) and chronic toxicity, including sub-lethal effects, to be conducted. Consequently in addition to the standard toxicity studies performed with adult bees (OECD 213 and 214) the following additional studies are also provided:

- Acute oral and contact toxicity of prothioconazole and the representative formulation Prothioconazole FS 100,
- Acute oral and contact toxicity of JAU 6476-desthio (metabolite of prothioconazole),
- Acute contact toxicity of prothioconazole to adult bumble bees under laboratory conditions,
- Chronic 10 day toxicity test with of Prothioconazole SC 480 on adult bees under laboratory conditions,
- Colony feeding study with Prothioconazole SC 480 according to Oomen *et al.* 1992 (using a realistic worse case spray solution concentration and covering exposure for effects on brood (eggs, young and old larvae) and their development, nurse bee on-going behaviour in brood care and colony strength),
- Semi-field brood feeding study with Prothioconazole EC 250 following OECD guidance document 75 (using a more realistic spray scenario onto flowering *Phacelia tanacetifolia* at the maximum application rate for the approval renewal of prothioconazole and covering exposure for effects on brood (eggs) and their development and colony parameters).

Details of the honey bee testing with prothioconazole and its metabolite JAU 6476-desthio are presented together with the ecotoxicological endpoints in MCA, Section 8, Point 8.3.1, as well as within the existing Review Report for prothioconazole (SANCO/3923/07 – 10.December 2007, for Annex I inclusion under Directive 94/414/EEC). Furthermore, contact laboratory toxicity data for bumble bees

indicated that non-*Apis* bees are not more sensitive than honey bees and consequently the risk assessment for honey bees is considered to protective to other bees.

The acute toxicity test conducted with the formulation Prothioconazole FS 100 is presented in this MCP document.

A summary of the critical endpoints of prothioconazole, its metabolite JAU 6476-desthio and the formulated product Prothioconazole FS 100 are provided in the following tables. Endpoints shown in bold are considered relevant for risk assessment.

Table CP 10.3.1- 1: Critical endpoints for prothioconazole, JAU 6476-desthio and Prothioconazole FS 100 – acute toxicity to adult bees

Test substance	Test species	Endpoint	Reference
Prothioconazole	Honey bee (contact 48 h) Honey bee (oral 48 h)	LD_{50} >100 µg a.s./bee LD_{50} **>105.1 µg a.s./bee**	[redacted] (2014) M-505379-01-1 KCA 8.3.1.1.1/02 KCA 8.3.1.1.2/02
	Honey bee (contact 48 h) Honey bee (oral48 h)	LD_{50} **>200 µg a.s./bee** LD_{50} >71 µg a.s./bee	[redacted] (1999) M-023105-01-1 KCA 8.3.1.1.1/01 KCA 8.3.1.1.2/01
	Bumble bee (contact 48 h) (*Bombus terrestris*)	LD_{50} >100 µg a.s./bumble bee	[redacted] (2015) M-521802-01-1 KCA 8.3.1.1.2/04
JAU 6476-desthio	Honey bee (oral 48 h)	LD_{50} **>106.5 µg p.m./bee**	[redacted] (2015) M-528139-01-1 KCA 8.3.1.1.1/03 KCA 8.3.1.1.2/03
	Honey bee (contact 48 h)	LD_{50} **>100 µg p.m./bee**	
Prothioconazole FS 100	Honey bee (oral 48 h)	LD_{50} **>106.1 µg a.s./bee**	[redacted] (2015) M-521546-01-1 KCP 10.3.1.1.1/01 KCP 10.3.1.1.2/01
	Honey bee (contact 48 h)	LD_{50} **>100.0 µg a.s./bee**	

Bold values used in risk assessment
a.s.: active substance; p.m.: pure metabolite

Table CP 10.3.1- 2: Critical endpoints for prothioconazole – chronic toxicity to adult bees

Test substance	Test species	Endpoint		Reference
Prothioconazole SC 480	Honey bee Laboratory chronic (10 d) (adults)	LC_{50} LDD_{50} NOEC NOEDD	> 100 mg a.s./kg >3.8 µg a.s./bee/day 100 mg a.s./kg 3.8 µg a.s./bee/day	[redacted] (2015) M-528888-01-1 KCA 8.3.1.2./01

a.s. = active substance

Table CP 10.3.1- 3: Critical endpoints for prothioconazole– toxicity to bee brood

Test substance	Test species	Endpoint	Reference
Prothioconazole SC 480	Bee brood feeding test (Oomen *et al.*)	No adverse effects on brood development, mortality and behaviour after feeding honey bee colonies sugar syrup at 0.47 g a.s./L.	[redacted] & [redacted] (2014) M-478670-01-1 KCA 8.3.1.3/01
Prothioconazole EC 250	Semi-field brood study, OECD 75	No adverse effects on brood development, mortality, foraging activity, behaviour, colony condition and strength after application of 187.5 g a.s./ha onto flowering *Phacelia tanacetifolia*.	[redacted] (2015) M-532419-01-1 KCA 8.3.1.3/02

a.s. = active substance

Risk assessment for bees

The risk assessment for bees is based on the maximum single application rate of prothioconazole 18 g a.s./ha and 180 mL Prothioconazole FS 100/ha in cereals.

Hazard Quotients

The risk assessment is based on Hazard Quotient approach (Q_H) by calculating the ratio between the application rate (expressed in g a.s./ha or in g total substance/ha) and the laboratory contact and oral LD_{50} (expressed in µg a.s./bee or in µg total substance/bee).

Q_H values can be calculated using data from the studies performed with the active substance and with the formulation. Q_H values higher than 50 indicate the need of higher tiered activities to clarify the actual risk to honey bees.

According to the use pattern of Prothioconazole FS 100 as a seed treatment, direct oral or contact exposure of bees to the product in bee relevant matrices like nectar and pollen is not to be anticipated. Since honeybees are not exposed to bee relevant matrices like nectar or pollen, no unacceptable risk is to be expected and therefore the calculation of hazard quotients (Q_{HO} and Q_{HC}) is considered to be an unrealistic worst case assumption.

Hazard Quotient, oral: $$Q_{HO} = \frac{\text{max. appl. rate}}{LD_{50}\ \text{oral}} = \frac{[\text{g a.s./ha or g total substance/ha}]}{[\mu\text{g a.s./bee or } \mu\text{g total substance/bee}]}$$

Hazard Quotient, contact: $$Q_{HC} = \frac{\text{max. appl. rate}}{LD_{50}\ \text{contact}} = \frac{[\text{g a.s./ha or g total substance/ha}]}{[\mu\text{g a.s./bee or } \mu\text{g total substance/bee}]}$$

Table CP 10.3.1- 4: Hazard quotients for bees – oral exposure

Test substance	Crop	LD_{50} [µg/bee]	Application rate [g/ha]	Hazard quotient Q_{HO}	Trigger
Prothioconazole FS 100	Cereals	>106.1	18	<0.2	50
Prothioconazole	Cereals	>105.1	18	<0.2	50
JAU 6476-desthio	Cereals	>106.5	18[A]	<0.2	50

[A] The hazard quotient for the metabolite JAU-6476-desthio was calculated with the application rate of the parent

compound prothioconazole – representing a worst-case

The hazard quotients for oral exposure are below the validated trigger value for higher tier testing (i.e. $Q_{HO} < 50$).

