Bayer CropScience

Document Title

Summary of the ecotoxicological studies Bixafen + Prothioconazole EC 225 (75 + 150 g/L)

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Section 10: Ecotoxicological studies

According to the guidance document, SANCO 10181/2013, for preparing dossiers for the approval of a chemical active substance

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Version history

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.

The representative formulation (spray use) submitted in the first Annex I listing process is no longer considered as a representative formulation for the renewal of approval of prothioconazole. One of the representative formulations used for the submission of the renewal of the approval of prothioconazole is the spray formulation Bixafen + Prothioconazole EC 225. The summaries of formulation studies and the risk assessment will be presented in this dossier.

Ecotoxicological endpoints used in the following risk assessment were derived from studies with the formulated product Bixafen + Prothioconazole EC 225, the active substance prothioconazole and its 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 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	F G or I (b)	Application				Application rate per treatment			Remarks: (m)
		method kind (f-h)	growth stage & season (j)	number min max (k)	interval between applications (min)	g a.s./hL min max	water L/ha min max	g a.s./ha min max	
Wheat Triticale Rye Spelt	**F**	**Foliar spray**	**BBCH 25-69**	**1-2**	**14-21**	**23.4-93.75 BIX + 46.9-187.5 PTZ**	**100-400**	**93.75 BIX + 187.5 PTZ**	**1.25 L/ha**
Barley Oat	**F**	**Foliar spray**	**BBCH 25-61**	**1-2**	**14-21**	**18.8-75 BIX + 37.5-150 PTZ**	**100-400**	**75 BIX + 150 PTZ**	**1.0 L/ha**

In this document, the risk assessment is conducted for the active substance prothioconazole only. A risk envelope approach is presented, using the most critical use (2 x 187.5 g a.s./ha at BBCH 25-69), which will cover the less critical use (2 x 150 g a.s./ha at BBCH 25-61, 1 x 187.5 g/ha at BBCH 25-69, and 1 x 150 g a.s./ha at BBCH 25-61), if not stated otherwise.

Definition of the residue for risk assessment for prothioconazole

Due to changes in triggers for metabolites to be further assessed as well as 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 compartments. The residue definition is included in Table CP 10-2.

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

Plant metabolites

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 CP 10.1.1 Effects on birds

Table CP 10.1.1- 1: Endpoints used in the 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] (1999) 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] (2004), 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-013315-01-1 KCA 8.1.1.1/02
	Reprod. 22 w dietary *Colinus virginianus* (Bobwhite quail)	NOEC NOEL	175 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

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

Table CP 10.1.1- 2: Relevant avian generic focal species for Tier 1 risk assessment

Crop	Growth stage (BBCH)	Generic focal species	Representative species	Shortcut value	
				For long-term RA based on RUD_m	For acute RA based on RUD_{90}
Cereals	early (shoots) (10-29)	Large herbivorous bird "goose"	Pink-foot goose (*Anser brachyrhynchus*)	**16.2**	**30.5**
	10 - 29	Small omnivorous bird "lark"	Woodlark (*Lullula arborea*)	**10.9**	**24.0**
	30 - 39			5.4	12.0
	≥ 40			3.3	7.2

In bold: For the acute and long-term Tier 1 risk assessments, only the maximum shortcut values were used for same generic focal species covering higher growth stages with lower SV. In case the trigger was not passed, the higher growth stages were included in the risk assessments.

Acute dietary risk assessment

Table CP 10.1.1- 3: Tier 1 acute risk assessment for birds

Crop	Generic focal species	LD_{50} [mg a.s./kg bw]	DDD			DDD	TER_A	Trigger
			Appl. rate [kg/ha]	SV_{90}	MAF_{90}			
Prothioconazole								
Cereals	Large herbivorous bird "goose" (10-29)	> 2000	0.1875	30.5	1.2	6.9	>291.4	10
	Small omnivorous bird "lark" (10-29)			24.0		5.4	>370.4	
JAU 6476-desthio								
Cereals	Large herbivorous bird "goose" (10-29)	> 2000	0.1875[A]	30.5	1.2	6.9	>291.4	10
	Small omnivorous bird "lark" (10-29)			24.0		5.4	>370.4	

[A] 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_A values calculated in the acute risk assessment on Tier 1 level exceed the *a-priori*-acceptability trigger of 10 for all evaluated scenarios. Thus, the acute risk to birds can be considered as low and acceptable without need for further, more realistic risk assessment.

Acute risk assessment for birds drinking contaminated water from pools in leaf whorls

In the EFSA GD (2009), section 5.5, step 1 the following guidance is given on the selection of relevant scenarios for assessing the risk of pesticides via drinking water to birds and mammals:
Leaf scenario: Birds taking water that is collected in leaf whorls after application of a pesticide to a crop and subsequent rainfall or irrigation.
Puddle scenario. Birds and mammals taking water from puddles formed on the soil surface of a field when a (heavy) rainfall event follows the application of a pesticide to a crop or bare soil.
For the crop under assessment in this evaluation (cereals), the leaf scenario is not considered relevant. The risk for birds from drinking water in puddles is addressed in Table CP 10.1.1- 5.

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 LDD50) 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_{AC} calculation with the single exposure LD_{50}.

Long-term reproductive risk assessment

Table CP 10.1.1- 4: Tier 1 reproductive risk assessment for birds

Crop	Generic focal species	NO(A)EL [mg a.s./kg bw/d]	DDD				DDD	TER_{LT}	Trigger
			Appl. rate [kg/ha]	SV_m	f_{TWA}	MAF_m			
Prothioconazole									
Cereals	Large herbivorous bird "goose" (10-29)	78	0.1875	16.2	0.53	1.4	2.3	34.6	5
	Small omnivorous bird "lark" (10-29)			10.9			1.5	51.4	
JAU 6476-desthio									
Cereals	Large herbivorous bird "goose" (10-29)	14.8	0.1875[A]	16.2	0.53	1.4	2.3	6.6	5
	Small omnivorous bird "lark" (10-29)			10.9			1.5	9.8	

[A] 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_{LT} values calculated in the reproductive risk assessment on Tier 1 level exceed the *a-priori*-acceptability trigger of 10 for all evaluated scenarios. Thus, the risk to birds can be considered as low and acceptable without need for further, more realistic risk assessment.

Long-term risk assessment for birds drinking contaminated water in puddles

Table CP 10.1.1- 5: Evaluation of potential concern for exposure of birds drinking water (escape clause)

Compound	Koc [L/kg]	Application rate * MAF [A] [g a.s./ha]	NO(A)EL [mg a.s./ kg bw/d]	Ratio (Application rate * MAF) / NO(A)EL	"Escape clause" No concern if ratio	Conclusion
Cereals						
Prothioconazole	1765	187.5 × 2	78	4.8	≤ 3000	No concern
JAU 6476-desthio	575	187.5 × 2	14.8	25.3	≤ 3000	No concern

[A] Simplified screening approach with full application rate of prothioconazole also for JAU 6476-desthio; no interception, MAF = 2 for 2 applications without dissipation

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 for 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- 6: Log Pow 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] (1983) M-045573-01-1
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- 7: 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- 6
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]	0.169	0.057	See MCP 9.1.3
PEC_{worm} [mg/kg]	0.206	0.268	
DDD calculation:			
FIR/bw	1.05	1.05	Default
DDD [mg/kg bw/d]	0.216	0.281	
TER_{LT} calculation:			
NO(A)EL [mg/kg bw/d]	14.8	7.8 [2)]	See Table CP 10.1.1- 1
TER_{LT}	69	28	
Trigger	5	5	
Refined risk assessment required?	No	No	

[1)] Worst case 21 d TWA_{soil} value based on 2 x 187.5 g/ha prothioconazole, 20% 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- 8: 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]	0.008626	0.001012	See MCP 9.2.5
PEC_{fish} [mg/kg]	0.561	0.323	
DDD calculation:			
FIR/bw	0.159	0.159	Default
DDD [mg/kg bw/d]	0.089	0.051	
TER calculation:			
NO(A)EL [mg a.s./kg bw/d]	14.8	7.8[3)]	See Table CP 10.1.1- 1
TER_{LT}	166	153	
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 8.2.2.3/01.
2) Worst-case 21d-TWA_{SW} (winter & spring cereals, 2 x 187.5 g a.s./ha, 20% interception, S-EU Multi)
[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

No additional studies are available or required as the toxicity can be derived from the studies on the active substance.

CP 10.1.1.2 Higher tier data on birds

The risk assessment indicates no risk at Tier 1; hence no higher tier studies are triggered. However additional data are presented to support the short half-life of prothioconazole and JAU 6476-desthio on plant matrices following spray treatment. These data are provided in chapter CP 10.1.2.2 and employed in the refined risk assessment for herbivorous mammals.

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
Prothio-conazole	acute, oral Rat	LD_{50}	**6200 mg a.s./kg bw**	[redacted] (1998) M-012312-01-1 KCA 5.2.1/1
	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 5.6.1/01
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 5.2.1/34
	Long-term (2-gen.-repro study) Rat	NOEL	**10 mg p.m./kg bw/d**	[redacted] & [redacted] (2000) 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 for Tier 1 risk assessment

Crop	Growth stage (BBCH)	Generic focal species	Representative species	Shortcut value	
				For long-term RA based on RUD_m	For acute RA based on RUD_{90}
Cereals	Early (shoots)	Large herbivorous mammal "lagomorph"	Rabbit (*Oryctolagus cuniculus*)	**22.3**	**42.1**
	10-19	Small insectivorous mammal "shrew"	Common shrew (*Sorex araneus*)	**4.2**	**7.6**
	≥ 20			1.9	5.4
	≥ 40	Small herbivorous mammal "vole"	Common vole (*Microtus arvalis*)	**21.7**	**40.9**
	10-29	Small omnivorous mammal "mouse"	Wood mouse (*Apodemus sylvaticus*)	**7.8**	**17.2**
	30-39			3.9	8.6
	≥ 40			2.3	5.2

In bold: For the acute and long-term Tier 1 risk assessments, only the maximum shortcut values were used for same generic focal species covering higher growth stages with lower SV.

Acute dietary risk assessment

Table CP 10.1.2- 3: Tier 1 acute DDD and TER calculation for mammals

Crop	Generic focal species (BBCH)	LD_{50} [mg a.s./kg bw]	DDD			DDD	TER_A	Trigger
			Appl. rate [kg/ha]	SV_{90}	MAF_{90}			
Prothioconazole								
Cereals	Large herbivorous mammal "lagomorph" (shoots)	> 6200	0.1875	42.1	1.2	9.5	654.5	10
	Small insectivorous mammal "shrew" (10-19)			7.6		1.7	3625.7	
	Small herbivorous mammal "vole" (≥ 40)			40.9		9.2	673.7	
	Small omnivorous mammal "mouse" (≥ 40)			17.2		3.9	1602.0	
JAU 6476-desthio								
Cereals	Large herbivorous mammal "lagomorph" (shoots)	2235	0.1875 [A]	42.1	1.2	9.5	235.9	10
	Small insectivorous mammal "shrew" (10-19)			7.6		1.7	1307.0	
	Small herbivorous mammal "vole" (≥ 40)			40.9		9.2	242.9	
	Small omnivorous mammal "mouse" (≥ 40)			17.2		3.9	577.5	

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

The TER_A values calculated in the acute risk assessment on Tier 1 level exceed the *a-priori* acceptability trigger of 10 for all evaluated scenarios. Thus, the acute risk to mammals can be considered as low and acceptable without need for further, more realistic risk assessment.

Long-term reproductive assessment

Table CP 10.1.2- 4: Tier 1 long-term DDD and TER calculation for mammals

Crop	Generic focal species (BBCH)	NO(A)EL [mg a.s./kg bw/d]	DDD				DDD	TER_{LT}	Trigger
			Appl. rate [kg/ha]	SV_m	f_{TWA}	MAF_m			
Prothioconazole									
Cereals	Large herbivorous mammal "lagomorph" (shoots)	95.6	0.1875	22.3	0.53	1.4	3.1	30.8	5
	Small insectivorous mammal "shrew" (10-19)			4.2			0.6	163.6	
	Small herbivorous mammal "vole" (≥ 40)			21.7			3.0	31.7	
	Small omnivorous mammal "mouse" (≥ 40)			7.8			1.1	88.1	
JAU 6476-desthio									
Cereals	Large herbivorous mammal "lagomorph" (shoots)	10	0.1875 [A]	22.3	0.53	1.4	3.1	**3.2**	5
	Small insectivorous mammal "shrew" (10-19)			4.2			0.6	17.1	
	Small herbivorous mammal "vole" (≥ 40)			21.7			3.0	**3.3**	
	Small omnivorous mammal "mouse" (≥ 40)			7.8			1.1	9.2	

[A)] The application rate is taken from the parent compound and represents an unrealistic worst-case scenario
Bold values do not meet the trigger

All calculated TER values for the active substance prothioconazole are above the required trigger of 5, indicating a low long-term risk for mammals. As the calculated TER value for JAU 6476-desthio for herbivorous generic focal species scenarios (rabbit and vole) are below the trigger, a refined risk assessment is presented below, based on the kinetic evaluation of cereal residue decline data ([redacted] et al. 2015, M-533352-02-1, KCP 10.1.2.2/01).

In this kinetic evaluation, the formation of JAU 6476-desthio, and its dissipation, was investigated based on measured residue values from samples taken at different time intervals after foliar application of prothioconazole on cereal plants.

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

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.
For the representative GAP, with two spray applications in a worst case 14-d interval, the refined MAF and refined 21-d f_{TWA} are calculated for each trial, employing a moving time-window calculator.

These MAF and refined 21-d f_{TWA} for JAU 6476-desthio per trial are then multiplied with the formation fraction determined in the respective trial. Where no valid determination of the formation fraction was possible, a worst case calculation was performed, employing the formation fraction of 1.0.

Table CP 10.1.2- 5: Adjustment factor calculation for JAU 6476-desthio (cereal foliage)

Trial	DT_{50}	MAF	21-d f_{TWA}	ff	adjustment factor: MAF x 21-d f_{TWA} x ff
J6118-01W	4.41	1.111	0.445	1.00 [a)]	0.494
J6119-01W	1.30	1.001	0.176	1.00	0.176
J6120-01W	1.54	1.002	0.207	0.58	0.120
J6121-01W	1.50	1.002	0.202	0.86	0.174
J6122-01W	2.40	1.018	0.302	1.00 [a)]	0.308
J6123-01W	1.64	1.003	0.219	0.12	0.026
13-2950-01	2.44	1.019	0.306	0.78	0.243
13-2950-02	5.56	1.175	0.491	0.27	0.156
13-2950-03	4.75	1.130	0.460	0.26	0.135
13-2950-04	3.11	1.044	0.364	1.00 [a)]	0.380
geomean					**0.176**

([a] worst case formation fraction included in calculation (no reliable estimate generated in the kinetic evaluation)

In the refined exposure assessment for JAU 6476-desthio, the adjustment factor is multiplied with the amount of parent applied (per single application) replaces then the terms MAF and 21-d f_{TWA}.

Table CP 10.1.2- 6: Refined long-term DDD and TER calculation for rabbits and voles

Crop	Generic focal species (BBCH)	NO(A)EL [mg a.s./kg bw/d]	DDD			DDD	TER_{LT}	Trigger
			Appl. rate [kg/ha]	SV_m	Adjustment factor			
JAU 6476-desthio								
Cereals	Large herbivorous mammal "lagomorph" (shoots)	10	0.1875	22.3	0.176	0.736	13.6	5
	Small herbivorous mammal "vole" (≥ 40)			21.7		0.716	14.0	

The TER value is above the trigger of 5 for reproductive/long-term exposure, indicating an acceptable risk for the use of the product according to the intended use pattern.

Long-term risk assessment for mammals drinking contaminated water

The puddle scenario is relevant for the long-term risk assessment.

Table CP 10.1.2- 7: Evaluation of potential concern for exposure via drinking water of mammals (escape clause)

Compound	Koc [L/kg]	Application rate × MAF [A] [g a.s./ha]	NO(A)EL [mg a.s./ kg bw/d]	Ratio (Application rate × MAF) / NO(A)ED	"Escape clause" No concern if ratio	Conclusion
Cereals						
Prothioconazole	1765	187.5 × 2	95.6	3.9	≤ 3000	No concern
JAU 6476-desthio	575	187.5 × 2	10	37.5	≤ 3000	No concern

[A] simplified screening approach with full application rate of prothioconazole also for JAU 6476-desthio; no interception, MAF = 2 for 2 applications without dissipation

This evaluation confirms that the risk for mammals from drinking water that may contain residues from the use of Bixafen + Prothioconazole EC 225 is acceptable.

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- 8: Tier I 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]	0.206	0.268	See Table CP 10.1.1- 7
DDD calculation:			
FIR/bw	1.28	1.28	Default
DDD [mg/kg bw/d]	0.264	0.343	
TER calculation:			
NO(A)EL [mg a.s./kg bw/d]	10	9.56 [1)]	See Table CP 10.1.2- 1
TER_{LT}	38	28	
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)

The TER values for all prothioconazole metabolites 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- 9: 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]	0.561	0.323	see Table CP 10.1.1- 8
DDD calculation:			
FIR/bw	0.142	0.142	Default
DDD [mg/kg bw/d]	0.080	0.046	
TER calculation:			
NO(A)EL [mg/kg bw/d]	10	9.56 [1]	See Table CP 10.1.2- 1
TER_{LT}	125	208	
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)

All 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

The acute oral toxicity of Bixafen + Prothioconazole EC 225 in rat was studied by [redacted] (2007, M-292722-01-1, KCP 7.1.1/01). According to OECD guideline 425, the result corresponds with LD_{50} >2000 mg prod./kg bw.

CP 10.1.2.2 Higher tier data on mammals

The kinetic evaluation of residue decline data for studies in cereals with determination of both prothioconazole and of its metabolite, JAU6476-desthio, is presented below ([redacted],; [redacted]; 2015; M-533352-02-1, KCP 10.1.2.2/01):

Report:	KCP 10.1.2.2/01 [redacted]; [redacted]; 2015; M-533352-02-1
Title:	Prothioconazole (PTZ) Foliar DT50 EUR - Residue dissipation of prothioconazole and its metabolite in or on wheat and barley: kinetic evaluation
Report No.:	EnSa-15-0199
Document No.:	M-533352-02-1
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 green material from the field of monocotyledonous plants (here: wheat, spring barley) that may represent food items for leaf-eating herbivorous birds or mammals. The residue decline data are available from regulatory plant residue studies ([redacted] and [redacted] 2002, M-042192-01-1, KCP Proline 10.1.2.2/01 and [redacted]s; [redacted]; 2013; M-471216-01-1, KCP 10.1.2.2/02).

The reliable single-first-order (SFO) and double-first-order-in-parallel (DFOP) half-lives of prothioconazole and JAU 6476-desthio derived in this evaluation are summarised in Table CP 10.1.2.2-

1 below, along with the formation fraction of prothioconazole to desthio. Where a reliable kinetic including parent and metabolite was not available, the half-life of desthio was obtained using only the data from the maximum occurrence.

Table CP 10.1.2.2- 1: Summary of DT_{50} values for prothioconazole and JAU6476-desthio, parent-to-metabolite kinetics (SFO or DFOP), or desthio alone fitted from maximum occurrence onwards

			SFO Fits		DFOP Fit			
Trial code	Compound	Model	DT_{50} [d]	DT_{50} [d]	$DT_{50\text{-fast}}$ [d]	$DT_{50\text{-slow}}$ [d]	"g"	Formation fraction
			JAU 6476	Desthio				
J6118-01W	JAU 6476	SFO	2.2					
	desthio	SFO from max.		4.41				NR [1]
J6119-01W	JAU 6476	DFOP			0.87	5.15	0.42	
	desthio	SFO		1.30				1.0
J6120-01W	JAU 6476	SFO	1.91					
	desthio	SFO		1.54				0.58
J6121-01W	JAU 6476	DFOP			0.11	3.93	0.32	
	desthio	SFO		1.50				0.86
J6122-01W	JAU 6476	SFO / DFOP	NR [1]					
	desthio	SFO from max.		2.40				NR [1]
J6123-01W	JAU 6476	SFO	0.23					
	desthio	SFO		1.64				0.12
13-2950-01	JAU 6476	SFO	0.41					
	desthio	SFO		2.44				0.78
13-2950-02	JAU 6476	SFO	0.34					
	desthio	SFO		5.56				0.27
13-2950-03	JAU 6476	SFO	0.29					
	desthio	SFO		4.75				0.26
13-2950-04	JAU 6476	SFO	0.53					
	desthio	SFO from max.		3.11				NR [1]

[1] Not reliable.

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Report: KCP 10.1.2.2/02 [redacted]; 2013; M-471216-01-1
Title: Determination of the residues of AE C656948 and prothioconazole in/on barley, spring after spray application of AE C656948 & JAU 6476 SE 250 in Germany, Belgium and the Netherlands
Report No.: 13-2950
Document No.: M-471216-01-1
Guideline(s): Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC,
EC Guidance working document 7029/VI/95 rev.5 (1997-07-22),
OECD 509 Adopted 2009-09-07, OECD GUIDELINE FOR THE TESTING OF CHEMICALS, Crop Field Trial,
US EPA OCSPP Guideline No. 860.1500
Guideline deviation(s): not specified
GLP/GEP: **yes**

The purpose of the study 13-2950 was to determine the magnitude of the relevant residues of AE C656948 (comprising AE C656948) and prothioconazole (comprising prothioconazole and JAU 6476-desthio) in/on barley, spring (green material) after one spraying application with AE C656948 & JAU 6476 SE 250 (SE 250) an SE (suspo-emulsion) formulation containing 125 g/L AE C656948 and 125 g/L prothioconazole.
The study included four supervised residue trials conducted in Northern Europe (Germany, Belgium and Netherlands) during the 2013 season.
The actual application data are presented in the following table. These data reflect the intended application scheme, or, if minor deviations occurred, these were within the acceptable range.

