

FINAL REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: FF-075

Product name(s): EUSKATEL PRO

Chemical active substance:

Prothioconazole, 200 g/L

Azoxystrobin 150 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(New Product Authorization)

Applicant: Rotam Agrochemical Europe Limited

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Updated: March 2022

MS Finalisation date: April 2022, August 2022

Version history

When	What
1 June 2021	New product application in accordance with Article 33 of Regulation (EC) No. 1107/2009.
March 2022	Updated at the request of zRMS Poland
April 2022	Finalization of the assessment by zRMS.
August 2022	Final version of Core Dossier after commenting period process

Table of Contents

9	Ecotoxicology (KCP 10).....	6
9.1	Critical GAP and overall conclusions.....	6
9.1.1	Overall conclusions.....	11
9.1.1.1	Effects on birds (KCP 10.1.1), Effects on terrestrial vertebrates other than birds (KCP 10.1.2), Effects on other terrestrial vertebrate wildlife (reptiles and amphibians) (KCP 10.1.3)	11
9.1.1.2	Effects on aquatic organisms (KCP 10.2).....	12
9.1.1.3	Effects on bees (KCP 10.3.1).....	14
9.1.1.4	Effects on arthropods other than bees (KCP 10.3.2)	15
9.1.1.5	Effects on non-target soil meso- and macrofauna (KCP 10.4), Effects on soil microbial activity (KCP 10.5)	15
9.1.1.6	Effects on non-target terrestrial plants (KCP 10.6)	15
9.1.1.7	Effects on other terrestrial organisms (flora and fauna) (KCP 10.7)	15
9.1.2	Grouping of intended uses for risk assessment.....	16
9.1.3	Consideration of metabolites	16
9.2	Effects on birds (KCP 10.1.1).....	17
9.2.1	Toxicity data	17
9.2.1.1	Justification for new endpoints	19
9.2.2	Risk assessment for spray applications.....	20
9.2.2.1	First-tier assessment (screening/generic focal species)	26
9.2.2.1	Higher-tier risk assessment	38
9.2.2.2	Drinking water exposure.....	38
9.2.2.3	Effects of secondary poisoning.....	40
9.2.2.4	Biomagnification in terrestrial food chains.....	49
9.2.3	Risk assessment for baits, pellets, granules, prills or treated seed.....	49
9.2.4	Overall conclusions.....	49
9.3	Effects on terrestrial vertebrates other than birds (KCP 10.1.2).....	49
9.3.1	Toxicity data	20
9.3.1.1	Justification for new endpoints	21
9.3.2	Risk assessment for spray applications.....	49
9.3.2.1	First tier assessment (screening/generic focal species).....	54
9.3.2.1	Higher-tier risk assessment.....	60
9.3.2.2	Drinking water exposure.....	65
9.3.2.3	Effects of secondary poisoning.....	66
9.3.2.4	Biomagnification in terrestrial food chains.....	74
9.3.3	Risk assessment for baits, pellets, granules, prills or treated seed.....	75
9.3.4	Overall conclusions.....	75
9.4	Effects on other terrestrial vertebrate wildlife (reptiles and amphibians) (KCP 10.1.3)	75
9.5	Effects on aquatic organisms (KCP 10.2).....	75
9.5.1	Toxicity data	75
9.5.1.1	Justification for new endpoints	86
9.5.2	Risk assessment	87
9.5.2.1	Prothiconazole and its metabolites.....	139
9.5.2.2	Azoxystrobin and its metabolites.....	153
9.5.2.3	The aquatic risk assessment for the formulated product.....	167
9.5.3	Overall conclusions.....	169