Table CP 10.3.1- 5: Hazard quotients for bees – contact exposure

Test substance	Crop	LD_{50} [µg/bee]	Application rate [g/ha]	Hazard quotient Q_{HO}	Trigger
Prothioconazole FS 100	Cereals	>100	18	<0.2	50
Prothioconazole	Cereals	>200	18	<0.1	50
JAU 6476-desthio	Cereals	>100	18[A]	<0.2	50

[A] The hazard quotient for the metabolite JAU-6476-desthio was calculated with the application rate of the parent compound prothioconazole – representing a worst-case

The hazard quotients for contact exposure are below the validated trigger value for higher tier testing (i.e. $Q_{HC} < 50$).

Further considerations for the risk assessment

In addition to acute laboratory studies with adult honey bees, prothioconazole was further subjected to topical acute bumble bee testing ([redacted], S.; 2015; M-521802-01-1, KCA 8.3.1.1.2/04). The study resulted in an LD_{50} of > 100 µg a.s./bumble bee and did not reveal sensitivity differences between honey bee and bumble bee foragers.

Moreover, prothioconazole was further subjected to chronic laboratory testing with adult honey bees ([redacted], S.; 2015; M-528888-01-1, KCA 8.3.1.2/01).

This chronic study was designed as a limit test by exposing adult honey bees for 10 consecutive days to a nominal concentration of 100 mg prothioconazole/kg feeding solution. The actual test was conducted by using the formulated product Prothioconazole SC 480. After exposing honey bees for ten consecutive days exclusively to sugar solution containing prothioconazole, the 10 day LC_{50} (Lethal Concentration) was determined to be > 100 mg prothioconazole/kg, which corresponds to a LDD_{50} (Lethal Dietary Dose) of > 3.8 µg a.s./bee/day. The respective NOEC (No Observed Effect Concentration) for mortality was determined to be 100 mg prothioconazole/kg, which corresponds to the NOEDD (No Observed Effect Dietary Dose) of > 3.8 µg a.s./bee/day.

In order to reveal whether prothioconazole poses a risk to immature honey bee life stages, a bee brood feeding study ([redacted], S.; [redacted], A.; 2014; M-478670-01-1, KCA 8.3.1.3/01) has been conducted by following the provisions/method of [redacted] (OEPP/EPPO Bulletin 22:613-616 (1992)), which require, amongst other parameters to "…use formulated products only… products are fed at a concentration recommended for high-volume use…". The honey bee brood feeding test is a worst-case screening test, by feeding the honey bees directly in the hive with a treated sugar solution which contains the test substance at a concentration typically present in the spray tank (and as such at a very high concentration) and by investigating the development of eggs, young and old larvae by employing digital photo imaging technology.

This particular study was conducted with Prothioconazole SC 480. The administration of prothioconazole at a concentration of 0.47 g a.s to honeybee colonies via feeding of 1 litre spiked sucrose solution has neither resulted in adverse effects on brood development, worker or pupal mortality, nor in behavioural abnormalities as compared to the control. Regarding brood development, the brood termination rates of the test item treatment were overall on a low level with 16.0, 12.4 and 3.6% for

eggs, young larvae and old larvae, respectively, which were not statistically significant different to the control with brood termination rates of 17.8, 10.2 and 6.47% for eggs, young larvae and old larvae, respectively at the end of the brood observation period.

In order to clarify whether prothioconazole poses a risk to honey bee brood and colony development in particular as well as on honey bees in general under realistic worst-case conditions, a higher tier semi-field honey bee brood study (according to the provisions of the OECD Guidance Document 75) was conducted under forced/confined exposure conditions using the formulation Prothioconazole EC 250, by application of 187.5 g a.s./ha under tunnel conditions to the full flowering and highly bee attractive surrogate crop Phacelia tanacetifolia ([redacted], R.; 2015, M-532419-01-1, KCA 8.3.1.3/02).
The study included three treatment groups: Control (tap water), Test item (187.5 g a.s./ha and Reference item (300 g fenoxycarb/ha) with all applications being carried out with a spray volume of 400 L water/ha. For all treatment groups, four replicates (tunnels) were set up. The application of all treatments was conducted during daily bee flight activity at the time of full flowering of the crop. Thereafter, the bees were kept for 7 days within the tunnels (confined exposure phase) and were then relocated out of the tunnels and transferred to a monitoring site without flowering crops and intensive agricultural area for further monitoring (day 8 to day 26 after treatment). Daily, throughout the confined exposure phase, mortality of worker bees, larvae and pupae was assessed along with assessments of foraging activity and behaviour. Daily mortality assessments were continued along with behaviour around the hive during the post-exposure observation period (day 8 to day 26 after treatment). Colony assessments (food stores, brood areas, colony strength) were made before confinement, after confinement and at the end of the study. Detailed brood assessments (brood termination rate, brood index and brood compensation index) by employing digital photo imaging technology, investigating the fate of more than 200 individually marked cells was performed on 5 occasions throughout the study, covering an entire brood cycle of honey bees.
The application of prothioconazole at the rate of 187.5 g a.s./ha under tunnel conditions to the full flowering and highly bee attractive surrogate crop Phacelia tanacetifolia did not cause any adverse effects on mortality, flight intensity, brood development (brood termination rate: 46.6%, brood index: 2.7, compensation index: 3.8 in test item compared to the control with brood termination rate: 30.6%, brood index: 3.5, compensation index: 4.0), as well as on colony strength and condition. Neither brood termination rate nor brood or compensation index were significantly different in the test item as compared to the control, indicating that these indices performed comparable to the control, including compensations of previous brood losses.

All in all, it can be concluded from the acute and chronic laboratory studies in adult honey bees as well as from the bee brood feeding study (Oomen et al. and OECD Guidance Document 75) investigating side-effects on immature honey bee life stages, that prothioconazole is of low general intrinsic toxicity to honey bees.

Synopsis
Prothioconazole is of low acute toxicity to honey bees, with LD_{50} (oral and contact) above the highest tested dose levels.
The calculated Hazard Quotients for prothioconazole are below the validated trigger value which would indicate the need for a refined risk assessment; no adverse effects on honey bee mortality are to be expected at the maximum envisaged application rate. This conclusion is confirmed by the results of the bee brood feeding study as well as by the results of the bee brood semi-field study, which covered the maximum application rate of 187.5 g a.s./ha.

The acute laboratory study conducted with bumble bees revealed no sensitivity differences between honey bee and bumble bee foragers.

It can be concluded from the acute and chronic laboratory studies in adult honey bees as well as from the bee brood feeding study ([redacted].) and bee brood semi-field study (OECD 75), investigating side-effects on immature honey bee life stages that prothioconazole is of low general intrinsic toxicity to honey bees.

Regarding potential side effects of prothioconazole on immature honey bee life stages, the conducted bee brood feeding study ([redacted]., 1992) found no statistically significant differences between test item and control in brood termination rates of eggs, young and old larvae at 0.47 g a.s./L. Overall the study revealed no adverse effects on the survival of adult bees and pupa and bee behaviour. Thus, when considering the severity of the exposure situation in this worst-case screening test in combination with the absence of effects on the overall development of bee brood, it can be concluded even on the basis of this worst-case screening study that the use of prothioconazole does not pose an unacceptable risk for adult honey bees, immature honey bee life stages and honey bee colonies.