Table CP 10.1.2.2- 2: Application summary

Trial no. Country	Formulation	Application							
		Appl. mode	Treated area Reference	No. of appl.	Growth stage (BBCH code)	Test item rate (L/ha)	Water rate (L/ha)	a.s.	Appl. rate (kg a.s./ha)
13-2950-01 Germany	AE C656948 & JAU 6476 SE 250	SPI	GF	1	30	1.0	300	prothioconazole	0.125
								AE C656948	0.125
13-2950-02 Germany	AE C656948 & JAU 6476 SE 250	SPI	GF	1	31	1.0	300	prothioconazole	0.125
								AE C656948	0.125
13-2950-03 Belgium	AE C656948 & JAU 6476 SE 250	SPI	GF	1	30	1.0	300	prothioconazole	0.125
								AE C656948	0.125
13-2950-04 Netherlands	AE C656948 & JAU 6476 SE 250	SPI	GF	1	30	1.0	300	prothioconazole	0.125
								AE C656948	0.125

a.s.: Active substance GF: Whole Area
Appl.: Application
SPI: Spraying

The analyses were conducted according to the following analytical method(s):

Table CP 10.1.2.2- 3: Summary of analytical method criteria relevant to this study

Active substance	Analytes	Method number	Limit of quantitation [mg/kg]	Sample material	Measurement principle
Prothioconazole	Prothioconazole	01013	0.01	green material	HPLC-MS/MS
	JAU 6476-desthio			green material	HPLC-MS/MS
AE C656948	AE C656948	00984/M003	0.01	green material	HPLC-MS/MS

The average recoveries were within the acceptable range of 70 – 110%. RSD values are below 20%. .
The level of residues of AE C656948 (comprising AE C656948) and prothioconazole (comprising prothioconazole and JAU 6476-desthio) in the treated samples are summarised in the table below.
Residues above the LOQ were found in some of the control samples and they were corrected for concurrent recoveries.

Table CP 10.1.2.2- 4: Residue summary in/on barley, spring

Trial No. Country	Sample material	DALT	Residues [mg/kg] a.s. prothioconazole	
			prothioconazole	JAU 6476-desthio
13-2950-01 Germany	green material	0	4.4	0.67
	green material	1	0.89	2.9
	green material	2	0.36	2.4
	green material	3	0.16	1.7
	green material	5	0.088	1.5
	green material	7	0.034	0.71
	green material	10	0.022	0.40
13-2950-02 Germany	green material	0	2.4	0.33
	green material	1	0.29	0.58
	green material	2	0.16	0.60
	green material	3	0.10	0.53
	green material	5	0.052	0.44
	green material	7	0.033	0.34
	green material	10	0.018	0.20
13-2950-03 Belgium	green material	0	3.6	1.5
	green material	1	0.40	1.1
	green material	2	0.27	1.0
	green material	3	0.17	0.98
	green material	5	0.057	0.75
	green material	7	0.039	0.43
	green material	10	0.026	0.34
13-2950-04 Netherlands	green material	0	3.6	1.5
	green material	1	1.5	2.7
	green material	2	0.14	1.6
	green material	3	0.15	1.5
	green material	5	0.052	0.96
	green material	7	0.030	0.74
	green material	10	0.016	0.53

DALT = Days after last treatment a.s. Active substance

Analyte:	Final determination as:	Residues calculated as:
prothioconazole	prothioconazole	prothioconazole
JAU 6476-desthio	JAU 6476-desthio	JAU 6476-desthio

Table CP 10.1.2.2- 5: Residue summary in/on barley, spring

Trial No. Country	Sample material	DALT	Residues [mg/kg] a.s. AE C656948 AE C656948
13-2950-01 Germany	green material	0	8.6
	green material	1	6.8
	green material	2	5.0
	green material	3	2.3
	green material	5	1.5
	green material	7	0.53
	green material	10	0.24
13-2950-02 Germany	green material	0	4.5
	green material	1	0.96
	green material	2	0.83
	green material	3	0.75
	green material	5	0.61
	green material	7	0.44
	green material	10	0.25
13-2950-03 Belgium	green material	0	7.3
	green material	1	1.4
	green material	2	1.2
	green material	3	1.0
	green material	5	0.54
	green material	7	0.30
	green material	10	0.20
13-2950-04 Netherlands	green material	0	8.0
	green material	1	6.3
	green material	2	1.5
	green material	3	1.4
	green material	5	0.67
	green material	7	0.41
	green material	10	0.20

DALT = Days after last treatment a.s. = Active substance

Analyte:	Final determination as:	Residues calculated as:
AE C656948	AE C656948	AE C656948

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. Therefore, the overall highest PEC_{sw} values were used to calculate the risk to aquatic organisms. This clearly represents the worst-case situation, covering all other intended uses of the product. Worst-case FOCUS STEP 3 & 4 PEC values were used as refinement until a safe use of each intended application could 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}	**2.3 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

Test substance	Test species	Endpoint	Reference
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 8.2.5.1/04
	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
	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 8.2.6.1/03
	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

Test substance	Test species	Endpoint	Reference
	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 8.2.1/01
	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
	Invertebrate, chronic *Daphnia magna* (Cladoceran)	NOEC **0.56 mg a.s./L** [4)]	[redacted] & [redacted] (2001) M-055997-01-1 KCA 8.2.5.1/01
	Pseudokirchneriella subcapitata (green alga)	E_rC_{50} **2.18 mg a.s./L** [4)]	[redacted] (2000) M-027625-01-1 KCA 8.2.6.1/01
BIX+PTZ EC 225 (75+150) G	Fish, acute *Oncorhynchus mykiss* (Rainbow trout)	**LC_{50} 1.55 mg prod./L**	[redacted] (2007) M-293311-02-1 KCP 10.2.1/01
	Invertebrate, acute *Daphnia magna* (Cladoceran)	**EC_{50} 3.0 mg prod./L**	[redacted] (2007) M-288432-01-1 KCP 10.2.1/02
	Pseudokirchneriella subcapitata (Green alga)	**E_rC_{50} 1.52 mg prod./L**	[redacted] (2007) M-289495-01-1 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} = 4.5 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 are 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 9.2.5/01 and KCP 9.2.5/02).

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

FOCUS scenario	Prothio-conazole	JAU 6476-desthio	JAU 6476-S-methyl	1,2,4-triazole	JAU 6476-thiazocine	JAU 6476-triazolylketone
	PEC_{sw} [µg/L]	PEC_{sw} [µg/L]	PEC_{sw} [µg/L]	PEC_{sw} [µg/L]	PEC_{sw} [µg/L]	PEC_{sw} [µg/L]
Winter & spring cereals, 2 × 187.5 g a.s./ha						
Step 1	40.73	73.39	8.468	9.732	14.39	6.297
Step 2						
N-EU Multi	1.551	6.011	0.730	0.341	0.501	0.216
S-EU Multi	1.551	**11.28**	**1.372**	**0.445**	**0.655**	**0.284**
N-EU Single	**1.724**	6.747	0.790	0.343	0.509	0.220
S-EU Single	1.724	6.747	0.790	0.343	0.509	0.220
Winter & spring cereals, 2 × 150 g a.s./ha						
Step 1	32.58	58.72	6.774	7.786	11.51	5.038
Step 2						
N-EU Multi	1.241	5.862	0.712	0.293	0.431	0.186
S-EU Multi	1.241	11.13	1.355	0.398	0.585	0.254
N-EU Single	1.380	6.646	0.778	0.316	0.469	0.203
S-EU Single	1.380	6.646	0.778	0.316	0.469	0.203

Bold values were considered in risk assessment

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) - 2 x 187.5 g a.s./ha**

Compound	FOCUS Scenario	2 × 187.5 g a.s./ha, BBCH 25-69			
		Winter cereals		Spring cereals	
		Single appl.	Multiple appl.	Single appl.	Multiple appl.
		$PEC_{sw, max}$ [µg/L]	$PEC_{sw, max}$ [µg/L]	$PEC_{sw, max}$ [µg/L]	$PEC_{sw, max}$ [µg/L]
JAU 6476-desthio	D1 (ditch)	0.0057	0.0312	0.1284	**0.2392**
	D1 (stream)	0.0235	0.0282	0.0325	0.0328
	D2 (ditch)	0.0362	**0.1388**	-	-
	D2 (stream)	0.0594	0.0887	-	-
	D3 (ditch)	0.0122	0.0116	0.0059	0.0107
	D4 (pond)	0.0049	0.0086	0.0084	0.0137
	D4 (stream)	0.0091	0.0122	0.0098	0.0210
	D5 (pond)	0.0054	0.0100	0.0077	0.0132
	D5 (stream)	0.0154	0.0140	0.0162	0.0154
	D6 (ditch)	0.0030	0.0144	-	-
	R1 (pond)	0.0284	0.0692	-	-
	R1 (stream)	0.4046	**0.6404**	-	-
	R3 (stream)	0.3580	**0.3580**	-	-
	R4 (stream)	0.7558	**0.9976**	**0.6119**	0.5745

Bold values were considered in risk assessment (overall worst-case of single or multiple applications over all scenarios).

Table CP 10.2- 4: **Initial max. PEC_{sw} values for the prothioconazole metabolite JAU 6476-desthio – use in winter and spring cereals (FOCUS Step 3) - 2 x 150 g a.s./ha**

Compound	FOCUS Scenario	2 × 150 g a.s./ha, BBCH 25-61			
		Winter cereals		Spring cereals	
		Single appl.	Multiple appl.	Single appl.	Multiple appl.
		$PEC_{sw, max}$ [µg/L]	$PEC_{sw, max}$ [µg/L]	$PEC_{sw, max}$ [µg/L]	$PEC_{sw, max}$ [µg/L]
JAU 6476-desthio	D1 (ditch)	0.0034	0.0173	0.1019	**0.1906**
	D1 (stream)	0.0184	0.0218	0.0260	0.0246
	D2 (ditch)	0.0255	0.1021	-	-
	D2 (stream)	0.0475	0.0642	-	-
	D3 (ditch)	0.0098	0.0093	0.0047	0.0085
	D4 (pond)	0.0039	0.0068	0.0067	0.0111
	D4 (stream)	0.0073	0.0091	0.0078	0.0100
	D5 (pond)	0.0043	0.0080	0.0062	0.0105
	D5 (stream)	0.0123	0.0112	0.0130	0.0123
	D6 (ditch)	0.0024	0.0117	-	-
	R1 (pond)	0.0222	0.0509	-	-
	R1 (stream)	0.3096	**0.4642**	-	-
	R3 (stream)	0.2749	**0.2749**	-	-
	R4 (stream)	0.5823	**1.0650**	0.4738	**1.2860**

Bold values were considered in risk assessment (worst-case of single or multiple applications over all scenarios).

Table CP 10.2- 5: Maximum PEC_{sw} values of single/multiple applications in winter & spring cereals for the prothioconazole metabolite JAU 6476-desthio (FOCUS Step 4)

Use pattern		2 x 187.5 g a.s./ha, BBCH 25-69 (0% drift reduction)		2 x 150 g a.s./ha, BBCH 25-61 (0% drift reduction)	
Buffer Width & Type#	**FOCUS scenario**	**Winter cereals**	**Spring cereals**	**Winter cereals**	**Spring cereals**
		$PEC_{sw\ max}$ [µg/L]		**$PEC_{sw\ max}$ [µg/L]**	
10m SD & RO	D1 (Ditch)	0.0312	0.0525	0.0173	0.0344
	D1 (Stream)	0.0198	0.0328	0.0110	0.0216
	D2 (Ditch)	0.1388	-	0.1021	-
	D2 (Stream)	0.0887	-	0.0642	-
	D3 (Ditch)	0.0018 *	0.0014	0.0014 *	0.0011
	D4 (Pond)	0.0052	0.0083	0.0041	0.0067
	D4 (Stream)	0.0122	0.0210	0.0091	0.0100
	D5 (Pond)	0.0060	0.0080	0.0048	0.0064
	D5 (Stream)	0.0030 *	0.0031 *	0.0024 *	0.0025 *
	D6 (Ditch)	0.0019	-	0.0016	-
	R1 (Pond)	0.0298	-	0.0220	-
	R1 (Stream)	**0.2909**	-	**0.2108**	-
	R3 (Stream)	0.1582	-	0.1215	-
	R4 (Stream)	**0.4549**	**0.2783** *	**0.4699**	**0.5849**
20m SD & RO	D1 (Ditch)	0.0312	0.0525	0.0173	0.0344
	D1 (Stream)	0.0198	0.0328	0.0110	0.0216
	D2 (Ditch)	0.1388	-	0.1021	-
	D2 (Stream)	0.0887	-	0.0642	-
	D3 (Ditch)	0.0009 *	0.0007	0.0007	0.0006
	D4 (Pond)	0.0038	0.0066	0.0028	0.0044
	D4 (Stream)	0.0122	0.0210	0.0091	0.0100
	D5 (Pond)	0.0039	0.0052	0.0031	0.0041
	D5 (Stream)	0.0016 *	0.0016 *	0.0012 *	0.0013 *
	D6 (Ditch)	0.0010	-	0.0008	-
	R1 (Pond)	0.0158	-	0.0117	-
	R1 (Stream)	0.1523	-	0.1104	-
	R3 (Stream)	0.0819	-	0.0629	-
	R4 (Stream)	**0.2386**	0.1458 *	**0.2444**	**0.3064**

Entries marked with * result from single applications
SD and RO denote spray drift- and runoff buffer
Bold values are considered in risk assessment

Acute risk assessment for aquatic organisms

Based on the risk envelope approach, the highest PEC_{sw} 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. Worst-case FOCUS STEP 3 & 4 PEC values were used as refinement until a safe use of each intended application could be considered.

Table CP 10.2- 6: TER_A calculations based on FOCUS Step 2 (PEC values based on worst-case GAP 2 × 187.5 g a.s./ha)

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	1.724	1061	100
	Invertebrate, acute *Daphnia magna*	EC_{50} 1300	1.724	754	100
	Invertebrate, acute *Americamysis bahia*	EC_{50} 2400	1.724	1392	100
JAU 6476-desthio	Fish, acute *Oncorhynchus mykiss*	LC_{50} 6630	11.28	588	100
	Invertebrate, acute *Daphnia magna*	EC_{50} >10000	11.28	> 887	100
	Invertebrate, acute *Americamysis bahia*	LC_{50} >1009	11.28	**> 89.5**	100
JAU 6476-S-methyl	Fish, acute *Oncorhynchus mykiss*	LC_{50} 1790	1.372	1305	100
	Invertebrate, acute *Daphnia magna*	EC_{50} 2800	1.372	2041	100
1,2,4-Triazole	Fish, acute *Oncorhynchus mykiss*	LC_{50} 498000	0.445	1119101	100
	Invertebrate, acute *Daphnia magna*	EC_{50} >100000	0.445	> 224719	100
JAU 6476-thiazocine	Fish, acute *Oncorhynchus mykiss*	LC_{50} 1830*	0.655	2794	100
	Invertebrate, acute *Daphnia magna*	EC_{50} 1300*	0.655	1985	100
JAU 6476-triazolylketone	Fish, acute *Oncorhynchus mykiss*	LC_{50} >100000	0.284	> 352113	100
	Invertebrate, acute *Daphnia magna*	EC_{50} >100000	0.284	> 352113	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)

Bold values do not meet the trigger

Table CP 10.2- 7: $RAC_{sw;\ ac}$ calculations based on FOCUS Step 2 (PEC values based on worst-case GAP 2 × 187.5 g a.s./ha) (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	1.724	0.09
	Invertebrate, acute *Daphnia magna*	EC_{50} 1300	13.0	1.724	0.13
	Invertebrate, acute *Americamysis bahia*	EC_{50} 2400	24.0	1.724	0.07
JAU 6476-desthio	Fish, acute *Oncorhynchus mykiss*	LC_{50} 6630	66.3	11.28*	0.17
	Invertebrate, acute *Daphnia magna*	EC_{50} >10000	>100	11.28	<0.11
	Invertebrate, acute *Americamysis bahia*	LC_{50} >1009	>10.09	11.28	**1.12**
JAU 6476-S-methyl	Fish, acute *Oncorhynchus mykiss*	LC_{50} 1790	17.9	1.372	0.08
	Invertebrate, acute *Daphnia magna*	EC_{50} 2800	28.0	1.372	0.05
1,2,4-Triazole	Fish, acute *Oncorhynchus mykiss*	LC_{50} 498000	4980	0.445	0.0001
	Invertebrate, acute *Daphnia magna*	EC_{50} >100000	>1000	0.445	< 0.0004
JAU 6476-thiazocinc	Fish, acute *Oncorhynchus mykiss*	LC_{50} 1830*	18.3	0.655	0.04
	Invertebrate, acute *Daphnia magna*	EC_{50} 1300*	13.0	0.655	0.05
JAU 6476-triazolylketone	Fish, acute *Oncorhynchus mykiss*	LC_{50} >100000	>1000	0.284	< 0.0003
	Invertebrate, acute *Daphnia magna*	EC_{50} >100000	>1000	0.284	< 0.0003

* 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 acute trigger was not met for the invertebrate *A. bahia* and a refined risk assessment is therefore required. The consideration of the more realistic FOCUS STEP 3 surface water concentrations is presented below.

Table CP 10.2- 8: TER_A calculations for winter and spring cereals based on FOCUS Step 3

Test species	Endpoint [µg/L]	$PEC_{sw, max}$ [µg/L]	FOCUS scenario (risk envelope)	TER_A	Trigger
JAU 6476-desthio, 2 × 187.5 g a.s./ha					
Invertebrate, acute *Americamysis bahia*	LC_{50} >1009	0.998	Winter cereals R4 (stream)	> 1011	100
		0.612	Spring cereals, R4 (stream)	> 1649	100
JAU 6476-desthio, 2 × 150 g a.s./ha					
Invertebrate, acute *Americamysis bahia*	LC_{50} >1009	1.065	Winter cereals R4 (stream)	> 947	100
		1.286	Spring cereals, R4 (stream)	> 785	100

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

Test species	Endpoint [µg/L]	$RAC_{sw; ac}$ (LC_{50}/100)	$PEC_{(sw, max}$ [µg/L]	FOCUS scenario (risk envelope)	PEC/RAC
JAU-6476-desthio, 2 × 187.5 g a.s./ha					
Invertebrate, acute *Americamysis bahia*	LC_{50} >1009	>10.09	0.998	Winter cereals R4 (stream)	< 0.10
			0.612	Spring cereals, R4 (stream)	< 0.06
JAU-6476-desthio, 2 × 150 g a.s./ha					
Invertebrate, acute *Americamysis bahia*	LC_{50} >1009	>10.09	1.065	Winter cereals R4 (stream)	< 0.11
			1.286	Spring cereals, R4 (stream)	< 0.13

The trigger is met for all evaluated scenarios. Consequently, a safe use can be assumed according to the proposed GAP.

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)[1]), 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] and [redacted] 2015, M-536695-01-1, KCP 10.2/01). This EFSA stepwise approach was placed in a stand-alone document because, as this approach is new, there is currently no agreed

[1] 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).

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 & 4 PEC values were used as refinement until a safe use of each intended application could be assumed.