9.6	Effects on bees (KCP 10.3.1).....	174
9.6.1	Toxicity data	174
9.6.1.1	Justification for new endpoints	175
9.6.2	Risk assessment	176
9.6.2.1	Hazard quotients for bees.....	176
9.6.2.2	Higher-tier risk assessment for bees (tunnel test, field studies).....	179
9.6.3	Effects on bumble bees	180
9.6.4	Effects on solitary bees	180
9.6.5	Overall conclusions.....	180
9.7	Effects on arthropods other than bees (KCP 10.3.2)	180
9.7.1	Toxicity data	180
9.7.1.1	Justification for new endpoints	182
9.7.2	Risk assessment	182
9.7.2.1	Risk assessment for in-field exposure.....	182
9.7.2.2	Risk assessment for off-field exposure	184
9.7.2.3	Additional higher-tier risk assessment.....	186
9.7.2.4	Risk mitigation measures	186
9.7.3	Overall conclusions.....	186
9.8	Effects on non-target soil meso- and macrofauna (KCP 10.4)	186
9.8.1	Toxicity data	186
9.8.1.1	Justification for new endpoints	189
9.8.2	Risk assessment	190
9.8.2.1	First-tier risk assessment.....	190
9.8.2.2	Higher-tier risk assessment	192
9.8.3	Overall conclusions.....	192
9.9	Effects on soil microbial activity (KCP 10.5).....	192
9.9.1	Toxicity data	192
9.9.1.1	Justification for new endpoints	194
9.9.2	Risk assessment	194
9.9.3	Overall conclusions.....	195
9.10	Effects on non-target terrestrial plants (KCP 10.6)	196
9.10.1	Toxicity data	196
9.10.1.1	Justification for new endpoints	197
9.10.2	Risk assessment	197
9.10.2.1	Tier-1 risk assessment (based screening data)	197
9.10.2.2	Tier-2 risk assessment (based on dose-response data).....	198
9.10.2.3	Higher-tier risk assessment	199
9.10.2.4	Risk mitigation measures	199
9.10.3	Overall conclusions.....	200
9.11	Effects on other terrestrial organisms (flora and fauna) (KCP 10.7)	200
9.11.1	Overall conclusions.....	200
9.12	Monitoring data (KCP 10.8)	200
9.13	Classification and Labelling	201
Appendix 1	Lists of data considered in support of the evaluation.....	203
Appendix 2	Detailed evaluation of the new studies	230
A 2.1	KCP 10.1 Effects on birds and other terrestrial vertebrates.....	230
A 2.2	KCP 10.2 Effects on aquatic organisms	230

A 2.3	KCP 10.3 Effects on arthropods	246
A 2.4	KCP 10.4 Effects on non-target soil meso- and macrofauna.....	304
A 2.5	KCP 10.5 Effects on soil nitrogen transformation.....	320
A 2.6	KCP 10.6 Effects on terrestrial non-target higher plants.....	326
A 2.7	KCP 10.7 Effects on other terrestrial organisms (flora and fauna).....	344
A 2.8	KCP 10.8 Monitoring data.....	344

9 Ecotoxicology (KCP 10)

This document reviews the ecotoxicological effects of FF-075, an suspension concentrate (SC) fungicide containing the active substance prothioconazole at the concentration 200g /L and azoxystrobin at the concentration of 150 g/L to control a wide range of fungal diseases in cereals and oilseed rape.

9.1 Critical GAP and overall conclusions

Table 9.1-1: Critical use pattern of the formulated product

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	17	18	19	20	21	22	23	
Use-No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf-ener/ synergist per ha	Conclusion								
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Groundwater	Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms	Non-target plants	
Zonal uses (field or outdoor uses, certain types of protected crops)																						
1	PL, DE, CZ	Winter oilseed rape	F	<i>Sclerotinia sclerotiorum</i> White mould (SCLESC) <i>Alternaria brassicae</i> Dark leaf spot (ALTEBA)	Foliar spray	BBCH 55-69	a) 1 b) 2	14	a) 0.8 L b) 1.6 L	a) Prothio: 0.16 Azoxy: 0.12 b) Prothio: 0.32 Azoxy: 0.24	100-400	35	Max. individual dose 0.8 L/ha. Max. total dose per season 1.6 L/ha									
2	DE, PL, CZ, IE	Winter wheat, durum, spelt, triticale	F	<i>Septoria tritici</i> (SEPTTR)	Foliar Spray	BBCH 30-59	a) 1 b) 2	14	a) 1.0 L b) 2.0 L	a) Prothio: 0.20 Azoxy: 0.15	100-400	35	Max. individual dose 1.0 L/ha. Max. total dose									