In order to clarify whether the conclusions on the basis of lower tiered honey bee studies are correct, prothioconazole was subjected to confined semi-field testing (according to the provisions of OECD Guidance Document No. 75), by applying the rate of 187.5 g a.s./ha to full-flowering Phacelia during honey bees actively foraging on the crop. This study design is from an apidological and apicultural point of view more realistic than an in-hive feeding of the test compound via a treated sugar solution, which contains the test substance at a concentration typically present in the spray tank (and as such at a very high concentration). The results of this higher tier semi-field study confirmed the conclusions made above on the basis of the outcome of the lower-tiered studies, as no adverse direct or delayed effects on mortality of worker bees or pupae, foraging activity, behaviour, colony strength and colony development as well as the development of bee brood were observed, even under aggravated, forced exposure conditions and by digitally following-up in a very detailed manner the fate of individually marked brood cells (digital photographic assessment) from egg stage until emergence.

Conclusion

Overall, it can be concluded that prothioconazole, when applied as a seed treatment in cereals at the maximum application rate of 18 g a.s./ha for Prothioconazole FS 100 does not pose an unacceptable risk to honey bees and honey bee colonies.

CP 10.3.1.1 Acute toxicity to bees

CP 10.3.1.1.1 Acute oral toxicity to bees

Report:	KCP 10.3.1.1.1/01 [redacted]=; 2015; M-521546-01-1
Title:	Prothioconazole FS 100 G: Effects (Acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory
Report No.:	100051035
Document No.:	M-521546-01-1
Guideline(s):	(GLP compliant study based on OECD 213 and 214 (1998))
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Objective:

The purpose of this study was to determine the acute contact and oral toxicity of prothioconazole FS 100 G to the honey bee (*A. mellifera* L.). Mortality of the bees was used as the toxic endpoint. Sublethal effects, such as changes in behaviour, were also assessed.

Material and methods:
Tetst item: Prothioconazole FS 100 G, analysed a.s. content: 97.86 g/L (8.47% w/w); Batch No. 2015-001031, TOX10850-00; Specification No.: 102000006421.

Under laboratory conditions *Apis mellifera* 50 worker bees per dose were exposed for 48 hours to a single dose of 100.0 µg a.s. per bee by topical application (contact limit test) and 50 worker bees per dose were exposed for 48 hours for feeding (oral limit test, value based on the actual intake of the test item) to a single dose of 106.1 µg a.s. per bee. During the test, the bees were kept in an incubactor in complet darkness (except during observation). The temperature was 25°C and the relative humidity was between 38 and 70%.

Findings:
Validity criteria:
All validity criteria were met as presented below:

Table CP 10.3.1.1.1- 1: Validity criteria

Validity criteria	Recommended	Obtained
Control mortality	≤ 10 %	Contact test: 6.0 % Oral test: 0.0%
LD_{50} of Reference item	Contact test (24h): 0.10 – 0.30 µg a.s./bee Oral test (24h): 0.10-0.35 µg a.s./bee	Contact test: 0.23 µg a.s./bee Oral test: 0.12 µg a.s./bee

Biological results:
Contact Test: At the end of the contact toxicity test (48 hours after application), 2.0% mortality occurred at 100.0 µg a.s./bee. There was 6.0 % mortality in the control group (water + 0.5% Adhäsit). No test item induced behavioural effects were observed at any time in the contact toxicity test.

Oral Test: In the oral toxicity test, the maximum nominal test level of prothioconazole FS 100 G (i.e. 100 µg a.s./bee) corresponded to an actual intake of 106.1 µg a.s./bee. This dose level led to 16.0% mortality after 48 hours. In the control group (50 % w/v sucrose solution = 500 g sucrose/L tap water), no mortality occurred. During the 4, 24 and 48-hrs assessments 1 - 3 bees were behaving abnormal (e.g. moribund), respectively.

Table CP 10.3.1.1.1- 2: Effect of Prothioconazole FS 100 G on Honey bees (*Apis mellifera*) – contact & oral test

Test item	Prothioconazole FS 100 G	
Test species	*Apis mellifera*	
Exposure	Contact (Solution Adhäsit (0.5%/water)	Oral (sucrose solution)
Application dose [µg a.s./bee]	100	106.1
LD_{50} [µg a.s./bee]	>100	>106.1

Conclusion:

The toxicity of prothioconazole FS 100 G was tested in both, an acute contact and an acute oral toxicity test on honey bees. The contact LD_{50} (48 h) was > 100.0 µg a.s./bee. The oral LD_{50} (48 h) was > 106.1 µg a.s./bee.

CP 10.3.1.1.2 Acute contact toxicity to bees

Report:	KCP 10.3.1.1.2/01 [redacted], 2015; M-521546-01-1
Title:	Prothioconazole FS 100 G: Effects (Acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory
Report No.:	100051035
Document No.:	M-521546-01-1
Guideline(s):	(GLP compliant study based on OECD 213 and 214 (1998))
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Same study as mentioned above. Please refer to CP 10.3.1.1.1.

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Additionally, an acute contact toxicity study was conducted on bumble bees with prothioconazole; the corresponding summary is provided in Document MCA, Section 8.3.1.1.2 ([redacted], S.; 2015; M-521802-01-1, KCA 8.3.1.1.2/04).

CP 10.3.1.2 Chronic toxicity to bees

A 10 day chronic oral toxicity study was conducted with Prothioconazole SC 480; the corresponding summary is provided in Document MCA, Section 8.3.1.2 ([redacted], S.; 2015; M-528888-01-1, KCA 8.3.1.2/01).

CP 10.3.1.3 Effects on honey bee development and other honey bee life stages

A honey bee brood feeding study according to the method of Oomen *et al.* 1998 ([redacted], S.; [redacted], A.; 2014; M-478670-01-1, KCA 8.3.1.3/01) has been conducted with Prothioconazole SC 480 and is included in Document MCA, Section 8.3.1.3.

A semi-field honey bee brood study (according to OECD 75) ([redacted], R.; 2015; M-532419-01-1, KCA 8.3.1.3/02) has been conducted with the Prothioconazole EC 250 and is included in Document MCA, Section 8.3.1.3.

CP 10.3.1.4 Sub-lethal effects

There is no particular study design / test guideline to assess “sub-lethal effects” in honey bees. However, in each laboratory study as well as in any higher-tier study, sub-lethal effects, if occurring, are described and in the reports under other points of chapter CP10.3.1.

CP 10.3.1.5 Cage and tunnel tests

Based on the findings presented above, a study with formulated product is not required.

CP 10.3.1.6 Field tests with honeybees

Based on the findings presented above, a study with formulated product is not required.

CP 10.3.2 Effects on non-target arthropods other than bees

Prothioconazole FS 100 is a seed dressing product that is applied on cereals. The maximum recommended rate is 0.18 L product/ha which corresponds to 18 g prothioconazole/ha. In the case of a seed treatment the Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002-final) recommends that the risk assessment for non-target arthropods should be covered with studies and the corresponding risk assessment for soil macro-invertebrates *Hypoaspis aculeifer* and/or *Folsomia candida.* Data for *H. aculeifer* and *F. candida* and the corresponding risk assessment are presented in chapter CP 10.4.2.

The study results for soil dwelling non-target arthropods for the spray formulation Prothioconazole EC 250 are presented below (see Table CP 10.3.2- 2) as additional information to the data from formulation Prothioconazole FS 100 (see Table CP 10.3.2- 1). All these studies were evaluated during the last EU evaluation and summaries of these studies are available in the original DAR for the first Annex I inclusion.