Table CP 10.2- 10: TER_{LT} calculations for winter and spring cereals based on FOCUS Step 2 (PEC values based on worst-case GAP 2 × 187.5 g a.s./ha)

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	1.724	284	10
	Invertebrate, chronic *Daphnia magna*	NOEC	560	1.724	325	10
	Sediment dweller, chronic *Chironomus riparius*	NOEC	9140	1.724	5302	10
	Marine diatom, chronic *Skeletonema costatum*	E_rC_{50}	46	1.724	27	10
	Aquatic plant, chronic *Lemna gibba*	E_rC_{50}	> 404	1.724	> 234	10
JAU 6476-desthio	Fish, early life stage *Oncorhynchus mykiss*	NOEC	3.34	11.28	**0.3**	10
	Invertebrate, chronic *Daphnia magna*	NOEC	100	11.28	**8.9**	10
	Invertebrate, chronic *Americamysis bahia*	NOEC	64	11.28	**5.7**	10
	Sediment dweller, chronic *Chironomus riparius*	NOEC	2000	11.28	177	10
	Green alga, chronic *Scenedesmus subspicatus*	E_rC_{50}	550	11.28	49	10
	Aquatic plant, chronic *Lemna gibba*	E_rC_{50}	80.9	11.28	**7.2**	10
JAU 6476-S-methyl	Sediment dweller, chronic *Chironomus riparius*	NOEC	100	1.372	73	10
	Green alga, chronic *Pseudokirchneriella subcapitata*	E_rC_{50}	47400	1.372	34548	10
1,2,4-Triazole	Fish, juvenile growth *Oncorhynchus mykiss*	NOEC	3200	0.445	7191	10
	Green alga, chronic *Pseudokirchneriella subcapitata*	E_rC_{50}	> 31000	0.445	> 69663	10

Compound	Test species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	TER_{LT}	Trigger
JAU 6476-thiazocine	Fish, early life stage *Oncorhynchus mykiss*	NOEC	490*	0.655	748	10
	Invertebrate, chronic *Daphnia magna*	NOEC	560*	0.655	855	10
	Green alga, chronic *Pseudokirchneriella subcapitata*	E_rC_{50}	2180*	0.655	3328	10
JAU 6476-triazolylketone	Green alga, chronic *Pseudokirchneriella subcapitata*	E_rC_{50}	> 100000	0.284	> 352113	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- 11: $RAC_{sw, ch}$ calculations for winter and spring cereals based on FOCUS Step 2 (PEC values based on worst-case GAP 2 × 187.5 g a.s./ha) (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	1.724	0.04
	Invertebrate, chronic *Daphnia magna*	NOEC 560	56.0	1.724	0.03
	Sediment dweller, chronic *Chironomus riparius*	NOEC 9140	914	1.724	0.002
	Marine diatom, chronic *Skeletonema costatum*	E_rC_{50} 46	4.6	1.724	0.37
	Aquatic plant, chronic *Lemna gibba*	E_rC_{50} > 404	> 40.4	1.724	< 0.04
JAU 6476-desthio	Fish, early life stage *Oncorhynchus mykiss*	NOEC 3.34	0.334	11.28	**33.77**
	Invertebrate, chronic *Daphnia magna*	NOEC 100	10.0	11.28	**1.13**
	Invertebrate, chronic *Americamysis bahia*	NOEC 64	6.4	11.28	**1.76**
	Sediment dweller, chronic *Chironomus riparius*	NOEC 2000	200	11.28	0.06
	Green alga, chronic *Scenedesmus subspicatus*	E_rC_{50} 550	55.0	11.28	0.21
	Aquatic plant, chronic *Lemna gibba*	E_rC_{50} 80.9	8.09	11.28	**1.39**
JAU 6476-S-methyl	Sediment dweller, chronic *Chironomus riparius*	NOEC 100	10.0	1.372	0.14
	Green alga, chronic *Pseudokirchneriella subcapitata*	E_rC_{50} 47400	4740	1.372	0.0003
1,2,4-Triazole	Fish, juvenile growth *Oncorhynchus mykiss*	NOEC 3200	320	0.445	0.001
	Green alga, chronic *Pseudokirchneriella subcapitata*	E_rC_{50} >31000	> 3100	0.445	< 0.0001
JAU 6476-thiazocine	Fish, early life stage *Oncorhynchus mykiss*	NOEC 490*	49.0	0.655	0.01
	Invertebrate, chronic *Daphnia magna*	NOEC 560*	56.0	0.655	0.01
	Green alga, chronic *Pseudokirchneriella subcapitata*	E_rC_{50} 2180*	218	0.655	0.003
JAU 6476-triazolylketone	Green alga, chronic *Pseudokirchneriella subcapitata*	E_rC_{50} > 100000	> 10000	0.284	< 0.00003

* 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, invertebrates and aquatic plants. 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- 12: 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 (risk envelope)	TER_{LT}	Trigger
JAU 6476-desthio, winter cereals 2 × 187.5 g a.s./ha					
Fish, early life stage *Oncorhynchus mykiss*	NOEC 3.34	0.998	Winter cereals, R4 (stream)	**3.3**	10
		0.640	Winter cereals R1 (stream)	**5.2**	10
		0.358	Winter cereals, R3 (stream)	**9.3**	10
		0.139	Winter cereals, D2 (ditch)	24	10
		0.612	Spring cereals, R4 (stream)	**5.5**	10
		0.239	Spring cereals, D1 (ditch)	14	10
Invertebrate, chronic *Daphnia magna*	NOEC 100	0.998	Winter cereals, R4 (stream)	100	10
		0.612	Spring cereals, R4 (stream)	163	10
Invertebrate, chronic *Americamysis bahia*	NOEC 64	0.998	Winter cereals, R4 (stream)	64	10
		0.612	Spring cereals, R4 (stream)	105	10
Aquatic plant, chronic *Lemna gibba*	E_rC_{50} 80.9	0.998	Winter cereals, R4 (stream)	81	10
		0.612	Spring cereals, R4 (stream)	132	10
JAU 6476-desthio, 2 × 150 g a.s./ha					
Fish, early life stage *Oncorhynchus mykiss*	NOEC 3.34	1.065	Winter cereals, R4 (stream)	**3.1**	10
		0.464	Winter cereals, R1 (stream)	**7.2**	10
		0.275	Winter cereals, R3 (stream)	12.1	10
		1.286	Spring cereals, R4 (stream)	**2.6**	10
		0.191	Spring cereals, D1 (ditch)	17	10
Invertebrate, chronic *Daphnia magna*	NOEC 100	1.065	Winter cereals, R4 (stream)	94	10
		1.286	Spring cereals, R4 (stream)	78	10

Test species	Endpoint [µg/L]	$PEC_{sw, max}$ [µg/L]	FOCUS scenario (risk envelope)	TER_{LT}	Trigger
Invertebrate, chronic *Americamysis bahia*	NOEC 64	1.065	Winter cereals, R4 (stream)	60	10
		1.286	Spring cereals, R4 (stream)	50	10
Aquatic plant, chronic *Lemna gibba*	E_rC_{50} 80.9	1.065	Winter cereals, R4 (stream)	76	10
		1.286	Spring cereals, R4 (stream)	63	10

Bold values do not meet the trigger

Table CP 10.2- 13: $RAC_{sw; ch}$ 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 (Risk envelope)	PEC/RAC
JAU 6476-desthio, 2 × 187.5 g a.s./ha					
Fish, early life stage *Oncorhynchus mykiss*	NOEC 3.34	0.334	0.998	Winter cereals, R4 (stream)	**2.99**
			0.640	Winter cereals R1 (stream)	**1.92**
			0.358	Winter cereals, R3 (stream)	**1.07**
			0.139	Winter cereals, D2 (ditch)	0.42
			0.612	Spring cereals, R4 (stream)	**1.83**
			0.239	Spring cereals, D1 (ditch)	0.72
Invertebrate, chronic *Daphnia magna*	NOEC 100	10	0.998	Winter cereals, R4 (stream)	0.10
			0.612	Spring cereals, R4 (stream)	0.06
Invertebrate, chronic *Americamysis bahia*	NOEC 64	6.4	0.998	Winter cereals, R4 (stream)	0.16
			0.612	Spring cereals, R4 (stream)	0.10
Aquatic plant, chronic *Lemna gibba*	E_rC_{50} 80.9	8.09	0.998	Winter cereals, R4 (stream)	0.12
			0.612	Spring cereals, R4 (stream)	0.08

Test species	Endpoint [µg/L]	$RAC_{sw;\ ch}$ (NOEC/10) (E_rC_{50}/10)	$PEC_{sw,\ max}$ [µg/L]	FOCUS scenario (Risk envelope)	PEC/RAC
JAU-6476-desthio, 2 × 150 g a.s./ha					
Fish, early life stage *Oncorhynchus mykiss*	NOEC 3.34	0.334	1.065	Winter cereals, R4 (stream)	**3.19**
			0.464	Winter cereals, R1 (stream)	**1.39**
			0.275	Winter cereals, R3 (stream)	0.82
			1.286	Spring cereals, R4 (stream)	**3.85**
			0.191	Spring cereals, D1 (ditch)	0.57
Invertebrate, chronic *Daphnia magna*	NOEC 100	10	1.065	Winter cereals, R4 (stream)	0.11
			1.286	Spring cereals, R4 (stream)	0.13
Invertebrate, chronic *Americamysis bahia*	NOEC 64	6.4	1.065	Winter cereals, R4 (stream)	0.17
			1.286	Spring cereals, R4 (stream)	0.20
Aquatic plant, chronic *Lemna gibba*	E_rC_{50} 80.9	8.09	1.065	Winter cereals, R4 (stream)	0.13
			1.286	Spring cereals, R4 (stream)	0.16

The trigger is not met for chronic fish for some evaluated scenarios regarding the FOCUS STEP 3 risk assessment. Consequently, further refinement is needed. A refined risk assessment for this organism and scenario based on FOCUS STEP 4 calculations is presented below.

Table CP 10.2- 14: TER_{LT} calculations for fish (long-term) based on FOCUS Step 4 including mitigation measures

Test species	Endpoint [µg/L]	$PEC_{sw, max}$ [µg/L]	FOCUS scenario (Risk envelope)	TER_{LT}	Trigger
JAU 6476-desthio, 2 × 187.5 g a.s./ha					
10 m buffer zone, 0% drift reduction					
Fish, early life stage *Oncorhynchus mykiss*	NOEC 3.34	0.455	Winter cereals, R4 (stream)	**7.3**	10
		0.291	Winter cereals R1 (stream)	11.5	10
		0.278	Spring cereals, R4 (stream)	12.0	10
20 m buffer zone, 0% drift reduction					
Fish, early life stage *Oncorhynchus mykiss*	NOEC 3.34	0.239	Winter cereals, R4 (stream)	14.0	10
JAU 6476-desthio, 2 × 150 g a.s./ha					
10 m buffer zone, 0% drift reduction					
Fish, early life stage *Oncorhynchus mykiss*	NOEC 3.34	0.470	Winter cereals, R4 (stream)	**7.1**	10
		0.211	Winter cereals R1 (stream)	16	10
		0.585	Spring cereals, R4 (stream)	**5.7**	10
20 m buffer zone, 0% drift reduction					
Fish, early life stage *Oncorhynchus mykiss*	NOEC 3.34	0.244	Winter cereals, R4 (stream)	13.7	10
		0.306	Spring cereals, R4 (stream)	10.9	10

Bold values do not meet the trigger

Table CP 10.2- 15: $RAC_{sw;\ ch}$ calculations for fish (long-term) based on FOCUS Step 4 including mitigation measures (acceptability of risk: PEC/RAC < 1)

Test species	Endpoint [µg/L]	$RAC_{sw;\ ch}$ (NOEC/10)	$PEC_{sw,\ max}$ [µg/L]	FOCUS scenario (Risk envelope)	PEC/RAC
JAU 6476-desthio, 2 × 187.5 g a.s./ha, winter & spring cereals					
10 m buffer zone, 0% drift reduction					
Fish, early life stage *Oncorhynchus mykiss*	NOEC 3.34	0.334	0.455	Winter cereals, R4 (stream)	**1.36**
			0.291	Winter cereals, R1 (stream)	0.87
			0.278	Spring cereals, R4 (stream)	0.83
20 m buffer zone, 0% drift reduction					
Fish, early life stage *Oncorhynchus mykiss*	NOEC 3.34	0.334	0.239	Winter cereals, R4 (stream)	0.72
JAU 6476-desthio, 2 × 150 g a.s./ha, winter & spring cereals					
10 m buffer zone, 0% drift reduction					
Fish, early life stage *Oncorhynchus mykiss*	NOEC 3.34	0.334	0.470	Winter cereals, R4 (stream)	**1.41**
			0.211	Winter cereals R1 (stream)	0.63
			0.585	Spring cereals, R4 (stream)	**1.75**
20 m buffer zone, 0% drift reduction					
Fish, early life stage *Oncorhynchus mykiss*	NOEC 3.34	0.334	0.244	Winter cereals, R4 (stream)	0.73
			0.306	Spring cereals, R4 (stream)	0.92

The TER trigger is exceeded considering 20 m buffer without drift reduction in winter cereals at an application rate of 2 × 187.5 g a.s./ha. For the same application rate in spring cereals, a buffer zone of 10 m without drift reduction is sufficient to assume a safe use of the product.
Regarding the lower application rate of 2 × 150 g a.s./ha in spring and winter cereals, a safe use can be predicted considering 20 m drift buffer without drift reduction.

Stepwise approach (EFSA AGD 2013)

Report:	KCP 10.2/01 [redacted]; [redacted]; 2015; M-536695-01-1
Title:	Stepwise approach for the risk assessment of major aquatic metabolites of prothioconazole (formulated as bixafen + prothioconazole EC 225 (75 + 150 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-536695-01-1
Document No.:	M-536695-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**no**

The EFSA AGD (2013) stepwise approach was used for all metabolites where chronic data are 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

A chronic risk assessment of all major aquatic metabolites of prothioconazole was provided, addressing the risk 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 stepwise approach described in the EFSA AGD (2013) (see [redacted]; [redacted]; 2015; M-536695-01-1, KCP 10.2/01). 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, for the proposed GAP a safe use can be concluded.

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

New studies for the representative formulation are summarized below. No additional acute toxicity studies are required.

Report:	KCP 10.2/1-01 [redacted]; 2007; M-293311-02-1
Title:	Acute toxicity of bixafen + prothioconazole EC 225 (75+150) G to fish (Oncorhynchus mykiss) under static conditions
Report No.:	EBDRP048
Document No.:	M-293311-02-1
Guideline(s):	EPA-FIFRA § 72-1/SEP-EPA-540/9-85-006 (1982/1985) OPPTS 850.1075 (Public Draft, 1996) Directive 92/69/EEC, C.1 (1992) OECD No. 203 (rev.1992)
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:

The aim of the study was to determine the acute toxicity of Bixafen + Prothioconazole EC 225 (75.+ 150) G to the Rainbow trout (*Oncorhynchus mykiss*), expressed as 96h-LC_{50} for mortality.

Material and methods:
Test item: Bixafen & Prothioconazole EC 225 (75+150) G, analyzed a.s. contents: Bixafen 7.7% and Prothioconazole 14.7%, Batch No. 2007-002622, TOX07852-00, Specification No. 102000013869.

The test was conducted according to the following guidelines: FIFRA 72-1, OPPTS 850.1075 and OECD 203 and the Directive 92/69/EEC, C.1. Rainbow trout (*Oncorhynchus mykiss*) was used as test species, with a mean body length 4.3 cm, a mean body weight 0.7 g. The biomass loading during testing was 0.18 g fish/ L test medium. Ten fish in each test level were exposed for 96 h under static conditions to nominal concentrations of 0, 0.313, 0.625, 1.25, 2.50 and 5.00 mg prod./L. Dissolved oxygen concentrations ranged from 91.4 to 98.4 % oxygen saturation, the pH values ranged from 6.8 to 7.2 and the water temperature ranged from 11.6°C to 12.3°C in all aquaria over the whole testing period. In order to confirm that nominal concentrations of the formulation were actually reached, common practice is to monitor the concentration of one of the active ingredient that compose the formulation. In the present study, bixafen was chosen as the reference active ingredient. Water samples were analyzed in all test levels after 0 h, on day 2 and on day 4 of the exposure period. In the event that 100% mortality was observed in test concentrations prior to the end of the test, the analytical determinations were made at those times.
Daily observations were made for mortality and sublethal effects.

Findings:
Validity criteria:
The test conditions met all validity criteria, given by the mentioned guidelines: less than 5% mortality within the 48-hour settling-in period, < 10% mortality in the control(s) and dissolved oxygen saturation > 60% throughout the test.

Analytical results:
The chemical analysis of bixafen revealed recoveries between 83% and 107% (mean) of nominal values over the entire test period of 96h. No contamination was detected in samples from untreated water control. As the toxicity has to be attributed to the tested formulation as a whole, all results submitted in this report are related to nominal test concentrations of the formulated product.

Biological results:
There were neither any sub-lethal effects nor any mortality in the control group. There was no behavioural effect on fish caused by the test item over the whole exposure period in all test levels below 0.625 mg prod./L. At 0.625 mg prod./L fish showed the following symptoms after 96h: they remained for unusually long periods on the bottom of the aquarium and had turn dark in coloration. The highest concentration without sublethal effects is considered to be 0.313 mg prod./L. Furthermore, mortality was observed after 96h in all test levels above 1.25 mg prod./L as shown below:

Table CP 10.2.1- 1: Cumulative mortality of Rainbow trout was observed as follows (with a total number of 10 fish tested in each test level)

Exposure time	4 h		24 h		48 h		72 h		**96 h**	
Test level mg prod./L	no. of dead	% dead	no. of dead	% dead	no. of dead	% dead	no. of dead	% dead	**no. of dead**	**% dead**
control	0	0	0	0	0	0	0	0	**0**	**0**
0.313	0	0	0	0	0	0	0	0	**0**	**0**
0.625	0	0	0	0	0	0	0	0	**0**	**0**
1.25	0	0	0	0	0	0	1	10	**1**	**10**
2.50	1	10	10	100	10	100	10	100	**10**	**100**
5.00	6	60	10	100	10	100	10	100	**10**	**100**

no. = number

Based on nominal concentrations, the 96h LC_{50} was estimated (by probit analysis) to be 1.55 mg prod./L (C.I. 95%: 1.29 - 1.87 mg / L). The minimum concentration causing 100% mortality (96 h) was 2.50 mg prod./L. The maximum concentration which did not cause any mortality (no-observed-effect concentration = NOEC) after 96h was 0.625 mg prod./L.

Conclusions:
Based on nominal concentrations, the 96h-LC_{50} of the tested formulation (Bixafen + Prothioconazole EC 75 + 150) G for the Rainbow trout (*Oncorhynchus mykiss*) was 1.55 mg prod./L (C.I. 95%: 1.29 - 1.87 mg prod./L).

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Report: KCP 10.2.1/02 [redacted]; 2007; M-288432-01-1
Title: Acute toxicity of BYF 00587 & Prothioconazole EC 075+150 to the waterflea Daphnia magna in a static laboratory test system
Report No.: EBDRP049
Document No.: M-288432-01-1
Guideline(s): OECD guideline 202,(2004); EEC Directive 92/69/EWG, part C.2 (1992); U.S. EPA Pesticide Assessment Guidelines, 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 objective of the study was a determination of possible effects of Bixafen + Prothioconazole EC 225 (75+150) G on the mobility of *Daphnia magna* after 48 hours of exposure in a static laboratory test system, expressed as EC_{50} for immobilisation.

Material and methods:
Test item: Bixafen & Prothioconazole EC 225 (75+150) G, analyzed a.s. contents: Bixafen 7.49% and Prothioconazole 14.8%, Batch No. 2006-001178, TOX07660-00, Specification No.: 102000013869.

The test was conducted according to the following guidelines: US EPA Pesticide Assessment Guidelines 72-2, OPPTS 850.1010, JMAFF 12 Nousan No. 8147, OECD 202 and EEC Directive 92/69/EWG, part C.2. *Daphnia magna* (1st instars <24 h old) were exposed in a static test system for 48 hours to nominal concentrations of 0**,** 1, 2, 4, 8 and 16 mg prod./L. In order to confirm that nominal concentrations of the formulation were actually reached, common practice is to monitor the concentration of one of the active

ingredient that compose the formulation. In the present study, bixafen was chosen as the reference active ingredient. Water samples were analyzed in all test levels after 0h and 48h of exposure.
The test vessels consisted of chemically clean 100 ml glass beakers filled with 50 ml of the test solution. Six vessels (replicates), each provided with five daphnids were used per treatment group and control (=30 animals per study group). The water fleas were not fed and 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. Additionally all possible signs of sublethal effects were recorded.
For water quality monitoring, temperature, pH values and O_2 concentrations of the test solutions, as well as conductivity, hardness and alkalinity of the used dilution water were controlled during the course of the study. The measured values for these physical chemical parameters met the required range and yielded no deviation from guideline recommendations.

Findings:

Validity criteria:

The test conditions met all validity criteria, given by the mentioned guidelines: ≤ 10.0% mortality in the control(s).

Analytical results:

The chemical analysis of Bixafen in the freshly prepared test solutions at test initiation revealed concentrations between 103% and 107% (mean: 105%) of the corresponding nominal concentrations. The corresponding concentrations of the aged test solutions at the end of the 48 hours exposure period ranged between 92% and 103% (mean: 101%) of nominal. These data confirm that targeted nominal concentrations were met. No contamination was detected in samples from untreated water control. As the toxicity has to be attributed to the tested formulation as a whole, all results are related to nominal test concentrations of the formulated product.

Biological results:

No immobilities or other effects on behaviour occurred in untreated control within 48 hours of exposure. Immobilisation of some water fleas was observed in test levels equal or higher than 2 mg prod./L, as shown below:

Table CP 10.2.1- 2: Toxicity to *Daphnia magna* (based on nominal concentrations)

Nominal Concentrations mg formulation/L	Exposed Daphnids (=100%)	Immobilised Daphnids			
		24 h		48 h	
		no	%	no	%
Control	30	0	0	0	0
1.0	30	0	0	0	0
2.0	30	1	3.3	2	6.7
4.0	30	5	16.7	28	93.3
8.0	30	11	36.7	29	96.7
16.0	30	29	96.7	30	100

no = number of immobilised animals

The EC_{50} for immobilisation after 24 and 48 hours of static exposure were determined using probit analysis based on nominal concentrations. The following results were found:

Table CP 10.2.1- 3: Statistical results of probit analysis conducted for determination of EC_{50} values

Probit analysis for data obtained after	EC_{50} mg formulation/L nominally	lower 95% CI mg formulation/L nominally	upper 95% CI mg formulation/L nominally
24 hours	7.7	n.d.	n.d.
48 hours	3.0	n.d.	n.d.

n.d.= not determined due to mathematical reasons

Conclusions:
Based on nominal concentrations, the 48h EC_{50} of the tested formulation (Bixafen + Prothioconazole EC 225 (75 + 150) G for *Daphnia magna* was 3.0 mg prod./L.

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Report:	KCP 10.2.1/03 [redacted]., 2007; M-289495-01-1
Title:	Pseudokirchneriella subcapitata growth inhibition test with bixafen & prothioconazole EC 225 (75 + 150) G
Report No.:	EBDRP050
Document No.:	M-289495-01-1
Guideline(s):	OECD Guideline 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test (March 23, 2006)
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:
The aim of the study was to determine the influence of Bixafen + Prothioconazole EC 225 (75+150) G on the exponentially growing green alga *Pseudokirchneriella subcapitata* expressed as NOEC, LOEC and EC_x for growth rate of algal biomass (cells per volume).

Material and methods:
Test item: Bixafen + Prothioconazole EC 225 (75 + 150) G; analysed a.s. contents: Bixafen: 7.49% and Prothioconazole: 14.8%, Batch No. 2006-001178, TOX07660-00, Specification No. 102000013869.

The test was conducting according to the OECD Guideline 201. *Pseudokirchneriella subcapitata* (freshwater microalgae, formerly known as *Selenastrum capricornutum*) was exposed in a chronic multigeneration test for 3 days under static exposure conditions to the nominal concentrations of 0, 0.162, 0.404, 1.01, 2.53 and 6.32 mg prod./L in comparison to a negative control. In order to confirm that nominal concentrations of the formulation were actually reached, common practice is to monitor the concentration of one of the active ingredient that compose the formulation. In the present study, Bixafen was chosen as the reference active ingredient. Concentrations in Bixafen were measured in all treatment groups and in the control on day 0 and day 3 of the exposure period.
The pH values ranged from 7.9 to 8.1 in the controls and the incubation temperature ranged from 21.7°C to 22.2°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 6956 lux.
The number of algal cell per volume was monitored daily by direct counting of algae cells per volume or indirect by calculation of cell numbers after measurement of optical cell density, and used as response parameter in order to determine the growth rate of algal biomass.