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	17	18	19	20	21	22	23
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, G, Gn, Gpn or I **	Pests or Group of pests con- trolled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion							
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. inter- val between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Groundwater	Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms	Non-target plants
										b) Prothio: 0.40 Azoxy: 0.30			per season 2.0 L/ha								
3	DE, PL, CZ, IE	Winter wheat, durum, spelt, triticale	F	<i>Puccinia strii- formis</i> Yellow Rust (PuccST)	Foliar spray	Up to BBCH 69	a) 1 b) 2	14	a) 1.0 L b) 2.0 L	a) Prothio: 0.20 Azoxy: 0.15 b) Prothio: 0.40 Azoxy: 0.30	100-400	35	Max. individual dose 1.0 L/ha. Max. total dose per season 2.0 L/ha								
4	DE, PL, CZ, IE	Winter wheat, durum, spelt, triticale	F	<i>Puccinia recon- dita</i> Brown rust (PuccRT)	Foliar spray	BBCH 30-69	a) 1 b) 2	14	a) 1.0 L b) 2.0 L	a) Prothio: 0.20 Azoxy: 0.15 b) Prothio: 0.40 Azoxy: 0.30	100-400	35	Max. individual dose 1.0 L/ha. Max. total dose per season 2.0 L/ha								
5	DE, PL, CZ, IE	Spring barley	F	<i>Rhynchosporium secalis</i> Leaf blotch (RHYNSE) <i>Pyrenophora</i>	Foliar spray	BBCH 30-69	a) 1 b) 2	14	a) 1.0 L b) 2.0 L	a) Prothio: 0.20 Azoxy: 0.15	100-400	35	Max. individual dose 1.0 L/ha. Max. total dose								

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	17	18	19	20	21	22	23
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, G, Gn, Gpn or I **	Pests or Group of pests con- trolled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion							
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. inter- val between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Groundwater	Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms	Non-target plants
				<i>teres</i> Net blotch (PYRNTE)						b) Prothio: 0.40 Azoxy: 0.30			per season 2.0 L/ha								
6	DE, IE	Winter barley	F	<i>Rhynchosporium secalis</i> Leaf blotch (RHYNSE) <i>Pyrenophora teres</i> Net blotch (PYRNTE)	Foliar spray	BBCH 30-69	a) 1 b) 2	14	a) 1.0 L b) 2.0 L	a) Prothio: 0.20 Azoxy: 0.15 b) Prothio: 0.40 Azoxy: 0.30	100-400	35	Max. individual dose 1.0 L/ha. Max. total dose per season 2.0 L/ha								

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	17	18	19	20	21	22	23
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, G, Gn, Gpn or I **	Pests or Group of pests con- trolled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion							
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. inter- val between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Groundwater	Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms	Non-target plants
7	DE, PL, CZ, IE	Rye	F	<i>Puccinia strii- formis</i> Yellow Rust (PUCCST) <i>Puccinia recon- dita</i> Brown rust (PUCCRT)	Foliar spray	BBCH 30-69	a) 1 b) 2	14	a) 1.0 L b) 2.0 L	a) Prothio: 0.20 Azoxy: 0.15 b) Prothio: 0.40 Azoxy: 0.30	100-400	35	Max. individual dose 1.0 L/ha. Max. total dose per season 2.0 L/ha								
8	DE, PL, CZ, IE	Oats	F	<i>Puccinia coro- nata</i> Crown rust (PUCCCA)	Foliar spray	BBCH 30-69	a) 1 b) 2	14	a) 1.0 L b) 2.0 L	a) Prothio: 0.20 Azoxy: 0.15 b) Prothio: 0.40 Azoxy: 0.30	100-400	35	Max. individual dose 1.0 L/ha. Max. total dose per season 2.0 L/ha								
Interzonal uses (use as seed treatment, in greenhouses (or other closed places of plant production), as post-harvest treatment or for treatment of empty storage rooms)																					
Minor uses according to Article 51 (zonal uses)																					
Minor uses according to Article 51 (interzonal uses)																					

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

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

Explanation for column 15 – 21 “Conclusion”

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

9.1.1 Overall conclusions

zRMS comment:

Since report in dRR format is prepared by the applicant, all remarks, comments, additional calculations and assessment done by the ZRMS are included in the commenting boxes or indicated in blue.

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

Regulatory testing with birds has been conducted with prothioconazole, azoxystrobin and the relevant metabolite, prothioconazole-desthio (M04) in accordance with EU requirements for quail (*Colinus virginianus*) and the mallard duck (*Anas platyrhynchos*). Results from these studies, summarised in the EFSA Review Reports for prothioconazole and azoxystrobin show that the active substances and prothioconazole-desthio (M04) have low toxicity to birds.