Table CP 10.3.2- 1: Prothioconazole FS 100 g/L: Ecotoxicological endpoints for soil dwelling non-target arthropods

Test organisms, Reference	Tested formulation, study type, exposure	Ecotoxicological endpoint		
Poecilus cupreus M-038376-03-1 Rep.No: [redacted]/RR/2009 [redacted], 2002 KCP 10.3.2.2/01	PTZ FS 100 Extended lab., dressed winter wheat seeds in standard soil (LUFA 2.1) 2.47 g a.s./ha	Corr. Mortality [%] 0	Effect on Feeding Rate [%] (day 1-7) 5.6	Effect on Feeding Rate [%] (day 8-14) 8.6
Aleochara bilineata M-058111-01-1 Rep. No: 12342071 [redacted], 2002 KCP 10.3.2.2/04	PTZ FS 100 Extended lab., dressed wheat seeds in standard soil (LUFA 2.1) 19.3 g a.s./ha	Effect on Reproduction [%] 11.2		
Pardosa spec. M-030622-01-2 Rep. No: IR/72501 [redacted], 2000 KCP 10.3.2.2/02	PTZ FS 100 Extended lab., dressed wheat seeds in standard soil (LUFA 2.1) 22.5 g a.s./ha	Corrected Mortality [%] -3.1[A]	Effect on Feeding Rate [%] -18[B]	

[A]: A negative value indicates a lower mortality rate in the treatment than in the control.
[B]: A negative value indicates a higher feeding rate in the treatment than in the control.

Table CP 10.3.2- 2: Prothioconazole EC 250 g/L: Ecotoxicological endpoints for soil dwelling non-target arthropods

Test species, Dossier-file-No., reference	Tested Formulation, study type, exposure	Ecotoxicological Endpoint
Aleochara bilineata M-066029-03-1 Rep.No.: 10191070 [redacted], 2001 KCA 8.3.2/02	PTZ EC 250 Laboratory, spray deposits on quartz sand, exposure: 28 d	ER_{50}: >400 g a.s./ha
		Effect on Reproduction [%]
	42 g a.s./ha	2.5
	200 g a.s./ha	9.9
	400 g a.s./ha	24.6
Poecilus cupreus M-032402-01-1 Rep.No.: [redacted] [redacted]/PC001 [redacted], 2000 KCA 8.3.2/01	PTZ EC 250 Laboratory, spray deposits on quartz sand, exposure: 14 d	
		Corr. Mortality [%]	Food Consumption (pupae/beetle)
	control	-	0.348
	400.5 g a.s./ha	0	0.349
	600.7 g a.s./ha	3.3	0.349

The available data on ground dwelling arthropods indicate that no unacceptable adverse effects on soil dwelling non-target arthropods are to be expected from exposure rates even exceeding the maximum intended application rate of 18 g a.s./ha for Prothioconazole FS 100.

CP 10.3.2.1 Standard laboratory testing for non-target arthropods

Additional laboratory studies are not required for non-target arthropods.

CP 10.3.2.2 Extended laboratory testing, aged residue studies with non-target arthropods

Additional extended laboratory studies are not required for non-target arthropods.

CP 10.3.2.3 Semi-field studies with non-target arthropods

Semi-field studies are not required.

CP 10.3.2.4 Field studies with non-target arthropods

Field studies are not required for non-target arthropods.

CP 10.3.2.5 Other routes of exposure for non-target arthropods

The exposure of soil-dwelling non-target arthropod as assessed in chapter CP 10.3.2 is considered the main route of exposure for non-target arthropods.

CP 10.4 Effects on non-target soil meso- and macrofauna

The risk assessment procedure follows the requirements as given in the EU Regulation 1107/2009 and the Guidance Document on Terrestrial Ecotoxicology.

Predicted environmental concentrations in soil (PEC_{soil}) values were calculated for the formulation, based on the standard assumptions of distribution in a soil layer of 5 cm with a bulk density of 1.5 g/cm^3; a crop interception of 0% was taken into account.

The relevant PEC values considered for TER calculations are summarised in the tables below. Maximum values are used for risk assessments.

Table CP 10.4- 1: Initial max PEC_{soil} values

Compound	Cereals	
	$PEC_{soil, max}$ [mg/kg]	$PEC_{soil\ accu}$ [mg/kg]
Prothioconazole	0.024	0.024
JAU 6476-desthio	0.012	0.012
JAU 6476-S-methyl	0.004	0.004

CP 10.4.1 Earthworms

Table CP 10.4.1- 1: Endpoints used in risk assessment

Test substance	Test species	Ecotoxicological endpoint	Reference
Prothioconazole EC 250	*Eisenia fetida* reproduction 56 d, sprayed	NOER ≥ 4.0 kg prod./ha NOER > 1.0 kg a.s./ha	[redacted] (2002) M-033501-02-1 RCA 8.4.1/04
Prothioconazole FS 300	*Eisenia fetida* reproduction 56 d, mixed	NOEC ≥ 1000 mg prod./kg dws **≥ 257 mg a.s./kg dws**	[redacted] (2007) M-287144-01-1 KCA 8.4.1/09
JAU 6476-desthio	*Eisenia fetida* reproduction 56 d, mixed	NOEC **0.5 mg p.m./kg dws***	[redacted] (2000) M-026193-01-2 KCA 8.4.1/05
JAU 6476-S-methyl	*Eisenia fetida* reproduction 56 d, mixed	NOEC **50 mg a.m./kg dws***	[redacted] (2000) M-021370-01-1 KCA 8.4.1/06
Prothioconazole FS 100	*Eisenia fetida* reproduction 56 d, treated seed	NOER ≥ 1150 kg prod./ha **≥ 122 g a.s./ha**	[redacted] & [redacted] (2001) M-088126-01-1 KCP 10.4.1.1/01
Prothioconazole EC 250	Natural earthworm populations, Field study up to 12 months, spraying	NOEAER ≥ 3 × 200 g a.s./ha	[redacted] (2005) M-040814-03-1 KCA 8.4.1/08

***** Adjusted by a factor of 2 to address the log P_{ow} > 2 and the high peat content of 10% in the artificial soil
Bold values: Endpoints considered relevant for risk assessment

Risk assessment for earthworms
Based on the endpoints in the table above the TER values are calculated using the following equations:

$TER_{LT} = NOEC / PEC_{soil}$

The risk is considered acceptable if the TER_{LT} is >5.

For lipophilic substances (log P_{OW} > 2) the Terrestrial Guidance Document recommends to apply an additional assessment factor of 2 for the ecotoxicological endpoints (LC_{50}, NOEC), if the study was

conducted in artificial soil with a high content of organic matter (i.e. 10 % peat), to consider the possible sorption of these compounds to the organic matter.

The log P_{OW} trigger was exceeded by the prothioconazole metabolites JAU 6476-desthio (log P_{OW} = 3.04) and JAU 6476-S-methyl (log P_{OW} = 4.3). Additionally, the chronic earthworm studies with these metabolites were performed with 10 % peat within the artificial soil. Therefore, in the risk assessment for those two metabolites an additional adjustment factor of 2 is applied on the respective endpoint.