Findings:

Validity criteria:

All validity criteria given by the mentioned guideline were met: the biomass in the control cultures had increased exponentially by a factor of at least 16 within the 72-hour test period, the mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3) in the controls did not exceed 35%, and the coefficient of variation of average specific growth rates during the whole test period between the control replicates did not exceed 7%.

Analytical results:

The analytical findings showed that Bixafen concentrations in the treatment levels on day 0 were between 94 and 100% of nominal (average 98.2%). On day 3: 93 to 101% of nominal concentrations (average 98.4%) were found. No contamination was detected in samples from untreated water control. As the toxicity has to be attributed to the tested formulation as a whole, all results are related to nominal test concentrations of the formulated product.

Biological results:

The static algae growth inhibition test provided the following tabulated results after 72 hours:

Table CP 10.2.1- 4: Effects of the static 72 hour algae growth inhibition test

Nominal Concentration [mg formulation/L]	Cell Number after 72 h (means) per mL*	(0-72 h)-Average Specific Growth Rates [days^{-1}]	Inhibition of Average Specific Growth Rate [%]	Doubling time of algae cells [days]
control	364000	1.197	-	0.579
0.162	289000	1.115	6.9	0.622
0.404	183000	0.969	19.1	0.715
1.01	77000	0.681	43.2	1.02
2.53	34000	0.409	65.9	1.69
6.32	24000	0.298	75.1	2.33

* test initiation with 10,000 cells/mL

Based on these results, the 72h-E_rC_{50} was estimated (using probit analysis) to be 1.52 mg prod./L. (C.I. 95%: 1.07-2.21 mg prod./L). The NOE_rC was < 0.162 mg prod./L and the LOE_rC was ≤0.162 mg prod./L.

Conclusions:

The 72h-E_rC_{50} of the tested formulation (Bixafen + Prothioconazole EC 75 + 150) G for the green alga *Pseudokirchneriella subcapitata* was 1.52 mg prod./L (95% C.I.: 1.07-2.21 mg prod./L), based on nominal concentrations.

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

No further testing of the formulation is available or required.

CP 10.2.3 Further testing on aquatic organisms

No further testing of the formulation is available or required.

CP 10.3 Effects on arthropods

CP 10.3.1 Effects on bees

Risk assessment for 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 Bixafen + Prothioconazole EC 225,
- 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 Bixafen + Prothioconazole EC 225 is presented in this MCP document.

A summary of the critical endpoints of prothioconazole, its metabolite JAU 6476-desthio and the formulated product Bixafen + Prothioconazole EC 225 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 Bixafen + Prothioconazole EC 225 – acute toxicity to adult bees

Test substance	Test species	Endpoint	Reference
Prothioconazole	Honey bee (oral 48 h) Honey bee (contact 48 h)	LD_{50} **> 105.1 µg a.s./bee** LD_{50} >100.0 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 (oral 48 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**	
Bixafen + Prothioconazole EC 225	Honey bee (oral 48 h)	LD_{50} **> 217.6 µg prod./bee**	[redacted] (2015) M-510508-01-1 KCP 10.3.1.1.1/01 KCP 10.3.1.1.2/01
	Honey bee (contact 48 h)	LD_{50} **> 200.0 µg prod./bee**	

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

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 feeding) (adults)	LC_{50} > 100 mg a.s./kg LDD_{50} >3.8 µg a.s./bee/day NOEC 100 mg a.s./kg NOEDD 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 187.5 g a.s./ha and 1.25 L Bixafen + Prothioconazole EC 225/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.

Hazard Quotient, oral:

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

Hazard Quotient, contact:

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

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

Test substance	Crop	$LD_{50, oral}$ [µg/bee]	Application rate [g/ha]	Hazard quotient Q_{HO}	Trigger
Bixafen + Prothioconazole EC 225	Cereals	> 217.6	1255 [A]	< 5.8	50
Prothioconazole	Cereals	>105.1	187.5	< 1.8	50
JAU 6476-desthio	Cereals	> 106.5	187.5 [B]	< 1.8	50

[A] Based on a product density of 1.004 g/mL and 1.25 L product/ha

[B] 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	$LD_{50, contact}$ [µg/bee]	Application rate [g/ha]	Hazard quotient Q_{HC}	Trigger
Bixafen + Prothioconazole EC 225	Cereals	> 200	1255 [A]	< 6.3	50
Prothioconazole	Cereals	> 200	187.5	< 0.9	50
JAU 6476-desthio	Cereals	> 100	187.5 [B]	< 1.9	50

[A] Based on a product density of 1.004 g/mL and 1.25 L product/ha

[B] 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 ([redacted], 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 pupae 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 in cereals at the maximum application rate of 187.5 g a.s./ha, as foreseen for the use of Bixafen + Prothioconazole EC 225, 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 10.3.1.1.2/01 [redacted].; 2015; M-510508-01-1
Title:	Effects of bixafen + prothioconazole EC 225 (75+150) G (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory
Report No.:	89711035
Document No.:	M-510508-01-1
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 bixafen + prothioconazole EC 225 (75+150) 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:

Test item: Bixafen + Prothioconazole EC 225 (75+150) G, analyzed a.s. contents: Bixafen (BYF 00587): 7.52% w/w, 75.50 g/L, prothioconazole (JAU 6476): 14.8% w/w, 148.6 g/L; Batch No. ECE2101898; TOX10490-00; Specification No.: 102000013869; Density: 1.004 g/mL (20 °C).
Reference item: Dimethoate 400.9 g/L (analytical)

Under laboratory conditions *Apis mellifera* 50 worker bees were exposed for 72 hours to a single dose of 200.0 µg product per bee by topical application (contact limit test, 5 µL droplet on the dorsal thorax) and 50 worker bees were exposed for 48 hours to a single dose of 217.6 µg product per bee by feeding (oral limit test, value based on the actual intake of the test item, 50% w/v sucrose solution).

Findings:

Validity criteria:

All validity criteria were met as presented in the table below:

Table CP 10.3.1.1.1- 1: Validity criteria

Validity criteria	Recommended	Obtained
Control Mortality	≤ 10%	0%
LD_{50} of Reference Item	Contact test (24 h): 0.10-0.30 µg a.s./bee Oral test (24 h): 0.10-0.35 µg a.s./bee	Contact test (24 h): 0.19 µg a.s./bee Oral test (24 h): 0.14 µg a.s./bee

Biological results:

Contact Test:

Enduring behavioural abnormalities during the 48 hrs assessment led to prolongation of the contact test for further 24 hours up to 72 hours in order to detect possible late-onset effects of the test item, although this is strictly seen not a guideline requirement. As mortality was not increasing between 48 and 72 hours a late-onset effect on the test item could be excluded.

During the 4 hours assessment 40 out of the 50 test item treated bees showed moving coordination problems. A few bees were found to be affected during the 24, 48 and 72 hours assessments, respectively. Beside this, some bees in 2-3 of the 5 replicates showed an unusual position of the wings during the assessments.

At the end of the contact toxicity test (72 hours after application), there was 8.0% mortality in the 200.0 µg product/bee treatment group. No mortality occurred in the control group (water + 0.5% Adhäsit).

Oral Test:

In the oral toxicity test, the maximum nominal test level of bixafen + prothioconazole EC 225 (75+150) G (i.e. 200 µg product/bee) corresponded to an actual intake of 217.6 µg product/bee. No mortality occurred in the 217.6 µg product/bee treatment group and in the control group (50% w/v sucrose solution = 500 g sucrose/L tap water), respectively. Four hours after application 6 out of 50 bees were apathetic, moribund or affected in the 217.6 µg product/bee treatment group. No further behavioural abnormalities were observed until the end of the oral toxicity test.

Table CP 10.3.1.1.1- 2: Toxicity to Honey Bees, laboratory tests

Test Item	Bixafen + Prothioconazole EC 225 (75 + 150) G	
Test Species	*Apis mellifera*	
Exposure	**contact (solution in Adhäsit (0.5%)/water)**	**oral (50% w/v sucrose solution)**
Application dose [µg product/bee]	200.0	217.6
LD_{50} [µg product/bee]	> 200.0	> 217.6

Conclusion:

The toxicity of Bixafen + Prothioconazole EC 225 (75+150) G was tested in both, an acute contact and an acute oral toxicity test on honey bees.

The contact LD_{50} (48 + 72 h) was > 200.0 µg product/bee.

The oral LD_{50} (48 h) was > 217.6 µg product/bee.

CP 10.3.1.1.2 Acute contact toxicity to bees

Report:	KCP 10.3.1.1.2/01 [redacted],; 2015; M-510508-01-1
Title:	Effects of bixafen + prothioconazole EC 225 (75+150) G (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory
Report No.:	89711035
Document No.:	M-510508-01-1
Guideline(s):	GLP compliant study based on OECD 213 and 214 (1998)
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

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 Qomen *et al.* 1998 ([redacted], S.; [redacted]; 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 reported.

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

Toxicity tests on non-target arthropods have been performed with Bixafen + Prothioconazole EC 225 on the species *Aphidius rhopalosiphi*, *Typhlodromus pyri*, *Coccinella septempunctata* and *Chrysoperla carnea*.

Table CP 10.3.2- 1: Bixafen + Prothioconazole EC 225: Ecotoxicological endpoints for arthropods other than bees

Test species, Dossier-file-No. Reference	Tested Formulation, study type, exposure	Ecotoxicological Endpoint
Aphidius rhopalosiphi M-489147-01-1 Rep.No: 14 10 48 039 A [redacted], 2014 KCP 10.3.2.1/01	BIX + PTZ EC 225 Laboratory, glass plate 50.4 mL prod./ha 89.4 mL prod./ha 159 mL prod./ha 283 mL prod./ha 504 mL prod./ha	LR_{50} 98.1 mL prod./ha % Corr. Mortality [%] 18.4 50.0 80.5 100 100
Typhlodromus pyri M-489138-01-1 Rep.No: 14 10 48 040 A [redacted], 2014 KCP 10.3.2.1/02	BIX + PTZ EC 225 Laboratory, glass plate 40.2 mL prod./ha 71.5 mL prod./ha 127 mL prod./ha 227 mL prod./ha 402 mL prod./ha	LR_{50} >402 mL prod./ha Corr. Mortality [%] 1.0 0 7.1 16 21.4
Aphidius rhopalosiphi M-282592-02-1 Rep.No: 31204002 [redacted], 2007 KCP 10.3.2.2/01	BIX + PTZ EC 225 Extended Lab., exposure on potted barley plants 40.3 mL prod./ha 139 mL prod./ha 417 mL prod./ha 1250 mL prod./ha 3750 mL prod./ha	LR_{50} 3485.16 mL prod./ha; ER_{50} >3750 mL prod./ha Corr. Mortality [%] 0 0 6.7 0 53.3 Effect on Reproduction [%] -4.5 [A] 19 29.7 13.4 31.9 Repellency rel. to control [%] -3 [B] 2.1 -4.2 [B] 5.5 25.4
Typhlodromus pyri M-280528-01-1 Rep.No: 31205062 [redacted], 2006 KCP 10.3.2.2/02	BIX + PTZ EC 225 Extended Lab., exposure on detached bean leaves 250 mL prod./ha 500 mL prod./ha 1000 mL prod./ha 2000 mL prod./ha 4000 mL prod./ha	LR_{50} 1296 mL prod./ha; ER_{50} >1000 mL prod./ha Corr. Mortality [%] 1.8 12.3 17.5 87.7 98.2 Effect on Reproduction [%] -35 [A] 35.9 31.3 n.a. n.a.
Coccinella septempunctata M-287283-01-1 Rep.No: 31206012 [redacted], 2007 KCP 10.3.2.2/03	BIX + PTZ EC 225 Extended Lab., exposure on detached bean leaves Control 417 mL prod./ha 722 mL prod./ha 1250 mL prod./ha 2165 mL prod./ha 3750 mL prod./ha	LR_{50} 3391 mL prod./ha; no effect on reproduction Corr. Mortality [%] - -10.3 [C] 6.9 0 27.6 55.2 Fertile Eggs/Female/Day 29.4 24.7 14.2 17 23.3 n.a.

Test species, Dossier-file-No. Reference	Tested Formulation, study type, exposure	Ecotoxicological Endpoint
Chrysoperla carnea M-290530-01-1 Rep.No: 31207047 [redacted], 2007 KCP 10.3.2.2/04	BIX + PTZ EC 225 Extended Lab., exposure on detached bean leaves Control 46.3 mL prod./ha 139 mL prod./ha 417 mL prod./ha 1250 mL prod./ha 3750 mL prod./ha	LR_{50} > 3750 mL prod./ha; no effect on reproduction Corr. Mortality [%] / Eggs/Female/Day / Hatching [%] - / 18.9 / 95.5 11.4 / 27.1 / 85.4 2.3 / 17 / 57.5 6.8 / 17.2 / 81.7 4.5 / 25 / 72.4 0 / 19 / 76.8
Typhlodromus pyri M-307529-01-1 Rep.No: 38631060 [redacted], 2008 KCP 10.3.2.3/01	BIX + PTZ EC 225 Aged residues on bean plants (2nd trial), 3 x 1250 mL prod./ha, interval 14 d residues aged for 0 d: residues aged for 7 d: residues aged for 14 d:	Corr. Mortality [%] / Effect on Reproduction [%] 39.8 / 31.8 32.2 / 27.7 21.2 / n.a.
Typhlodromus pyri M-534076-01-1 Rep.No. CW14/017 [redacted], 2015 KCP 10.3.2.3/02	BIX + PTZ EC 225 Aged residues on apple leaves, 2 x 1250 mL prod./ha, interval 14 d residues aged for 0 d: residues aged for 14 d:	Corr. Mortality [%] / Effect on Reproduction [%] 0.0 / -6.5[A] 6.0 / 13.2
Aphidius rhopalosiphi M-512453-01-1 Rep.No. CW14/018 [redacted], 2015 KCP 10.3.2.3/03	BIX + PTZ EC 225 Aged residues spray deposit on maize plants 2 x 1250 mL prod./ha, interval 14 d residues aged for 0 d: residues aged for 14 d:	Corr. Mortality [%] / Effect on Reproduction [%] / Repellency rel. to control [%] 3.3 / 15.0 / -10.6[B] -3.3[C] / -27.1[A] / -13.5[B]

[A]: A negative value indicates a higher reproduction rate in the treatment than in the control.
[B]: A negative value indicates a higher percentage of wasps found on plants in the treatment than in the control.
[C]: A negative value indicates a higher mortality rate in the control than in the treatment.
n.a.: not assessed

Risk assessment for other non-target arthropods

The risk assessment was performed according to Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002) and to the Guidance Document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods (ESCORT 2, Candolfi *et al*. 2000[2]).

Tier 1 risk assessment

The product BIX + PTZ EC 225 is applied at max. 2 × 1.25 L prod./ha in cereals (worst case use). In addition, a lower rate of 2 × 1.0 L prod./ha is also intended (see Table CP 10-1).

2 [redacted].: Guidance document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods; ESCORT 2 workshop (European Standard Characteristics Of Non-Target Arthropod Regulatory Testing), Wageningen, NL, March 21-23, 2000, SETAC Europe; SETAC publication August 2001

Table CP 10.3.2- 2: Tier 1 in-field risk assessment for non-target arthropods

Crop	Species	Appl. rate [mL prod./ha]	MAF	LR_{50} [mL prod./ha]	HQ	Trigger
Cereals	*T. pyri*	1250	1.7	98.1	21.7	2
	A. rhopalosiphi	1250	1.7	> 402	< 5.3	2
Cereals	*T. pyri*	1000	1.7	98.1	17.3	2
	A. rhopalosiphi	1000	1.7	> 402	< 4.2	2

The calculated HQ values for *Aphidius rhopalosiphi* and *Typhlodromus pyri* for the in-field scenario are above the trigger of concern (2) for both use rates (2 x 1.25 L prod./ha and 2 x 1.0 L prod./ha), indicating the need for a refined risk assessment for the in-field scenario which is presented below.

Table CP 10.3.2- 3: Tier 1 off-field risk assessment for non-target arthropods

Crop	Species	Appl. rate [mL prod./ha]	MAF	Drift [%]	VDF	Correction factor	LR_{50} [mL prod./ha]	HQ	Trigger
Cereals	*T. pyri*	1250	1.7	2.38	10	10	98.1	0.52	2
	A. rhopalosiphi	1250	1.7	2.38	10	10	> 402	< 0.13	2
Cereals	*T. pyri*	1000	1.7	2.38	10	10	98.1	0.41	2
	A. rhopalosiphi	1000	1.7	2.38	10	10	> 402	< 0.10	2

The calculated HQ values for *Aphidius rhopalosiphi* and *Typhlodromus pyri* for the off-field scenario are below the trigger of concern for both use rates, indicating an acceptable risk for non-target arthropods in off-field habitats.

Tier 2 in-field risk assessment

Potential exposure

The exposure scenario is based on the intended use pattern as given in Table CP 10-1.
The product BIX + PTZ EC 225 is applied at max. 2 × 1.25 L prod./ha (worst case use) in cereals. In addition, a lower rate of 2 × 1.0 L prod./ha is also intended.

The following equation was used to calculate the in-field PECs for the tier 2 risk assessment. The risk is considered acceptable if effects are < 50%.

PEC in-field: Application rate × MAF

- Application rate: 1.25 L product/ha (1.0 L prod./ha)
- MAF (multiple application factor) = 1.7 (leaf default, 2 applications; ESCORT 2)

Table CP 10.3.2- 4: Exposure calculation for in-field assessment

Crop / no. of appl.	Appl. rate [mL prod./ha]	MAF	in-field $PEC_{max.}$ [mL prod./ha]
Cereals / 2	1250	1.7	2125
Cereals / 2	1000	1.7	1700

In-field risk assessment

Table CP 10.3.2- 5: In-field risk assessment based on extended laboratory studies

Test species	in-field PEC_{max} [mL prod./ha]	$LR_{50, EXT}$; $ER_{50, EXT}$ [mL prod./ha]	Refined assessment required? (if effects > 50%)
Cereals, 2 × 1250 mL prod./ha, 14 d interval			
A. rhopalosiphi	2125	3485	No
T. pyri		> 1000	**Yes**
C. septempunctata		3391	No
C. carnea		> 2165	No
Cereals, 2 × 1000 mL prod./ha, 14 d interval			
A. rhopalosiphi	1700	3485	No
T. pyri		> 1000	**Yes**
C. septempunctata		3391	No
C. carnea		> 2165	No

The tier 2 in-field risk assessment for *A. rhopalosiphi*, *C. carnea* and *C. septempunctata* indicates that no unacceptable adverse effects are to be expected in the in-field area for arthropod species with a similar sensitivity as these species. The risk assessment for *T. pyri* indicates that initial effects in the in-field area cannot be excluded. Therefore, further refinement is needed for *T. pyri*.

Refined in-field risk assessment
Since the tier 2 risk assessment based on the extended laboratory study for *Typhlodromus pyri* indicated, that initial effects on non-target arthropods with a similar sensitivity like *Typhlodromus pyri* cannot be excluded, two aged residue studies were conducted with *Typhlodromus pyri* in order to demonstrate the potential for recovery.
In the first study conducted by [redacted] (2008, M-307529-01-1, KCP 10.3.2.3/01), bean plants were treated 3 times with 1.25 L product/ha with a spray interval of 14 days. Already in the first bioassay that was started on the day of the last application, the effects on mortality (corrected mortality: 39.8%) and reproduction (effect on reproduction: 31.8%) were below the trigger value of 50%. These results were confirmed by the second (corr. mortality: 32.2%, effect on reproduction: 27.7%) and third bioassay (corr. mortality: 21.2%, reproduction: not assessed).
A second aged residue study with *Typhlodromus pyri* was conducted by [redacted] (2015, M-534076-01-1, KCP 10.3.2.3/02) on potted apple seedlings which were sprayed 2 times with 1.25 L prod./ha at a 14 days interval. In line with the results of the first study (M-307529-01-1), already in the first bioassay that was started on the day of the last application no mortality occurred and no reduction in reproductive success was detected. These results were confirmed by the second bioassay that started 14 days later.
The results of the two aged residue studies indicate that under more realistic exposure conditions, no adverse effects >50% are to be expected from the use of the product according to the intended use pattern in cereals indicating an acceptable risk for non-target arthropods in the in-field habitat.
The results of the two aged residue studies for *Typhlodromus pyri* are fully in line with the results of the aged residues study for *Aphidius rhopalosiphi* ([redacted], 2015, M-512453-01-1, KCP 10.3.2.3/03) which indicated also no relevant adverse effects after an application of 2 × 1.25 L prod/ha at a 14 days interval.

Overall conclusion:

Considering the results of laboratory, extended laboratory and aged residue studies, it can be concluded that the application of the product BIX + PTZ EC 225 according to the intended use pattern in cereals will not pose unacceptable effects to non-target arthropods in the in-field or off-field habitat.

CP 10.3.2.1 Standard laboratory testing for non-target arthropods

Report:	KCP 10.3.2.1/01 [redacted], 2014; M-489147-01-1
Title:	Effects of prothioconazole + bixafen EC 225 g/L (150 + 75 g/L) on the parasitic wasp Aphidius rhopalosiphi (DESTEFANI-PEREZ) in a laboratory test - Rate-Response-Test (LR50) -
Report No.:	14 10 48 039 A
Document No.:	M-489147-01-1
Guideline(s):	[redacted], 2000)
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:

The purpose of this study was to determine a rate-response relationship for mortality of the parasitic wasp *Aphidius rhopalosiphi* (DESTEFANI-PEREZ) in a laboratory test. Wasps were exposed to dried spray residues of different application rates of the test item applied on glass plates. Survival of the parasitic wasps was used as test endpoint with the aim to estimate the LR_{50}.

The test was performed following the IOBC Guideline ([redacted], 2000) taking account of the recommendations given by [redacted] (2001) but without performance of a post-exposure assessment of wasp reproduction.

Material and methods:

Test item: Prothioconazole + Bixafen EC 225 (150 + 75 g/L), analysed a.s. contents:
14.8 % w/w (148.6 g/L) prothioconazole (JAU 6476); 7.52 % w/w (75.50 g/L) bixafen (BYF 00587); Batch No. ECE2101898, Specification No. 102000013869, TOX10490-00, density (20 °C): 1.004 g/mL (according to Certificate of Analysis)

The effect of Prothioconazole + Bixafen EC 225 was tested under laboratory conditions after contact exposure of adults of the parasitic wasp *Aphidius rhopalosiphi* (DESTEFANI-PEREZ) to dried spray residues of the test item with rates of 50.4, 89.4, 159, 283 and 504 mL product/ha in 200 L deionised water/ha applied on glass plates. The control was treated with deionised water (200 L/ha). Dimethoate EC 400 (0.3 mL product/ha, nominally equivalent to 0.12 g a.s./ha, in 200 L deionised water/ha) was used as a toxic reference item.

Adults of the parasitic wasp *Aphidius rhopalosiphi* (DESTEFANI-PEREZ) were exposed in 4 replicates per treatment group and 7 females and 3 males per replicate to the residues of the test item, reference item and control treatments, respectively. During the exposure phase the adult wasps were fed with 25 % w/w aqueous fructose solution. The number of surviving, affected, moribund and dead wasps was recorded over a period of 48 hours. From these data the endpoint mortality was calculated.

The temperature ranged between 19-22°C and the relative humidity was 68-72%-The photoperiod was 16 h light and 8 h darkness (light intensity > 3000 lx).

Findings:

Validity criteria:
All validity criteria were met as presented in the table below:

Table CP 10.3.2.1- 1: Validity criteria

Validity criteria	Recommended	Obtained
Mortality in control group	≤ 13% (48 h)	5%
Corrected mortality in reference group	> 50% (48 h)	100%

Biological results:
After 48 h, the corrected mortality for the different rates was between 18.4% and 100%. Statistically significant differences compared to the control were observed at all test item rates and the NOER (no observed effect rate) for mortality was estimated to be < 50.4 mL product/ha. The LR_{50} for Prothioconazole + Bixafen EC 225 (150 + 75 g/L) was calculated to be 98.1 mL product/ha in 200 L water/ha.

Table CP 10.3.2.1- 2: Effect of Prothioconazole + Bixafen EC 225 on *Aphidius rhopalosiphi* (DESTEFANI-PEREZ)

Test item	Prothioconazole + Bixafen EC 225 (150 + 75 g/L)	
Test object	*Aphidius rhopalosiphi* (DESTEFANI-PEREZ)	
Exposure	dried spray deposits on glass plates	
Treatment [mL product/ha][1)]	**Mortality** [2)] **[%]**	**Corrected mortality** [3)] **[%]**
Control	5.0	
50.4	22.5*	18.4
89.4	52.5*	50.0
159	62.5*	60.5
283	100*	100
504	100*	100
Dimethoate EC 400 (0.3 mL product/ha)	100*	100
LR_{50} [95 % CL]	**98.1 mL product/ha** [63.8 – 150.8 mL product/ha]	

[1)] Application rate in 200 L water/ha
[2)] Mortality after exposure to residues on treated glass plates. The results for mortality in individual treatments were compared to that in the control using FISHER´S Exact Binomial test ($\alpha = 0.05$).
[3)] Corrected mortality according to ABBOTT (1925)
* statistically significantly different compared to the control
95 % CL means lower and upper 95 % confidence limits

Conclusion:
In a worst-case laboratory study with Prothioconazole + Bixafen EC 225 (150 + 75 g/L) the LR_{50} for *Aphidius rhopalosiphi* was estimated to be 98.1 mL product/ha in 200 L water/ha.
The NOER (no observed effect rate) for mortality was estimated to be < 50.4 mL product/ha.

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Report:	KCP 10.3.2.1/02[redacted]G; 2014; M-489138-01-1
Title:	Effects of Prothioconazole + Bixafen EC 225 (150 + 75 g/L) on the predatory mite Typhlodromus pyri SCHEUTEN in a laboratory test - Rate-response-Test (LR50) -
Report No.:	14 10 48 040 A
Document No.:	M-489138-01-1
Guideline(s):	IOBC ([redacted]. 2000)
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:
The purpose of this study was to determine a rate-response relationship for mortality of the predatory mite *Typhlodromus pyri* SCHEUTEN in a laboratory test. Mites were exposed to dried spray residues of different application rates of the test item applied on glass plates. Survival of the predatory mites was used as test endpoint, with the aim to estimate the LR50.
The test was performed following the IOBC Guideline ([redacted]. 2000) and taking account of the recommendations given by [redacted] (2001) but without performance of a post-exposure assessment of mite reproduction.

Material and methods:
Test item: Prothioconazole + Bixafen EC 225 (150 + 75 g/L) analysed a.s. contents: 14.8% w/w (148.6 g/L) prothioconazole (JAU 6476); 7.52% w/w (75.50 g/L) bixafen (BYF 00587); Batch No. ECE2101898, TOX10490-00, Specification No.: 102000013869, density (20 °C): 1.004 g/mL (according to Certificate of Analysis)

The effect of Prothioconazole + Bixafen EC 225 was tested under laboratory conditions after contact exposure of protonymphs of the predatory mite *Typhlodromus pyri* SCHEUTEN to dried spray residues of the test item with rates of 40.2, 71.5, 127, 227 and 402 mL product/ha in 200 L deionised water/ha applied on glass plates.
The control was treated with deionised water (200 L/ha). Dimethoate EC 400 (15 mL product/ha, nominally equivalent to 6 g a.s./ha, in 200 L deionised water/ha) was used as a toxic reference item.
Protonymphs of the predatory mite *Typhlodromus pyri* SCHEUTEN were exposed in 5 replicates per treatment group and 20 mites per replicate to the residues of the test item, reference item and control treatments, respectively. During the assessments the mites were fed with a mix of pollen pine (*Pinus nigra*) and birch (*Betula pendula*), l.i. The number of surviving, dead, trapped and escaped predatory mites was recorded over a period of 7 days. From these data the endpoint mortality was calculated.

The temperature ranged between 23-27°C and the relative humidity was 67-74%-The photoperiod was 16 h light and 8 h darkness (light intensity > 3000 lx).

Findings:

Validity criteria:
All validity criteria were met as presented in the table below:

Table CP 10.3.2.1- 3: Validity criteria

Validity criteria	Recommended	Obtained
Mortality in control group	≤ 20% on day 7	2%
Corrected mortality in reference group	≥ 50% on day 7	81.6%

Biological results:
In the test item treatments, corrected mortality rates were between 0% and 21.4%. No statistically significant effects on mortality were determined at rates of 40.2, 71.5 and 127 mL product/ha (FISHER`s Exact Binomial test, a = 0.05) and the NOER (no observed effect rate) for mortality was 127 mL product/ha. The LR50 for Prothioconazole + Bixafen EC 225 (150 + 75 g/L) was estimated to be higher than 402 mL product/ha in 200 L water/ha, the highest rate tested.

Table CP 10.3.2.1- 4: Effect of Prothioconazole + Bixafen EC 225 on *Typhlodromus pyri* SCHEUTEN

Test item	Prothioconazole + Bixafen EC 225 (150 + 75 g/L)	
Test object	*Typhlodromus pyri* SCHEUTEN	
Exposure	dried spray deposits on glass plates	
Treatment [mL product/ha] [1)]	**Mortality [2)] [%]**	**Corrected mortality [3)] [%]**
Control	2.0	-
40.2	3.0 (n.s.)	1.0
71.5	2.0 (n.s.)	0
127	9.0 (n.s.)	7.1
227	13.0*	11.2
402	23.0*	21.4
Dimethoate EC 400 (15 mL product/ha)	82.0*	81.6
LR_{50}	**> 402 mL product/ha**	

[1)] Application rate in 200 L water/ha
[2)] Mortality after exposure to residues on treated glass plates after 7 days. The results for mortality in individual treatments were compared to that in the control using FISHER´S Exact Binomial test ($\alpha = 0.05$).
[3)] Corrected mortality according to ABBOTT (1925)
(n.s.) not statistically significantly different compared to the control
* statistically significantly different compared to the control

Conclusion:
In a worst-case laboratory study with Prothioconazole + Bixafen EC 225 (150 + 75 g/L) the LR_{50} for *Typhlodromus pyri* was estimated to be > 402 mL product/ha in 200 L water/ha, the highest rate tested. The NOER (no observed effect rate) for mortality was 127 mL product/ha.

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

Report: KCP 10.3.2.2/01 [redacted]; 2012; M-282592-02-1
Title: Effects of BYF 00587 + PTZ EC 75 + 150 G on the parasitoid Aphidius rhopalosiphi, extended laboratory study - dose response test -
Report No.: 31204002
Document No.: M-282592-02-1
Guideline(s): [redacted] 2000, [redacted]. 2006
Guideline deviation(s): not specified
GLP/GEP: yes

Objective:
The aim of the study was to determine the effects of freshly dried residues of BYF 00587 + PTZ EC 75 + 150 G applied onto barley seedlings to the parasitoid wasp *Aphidius rhopalosiphi*.

Materials and methods:
Test item: BYF 00587 + PTZ EC 75 + 150 G; analyzed a.s. contents: BYF 00587 (bixafen): 75.3 g/L; JAU 6476 (prothioconazole): 149 g/L; Batch No. 2006-001178, TOX07660-00, Specification No.: 102000013869.
Test organism: the parasitoid wasp *Aphidius rhopalosiphi*, less than 48 h old adults

Under extended laboratory conditions parasitoid wasps (5 females per replicate) were exposed to dried spray deposits of 46.3, 139, 417, 1250 and 3750 mL product/ha (diluted in 400 L deionised water/ha) on treated potted barley seedlings (6 replicates per treatment group). Deionised water was used as a control treatment and Perfekthion (10.0 mL product/ha diluted in 400 L deionised water/ha) as a reference treatment. The duration of the mortality part was 48 hours. The reproductive performance of the survivors was examined for another 24 hour period using females from the control and from those test item concentrations where corrected mortality was < 53.3%.

Dates of work: 2006-09-18 to 2006-11-14

Findings:
The results can be considered as valid, as all validity criteria of the test were met. Mortality in the water control was 0% (≤ 10% required), corrected mortality of the reference item was 96.7% (≥ 50% required), mean reproduction per female in water control was 41.6 (≥ 5 required) and not more than 2 wasps produced zero reproduction in the water control (0 wasps in this study).

Table CP 10.3.2.2- 1: *Aphidius rhopalosiphi*, extended laboratory testing - dose response test -

Test item	BYF 00587 + PTZ EC 75 + 150 G				
Test object	***Aphidius rhopalosiphi***				
Exposure	**Barley seedlings**				
Treatment	Mortality after 48 h [a] [%]	Corrected mortality after 48 h [%]	Settling rate [b] [% wasps on the plants]	Mummies per female [c]	Reduction of parasitisation efficiency relative to the control [d] [%]
Control	0.0	-	47.3	41.6	-
46.3 mL product/ha	0.0 n.s.	0.0	48.7 n.s.	43.5 n.s.	-4.5
139 mL product/ha	0.0 n.s.	0.0	46.3 n.s.	33.7 n.s.	19.0
417 mL product/ha	6.7 n.s.	6.7	49.3 n.s.	29.3 n.s.	29.7
1250 mL product/ha	0.0 n.s.	0.0	44.7 n.s.	36.1 n.s.	13.4
3750 mL product/ha	53.3 *	53.3	35.3 *	28.3 n.s.	31.9
10.0 mL Perfekthion/ha (Toxic Reference)	96.7 *	96.7	46.0 n.s.	n.a.	-
LR_{50} (CL 95 %)	**3485.16 mL product/ha (2398.42 - 5064.31 mL product/ha)**				

[a] n.s. = not significant, * = significant; Fisher Exact Test, $\alpha = 0.05$
[b] n.s. = not significant, * = significant;
test item: Dunnett-Test, $\alpha = 0.05$; reference item: Student-t-Test, $\alpha = 0.05$
[c] n.s. = not significant, Dunnett-Test, $\alpha = 0.05$
[d] negative value means increased reproductive capacity compared to the control
n.a. =not assessed
CL = Confidence Limits

Observations:

No repellent effect was observed in the test item treatment groups up to and including 1250 mL product/ha and in the reference item group compared to the control. At 3750 mL product/ha the settling rate was statistically significantly lower compared to the control. This might be an indication for a repellent effect of the test item at this rate.
The reproductive capacity of *Aphidius rhopalosiphi* was not affected up to and including 3750 mL product/ha compared to the control.

Conclusion:

Under extended laboratory conditions the LR_{50} of BYF 00587 + PTZ EC 75 + 150 G is 3485.16 mL product/ha (95% confidence limits: 2398.42 - 5064.31 mL product/ha).
No repellent effect was observed in the test item treatment groups up to and including 1250 mL product/ha and in the reference item group compared to the control.
At 3750 mL product/ha the settling rate was statistically significantly lower compared to the control. This might be an indication for a repellent effect of the test item at this rate.
The reproductive capacity of *A. rhopalosiphi* was not affected up to and including 3750 mL product/ha compared to the control (i.e. ER_{50} > 3750 mL product/ha).

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Report:	KCP 10.3.2.2/02 [redacted]; 2006; M-280528-01-1
Title:	Effects of BYF 00587 + PTZ EC 75 + 150 G on the predatory mite Typhlodromus pyri, extended laboratory study - dose response test -
Report No.:	31205062
Document No.:	M-280528-01-1
Guideline(s):	[redacted] 2000: Laboratory residual contact test with the predatory mite Typhlodromus pyri Scheuten (Acari: Phytoseiidae) for regulatory testing of plant protection products; Oomen 1988: Guideline for the evaluationof side-effects of pesticides on
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:

The aim of the study was to determine the toxicity of freshly dried residues of BYF 00587 + PTZ EC 75 + 150 G applied onto detached bean leaves, to the predatory mite *Typhlodromus pyri*.

Materials and methods:

Test item: BYF 00587 + PTZ EC 75 + 150 G, analyzed a.s. contents: BYF 00587 (bixafen): 75.3 g/L; JAU 6476 (prothioconazole), purity: 149 g/L, Batch No. 2006-001178, TOX07660-00, Specification No. 102000013869.
Test organism: the predatory mite *Typhlodromus pyri*, < 24 hours old protonymphs.

Under extended laboratory conditions protonymphs (10 mites per replicate) were exposed to air dried spray deposits of 250, 500, 1000, 2000 and 4000 mL product/ha (diluted in 200 L deionised water/ha) on bean leaves (6 replicates per treatment group). Deionised water was used as a control treatment and Perfekthion (40 mL product/ha diluted in 200 L deionised water/ha) as a reference treatment. Assessment of the number of living, escaped and dead mites was conducted 3 and 7 days after application. For the reproduction assessment surviving mites from the control and from all test item groups where corrected mortality was < 50% were sexed and the number of eggs per females was recorded at 3 assessment days within one week.

Findings:

The results can be considered as valid, as all validity criteria of the test were met. The control mortality on day 7 after exposure was < 20% (5.0% in this study), the corrected mortality in the reference item was > 50% at day 7 after exposure (98.2% in this study) and the average number of eggs/female in the control group exceeded 4 eggs per female for the second week (5.4 in this study).

Table CP 10.3.2.2- 2: Effects on mortality and reproduction of *Typhlodromus pyri*

Treatment	mL product/ha	Mortality [a] [%]	Corrected Mortality [%]	Reproduction [b] [eggs/ female]	Effect on reproduction [c] [%]
Control	0	5.0	-	5.4	-
Test item	250	6.7 n.s.	1.8	7.3 n.s.	-35.0
Test item	500	16.7 n.s.	12.3	3.5 n.s.	35.9
Test item	1000	21.7 *	17.5	3.7 n.s.	31.3
Test item	2000	88.3 *	87.7	n.a.	-
Test item	4000	98.3 *	98.2	n.a.	-
Reference item (Perfekthion)	40	98.3 *	98.2	n.a.	-
LR_{50} (CL 95%)	**1296 mL product/ha** (424 – 2348 mL product/ha)				

[a] n.s. = not significant, * = significant; Fisher Exact Test, α = 0.05
[b] n.s. = not significant, * = significant; Dunnett-Test, α = 0.05
[c] negative value means increased reproduction compared to the control
n.a. = not applicable
CL = Confidence Limits

Observations:

The reproductive capacity of *Typhlodromus pyri* was tested at 250, 500 and 1000 mL product/ha. There was no statistically significant effect on reproduction at these dose rates compared to the control.

Conclusion:

Under extended laboratory conditions the LR_{50} of BYF 00587 + PTZ EC 75 + 150 G to *Typhlodromus pyri* is 1296 mL product/ha (95% confidence limits: 424 - 2348 mL product/ha).
No adverse effects on reproduction were observed up to a dose rate of 1000 mL product/ha. Therefore, the ER_{50} is >1000 mL product/ha.

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Report:	KCP 10.3.2.2/03 [redacted]; 2007; M-287283-01-1
Title:	Effects of BYF 00587 + PTZ EC 75 + 150 G on the ladybird beetle Coccinella septempunctata, extended laboratory study - dose response test -
Report No.:	31206012
Document No.:	M-287283-01-1
Guideline(s):	[redacted] 2000; this guideline was modified for exposure of C. septempunctata on natural substrate.
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:

The aim of the study was to determine the toxicity of freshly dried residues of BYF 00587 + PTZ EC 75 + 150 G applied onto detached bean leaves, to the Ladybird Beetle *Coccinella septempunctata*.

Materials and methods:
Test item: BYF 00587 + PTZ EC 75 + 150 G; analyzed a.s. contents: BYF 00587 (Bixafen): 75.3 g/L, JAU 6476 (Prothioconazole): 149 g/L, Batch No. 2006- 001178, TOX07660-00, Specification No. 102000013869.

Test organism: the Ladybird Beetle *Coccinella septempunctata,* 3-4 day old larvae.

Under extended laboratory conditions approximately 3-4 day old larvae of *Coccinella septempunctata* (1 larva per replicate) were exposed to dried spray deposits of 417, 722, 1250, 2165 and 3750 mL product/ha (diluted in 200 L deionised water/ha) on treated bean leaves (*Phaseolus vulgaris*; 40 replicates per treatment group). Deionised water was used as a control treatment and Perfekthion (50 mL product/ha diluted in 200 L deionised water/ha) as a reference treatment. The duration of the pre-imaginal mortality part was 12-15 days (reference item only 2 days). The reproductive performance of the survivors was examined over 2 weeks (oviposition period) using adults from the control and from those test item concentrations where the corrected mortality was <50.0%. The reference item treatment caused 100% corrected mortality.
Dates of work: 2006-11-10 to 2007-03-22

Findings:
The results can be considered as valid, as all validity criteria of the test were met. The control mortality was ≤ 30% (27.5% in this study), the corrected mortality in the reference item was > 40% (100% in this study) and the average number of viable eggs per female per day in the control group was ≥ 2 (29.4 in this study).

Table CP 10.3.2.2- 3: Effects on mortality and reproduction of *Coccinella septempunctata*

Treatment	mL product/ha	Preimaginal mortality [a] [%]	Corrected mortality [b] [%]	Eggs per female per day [c]	Fertile eggs per female per day [c]	Larval hatching rate [c] [%]
Control	0	27.5	-	38.5	29.4	76.9
Test item	417	20.0 n.s.	-10.3	32.1 n.s.	24.7 n.s.	76.9 n.s.
Test item	722	32.5 n.s.	6.9	19.2 *	14.2 *	74.8 n.s.
Test item	1250	27.5 n.s.	0.0	20.2 *	17.0 *	84.1 n.s.
Test item	2165	47.5 n.s.	27.6	29.9 n.s.	23.3 n.s.	79.3 n.s.
Test item	3750	67.5 *	55.2	n.a. [d]	n.a. [d]	n.a. [d]
Reference item (Perfekthion)	50	100.0 *	100.0	n.a. [d]	n.a. [d]	n.a. [d]
LR_{50} (CL 95%)	**3391 mL product/ha** (2508 – 4585 mL product/ha)					

[a] n.s. = not significant, * = significant; Fisher Exact Test, $\alpha = 0.05$
[b] negative value means lower mortality compared to the control
[c] n.s. = not significant, * = significant; Dunnett-Test, $\alpha = 0.05$,
[d] n.a. = not applicable, CL = Confidence Limits

Observations:
Reproduction was > 2 fertile eggs per viable female per day at dose rates of 417, 722, 1250 and 2165 mL product/ha (the highest rate tested for effects on reproduction), so the reproductive output is within the

historical data base for control beetles and therefore this parameter is considered as not impacted by the treatment ([redacted]2000) up to and including 2165 mL product/ha.

Conclusion:
Under extended laboratory conditions the LR_{50} of BYF 00587 + PTZ EC 75 + 150 G to *Coccinella septempunctata* is 3391 mL product/ha (95% confidence limits = 2508 - 4585 mL product/ha). No adverse effects on reproduction were observed up to and including rates of 2165 mL product/ha.