Table CP 10.4.1- 2: TER calculations for earthworms

Compound test design	Endpoint	[mg a.s./kg soil]	PEC_{max} [mg/kg soil]	TER_{LT}	Trigger	Refined risk assessment ?
Prothioconazole chronic, mixed [1)]	NOEC	≥ 257	0.024	≥ 10708	5	No
JAU 6476-desthio chronic	NOEC	0.5 *	0.012	42	5	No
JAU 6476-S-methyl chronic	NOEC	50 *	0.004	12500	5	No

[1)] The endpoint from the earthworm reproduction study with PTZ FS 300 better reflects the overall low toxicity of prothioconazole to earthworms than the EU-agreed endpoint given in the EFSA conclusion (2007). The EU-agreed endpoint for prothioconazole was derived from a study where PTZ EC 250 was sprayed onto the soil surface and the NOEC represents the highest application rate tested. The study where PTZ FS 300 was mixed into is considered to better describe the low intrinsic toxicity of prothioconazole to *E. fenga*.

* Adjusted by a factor of 2 to address the log P_{ow} >2 and the high peat content of 10% in the artificial soil

All TER values calculated with the worst case $PEC_{soil,max}$ values exceed the trigger value of 5 indicating that no unacceptable adverse effects on earthworms are to be expected from the intended use of the product.

Additional higher tiered evidence supporting the conclusion of an accecptable risk to earthworms

A laboratory study on effects of seeds treated with Prothioconazole FS 100 on reproduction of *Eisenia fetida* was performed in order to assess the toxicity of prothioconazole to earthworms under more realistic exposure conditions. No effects on survival, biomass, and reproduction of *E. fetida* were observed up to 122 g prothioconazole/ha, the highest rate tested. This exceeds the recommended application rate by a factor of ≥6.8 (see table below) confirming the acceptable risk to earthworms.

Table CP 10.4.1- 3: TER calculations for earthworms (higher tiered evidence)

Compound test design	Endpoint	[mg a.s./kg soil]	PEC_{max} [mg/kg soil]	TER_{LT}	Trigger	Refined risk assessment ?
PTZ FS 100 chronic, treated seeds	NOEC	≥ 122 g a.s./ha	18 g a.s. /ha	≥ 6.8	5	No

An earthworm field study with Prothioconazole EC 250 ([redacted], C.; 2005; M-040814-03-1, KCA 8.4.1.08) is available. This study was evaluated during the last EU review and considered acceptable. Prothioconazole EC 250 did not reveal ecologically adverse effects up to an application rate of 3 × 200 g a.s./ha (NOEAER) on a grassland site. The total soil loading in this field study at the NOEAER of 3 × 200 g a.s./ha is 33 times higher than the proposed application rate for the use of

prothioconazole as a seed treatment in cereals (1 × 18 g a.s./ha). Thus, a low risk for earthworms is concluded if prothioconazole is applied as treated cereal seeds at a rate of 18 g a.s./ha in cereals.

CP 10.4.1.1 Earthworms sub-lethal effects

An earthworm reproduction study with Prothioconazole FS300 (test substance mixed into soil; [redacted] T.; 2007; M-287144-01-1, KCA 8.4.1/09 is presented in the active substance dossier which better reflects the overall low intrinsic toxicity of Prothioconazole to *Eisenia fetida* than the chronic EU agreed endpoint for *Eisenia fetida*. The EU-agreed endpoint was derived from a study where Prothioconazole EC 250 was sprayed onto soil surface and the NOEC in this study represents the highest tested rate of 1000 g a.s./ha. The NOEC of ≥257 mg a.s./kg from the Prothioconazole FS 300 study clearly demonstrates a low toxicity of prothioconazole to *Eisenia fetida*.

CP 10.4.1.2 Earthworms field studies

Not required as the risk to earthworms is acceptable.

CP 10.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

Table CP 10.4.2- 1: Endpoints used in risk assessment

Test substance	Test species	Ecotoxicological endpoint	Reference
Prothioconazole	*Folsomia candida* Reproduction 28 d, mixed	NOEC **≥ 1000 mg a.s./kg dws**	[redacted] (2011) M-405273-01-1 KCA 8.4.2.1/06
	Hypoaspis aculeifer Reproduction 34 d, mixed Lufa 2.1	NOEC **≥ 100 mg a.s./kg dws**	[redacted] (2000) M-037786-02-1 KCA 8.4.2.1/02
JAU 6476-desthio	*Folsomia candida* Reproduction 28 d, mixed	NOEC **31.3 mg p.m./kg dws***	[redacted] & [redacted] (2002) M-035070-03-1 KCA 8.4.2.1/03
	Hypoaspis aculeifer Reproduction 14 d, mixed	NOEC **≥ 100 mg p.m./kg dws**	[redacted] (2014) M-491764-01-1 KCA 8.4.2.1/07
JAU 6476-S-methyl	*Folsomia candida,* Reproduction 28 d, mixed	NOEC **≥ 15.8 mg p.m./kg dws***	[redacted] & [redacted] (2001) M-087207-01-1 KCA 8.4.2.1/04
	Hypoaspis aculeifer Reproduction 14 d, mixed	NOEC **≥ 100 mg p.m./kg dws**	[redacted] (2014) M-491804-01-1 KCA 8.4.2.1/08

Test substance	Test species	Ecotoxicological endpoint		Reference
Prothioconazole FS 100	*Folsomia candida* Reproduction 28 d, treated seeds	NOER	≥ 1150 kg prod./ha **≥ 112.4 g a.s./ha**[1)]	[redacted] & [redacted] (2002) M-033780-01-1 KCP 10.4.2/02
	Folsomia candida Reproduction 28 d, treated seeds	NOER	≥ 230 kg prod./ha ≥ 24 g a.s./ha	[redacted] (2001) M-073191-04-1 KCP 10.4.2/01
	Hypoaspis aculeifer Reproduction 14 d, mixed	NOEC	≥ 1000 mg prod./kg dws **≥ 84.7 mg a.s./kg dws**	[redacted] (2015) M-532652-01-1 KCP 10.4.2.1/01
Prothioconazole FS 300	*Folsomia candida* Reproduction 28 d, mixed	NOEC	≥ 30 mg prod./kg dws[2)] **≥ 7.7 mg a.s./kg dws**	[redacted] (2007) M-287951-01-1 KCP 10.4.2.1/02

* Adjusted by a factor of 2 to address the log P_{ow} > 2 and the high peat content of 10% in the artificial soil

[1)] The NOEC of ≥ 24 g a.s./ha resulting from the *Folsomia candida* reproduction study with Prothioconazole FS 100 ([redacted], T.; 2001; M-073191-04-1, KCP 10.4.2/01) was set above the highest concentration tested. In a similar reproduction study with *Folsomia candida* and Prothioconazole FS 100, a NOEC of ≥ 112.4 g a.s./ha was gained, which is more suitable to describe the realistic toxic potential of Prothioconazole FS 100 to *Folsomia candida*.

[2)] A collembola reproduction study where the test item was mixed into soil is not available with Prothioconazole FS 100. However, a study with Prothioconazole FS 300 is presented which can be used in the risk assessment instead as the formulation is (apart from the active substance content) slightly different to Prothioconazole FS 100.

Risk assessment for other non-target soil meso- and macrofauna (other than earthworms)

Ecotoxicological endpoints and PEC_{soil} values used for TER calculations for soil non-target macro-organisms are summarised below. TER values were calculated using the equation:

$$TER = NOEC / PEC_{soil}$$

The risk is considered acceptable if the TER is >5.

For lipophilic substances (log P_{OW} > 2) the Terrestrial Guidance Document recommends to apply an additional assessment factor of 2 for the ecotoxicological endpoints (LC_{50}, NOEC), if the study was conducted in artificial soil with a high content of organic matter (i.e. 10 % peat), to consider the possible sorption of these compounds to the organic matter.

The log P_{OW} trigger was exceeded by the prothioconazole metabolites JAU 6476-desthio (log P_{OW} = 3.04) and JAU 6476-S-methyl (log P_{OW} = 4.3). Additionally, the collembolan studies with these metabolites were performed with 10 % peat within the artificial soil. Therefore, in the risk assessment for those two metabolites an additional adjustment factor of 2 is applied on the respective endpoint.

Table CP 10.4.2- 2: TER calculations for other non-target soil meso- and macrofauna

Compound Test design	Endpoint [mg a.s./kg soil]	PEC_{max}, PEC_{acc} [mg/kg soil]	TER_{LT}	Trigger	Refined risk assessment?
Folsomia candida					
Prothioconazole chronic	NOEC ≥ 1000	0.024	≥ 41667	5	No
Prothioconazole FS 300 chronic, mixed [A)]	NOEC ≥ 7.7	0.024	≥ 321	5	No
JAU 6476-desthio chronic	NOEC 31.3 *	0.012	2608	5	No
JAU 6476-S-methyl chronic	NOEC ≥ 15.8 *	0.004	≥ 3950	5	No
Hypoaspis aculeifer					
Prothioconazole chronic, mixed	NOEC ≥ 100	0.024	≥ 4167	5	No
Prothioconazole FS 100 chronic, mixed	NOEC ≥ 84.7	0.024	≥ 3529	5	No
JAU 6476-desthio chronic	NOEC ≥ 100	0.012	≥ 8333	5	No
JAU 6476-S-methyl chronic	NOEC ≥ 100	0.004	≥ 25000	5	No

* Adjusted by a factor of 2 to address the log P_{ow} > 2 and the high peat content of 10% in the artificial soil

[A)] A collembola reproduction study where the test item was mixed into soil is not available with Prothioconazole FS 100. However, a study with Prothioconazole FS 300 is presented which can be used in the risk assessment instead as the formulation is (apart from the active substance content) slightly different to Prothioconazole FS 100.

All TER values calculated with the worst case PEC_{soil} values clearly exceed the trigger value of 5 indicating that no unacceptable adverse effects on soil macro-organisms are to be expected from the intended use of the product.

Further higher tiered evidence on the conclusion of low risk to Collembola

The conclusion of a low risk of Prothioconazole FS 100 used as a seed treatment in cereals is further supported by a laboratory reproduction study with *Folsomia candida* considering a more realistic exposure, i.e. application of treated cereal seeds. No effectcs on reproduction and survival of *F. candida* was observed up to the highest rate tested (NOER ≥112.4 g a.s./ha). The TER value in this extended tier 1 risk assessment reaches the value of ≥ 6.2, see table below.

Table CP 10.4.2- 3: TER calculations for further higher tier evidence on the conclusion of low risk to Collembola

Compound Test design	Endpoint [g a.s./ha]	PEC_{max}, [g a.s./ha]	TER_{LT}	Trigger	Refined risk assessment?
Folsomia candida					
Prothioconazole FS 100 chronic, treated seeds	NOER ≥ 112.4	18	≥ 6.2	5	No

Please note that the NOER in this study represents the highest application rate tested and that the intrinsic toxicity of prothioconazole and the product to Collembola is considered very low (NOEC ≥ 1000 mg a.s./kg in the reproduction study with the active substance and NOEC ≥ 7.7 mg a.s./kg for the product Prothioconazole FS 300).

Thus, an overall low risk to Collembola and soil mites is concluded if prothioconazole is applied as treated cereal seeds at an application rate of 18 g a.s./ha.

CP 10.4.2.1 Species level testing

A new reproduction study with *Hypoaspis aculeifer* is available with Prothioconazole FS 100; a summary is presented below. A *Folsomia candida* reproduction study with Prothioconazole FS 100 (application of treated seeds) was evaluated during the EU review (2007) with a NOER of 112.4 g a.s./ha, however, a study where the test item was mixed into the soil is not available with Prothioconazole FS 100. However, a *F. candida* reproduction study is available with Prothioconazole FS 300 which is a slightly different formulation compared to Prothioconazole FS 100. This study can be taken into account to describe the intrinsic toxicity of the product to *F. candida*. A summary is presented below.

Report:	KCP 10.4.2.1/01 [redacted]., 2015; M-532652-01-1
Title:	Prothioconazole FS 100 G: Influence on mortality and reproduction of the soil mite species Hypoaspis aculeifer tested in artificial soil
Report No.:	E 428 4711-7
Document No.:	M-532652-01-1
Guideline(s):	US EPA OCSPP: Not Applicable; OECD 226 from October 03, 2008: OECD guideline for the Testing of Chemicals - Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil
Guideline deviation(s):	minor deviations
GLP/GEP:	**yes**

Objective:
The purpose of this study was to assess the effect of prothioconazole FS 100 G on mortality and reproduction of the soil mite species *Hypoaspis aculeifer* tested during an exposure of 14 days in artificial soil comparing control and treatment.

Material and methods:
Test item: Prothioconazole FS 100 G, analysed a.s. content: 8.47 % w/w (97.86 g/L), Batch No. 2015-001031, TOX10850-00, Specification No. 102000006421, Density: 1.155 g/mL (20°C).

Ten adult, fertilized, female *Hypoaspis aculeifer* per replicate (8 replicates for the control group and 4 replicates for each treatment group) were exposed to control and treatments. Concentrations of 100, 178, 316, 562, and 1000 mg test item/kg dry weight artificial soil were tested. During the test, the *Hypoaspis aculeifer* were fed with nematodes bred on watered oat flakes. During the study a temperature of 20 ± 2 °C and light regime of 400 – 800 Lux, 16 h light : 8 h dark was applied. The artificial soil was prepared according to the guideline with the following constituents (percentage distribution on dry weight basis): 75 % fine quartz sand, 5 % Sphagnum peat, air dried and finely ground, 20 % Kaolin clay.
After a period of 14 days, the surviving adults and the living juveniles were extracted by applying a temperature gradient using a MacFadyen-apparatus. Extracted mites were collected in a fixing solution

(20 % ethylene glycol, 80 % deionised water; 2 g detergent/L fixing solution were added). All *Hypoaspis aculeifer* were counted under a binocular.

Findings:

Validity criteria:

All validity criteria were met as presented below:

Table CP 10.4.2.1- 1: Validity criteria

Validity criteria	Recommended	Obtained
Mean adult mortality	≤ 20%	6.3%
Mean number of juveniles per replicate (with 10 mites introduced)	≥ 50	291.3
Coefficient of variation calculated for the number of juveniles per replicate	≤ 30%	3.5

The most recent non-GLP-test (Maria Lyonne [redacted], LAR/HR-O-16/14, January 05, 2015) with the reference item dimethoate was performed at test concentrations 1.0, 1.8, 3.2, 5.6 and 10.0 mg dimethoate/kg dry weight artificial soil.

Dimethoate showed a LC_{50} of 2.51 mg a.s./kg (95 % confidence limits from 0.85 mg a. s./kg to 3.30 mg a. s./kg) for mortality of the adult mites according Probit analysis using maximum likelihood regression. The reproduction of the soil mites was not significantly reduced in comparison to the control up to 3.2 mg a.s./kg dry weight artificial soil. Therefore the NOEC is calculated to be 3.2 mg a.s./kg and accordingly the LOEC is 5.6 mg a.s./kg. Since variances of the data were homogenous Williams-t test $\alpha = 0.05$, one-sided smaller was used. Dimethoate EC 400E G showed an EC_{50} of 5.47 mg a. s./kg (95 % confidence limits from 4.09 mg a. s./kg to 7.30 mg a. s./kg) for reproduction according Probit analysis using maximum likelihood regression.