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Report:	KCP 10.3.2.2/04[redacted].; 2007; M-290530-01-1
Title:	Effects of BYF 00587 + PTZ EC 75 + 150 G on the lacewing Chrysoperla carnea, extended laboratory study - dose response test -
Report No.:	31207047
Document No.:	M-290530-01-1
Guideline(s):	[redacted]., 2000: Laboratory method to test effects of plant protection products on larvae of Chrysoperla carnea (Neuroptera: Chrysopidae).
Guideline deviation(s):	For the 3rd and 4th check the total number of eggs was calculated on the counted numbers of eggs on the wall of the acrylic cylinder and of eggs on the gauze.
GLP/GEP:	**yes**

Objective:
The aim of the study was to determine the toxicity of freshly dried residues of BYF 00587 + PTZ EC 75 + 150 G applied onto detached bean leaves, to the lacewing *Chrysoperla carnea.*

Materials and methods:
Test item: BYF 00587 + PTZ EC 75 + 150 G; analyzed a.s. contents: 75.3 g/L BYF 00587, 149 g/L JAU 6476, Batch No. 2006-001178, TOX07660-00, Specification No. 102000013869.

Test organism: Lacewing *Chrysoperla carnea*, 2-3 days old larvae.

Under extended laboratory conditions lacewings (2-3 days old larvae) of *Chrysoperla carnea* (50 larvae per treatment group) were exposed to air dried spray deposits of 46.3 - 3750 mL/ha (diluted in 200 L deionised water/ha) on treated bean leaves (50 replicates each and each containing one larvae). Deionised water was used as a control treatment and Perfekthion (50 mL product/ha diluted in 200 L deionised water/ha) as a reference treatment.
Initial evaluation of the test item took place in a range finding test. Based on these results a main test was designed. Exposure time lasted as long as pupae were transferred to petri dishes for development of adults. Mortality checks were carried out regularly until hatching of adult lacewings. For the reproduction assessment surviving lacewings from the control and from all test item groups displaying less than 50% corrected mortality were sexed and egg deposition and larval hatching rate, was determined (2 assessments/week, 24 hours period each assessment). The toxic standard treatment caused 59.1% corrected mortality.

Dates of work: 2006-09-20 to 2006-12-19

Findings:
The results can be considered as valid, as all validity criteria of the test were met. The control mortality was ≤ 20% (12.0% in this study), the corrected mortality in the reference item was > 50% (64.0% in this

study), the average number of eggs per female per day in the control group was ≥ 15 (18.4 in this study) and the mean larval hatching rate in the control group ≥ 70% (95.5% in this study).

Table CP 10.3.2.2- 4: Effects on mortality and reproduction of *Chrysoperla carnea*

Treatment	mL product/ha	Mortality [a] [%]	Corrected mortality [%]	Reproduction [b] [eggs/female/day]	Hatching rate [b] [%]
Control	0.0	12.0		18.9	95.5
Test item	46.3	22.0 n.s.	11.4	27.1	85.4
Test item	139.0	14.0 n.s.	2.3	17.0	57.3
Test item	417.0	18.0 n.s.	6.8	17.2	81.7
Test item	1250.0	16.0 n.s.	4.5	25.0	72.4
Test item	3750.0	12.0 n.s.	0.0	19.0	76.8
Reference item (Perfekthion)	50.0	64.0 *	59.1	n.a.	n.a.
LR_{50}	**> 3750 mL product/ha**				

[a] n.s. = not significant, * = significant; Fisher Exact Test, $\alpha = 0.05$
[b] values of the 3rd and 4th fecundity check
n.a. = not applicable

Observations:
The reproduction of *Chrysoperla carnea* was not affected at all dose rates tested (46.3 - 3750 mL product/ha) with the exception of the hatching rate in the 139 mL/ha treated group. This effect on hatching rate is considered to be not test item related, because no effects occurred in the higher rates either on fertility or fecundity of *Chrysoperla carnea*.

Conclusion:
Under extended laboratory conditions the LR_{50} of BYF 00587 + PTZ EC 75 + 150 G to *Chrysoperla carnea* was determined to be > 3750 mL product/ha. No adverse effects on reproduction were observed.

CP 10.3.2.3 Semi-field studies with non-target arthropods

Report: KCP 10.3.2.3/01 [redacted], 2008; M-307529-01-1
Title: Effects of BYF 00587 + PTZ EC 75 + 150 G on the predatory mite Typhlodromus pyri, extended laboratory study - aged residue test -
Report No.: 38631060
Document No.: M-307529-01-1
Guideline(s): [redacted] 1988
Guideline deviation(s): none
GLP/GEP: **yes**

Objective:
The purpose of this study was to determine the duration and extent of effects of freshly dried or field aged residues of BYF 00587+PTZ EC 75+150G applied to bean plants on the predatory mite *Typhlodromus pyri* Scheuten in the laboratory. Therefore, different bioassays were started after different aging intervals of the residues on the bean plants. Additionally, an assessment for sublethal effects (reproduction assessment) was done when the effects on corrected mortality were below 50%.

Materials and methods:

Two trials were conducted. The first one was conducted on oil seed rape plants. Due to invalid bioassays and deviations to the study plan this first trial was considered invalid. The second trial was conducted with bean plants and is summarized below.

BYF 00587+PTZ EC 75+150G (active ingredients: BYF 00587 (Bixafen), analysed content of a.s.: 7.7% w/w (77.2 g BIX/L), JAU 6476 (Prothioconazole), analysed content of a.s.: 14.7% w/w (147 g PTZ/L). Batch No. 2007-002622, TOX07852-00, Specification No. 102000013869, Density 1.003 g/mL.

Test organism: Protonymphs (< 27 hours old) of *Typhlodromus pyri*.

Under extended laboratory conditions protonymphs (< 27 hours old) of *Typhlodromus pyri* (10 mites per replicate) were exposed to freshly dried and aged spray residues of 1.25 L product/ha (diluted in 400 L tap water/ha) on field treated bean plants (10 replicates per treatment group). The test item was applied under field conditions 3 times at a rate of 1.25 L product/ha with spray intervals of 2 weeks. Tap water was used as a control treatment and Dimezyl 40 EC (a.s.: dimethoate 400 g/L, 60.0 mL product/ha diluted in 400 L tap water/ha) as a toxic reference treatment.
Three bioassays were performed; the 1st bioassay was started on the day of the last application, the 2nd bioassay was started 7 days after the last application, and the 3rd (and last) bioassay was started 14 days after the last application. Assessment of the number of living, escaped and dead mites was conducted until day 7 for each bioassay. Reproduction assessment of surviving mites from the control and from the test item groups was examined in the bioassays where corrected mortality was < 50%. Mites were sexed and the number of eggs per females was recorded at 3 assessment days within one week.

Dates of work: 2007-10-29 to 2008-06-30

Findings:
The results can be considered as valid, as all validity criteria of the test were met. The control mortality was ≤ 20% at day 7, the corrected mortality in the reference item was ≥ 50% and the number of eggs per female in the control group was ≥ 4 for the second week.

Table CP 10.3.2.3- 1: Effects on mortality and reproduction of *Typhlodromus pyri*

Test Item	BYF 00587+PTZ EC 75+150G				
Test object	*Typhlodromus pyri*				
Exposure	Bean Plants				
1st bioassay: test start on the day of the last application					
Treatment	Mortality after 7 days[a] [%]		Corrected Mortality [%]	Reproduction[b] [eggs/female]	Effect on reproduction [%]
Control	17.0		--	4.3	--
3 x 1.25 L/ha	50.0	*	39.8	2.9 n.s.	31.8
60 mL Dimezyl 40 EC/ha (Reference Item)	69.0	*	62.7	1.1 n.s.	74.4
2nd bioassay: test start 7 days after the last application					
	Mortality after 7 days[a] [%]		Corrected Mortality [%]	Reproduction[b] [eggs/female]	Effect on reproduction [%]
Control	10.0		--	4.3	--
3 x 1.25 L/ha	39.0	*	32.2	3.1 n.s.	27.7
3rd bioassay: test start 14 days after the last application					
	Mortality after 7 days[a] [%]		Corrected Mortality [%]	Reproduction[b] [eggs/female]	Effect on reproduction [%]
Control	5.6		--	Not performed	--
3 x 1.25 L/ha	25.6	*	21.2		--

[a] n.s. = not significant, * = significant; Fisher Exact Test, $\alpha = 0.05$
[b] n.s. = not significant; Student-t-Test, $\alpha = 0.05$
n.a. = not assessed

Conclusion:

The duration and the extent of effects of fresh dried and aged residues of BYF 00587+PTZ EC 75+150G applied on bean plants (*Phaseolus vulgaris*) on the predatory mite *Typhlodromus pyri* were evaluated under extended laboratory conditions.

On the day of the last application survival was statistically significantly affected at 3 × 1.25 L product/ha (corrected mortality was 39.8%). No unacceptable effects of BYF 00587+PTZ EC 75+150 G on reproduction were observed (31.8%).

In the 2nd bioassay (7 days after the last application) the corrected mortality in the test item treated plot was 32.2%. Effect on reproduction in this bioassay was 27.7%.

In the 3rd bioassay (14 days after the last application) corrected mortality of the test item treated animals was 21.2% compared to the control. Since there were no effects of BYF 00587+PTZ EC 75+150G on reproduction of *T. pyri* in the 1st and 2nd bioassay (0 and 7 days after application), it was not necessary to do an additional reproduction assessment in the 3rd.

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Report:	KCP 10.3.2.3/02 [redacted].; 2015; M-534076-01-1
Title:	Toxicity to the predatory mite Typhlodromus pyri (Acari: Phytoseiidae) using an extended laboratory test with aged residues on apple - Prothioconazole + bixafen EC 225 (150 + 75 g/L)
Report No.:	CW14/017
Document No.:	M-534076-01-1
Guideline(s):	[redacted] (2000) modified: Use of treated apple seedlings; mites exposed to freshly applied and under semi-field conditions aged residues on detached leaves; [redacted]. (2001) US EPA OCSPP Not Applicable
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Objective:
The objective of this study was to investigate the lethal and sub lethal toxicity of Prothioconazole + Bixafen EC 225 to the predatory mite *Typhlodromus pyri* when exposed to fresh and aged residues of the test item on apple.

Material and methods:
Test item: Prothioconazole + Bixafen EC 225 (150 + 75 g/L); analyzed a.s. contents: prothiconazole 148.6 g/L, bixafen 75.50 g/L, Batch No. ECE2101898, TOX10490-00, Specification No. 102000013869.

The test item was applied two times with 1.25 L product/ha diluted in 400 L deionised water/ha on potted apple seedlings (*Malus sylvestris*). The application interval between was 14 days. The control was treated with deionised water in the same way as the test item.
The toxic reference dimethoate was applied at 0.0476 L product/ha (20 g a.s./ha) diluted in 400 L deionised water/ha on the day of the second application on potted apple seedlings as well. For the further exposure dates it was applied directly on detached apple leaves (with 0.0476 L diluted in 200 L deionised water/ha). It was included to indicate the relative susceptibility of the test organisms and the test system.
Aging of the spray deposits of the test item on the potted apple seedlings took place under semi-field conditions with UV permeable rain protection during the whole study. Two bioassays were performed, the first started on the day of the second application (0DAT2 = 0 days after treatment 2) and the last one fourteen days later (14DAT2).
The laboratory phase for each exposure date was performed in a controlled environment room (target range 25 ± 2 °C and 60 - 90% relative humidity).
Predatory mites (*Typhlodromus pyri*) were exposed to these residues on the treated leaf surfaces. Mortality of 100 protonymphs was assessed up to 14 days after exposure in both bioassays by counting the number of living and dead mites. The number of escaped mites was calculated as the difference from the total number exposed.
In both bioassays the reproduction rate of surviving mites was evaluated over the period of 7 - 14 days after exposure by counting the total number of offspring (eggs and larvae) produced.
From these data the endpoints mortality (after 7 days) and effects on reproduction were calculated.

Findings:
<u>Validity criteria:</u>
All validity criteria were met as presented in the table below:

Table CP 10.3.2.3- 2: Validity criteria

Validity criteria	Recommended	Obtained	
		0DAT2[a)]	14DAT2[a)]
Mort/Esc.-rate in control on day 7	≤ 20%	17.0%	17.0%
Average corr. mortality in reference item	≥ 50%	96.4%	100%
Average number of eggs/female (calculated as sum of 4 assessment dates - from day 7 on) in control	≥ 4	4.2	5.1

[a)] Days after second treatment

Biological results:

In this extended laboratory test the effects of Prothioconazole + Bixafen EC 225 residues (aged under semi-field conditions, with rain protection during the whole study) on the survival of the predatory mite *Typhlodromus pyri* were determined after two applications of 1.25 L product/ha with an application interval of 14 days onto apple seedlings (*Malus sylvestris*).

In the first bioassay started at the application day of the test item, no corrected mortality occurred. In the second bioassay started 14 days later, a corrected mortality of only 6% was found.

In the first bioassay no reduction in reproductive success relative to the control could be detected. A reduction of 13.2% was found in the second bioassay.

A summary of the effects observed in this study are given on the next page.

Table CP 10.3.2.3- 3: Effect of aged Prothioconazole + Bixafen EC 225 residues on *Typhlodromus pyri*

Test item:	Prothioconazole + Bixafen EC 225 (150 + 75 g/L)			
Application:	2 x 1.25 L product/ha (interval of 14 days)			
Test organism:	*Typhlodromus pyri*			
Exposure on:	Dried spray deposits on apple leaves (from treated apple seedlings)			
Start bioassay	0DAT2[a] (0 weeks)	14DAT2[a] (2 weeks)	0DAT2[a] (0 weeks)	14DAT2[a] (2 weeks)
	Mortality (%) after 48 h		**Reproduction - Number of eggs per female**	
Control:	17.0	17.0	4.2	5.1
Test item:	17.0	22.0	4.4	4.4
Reference item:	97	100.0	-	-
	Corrected mortality (%)		**Reduction relative to control (%)**	
Test item	**0.0** (p-value 0.575, not significant[b])	**6.0** (p-value 0.238, not significant[b])	**-6.5** (p-value 0.443, not significant[c])	**13.2** (p-value 0.180, not significant[d])
Reference item:	96.4	100.0	-	-

[a] DAT = days after treatment
[b] Fisher´s Exact test (one-sided, α = 0.05), p-values adjusted according to Bonferroni-Holm
[c] Welch test, α = 0.05
[d] one-way ANOVA, Williams test (one-sided, α = 0.05)

Conclusion:

Both bioassays (started on 0DAT2 and 14DAT2) resulted in a corrected mortality of < 50% as well as a reduction of reproduction of < 50%.

The figures obtained fulfil the validity criteria of the laboratory method for exposure on glass plates.

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Report:	KCP 10.3.2.3/03 [redacted],; 2015; M-512453-01-1
Title:	Toxicity to the parasitoid wasp aphidius rhopalosiphi (Hymenoptera: Braconidae) using an extended laboratory test with aged residues on maize prothioconazole + bixafen EC 225 (150 + 75 g/L)
Report No.:	CW14/018
Document No.:	M-512453-01-1
Guideline(s):	[redacted]. (2000), [redacted]. (2010) modified: Use of treated maize plants, wasps exposed to freshly applied and under semi-field conditions aged residues on detached leaves. [redacted] (2001)
Guideline deviation(s):	not applicable
GLP/GEP:	**yes**

Objective:

The objective of this study was to investigate the lethal and sub lethal toxicity of Prothioconazole + Bixafen EC 225 to the parasitoid wasp *Aphidius rhopalosiphi* when exposed to fresh and aged residues of the test item on maize.

Material and methods:

Test item: Prothioconazole + Bixafen EC 225 (150 + 75 g/L), analysed content of a.s.: prothioconazole: 148.6 g/L, bixafen: 75.50 g/L, Batch No. ECE2101898, TOX10490-00, Specification No. 102000013869, density: 1.004 g/mL.

The test item was applied two times at 1.25 L product/ha diluted in 400 L deionised water/ha on potted maize plants. The application interval was 14 days. The control was treated with deionised water in the same way as the test item.

A toxic reference (active substance: dimethoate) was applied at 0.0075 L product/ha (3 g a.s./ha) diluted in 400 L deionised water/ha on the second application day of the test item on potted maize plants as well. For the further exposure dates it was applied directly on detached maize leaves (with 0.0075 L product/ha diluted in 400 L deionised water/ha). It was included to indicate the relative susceptibility of the test organisms and the test system.

Aging of the spray deposits of the test item on the potted maize plants took place under semi-field conditions with UV permeable rain protection during the first four weeks of the study. Two bioassays were performed, the first started on the day of the second application (0DAT2 = 0 days after treatment 2) and the last 14 days later (14DAT2).

Parasitoid wasps (*Aphidius rhopalosiphi*) were exposed to these residues on the treated leaf surfaces under laboratory conditions (20 ± 2 °C and 60 – 90% relative humidity).

Mortality of 30 female wasps, not older than 48 hours at study start (6 replicates with 5 wasps per test group) was assessed 2, 24 and 48 hours, respectively, after exposure in all bioassays.

Repellency of the test item was assessed during the initial 3 hours after the release of the females. Five separate observations were made at 30-minute intervals starting 15 - 30 minutes after the introduction of all wasps.

The reproductive performance was assessed in both bioassays. For this 15 impartially chosen females from the water control and the test item group were each transferred to a cylinder containing untreated barley seedlings infested with *Rhopalosiphum padi* for a period of 24 h. The number of mummies was assessed 11 days later in the first bioassay and 10 days later in the second bioassay.

Findings:

Validity criteria:

All validity criteria were met as presented in the table below:

Table CP 10.3.2.3- 4: Validity criteria

Validity criteria	Recommended	Obtained	
		0DAT2[a]	14DAT2[a]
Mortality in water control	≤ 10%	0%	3.3
Corrected mortality reference item	≥ 50%	100%	96.6%
Mean reproduction per female in water control	≥ 5	46.3	18.2
Number of wasps in the water control producing zero values for reproduction	≤ 2	0	0

Biological results:

In this extended laboratory test the effects of Prothioconazole + Bixafen EC 225 residues (aged under semi-field conditions, with rain protection during the first four weeks of the study) on the parasitoid wasp *Aphidius rhopalosiphi* were determined after two applications of 1.25 L product/ha with an application interval of 14 days onto maize plants (*Zea mays*).

In the first bioassay that started on the day of the second application, a corrected mortality of 3.3% of the test item was found. A reduction in reproductive success of 15.0% was found in this bioassay.

In the second bioassay which was started 14 days after the second application, no mortality in the test item group was found anymore after 48 h of exposure. In this bioassay no reduction of reproductive success (-27.1%) was detected.

No repellent effect of the test item (settling of the wasps on plants <30%) was observed in all bioassays.

Table CP 10.3.2.3- 5: Effect of Prothioconazole + Bixafen Ec 225 residues on *Aphidius rhopalosiphi*

Test item:	Prothioconazole + Bixafen EC 225 (150 + 75 g/L)					
Application:	2 x 1.25 L product/ha (interval of 14 days)					
Test organism:	*Aphidius rhopalosiphi*					
Exposure on:	Dried spray deposits on maize leaves (from treated maize plants)					
Start bioassay	0DAT2[a]	14DAT2[a]	0DAT2[a]	14DAT2[a]	0DAT2[a]	14DAT2[a]
	Mortality (%) after 48 h		**Repellency** (mean values) - **% Wasps on plant**		**Reproduction - Number of mummies per female**	
Control:	0.0	3.3	59.0	56.7	46.3	18.2
Test item:	3.3	0.0	65.3	64.3	39.3	23.1
Reference item:	100.0	96.7	62.8	52.7	-	-
	Corrected mortality (%)		**Reduction relative to control (%)**		**Reduction relative to control (%)**	
Test item	**3.3** (p-value 0.500, not significant[b])	**-3.4** (p-value 1.000, not significant[b])	**-10.6** (p-value 0.141, not significant[c])	**-13.5** (p-value 0.060, not significant[c])	**15.0** (p-value 0.238, not significant[c])	**-27.1** (p-value 0.209, not significant[c])
Reference item	100.0	96.6	-6.5	7.1	-	-

[a] DAT = days after treatment

[b] Fisher´s Exact test (one-sided); [c] one-way ANOVA, Williams test (one-sided, α = 0.05)

Conclusion
Directly after the application of 2 x 1.25 L product/ha, with an application interval of 14 days, the effects on mortality and reproduction were ≤ 15%. No adverse effects on mortality and reproduction were found 14 days after the second application.

CP 10.3.2.4 Field studies with non-target arthropods

In view of the results presented above, no additional field studies were deemed necessary.

CP 10.3.2.5 Other routes of exposure for non-target arthropods

No relevant exposure of non-target arthropods is expected by other routes of exposure.

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 used in risk assessment
Predicted environmental concentrations in soil (PEC_{soil}) values were calculated and reported in MCP 9.1.3.

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: Maximum PEC_{soil} values

Compound	Cereals	
	$PEC_{soil, max}$ [mg/kg]	$PEC_{soil\ accu}$ [mg/kg]
BIX + PTZ EC 225	2.682[A]	-
Prothioconazole	0.200	0.200
JAU 6476-desthio	0.189	0.190
JAU 6476-S-methyl	0.058	0.068

[A] Based on formulation density of 1.006 g/mL and 2 applications at 14 d interval (no degradation between the 2 applications and 20% interception: worse case).

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	NOEC NOEC	≥ 4.0 kg prod./ha ≥ 1.0 kg a.s./ha	[redacted] (2002) M-033501-02-1 KCA 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 p.m./kg dws***	[redacted] (2000) M-021370-01-1 KCA 8.4.1/06
Bixafen + Prothioconazole EC 225	*Eisenia fetida* reproduction 56 d, sprayed	$NOEC_{repro}$	≥ 75 L prod./ha calc.: ≥ **286.33 mg prod./kg dws**	[redacted] (2006) M-281333-01-1 KCP 10.4.1.1/01
Prothioconazole EC 250	Natural earthworm populations, Field study up to 11 months, spraying	NOEAER	≥ 3 × 200 g a.s./ha	[redacted] - [redacted] (2005) M-040814-03-1 KCA 8.4.1/08

a.s.: active substance; p.m.: pure metabolite; dws: dry weight soil
* Adjusted by a factor of 2 to address the log P_{ow} > 2 and the high peat content of 10% in 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}, PEC_{acc} [mg/kg soil]	TER_{LT}	Trigger	Refined risk assessment?
BIX + PTZ EC 225 chronic	NOEC	≥ 286.33 mg prod./kg soil	2.682	≥ 107	5	No
Prothioconazole, chronic [1)]	NOEC	≥ 257	0.200	≥ 1285	5	No
JAU 6476-desthio chronic	NOEC	0.5 *	0.190	**2.6**	5	Yes
JAU 6476-S-methyl chronic	NOEC	50 *	0.068	735	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. fetida*.
* Adjusted by a factor of 2 to address the log P_{ow} > 2 and the high peat content of 10% in artificial soil

The TER values for the product, the active substance prothioconazole, and the metabolite JAU 6476-S-methyl are above the critical trigger of concern indicating a low risk for earthworms.
The TER value for the prothioconazole metabolite JAU 6476-desthio is below the trigger of concern. Therefore, further refinements are needed for the metabolite JAU 6476-desthio.