This is in the recommended range of the guideline, indicating that an EC_{50} based on the number of juveniles of 3.0 – 7.0 mg a. s./kg dry weight artificial soil shows that the test organisms are sufficiently sensitive.

Biological results:

In the control group 6.3 % of the adult *Hypoaspis aculeifer* died which is below the allowed maximum of ≤ 20 % mortality.

Concerning the adult mortality of the test organism statistical analysis (Fisher´s exact Binomial Test with Bonferroni Correction, one-sided-greater, α=0.05) revealed no significant difference between control and any treatment group.

Concerning the number of juveniles statistical analysis (William's-t test, one-sided smaller, $\alpha = 0.05$) revealed no significant difference between control and any treatment group.

Table CP 10.4.2.1- 2: Effect of Prothioconazole FS 100 G on *Hypoaspis aculeifer*

Test item	Prothioconazole FS 100 G				
Test species	*Hypoaspis aculeifer*				
Exposure	Artificial soil				
mg test item/kg dry weight artificial soil	**Adult mortality (%)**	**Significance (*)**	**Mean number of juveniles per test vessel ± standard dev.**	**Reproduction (% of control)**	**Significance (**)**
Control	6.3	--	291.3 ± 10.3	--	--
100	0.0	-	325.0 ± 23.7	111.6	-
178	0.0	-	326.0 ± 8.8	111.9	-
316	5.0	-	320.5 ± 26.8	110.0	-
562	2.5	-	319.5 ± 7.2	109.7	-
1000	7.5	-	318.0 ± 23.1	109.2	-
				Mortality	**Reproduction**
NOEC (mg test item/kg dry weight artificial soil)				≥ 1000	≥ 1000
LOEC (mg test item/kg dry weight artificial soil)				> 1000	> 1000
Overall NOEC (mg test item/kg dry weight artificial soil)				≥ 1000	
Overall LOEC (mg test item/kg dry weight artificial soil)				> 1000	

Calculations were done with un-rounded values.
(*) = Fisher´s exact Binomial Test with Bonferroni Correction, one-sided-greater, $\alpha=0.05$, "-": non-significant; "+": significant
(**) = William's-t.-test, one sided smaller; $\alpha=0.05$; "-": non-significant; "+": significant

Conclusion:
Overall NOEC: ≥ 1000 mg test item/kg dry weight artificial soil
Overall LOEC: > 1000 mg test item/kg dry weight artificial soil

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Report:	KCP 10.4.2.1/02 [redacted].; 2007; M-287951-01-1
Title:	Prothioconazole FS 300 G: Influence on the reproduction of the collembola species Folsomia candida tested in artificial soil with 5 % peat
Report No.:	FRM-COLL-51/07
Document No.:	M-287951-01-1
Guideline(s):	ISO 11267 (1999)
Guideline deviation(s):	With respect to the properties of the test item (Log pow ¡Ý 2) 5 % peat instead of 10 % peat was used considering the influence on bioavailability (EPPO 2002).
GLP/GEP:	**yes**

Objective:
The purpose of the study was to provide data for the registration of plant protection products on the lethal and sub lethal effects of the test item on the collembola species *Folsomia candida* as a representative of the soil fauna.

Material and methods:
Test item: Prothioconazole FS 300 G, analysed a.s. content: 296.8 g/L, Batch No. 2006-006218, TOX07688-00, Specification No. 102000014331, Density: 1.155 g/mL (20°C).

Ten Collembola (10-12 days old) per replicate (5 replicates per treatment group) were exposed to control (water treated), 1.0, 2.3, 5.5, 12.8 and 30.0 mg test item/kg artificial soil dry weight at 18 – 22°C, 400 –

800 Lux, 16h light : 8h dark, 5 % peat in the artificial soil. During the study, they were fed with granulated dry yeast. The study was conducted in artificial soil according to OECD 207 (1984) (5% sphagnum peat, 20% kaolin clay, 74.8% industrial quartz sand and 0.2% $CaCO_3$).
Mortality and reproduction were determined after 28 days.
To demonstrate the sensitivity of the test system Betosip (Phenmedipham 15.4 %) as a toxic standard is regularly tested (once a year) at concentrations of 89, 133, 200, and 300 mg test item/kg artificial soil dry weight.

Findings:
Validity criteria:
All validity criteria were met as presented below:

Table CP 10.4.2.1- 3: Validity criteria

Validity criteria	Recommended	Obtained
Average mortality oft he adults in the control after 28 days	< 20 %	14 %
Average reproduction rate in the control after 28 days	>100 juveniles/control vessel	590
Coefficient of variation of reproduction in the control after 28 days	< 30 %	9.2

In the most recent reference test with Betosip (Phenmedipham 15.4 %) ([redacted] 2006, Non-GLP) the mortality rate of adult collembola was 8 %, 14 %, 22 % and 32 % at 89, 133, 200 and 300 mg Betosip/kg artificial soil dry weight. In all treatment groups the number of juveniles was statistically significant reduced (William s-T Test, one-sided-smaller, $\alpha = 0.05$) in comparison to the control.
$NOEC_{reproduction}$ < 89 mg Betosip (13.7 mg a.s)/kg artificial soil dry weight
$LOEC_{reproduction}$ 89 mg Betosip (13.7 mg a.s)/kg artificial soil dry weight.
The study proves the sensibility of the test system.

Biological results:
In the control group 14 % of the adult Collembola died which is within the tolerated range of ≤ 20 % mortality recommended by the guideline. The highest mortality rate of 30 % was found in the test item concentration of 23 mg test item/kg soil dry weight. In all other treatment groups the mortality rate was lower than in the control group.
Concerning the number of juveniles statistical analysis (Dunnett's Test, one sided-smaller, $\alpha = 0.05$) reveals no significant differences between the control and any treatment group.

Table CP 10.4.2.1- 4: Effect of Prothioconazole FS 300 G on *Folsomia candida*

Test item	Prothioconazole FS 300 G		
Test species	*Folsomia candida*		
Exposure	Artificial soil		
mg test item/kg dry weight artificial soil	**Adult mortality (%)**	**Mean number of juveniles per test vessel ± standard dev.**	**Reproduction (% of control)**
Control	14	590 ± 54	-
1.0	6	692 ± 127	117
2.3	30	652 ± 164	110
5.5	8	704 ± 62	119
12.8	10	627 ± 137	106
30.0	8	763 ± 133	129
			Reproduction
NOEC (mg test item/kg dry weight artificial soil)			**≥** 30
LOEC (mg test item/kg dry weight artificial soil)			> 30

Conclusion:
$NOEC_{reproduction}$: ≥ 30 mg test item/kg dry weight artificial soil
Overall $LOEC_{reproduction}$: > 30 mg test item/kg dry weight artificial soil

CP 10.4.2.2 Higher tier testing

No higher tier testing was performed or required.