Refined risk assessment for JAU 6476-desthio for the use in cereals

An earthworm field study has been performed with the formulation Prothioconazole EC 250 ([redacted]-[redacted], C.; 2005; M-040814-03-1, KCA 8.4.1/08). In this study, the influence of repeated applications of JAU 6476 EC 250 on natural earthworm populations of a grassland area has been investigated. JAU 6476 EC 250 has been applied 3 times with an application rate of 200 g a.s./ha with a 14 d interval between the first and the second application and with a 21 d interval between the second and the third application.

The "***EFSA Scientific Report* (2007) 106, 1-98, Conclusion on the peer review of prothioconazole"** regarding this field study reads: "No adverse effects to be expected, see results of the field study. Desthio-metabolite confirmed as being present in field study: maximum concentration recorded 7 days after second application, was 0.106 mg/kg which is equivalent to 0.212 mg desthio/kg over the standard 5 cm depth".

The maximum PEC_{soil} determined for this metabolite and the intended application rates of BIX + PTZ EC 225 is 0.190 mg/kg (see Table CP 10.4-1). This is lower than the exposure in the earthworm field study where no ecologically adverse effects were seen. Consequently no unacceptable effects from JAU 6476-desthio are to be expected from the intended application of BIX + PTZ EC 225.

In conclusion, no unacceptable risk for earthworms is to be expected from the use of the product according to the intended use pattern in cereals.

CP 10.4.1.1 Earthworms sub-lethal effects

Report:	KCP 10.4.1.1/01 [redacted],; 2006; M-281333-01-1
Title:	BYF 00587 + PTZ EC 75 + 150: Effects on reproduction and growth of earthworms Eisenia fetida in artificial soil with 5% peat
Report No.:	31202022
Document No.:	M-281333-01-1
Guideline(s):	OECD, Guideline for the testing of chemicals Nr. 222 "Earthworm, Reproduction Test" (adopted April 13, 2004) ; ISO-Guideline 11268-2, "Soil quality - Effects of pollutants on earthworm (Eisenia fetida) - Part 2: Determination of effects on reproduction", International Organization for Standardization, 1998
Guideline deviation(s):	exposure temperature was up to 23°C for 3 days instead of a maximum of 22°C
GLP/GEP:	**yes**

Objective:
The aim of the study was to determine the effects of BYF 00587 + PTZ EC 75 + 150 on the reproduction and growth of the earthworm *Eisenia fetida*.

Materials and Methods:
Test item: BYF 00587 + PTZ EC 75 + 150 (g/L), analysed a.s. contents: BYF 00587 (bixafen): 75.3 g/L (7.49% w/w); prothioconazole (FAU 6476): 149 g/L (14.8% w/w). Batch No. 2006-001178, TOX07660-00, Specification No. 102000013869.
Reference Item: Brabant Carbendazim Flowable (500 g/L) (active ingredient carbendazim) is tested at least once a year in a dose response study.
Control: sprayed with deionised water.
Test organism: adult earthworms (*Eisenia fetida*), approximately 11 months old and with clitellum

BYF 00587 + PTZ EC 75 + 150 was sprayed onto artificial soil (dry weight) (containing approx. 74.8% quartz sand, 20% kaolinite clay, 5% sphagnum peat and approx. 0.2% $CaCO_3$) at rates corresponding to 4.688, 9.375, 18.75, 37.5 and 75 L test item/ha to which earthworms were exposed at 19°C - 23°C, a photoperiod of 16 h light: 8 h dark and a light intensity of 540 - 800 lux. Four replicates with 10 earthworms were used per treatment group and 8 replicates with 10 earthworms for the control. Earthworms were fed weekly with dried cattle manure.
The test vessel size was 18.3 cm x 13.6 cm x 6 cm; containing about 500 g dry artificial soil.
The initial soil water content was 51.8% to 52.8% of the maximum water holding capacity; the water content at experimental termination 58.8% to 63.8% of the maximum water holding capacity. The initial was pH 5.8, the pH at experimental termination 5.8 - 6.1.
Assessed endpoints were mortality (at day 28), body weight change (at day 28), feeding activity and reproduction (after 8 weeks).

Findings:
The results can be considered as valid, as all validity criteria of the test were met. Mortality in the control was ≤ 10% (0% in this study), reproduction of the control was ≥ 30 worms per container (203-391 worms in this study) and the coefficient of variation of reproduction in the control was ≤ 30% (18.3% in this study).

Table CP 10.4.1.1- 1: Effect of BYF 00587 + PTZ EC 75 + 150 on earthworm (*Eisenia fetida*) mortality, biomass and reproduction

	Control	BYF 00587 + PTZ EC 75 + 150				
		4.688 L prod./ha	9.375 L prod./ha	18.750 L prod./ha	37.500 L prod./ha	75.000 L prod./ha
Mortality (day 28) [%]	0.0	0.0	0.0	0.0	0.0	0.0
body weight change (day 28) [%]	33.4	26.9 n.s.	35.3 n.s.	35.9 n.s.	30.0 n.s.	32.1 n.s.
number of juveniles (day 56)	331	337 n.s.	325 n.s.	264 n.s.	264 n.s.	265 n.s.
reproduction in % of control [%]	-	101.7	98.1	79.9	79.7	80.0
n.s. = not significantly different compared to the control Dunnett-test, $\alpha = 0.05$, two sided for weight changes, one-sided smaller for reproduction						

Observations:
No mortality and no behavioural abnormalities were observed in any treatment group and none of the body weight changes of the test item treated groups were significantly different compared to the control (Dunnett test, $\alpha = 0.05$, two sided).
The reproduction rates were not significantly different compared to the control in any treatment group (Dunnett test, $\alpha = 0.05$, one sided smaller).

Conclusion:
In this study the lowest-observed-effect-concentration (LOEC) of BYF 00587 + PTZ EC 75 + 150 for mortality, growth and reproduction of the earthworm *Eisenia fetida* was estimated to be greater than 75 L product/ha.
The no-observed-effect-concentration (NOEC) of BYF 00587 + PTZ EC 75 + 150 for mortality, growth and reproduction of the earthworm *Eisenia fetida* was determined to be 75 L product/ha, i.e. the highest tested rate.

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] (2010) M-037756-02-1 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
BIX + PTZ EC 225	*Folsomia candida,* Reproduction 28 d, mixed	NOEC **104 mg prod./kg dws**	[redacted] (2007) M-291632-01-1 KCP 10.4.2.1/01
	Hypoaspis aculeifer Reproduction 14 d, mixed	NOEC **193 mg prod./kg dws**	[redacted] (2015) M-508746-01-1 KCP 10.4.2.1/02

a.s.: active substance; p.m.: pure metabolite; dws: dry weight soil
* adjusted by a factor of 2 to address the log P_{ow} and the organic matter content in the study
dw s = dry weight soil

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:

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

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 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						
BIX + PTZ EC 225 chronic	NOEC	104 mg prod./kg soil	2.682	39	5	No
Prothioconazole chronic	NOEC	≥ 1000	0.200	≥ 500	5	No
JAU 6476-desthio chronic	NOEC	31.3 *	0.190	165	5	No
JAU 6476-S-methyl chronic	NOEC	≥ 15.8 *	0.068	≥ 232	5	No
Hypoaspis aculeifer						
BIX + PTZ EC 225 chronic	NOEC	193 mg prod./kg soil	2.682	72	5	No
Prothioconazole chronic	NOEC	≥ 100	0.200	≥ 500	5	No
JAU 6476-desthio chronic	NOEC	≥ 100	0.190	≥ 526	5	No
JAU 6476-S-methyl chronic	NOEC	≥ 100	0.068	≥ 1471	5	No

* adjusted by a factor of 2 to address the log P_{ow} and the organic matter content in the study

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.

CP 10.4.2.1 Species level testing

Report: KCP 10.4.2.1/01 [redacted]; 2007; M-291632-01-1
Title: BYF 00587 + PTZ EC 75 + 150: effects on reproduction of the collembola Folsomia candida in artificial soil with 5% peat
Report No.: 31209016
Document No.: M-291632-01-1
Guideline(s): ISO 11267 Soil Quality - Inhibition of reproduction of Collembola (Folsomia candida) by soil pollutants, 1999
Guideline deviation(s): none
GLP/GEP: **yes**

Objective:
The purpose of the study was to determine the effects of BYF 00587 + PTZ EC 75 + 150 on mortality and reproduction of the collembola *Folsomia candida* in artificial soil.

Materials and methods:
Test item: BYF 00587 + PTZ EC 75 + 150, analyzed a.s. contents: Bixafen (BYF 00587): 75.3 g/L (7.49% w/w); Prothioconazole (JAU 6476): 149 g/L (14.8% w/w), Batch No. 2006-001178, TOX07660-00, Specification No. 102000013869.
Reference Item: Betosip (a.s. phenmedipham) is tested at least once a year in a dose response study.
Test organism: the collembola *Folsomia candida.*

BYF 00587 + PTZ EC 75 + 150 was mixed into soil (containing approx.74.8% quartz sand, 20% kaolinite clay, 5% sphagnum peat and approx. 0.2% $CaCO_3$) at 26, 52, 104, 208 and 416 mg BYF 00587 + PTZ EC 75 + 150/ kg dws, to which collembola *Folsomia Candida* (50 individuals per treatment group) were exposed for 28 days at 18 - 20°C and a photoperiod of 16 h light : 8 h dark with a light intensity of 410 to 540 lux. The initial soil water content was 21.9% to 22.7% equivalent to 53.5% to 55.3% of the maximum water holding capacity and the water content at experimental termination was 20.2% to 21.8% equivalent to 49.2% to 53.2%. The initial pH was 5.7 to 5.8 and the pH at experimental termination 5.5. Collembola were fed with dry yeast at start and after 14 days.
Endpoints were mortality and reproduction after 28 days.

Findings:
The results can be considered as valid, as all validity criteria of the test were met. The mean mortality in the control was ≤ 20% (12% in this study), the number of juvenile collembola per replicate was ≥ 100 (692-831 in this study) and the coefficient of variation of the control reproduction was ≤ 30% (7.6% in this study).

Table CP 10.4.2.1- 1: Effects of BYF 00587 + PTZ EC 75 + 150 on mortality and reproduction of *Folsomia candida*

	Control	BYF 00587 + PTZ EC 75 + 150 [mg/kg d.w.s.]				
		26	**52**	**104**	**208**	**416**
Mortality (day 28) [%] 1)	12	12 n.s.	10 n.s.	24 n.s.	18 n.s.	98 *
No. of juveniles (day 28) 2)	752	705 n.s.	712 n.s.	669 n.s.	369 *	2 *
Reproduction in % of control (day 28)	-	94	95	89	49	0
Endpoints [mg product/kg dws]						
NOEC (mortality)	208					
LC_{50} (mortality) 3)	271.4 (95% CL: 154.6 - 431.2)					
NOEC (reproduction)	104					
EC_{50} (reproduction) 3)	194.9 (95% CL 155.5 – 239.3)					

n.s. = not significantly different compared to the control
* = significantly different compared to the control
1) Fisher-exact test, α = 0.05, one-sided
2) Dunnett-test, α = 0.05, one-sided smaller
3) Probit analysis
CL = confidence limit

Observations:
In this study BYF 00587 + PTZ EC 75 + 150 caused statistically significant effects on mortality of *Folsomia candida* at 416 mg test item/kg soil dry weight.
A statistically significant reduction of reproduction occurred at 208 mg test item/kg soil dry weight.

Conclusion:
The overall NOEC was determined to be 104 mg test item/kg soil dry weight.
The overall LOEC was determined to be 208 mg test item/kg soil dry weight.

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Report:	KCP 10.4.2.1/02[redacted]; 2015; M-508746-01-1
Title:	Bixafen + prothioconazole EC 225 (75+150) G: Influence on mortality and reproduction of the soil mite species Hypoaspis aculeifer tested in artificial soil
Report No.:	LAR-HR-114/14
Document No.:	M-508746-01-1
Guideline(s):	US EPA OCSPP: Not Applicable OECD guideline for the Testing of Chemicals - Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:
The purpose of this study was to assess the effect of Bixafen + Prothioconazole EC 225 (75+150) 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 treatments.

Material and methods:
Test item: Bixafen + prothioconazole EC 225 (75+150) G, analyzed a.s. content: 7.52 % w/w bixafen (BYF 00587) equivalent to 75.50 g/L; 14.8 % w/w prothioconazole (JAU 6476) equivalent to 148.6 g/L, density: 1.004 g/mL (20°C), Batch No.: EC E2101898; TOX10490-00; Specification No. 102000013869.

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, 139, 193, 268, 372, 518, 719 and 1000 mg test item/kg artificial soil dry weight were tested. During the test, the *Hypoaspis aculeifer* were fed with cheese mites bred on brewer's yeast. 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 in the table below:

Table CP 10.4.2.1- 2: Validity criteria

Validity criteria	Recommended	Obtained
Mean adult mortality	≤ 20%	1.3%
Mean number of juveniles per replicate (with 10 mites introduced)	≥ 50	296.5
Coefficient of variation calculated for the number of juveniles per replicate	≤ 30%	5.8%

The most recent non-GLP-test ([redacted], LAR/HR-O-14/14, March 11, 2014) with the reference item Dimethoate EC 400E G showed an EC_{50} of 5.28 mg a.s./kg. This is in the recommended range of the guideline, indicating that an EC50 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:

Mortality:

In the control group 1.3 % of the adult *Hypoaspis aculeifer* died which is below the allowed maximum of ≤ 20 % mortality.

Reproduction:

Concerning the number of juveniles statistical analysis (William's-t test, one-sided smaller, $\alpha = 0.05$) revealed a significant difference between control and the five highest concentrations of the test item. Therefore the No-Observed-Effect-Concentration (NOEC) for reproduction is 193 mg test item/kg artificial soil dry weight. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is 268 mg test item/kg artificial soil dry weight. The EC_{10} is 248 mg test item/kg dry weight artificial soil (95% confidence limits: 10 - 412) and the EC_{20} is 528 mg test item/kg dry weight artificial soil (95% confidence limits: 229 - 1022).

Table CP 10.4.2.1- 3: Effect of Prothioconazole + Bixafen EC 225 on *Hypoaspis aculeifer*

Test item	Prothioconazole + Bixafen EC 225 (150 + 75 g/L)			
Test object	*Hypoaspis aculeifer*			
Exposure	Artificial soil			
Treatment [mg product/kg dws soil]	**% mortality (Adults)**	**Mean number of juveniles per test vessel ± standard dev.**	**Reproduction (% of control)**	**Significance (*)**
Control	1.3	296.5 ± 17.2	-	
100	0.0	306.3 ± 16.5	103.3	-
139	2.5	306.3 ± 11.5	103.3	-
193	2.5	309.3 ± 41.1	104.3	-
268	0.0	260.0 ± 24.9	87.7	+
372	0.0	235.3 ± 54.5	79.3	+
518	5.0	206.3 ± 14.9	69.6	+
719	0.0	223.3 ± 35.6	75.3	+
1000	0.0	222.3 ± 43.2	75.0	+
$NOEC_{reproduction}$	193 mg test item/kg dws			
$LOEC_{reproduction}$	268 mg test item/kg dws			
EC_{10} (95% confidence interval)**	248 mg test item/kg dws (10-412)			
EC_{20} (95% confidence interval)**	528 mg test item/kg dws (229-1022)			

* William's-t.-test one sided smaller; α=0.05; "-": non-significant; "+": significant

** Probit analysis

Conclusion:
$NOEC_{reproduction}$: 193 mg test item/kg artificial soil dry weight
$LOEC_{reproduction}$: 268 mg test item/kg artificial soil dry weight

CP 10.4.2.2 Higher tier testing

Not required as the risk for other non-target soil meso- and macro-organisms is acceptable.

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 kg a.s./ha **≥ 2.71 mg a.s./kg dws**	[redacted] (1999) M-024673-01-1 KCA 8.5/01
N-cycle	JAU 6476-S-methyl	28 d	no influence ≥ 2.0 kg p.m../ha **≥ 2.69 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 kg p.m../ha **≥ 1.37 mg p.m./kg dws**	[redacted] (2001) M-057459-01-1 KCA 8.5/06
N-cycle	Bixafen + Prothioconazole EC 225	28 d	no influence ≥ 12.5 kg prod.ha **≥ 16.8 mg prod./kg dws**	[redacted] (2006) M-281135-01-1 KCP 10.5/01

a.s.: active substance; p.m.: pure metabolite; dws: dry weight soil

Risk assessment for Soil Nitrogen Transformation

Table CP 10.5- 2: 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	0.200	No
JAU 6476-S-methyl	Soil micro-organisms	≥ 2.69 mg a.s./kg dws	0.068	No
JAU 6476-desthio	Soil micro-organisms	≥ 1.37 mg p.m./kg dws	0.190	No
Bixafen + Prothioconazole EC 225	Soil micro-organisms	≥ 16.8 mg prod./kg dws	2.682	No

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.

Report:	KCP 10.5/01 [redacted]; 2006; M-281135-01-1
Title:	Effects of BYF 00587 + PTZ EC 75 + 150 G on the activity of the soil microflora in the laboratory
Report No.:	31208080
Document No.:	M-281135-01-1
Guideline(s):	OECD-Guideline for the Testing of Chemicals, Soil Microorganisms: Carbon Transformation Test, Guideline 217, January 21, 2000. OECD-Guideline for the Testing of Chemicals, Soil Microorganisms: Nitrogen Transformation Test, Guideline 216, January 21, 2000.
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objectives:
The objective of the test was to determine the influence of 1.68 and 16.77 mg of BYF 00587 + PTZ EC 75 + 150 G /kg d.wt.s. on nitrogen transformation in an agricultural soil.

Materials and Methods:
Test item: BYF 00587 + PTZ EC 75 + 150 G; Batch No.: 2006-00117; analysed a.s. contents: BYF 00587 (bixafen): 75.3 g/L, JAU 6476 (prothioconazole): 149 g/L.

Nitrogen transformation:
A loamy sand soil was exposed for 42 d to concentrations of 1.68 mg and 16.77 mg BYF 00587 + PTZ EC 75 + 150 G /kg d.wt.s. (application rates were equivalent to 1x and 10x recommended field rate, respectively). Lucerne meal was added to the soil (5 g/kg dry weight soil) to stimulate nitrogen transformation.
Endpoint was the NO_3-nitrogen production after 42 days of exposure.

Findings:
The variation between the replicate control samples clearly matched the validity criterion of 15% for both the carbon and nitrogen transformation test (OECD test guidelines 216). The validity of the test system was further confirmed by the sensitivity established in positive control experiments.

Nitrogen transformation:
The soil nitrate formation rates were calculated incremental (i.e. between sampling dates). At the lower dose rate of 1.68 mg/kg d.wt.s. the soil nitrate formation rate was higher than the 25% trigger value given by the OECD 216 test guideline at the interval of 7 - 14 days. At the interval between days 28 and 42, the difference from control was -8.5%. At the higher dose rate of 16.77 mg/kg d.wt.s., the difference in nitrate formation rate was below the 25% trigger value within the test. On time interval between days 28 and 42, the difference was -6.2%. A statistically significantly difference of treated groups from control was found at the lower test concentration but not at the higher test concentration.

Table CP 10.5- 3: Effects of BYF 00587 + PTZ EC 75 + 150 G on soil nitrogen transformation (nitrate formation rate) in a loamy sand soil

NO₃-nitrogen formation rate [mg/ kg soil dry weight per day] [3]						
	Control		BYF 00587 + PTZ EC 75+150 G 1.68 mg/kg d.wt.s.		BYF 00587 + PTZ EC 75+150 G 16.77 mg/kg d.wt.s.	
Interval [3]	Nitrate-N Formation	Replicate Variation [1]	Nitrate-N Formation	Deviation [2]	Nitrate-N Formation	Deviation [2]
Day 7	-0.83	0.00	-0.83	0.00	-0.77	-7.2*
Day 14	0.72	10.42	0.42	-41.7*	0.68	-5.6
Day 28	1.02	4.31	0.77	-24.5*	0.91	-10.8*
Day 42	1.30	2.69	1.19	-8.5*	1.22	-6.2

[1] = % variation within control replicates (coefficient of variation, calculated as standard deviation / mean value * 100)
[2] = % deviation to control
[3] = related to intervals between test start and sampling
+ = stimulating effect; - = inhibitory effect
d.wt.s = dry weight soil
* statistically significant different from control (Student-t-test: $\alpha = 0.5$)

Conclusions:
Based on the results of this study, BYF 00587 + PTZ EC 75 + 150 G has no impact on soil nitrate formation rate of soil microflora when applied up to 16.77 mg/kg soil dry weight (corresponding to 10 times the maximum recommended application rate of 1.25 L BYF 00587 + PTZ EC 75 + 150 G per ha).
It can be concluded that BYF 00587 + PTZ EC 75 + 150 G does not have long term influence on soil microflora.