CP 10.5 Effects on soil nitrogen transformation

Table CP 10.5-1: Endpoints used in risk assessment

Test species	Test item	Test design	Ecotoxicological endpoint			Reference
N-cycle	Prothioconazole	28 d	no influence	≥ 2.0 ≥ **2.71**	kg a.s./ha **mg a.s./kg dws**	[redacted] (1999) M-024673-01-1 KCA 8.5/01
N-cycle	JAU 6476-desthio-methyl	28 d	no influence	≥ 2.0 ≥ **2.69**	kg p.m../ha **mg p.m./kg dws**	[redacted] (1999) M-024931-01-1 KCA 8.5/03
N-cycle	JAU 6476-desthio	28 d	no influence	≥ 1.0 ≥ **1.37**	kg p.m../ha **mg p.m./kg dws**	[redacted] (2001) M-057459-01-1 KCA 8.5/06
N-cycle	Prothioconazole FS 300 [a)]	28 d	no influence	≥ 0.1 ≥ 0.15 ≥ **0.0392**	L prod./ha mg prod./kg dws **mg a.s./kg dws**	[redacted] (2013) M-451627-01-1 KCP 10.5/01

[a)] A N-cycle study with Prothioconazole FS 100 is not available. However, a study with Prothioconazole FS 300 is presented which can be used in the risk assessment instead as the formulation is (apart from the active substance content) slightly different to Prothioconazole FS 100.

No nitrogen transformation study with Prothioconazole FS 100 is available, however, a study is available with Prothioconazole FS 300 which is a slightly different formulation compared to Prothioconazole FS 100. This study can be taken into account to describe the toxicity of the product to soil nitrogen transformation. A summary is presented below.

Report:	KCP 10.5/01 [redacted] E; 2013; M-451627-01-1
Title:	Prothioconazole FS 300 G: Effects on the activity of soil microflora (nitrogen transformation test)
Report No.:	13 10 48 010 N
Document No.:	M-451627-01-1
Guideline(s):	OECD 216 (2000)
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:

The purpose of this study was to determine the effects of Prothioconazole FS 300 on the activity of soil microflora with regard to nitrogen transformation in a laboratory test. The test was performed in accordance with OECD guideline 216 (2000) by measuring the nitrogen turnover.

Material and methods:

Test item: Prothioconazole FS 300 G, analysed a.s. content: 26.1% w/w (293.1 g/L), Batch No. 2011-004670, TOX09507-00, Specification No. 102000021339-01, Density: 1.123 g/mL (20°C).

A loamy sand soil (DIN 4220) was exposed for 28 days to 0.03 and 0.15 mg prod./kg soil dry weight. Application rates were equivalent to 0.02 and 0.10 L prod./ha. Determination of the nitrogen transformation (NO_3-nitrogen production) in soil enriched with lucerne meal (concentration in soil 0.5 %). NH_4-nitrogen, NO_3- and NO_2-nitrogen were determined using the Autoanalyzer (BRAN+LUEBBE) at different sampling intervals (0, 7, 14 and 28 days after treatment).

Findings:

Validity criteria:

The coefficients of variation in the control (NO_3-N) were maximum 7.4% and thus fulfilled the demanded range (≤15 %).

In a separate study the reference item Dinoterb caused a stimulation of nitrogen transformation of +33.7 % and +42.6 % at 16.00 mg and 27.00 mg Dinoterb per kg soil dry weight, respectively, 28 days after application.

Biological results:

No adverse effects of Prothioconazole FS 300 G on nitrogen transformation in soil could be observed at both test concentrations (0.03 mg/kg dry soil and 0.15 mg prod./kg dry soil) after 28 days. Differences from the control of +5.4 % (test concentration 0.03 mg prod./kg dry soil) and +5.4 % (test concentration 0.15 mg prod./kg dry soil) were measured at the end of the 28-day incubation period (time interval 14-28).

Table CP 10.5-2: Effect of Prothioconazole FS 300 G on Nitrogen transformation in soil

Time Interval (days)	Control	0.03 mg prod./kg soil dry weight equivalent to 0.02 L test item/ha		0.15 mg prod./kg soil dry weight equivalent to 0.10 L test item/ha	
	Nitrate –N[1)]	Nitrate –N[1)]	% difference to control	Nitrate –N[1)]	% difference to control
0-7	3.31 ± 0.33	3.47 ± 0.02	+4.6[n.w.]	3.13 ± 0.18	-5.5[n.s.]
7-14	1.11 ± 0.39	1.18 ± 0.17	+6.0[n.s.]	1.27 ± 0.09	+14.1[n.s.]
14-28	0.80 ± 0.08	0.84 ± 0.14	+5.4[n.s.]	0.84 ± 0.04	+5.4[n.s.]

The calculations were performed with unrounded values
1) Rate: Nitrate-N in mg/kg soil dry weight/time interval/day, mean of 3 replicates and standard deviation
n.s. = No statistically significant difference to the control (Student-t-test for homogeneous variances, 2-sided, $p \leq 0.05$)
n.w. = No statistically significant difference to the control (Welch-t-test for inhomogeneous variances, 2-sided, $p \leq 0.05$)

Conclusion:
Prothioconazole FS 300 G caused no adverse effects (difference to control < 25 %, OECD 216) on the soil nitrogen transformation (measured as NO_3-N production) at the end of the 28-day incubation period. The study was performed in a field soil at concentrations up to 0.15 mg test item/kg soil, which are equivalent to application rates up to 0.10 L prod./ha.

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Risk assessment for Soil Nitrogen Transformation

Table CP 10.5- 3: Risk assessment for soil micro-organisms

Compound	Species	Endpoint [mg/kg]	$PEC_{soil,max}$, $PEC_{soil,acc}$ [mg/kg]	Refinement required
Prothioconazole	Soil micro-organisms	≥ 2.71 mg a.s./kg dws	0.024	No
JAU 6476-S-methyl	Soil micro-organisms	≥ 2.69 mg p.m./kg dws	0.012	No
JAU 6476-desthio	Soil micro-organisms	≥ 1.37 mg p.m./kg dws	0.004	No
Prothioconazole FS 300[a)]	Soil micro-organisms	≥ 0.0392 mg a.s./kg dws	0.024	No

a) A N-cycle study with Prothioconazole FS 100 is not available. However, a study with prothioconazole FS 300 is presented which can be used in the risk assessment instead as the formulation is (apart from the active substance content) slightly different to Prothioconazole FS 100

According to current regulatory requirements the risk is considered acceptable if the effect on nitrogen mineralisation at the recommended application rate of a compound/product is ≤ 25% after 100 days.

In no case did deviations from the control exceed the threshold level of 25% at 28 days after application. The tested concentrations by far exceeded the maximum predicted environmental concentrations in soil of the respective components. This indicates acceptable risk to soil micro-organisms for the intended uses.

CP 10.6 Effects on terrestrial non-target higher plants

In the case of a seed treatment, exposure of non-target terrestrial plants to the product and its active ingredient(s) is not to be expected. Therefore, no risk assessment will be performed.

CP 10.6.1 Summary of screening data

Not necessary. Please refer to point CP 10.6.

CP 10.6.2 Testing on non-target plants

Not necessary. Please refer to point CP 10.6.

CP 10.6.3 Extended laboratory studies on non-target plants

Not necessary. Please refer to point CP 10.6.

CP 10.6.4 Semi-field and field tests on non-target plants

Not necessary. Please refer to point CP 10.6.

CP 10.7 Effects on other terrestrial organisms (flora and fauna)

Not necessary. Please refer to point CP 10.6.

CP 10.8 Monitoring data

Not necessary. Please refer to point CP 10.6.