CP 10.6 Effects on terrestrial non-target higher plants

Risk assessment for Terrestrial Non-Target Higher Plants
The risk assessment is based on the "Guidance Document on Terrestrial Ecotoxicology", (SANCO/10329/2002 rev2 final, 2002). It is restricted to off-field situations, as non-target plants are defined as non-crop plants located outside the treated area. Spray drift from treated areas may produce residues of a product in adjacent off-crop areas.

Overall, four Tier 1 limit tests have been conducted with the formulation Bixafen + Prothioconazole EC 225, including two seedling emergence and two vegetative vigour studies. In all studies the intended maximum application rate of 1.25 L prod./ha has been tested. An overview of the studies and the endpoints relevant for the non-target plant risk assessment is provided in the table below.

Table CP 10.6- 1: Survey of non-target terrestrial plant studies performed with Bixafen + Prothioconazole EC 225

Test organism	Study type, tested rate	Max. effects*	Most sensitive species	References
Terrestrial non-target plants; 10 species	Vegetative vigour, 21 days, 1.25 L prod./ha	37.2% reduction of shoot dry weight (stat. sig.)	Buckwheat (*Fagopyrum esculentum*)	[redacted] & [redacted] (2007) M-291578-01-1 KCP 10.6.2/03
Terrestrial non-target plants; 11 species	Vegetative vigour, 21 days, 1.25 L prod./ha	22% reduction of shoot dry weight (not sig.)	Buckwheat (*Fagopyrum esculentum*)	[redacted] (2014) M-501606-01-1 KCP 10.6.2/04
Terrestrial non-target plants; 10 species	Seedling emergence, 14 days, 1.25 L prod./ha	42.6% reduction of shoot dry weight (not sig.)	Sugar beet (*Beta vulgaris*)	[redacted] & [redacted] (2007) M-291576-01-1 KCP 10.6.2/01
Terrestrial non-target plants; 11 species	Seedling emergence, 21 days, 1.25 L prod./ha	13% reduction of shoot dry weight (not sig.)	Tomato (*Lycopersicon esculentum*)	[redacted] (2014) M-501602-01-1 KCP 10.6.2/02

* stat. sig.: stastistically significant ($p \leq 0.05$); not sig.: not statistically significant ($p > 0.05$)

In none of the studies conducted with Bixafen + Prothioconazole EC 225 phytotoxic effects >50% at the tested rate of 1.25 L prod./ha were found.

To demonstrate the low risk of the formulation to terrestrial non-target plants, TER calculations have been performed for the representative use in cereals. The tested rate of 1.25 L prod,/ha was used as most conservative endpoint estimate (i.e., ER_{50} > 1.25 L prod./ha).

Table CP 10.6- 2: Deterministic risk assessment based on the ER_{50} > 1.25 L prod./ha (vegetative vigour)

Crop	Use pattern	Distance from field edge [m]	Drift [%]	PER* [L prod./ha]	TER (Trigger = 5)
Cereals	2 × 1.25 L prod./ha	1	2.38 [1)]	0.042 [2)]	> 30

* Predicted environmental rate
[1)] Basic drift value for two applications in field crops
[2)] Considering MAF = 1.4 from EFSA GD Birds & Mammals (2009)

Table CP 10.6- 3: Deterministic risk assessment based on the ER_{50} > 1.25 L prod./ha (seedling emergence)

Crop	Use pattern	Distance from field edge [m]	Drift [%]	PER* [L prod./ha]	TER (Trigger = 5)
Cereals	2 × 1.25 L prod./ha	1	2.38 [1)]	0.021 [2) 3)]	> 60

* Predicted environmental rate
[1)] Basic drift value for two applications in field crops
[2)] Considering MAF = 1.4 from EFSA GD Birds & Mammals (2009)
[3)] Considering 50% interception by off-crop vegetation

From the calculations above, it is concluded that no unacceptable effects of the product on non-target terrestrial plants are to be expected.

CP 10.6.1 Summary of screening data

No screening tests were performed. Please refer to CP 10.6.2 for further information.

CP 10.6.2 Testing on non-target plants

Report:	KCP 10.6.2/01 [redacted]; [redacted]; 2007; M-291576-01-1
Title:	Non-target terrestrial plants: an evaluation of the effects of BYF 00587 + Prothioconazole EC 75 + 150 g/L in the seedling emergence and growth test (Tier 1)
Report No.:	SE07/10
Document No.:	M-291576-01-1
Guideline(s):	OECD 208 (July 2006): seedling emergence and growth test (Tier 1)
Guideline deviation(s):	none
GLP/GEP:	**no**

Objective:
The purpose of the study was to evaluate phytotoxic effects of 1.25 L BIX + PTZ 75+150 G/ha on ten species representing non-target terrestrial plants during seedling emergence and growth following a pre-emergence application of the product.

Materials and Methods:
Test item: BYF 00587 + Prothioconazole EC 75+150 g/L (BIX + PTZ 75+150 G), analyzed a.s. contents: 7.7% w/w BYF 00587, 14.7% w/w Prothioconazole. Batch No. 2007-002622, TOX07852-00, Specification No.: 102000013869
Test organisms: Ten species of terrestrial non-target plants (7 dicotyledonae and 3 monocotyledonae): cucumber (*Cucumis sativus*), oilseed rape (*Brassica napus*), soybean (*Glycine max*), sugar beet (*Beta vulgaris*), sunflower (*Helianthus annuus*), tomato (*Lycopersicon esculentum*), buckwheat (*Fagopyrum esculentum*), corn (*Zea mays*), oat (*Avena sativa*), and ryegrass (*Lolium perenne*).

The terrestrial non-target plants were treated at an application rate of 1.25 L product/ha (limit test).
All seeds were sown one day before application and test duration was 14 days after 70% emergence of the seedlings in the controls for each species. Spray treatments were applied once, at test initiation, with a sprayer set at the nominal spray volume of 200 L/ha. Control pots were sprayed with deionised water. Four replicates with five seeds per pot for each species were tested. All pots were individually contained in saucers and retained on benches within a greenhouse. Plants were assessed for emergence, survival and rated for phytotoxicity on days 7 and 14.
At study termination, biomass endpoint determinations were performed for plant dry weights. Statistical analysis was carried out using the Pairwise Mann-Whitney-U-test (one sided smaller).

Findings:
Biological results:
Germination was increased in cucumber and tomato by 25.0% and 5.3% respectively. Germination was reduced in oilseed rape, soybean, sugar beet, sunflower, buckwheat, oat and ryegrass by 5.0%, 21.1%, 13.3%, 5.3%, 5.6%, 5.0% and 16.7%, respectively.

Survival of emerged plants was not effected in any of the treated plant species. Most species did not show any symptoms of phytotoxicity. Phytotoxicity, visualized as chlorosis, necrosis, leaf deformation and stunting was observed in oilseed rape, soybean, sugar beet and sunflower. Severity of phytotoxicity varied and was most pronounced with soybean and sugar beet.
Biomass was increased in corn by 7.1%. Biomass was reduced in cucumber, oilseed rape, soybean, soybean, sugar beet, sunflower, tomato, buckwheat, oat and ryegrass at 19.8%, 38.5%, 24.8%, 42.6%, 21.0%, 32.7%, 10.8%, 15.3% and 36.7%, respectively. The differences in biomass were statistically significant for oilseed rape, tomato and ryegrass.

Table CP 10.6.2- 1: Effects of 1.25 L BIX + PTZ 75+150 G/ha on seedling emergence

	Cucum-ber	Oil-seed rape	Soy-bean	Sugar-beet	Sun-flower	Tomato	Buck-wheat	Corn	Oat	Rye-grass
Germination (% inhibition)	(25.0)	5.0	21.1	13.3	5.3	(5.3)	5.6	0	5.0	16.7
Survival * (% inhibition)	0	0	0	0	0	0	0	0	0	0
Phytotoxicity	0	B	A-C	A-C	0-A	0	0	0	0	0
Dry Weight ** (% inhibition)	19.8	**38.5**	24.8	42.6	21.0	**32.7**	10.8	(7.1)	15.3	**36.7**

* survival is a measure of treated plants that survived at the end of the study and is expressed as an inhibition compared to the untreated control.
** inhibition expressed on a per plant basis.
Figures in parentheses indicate that there was an increase when compared to the control.
Bold figures indicate statistically significant differences ($p \leq 0.05$).

Conclusion:
Applied at the nominal application rate of 1.25 L product/ha, BYF 00587 + Prothioconazole EC 75 + 150 g/L showed no adverse effects (i.e. greater than 50%) for all tested species in this seedling emergence test.

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Report:	KCP 10.6.2/02 [redacted], 2014; M-501602-01-1
Title:	Terrestrial plant test with Prothioconazole + Bixafen EC 225 (150 + 75 g/L): Seedling emergence and seedling growth test
Report No.:	14 10 48 003 P
Document No.:	M-501602-01-1
Guideline(s):	OECD 208 (2006)
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:
The purpose of the study was to determine potential effects of the test item on seedling emergence and early growth of higher terrestrial plants after soil application under controlled environmental conditions. Endpoints were seedling emergence, survival of emerged seedlings, shoot dry weight and visible detrimental effects (e.g. chlorosis and mortality). Statistical analysis was performed to determine significant differences between treatment and control.
The test was performed as a limit test in accordance with OECD Guideline 208 (2006).
Material and methods:

Test item: Prothioconazole + Bixafen EC 225 (150 + 75 g/L), Batch No.: ECE2101898, Spec. No.: 102000013869.

During a 21-day seedling emergence and seedling growth test, the phytotoxicity of Prothioconazole + Bixafen EC 225 (150 + 75 g/L) to 11 plant species was examined in comparison with water controls under greenhouse conditions at 14 – 31 °C, 17 – 73% relative humidity and a photoperiod of 16 h light : 8 h dark (314 – 394 µE/m²/s), using 10 pots with 2 seeds per replicate for corn, oilseed rape, sugar beet, sunflower, buckwheat, tomato, cucumber and soybean as well as 5 pots with 4 seeds for barley, perennial ryegrass and onion.
In the experiment Prothioconazole + Bixafen EC 225 (150 + 75 g/L) was applied onto the soil surface after sowing at a nominal application rate of 1.25 L test item/ha with a spray volume corresponding to 200 L water/ha (range of deviations from the nominal rate: 96 – 102 %). The measured concentration of the active ingredient prothioconazole in the analysed test solution amounted to 102 % of nominal value. During the observation period, i.e. up to 21 days after 50 % of the control plants had emerged, the plants were observed weekly for seedling emergence, survival/mortality and visual phytotoxicity. Endpoints observed on day 21 after 50 % seedling emergence were seedling emergence, visual phytotoxicity and shoot dry weight. Statistical analysis of data was performed using the software ToxRat Professional 2.10.06 (Ratte 2010).

Findings:

Validity criteria:

All validity criteria were met as presented in the table below:

Table CP 10.6.2- 2: Validity criteria

Validity criteria	Required	Obtained
Seedling emergence in the control	≥70%	94-100%
Mean survival of emerged control seedlings	≥ 90%	100%

Analytical results:

Analysis of prothioconazole in the spray solution of Prothioconazole + Bixafen EC 225 yielded 102.1% of nominal.

Biological results:

The soil application of Prothioconazole + Bixafen EC 225 (150 + 75 g/L) to corn, barley, perennial ryegrass, onion, oilseed rape, sugar beet, sunflower, buckwheat, tomato, cucumber and soybean caused no adverse effects on seedling emergence, survival of emerged seedlings and shoot dry weight at the tested rate of 1.25 L test item/ha. None of the inhibitions was found to be statistically significant.
No visible phytotoxic effects were observed on day 21 after 50 % control seedling emergence at the tested rate.

Table CP 10.6.2- 3: Effect of Prothioconazole + Bixafen EC 225 on seedling emergence (1.25 L test item/ha)

Species	Seedling emergence (% inhibition)[1]	Survival (% mortality)[1]	Shoot dry weight (% inhibition)[1]	BBCH (control / treated) min - max	Phytotoxicity (%inhibition)[1]
Monocotyledons					
Zea mays	6	0	8	15/15	0
Hordeum vulgare	0	0	-1	21-22/21-22	0
Lolium perenne	0	0	-1	22/22	0
Allium cepa	0	0	3	11-12/11-12	0
Dicotyledons					
Brassica napus	5	0	-5	14-15/14-15	0
Beta vulgaris	0	0	3	13-14/13-14	0
Helianthus annuus	0	0	1	14-16/14-16	0
Fagopyrum esculentum	0	0	2	51-53/51-53	0
Lycopersicon esculentum	6	0	13	13-14/13-14	0
Cucumis sativus	0	0	-1	12/12	0
Glycine max	5	0	4	13-14/13-14	0

[1] compared to control

No statistically significant differences between control and test item were calculated for seedling emergence and survival (Fisher´s Exact Binomial Test, $p > 0.05$) and for shoot dry weight (Student-t-test, $p > 0.05$)

Conclusion:
The soil application of Prothioconazole + Bixafen EC 225 (150 + 75 g/L) at a rate of 1.25 L test item/ha to eleven terrestrial plant species did not produce effects on seedling emergence, survival of emerged seedlings and shoot dry weight reaching or exceeding the 50 % threshold for further testing.

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Report: KCP 10.6.2/03 [redacted]; 2007; M-291578-01-1
Title: Non-target terrestrial plants: an evaluation of the effects of BYF 00587 + Prothioconazole EC 75 + 150 g/L in the vegetative vigour test (Tier 1)
Report No.: VV07/10
Document No.: M-291578-01-1
Guideline(s): OECD 227 (July 2006); vegetative vigour test (Tier 1)
Guideline deviation(s): none
GLP/GEP: **no**

Objective:
The purpose of the study was to evaluate phytotoxic effects of 1.25 L/ha BYF 00587 + Prothioconazole EC 75 + 150 g/L on ten species representing non-target terrestrial plant species during a vegetative vigour test following a post emergence application of the product onto the foliage of plants at the 2 to 4 -leaf stage.

Materials and Methods:

Test item: BYF 00587 + Prothioconazole EC 75+150 g/L (BIX + PTZ 75+150 G), analyzed a.s. contents: 7.7% w/w BYF 00587 (bixafen), 14.7% w/w prothioconazole, Batch No. 2007-002622, TOX07852-00, Specification No. 102000013869.

Test organisms: Ten species of terrestrial non-target plants (7 dicotyledonae and 3 monocotyledonae): cucumber (*Cucumis sativus*), oilseed rape (*Brassica napus*), soybean (*Glycine max*), sugar beet (*Beta vulgaris*), sunflower (*Helianthus annuus* L.), tomato (*Lycopersicon esculentum*), buckwheat (*Fagopyrum esculentum*), corn (*Zea mays*), oat (*Avena sativa*), and ryegrass (*Lolium perenne* L.).

Plants were treated at the 2-4-leaf stage with a foliar spray application of 1.25 L product/ha (limit test). Spray treatments were applied once, at test initiation, with a sprayer set at the nominal spray volume of 200 L/ha. Control pots were sprayed with deionized water. Four to five replicates with four to five plants per pot for each species were tested. All pots were individually contained in saucers and retained on benches within a greenhouse.

Plants were assessed for survival and phytotoxicity on days 7, 14 and 21. At study termination, endpoint determinations were performed for plant dry weights.

Statistical analysis was carried out using the Pairwise Mann-Whitney-U-test (one sided smaller).

Findings:

Biological results:

There was no effect of 1.25 L BIX + PTZ 75+150 G/ha on the survival of the ten species tested.

Phytotoxicity, visualized as chlorosis, necrosis, leaf deformation and stunting was observed in cucumber, oilseed rape, soybean, sugar beet, sunflower, tomato and buckwheat. Severity of phytotoxicity varied and was most pronounced with sunflower.

Biomass was increased in corn and oat by 15.7% and 26.4%, respectively. Biomass was reduced in cucumber, oilseed rape, soybean, sugar beet, sunflower, tomato, buckwheat and ryegrass by 31.9%, 30.9%, 35.3%, 12.7%, 24.7%, 35.9%, 37.2 and 5.1%, respectively. Differences were statistically significant for cucumber, oilseed rape, soybean, tomato and buckwheat.

Table CP 10.6.2- 4: Effects of 1.25 L BIX + PTZ 75+150 G/ha in the 21 days vegetative vigour test

	Cucumber	Oil-seed rape	Soy-bean	Sugar-beet	Sun-flower	Tomato	Buck-wheat	Corn	Oat	Rye-grass
Survival * (% inhibition)	0	0	0	0	0	0	0	0	0	0
Phytotoxicity	B	A	A	A	B-D	B	B	0	0	0
Dry Weight ** (% inhibition)	**31.9**	**30.9**	**35.3**	12.7	24.7	**35.9**	**37.2**	(15.7)	(26.4)	5.1

* Survival is a measure of treated plants that survived at the end of the study and is expressed as an inhibition compared to the untreated control.

** Inhibition expressed on a per plant basis.

Figures in parentheses indicate that there was an increase when compared to the control.

Bold figures are statistically significant (p ≤ 0.05).

Conclusion:

Applied at the nominal application rate of 1.25 L product/ha, BYF 00587 + Prothioconazole EC 75 + 150 g/L showed no adverse effect (i.e. greater than 50%) for all the tested species in this vegetative vigour test.

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Report:	KCP 10.6.2/04 [redacted] F; 2014; M-501606-01-1
Title:	Terrestrial plant test with Prothioconazole + Bixafen EC 225 (150 + 75 g/L): Vegetative vigour test
Report No.:	14 10 48 004 P
Document No.:	M-501606-01-1
Guideline(s):	OECD 227 (2006)
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:

The purpose of the study was to determine potential effects of the test item on vigour and growth of higher terrestrial plants after foliar application under controlled environmental conditions.
Endpoints were shoot dry weight, survival and visible detrimental effects (e.g. chlorosis). Statistical analysis was performed to determine significant differences between treatment and control. The test was performed as a limit test in accordance with OECD Guideline 227 (2006).

Material and methods:

Test item: Prothioconazole + Bixafen EC 225 (150 + 75 g/L), analyzed a.s. contents: 7.52% w/w bixafen, 14.8% w/w prothioconazole, Batch No. ECE2101898, Spec. No. 102000013869.

During a 21-day vegetative vigour test, the phytotoxicity of Prothioconazole + Bixafen EC 225 (150 + 75 g/L) to 11 plant species was examined in comparison with water controls under greenhouse conditions at 14 – 31 °C, 17 – 72 % relative humidity and a photoperiod of 16 h light : 8 h dark (311 – 392 µE/m2/s), using 10 pots with 2 plants per replicate for corn, oilseed rape, sugar beet, sunflower, buckwheat, cucumber, tomato and soybean as well as 5 pots with 4 plants for barley, perennial ryegrass and onion.
In the experiment Prothioconazole + Bixafen EC 225 (150 + 75 g/L) was applied onto the foliage of plants at the 2 - 4 leaf stage at a nominal application rate of 1.25 L test item/ha with a spray volume corresponding to 200 L water/ha (range of deviations from the nominal rate: 98 - 104 %). The measured concentration of the active ingredient prothioconazole in the analysed test solution amounted to 104 % of nominal value.
During the observation period, i.e. up to 21 days after application, the plants were observed weekly for survival/mortality and visual phytotoxicity. Endpoints observed on day 21 after application were survival (mortality), visual phytotoxicity and shoot dry weight. Statistical analysis of data was performed using the software ToxRat Professional 2.10.06 ([redacted] 2010).

Findings:

Validity criteria:

All validity criteria were met as presented in the table below:

Table CP 10.6.2- 5: Validity criteria

Validity criteria	Required	Obtained
Seedling emergence	≥70%	90-99%
Mean survival of control plants	≥ 90%	100%

Analytical results:
Analysis of prothioconazole in the spray solution of Prothioconazole + Bixafen EC 225 yielded 104.0% of nominal.

Biological results:
No mortality was found for any species tested. Phytotoxic effects were lower than 10% with the exception of *Fagopyrum esculentum*, *Lycopersicon esculentum* and *Cucumis sativus* with maximum phytotoxicity of 17%. Statistically significant effects on shoot dry weight were found for *Beta vulgaris*, *Helianthus annuus*, *Fagopyrum esculentum*, *Lycopersicon esculentum* and *Cucumis sativus*. The maximum effect on shoot dry weight was 22% for *Fagopyrum esculentum*.

Table CP 10.6.2- 6: Effect of Prothioconazole + Bixafen EC 225 on vegetative vigour (1.25 L test item/ha)

Species	Survival (% mortality)	Phytotoxicity (% inhibition) [1]			Shoot dry weight (% inhibition) [1]	BBCH (control / treated) min - max
		Chlorosis	Necrosis	Growth inhibition		
Monocotyledons						
Zea mays	0	0	1	0	14	16-17/16-17
Hordeum vulgare	0	0	0	0	4	31/31
Lolium perenne	0	0	0	0	0	25/25
Allium cepa	0	0	0	0	-4	14/14
Dicotyledons						
Brassica napus	0	0	<1	7	2	16-17/16-17
Beta vulgaris	0	2	3	8	19*	16/16
Helianthus annuus	0	3	2	7	17*	51/51
Fagopyrum esculentum	0	3	3	14	22*	61-63/61-63
Lycopersicon esculentum	0	2	3	13	17*	61/61
Cucumis sativus	0	6	12	17	19*	61/61
Glycine max	0	2	3	4	9	21-22/21-22

[1] compared to control
* statistically significantly different from control (Student t-test, $p \le 0.05$)

Conclusion:
The foliar application of Prothioconazole + Bixafen EC 225 (150 + 75 g/L) at a rate of 1.25 L test item/ha to eleven terrestrial plant species at the 2 to 4 leaf stage did not produce effects on survival and shoot dry weight reaching or exceeding the 50% threshold for further testing.

CP 10.6.3 Extended laboratory studies on non-target plants

In view of the results presented above, no further studies are deemed necessary.

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

Please refer to Point CP 10.6.3.

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

No studies are required.

CP 10.8 Monitoring data

No monitoring data are available.