The estimated toxicity for FF-075, derived from the combined assessment of the active ingredients, demonstrates low risk to birds from applications of the formulated product.

The assessment of risk from secondary poisoning of earthworm-eating birds and fish-eating birds via exposure to prothioconazole, prothioconazole-desthio (M04) and prothioconazole-S-methyl (M01) via bioaccumulation in earthworms and fish indicates low risk to birds following applications of FF-075 in accordance with the proposed GAP.

An acceptable acute and long-term risk to birds is expected from the proposed uses of FF-075 in winter oilseed rape (BBCH 55-69) and winter and spring cereals (BBCH 30-69).

Regulatory testing with mammals has been conducted with azoxystrobin, prothioconazole and the relevant metabolite, prothioconazole-desthio (M04), in accordance with EU requirements for rat and mouse. Results from these studies, summarised in the EFSA Review Reports for azoxystrobin and prothioconazole, show that the active substances and metabolite, prothioconazole-desthio (M04), have low toxicity to mammals.

The TER_{it} values do not exceed the Annex VI trigger of 5 for prothioconazole-desthio (M04) when considering both relevant mammalian species in winter and spring cereals and winter oilseed rape, and when deposition factors in cereals are refined, according to the guidance in EFSA, (2009).

The estimated toxicity for FF-075, derived from the combined assessment of the active ingredients, demonstrates low risk to mammals from applications of the formulated product in accordance with the GAP.

The assessment of risk from secondary poisoning of earthworm-eating mammals and fish-eating mammals via exposure to prothioconazole, prothioconazole-desthio (M04) and prothioconazole-S-methyl (M01) via bioaccumulation in earthworms and fish indicates low risk to mammals following applications of FF-075 in accordance with the proposed GAP.

Low risk to mammalian species is expected from applications of FF-075 in accordance with the GAP.

No data on reptiles and terrestrial amphibians are available for FF-075. In the absence of a specific framework, the data and risk assessment for birds and mammals are considered an adequate surrogate for other terrestrial vertebrates. No overt toxicity has been observed in any of the avian and mammalian studies relevant for the ecotoxicological risk assessment. In addition, low acute and long-term risks were concluded for birds and mammals. Since terrestrial amphibians and reptiles' diets have generally a lower vegetation content than those of the focal bird and mammal species considered in the previous risk assessment, it is expected that exposure to feed items possibly contaminated with FF-075 will be lower for terrestrial amphibians and reptiles than birds and mammals. As such, no adverse effects or risks are expected for reptiles and terrestrial amphibians exposed to prothioconazole or prothioconazole-desmethio (M04) following applications of FF-075.

9.1.1.2 Effects on aquatic organisms (KCP 10.2)

Regulatory testing with fish, aquatic invertebrates, algae and aquatic macrophytes has been conducted with prothioconazole, azoxystrobin, the relevant metabolites and FF-075 in accordance with EU requirements.

Acute and chronic mixture toxicity assessment has been conducted. The acute mixture toxicity assessment for uses in winter oilseed rape (BBCH 55-69) concludes acceptable risk in the ETR_{mix-CA}/RQ_{mix} assessments for fish, aquatic invertebrates and algae when risk mitigation is applied as a 10m vegetative strip and a 10m no spray. An assessment of the "driver" of the risk assessment indicates that both active substances drive the risk assessments for different FOCUS scenarios. The risk to aquatic invertebrates, algae and fish from acute exposure to prothioconazole and azoxystrobin from applications of FF-075 is, therefore, covered by the calculation of the ETR_{mix-CA}/RQ_{mix} and the risk assessment for the individual active substances for use in winter oilseed rape.

The acute mixture toxicity assessment for uses of FF-075 in winter cereals (BBCH 30-69) concludes acceptable risk to algae at FOCUS Step 2 for all scenarios. An acceptable risk to fish from applications of FF-075 were concluded at FOCUS Step3 for all relevant scenarios. An acceptable risk to aquatic invertebrates was concluded at FOCUS Step 4 for all relevant scenarios when a 10 m vegetative buffer and a 10 m no spray buffer were applied. Outstanding risks for D1 ditch, D2 ditch and D2 stream scenarios were identified for aquatic invertebrates. However, D1 and D2 scenarios are not considered relevant for national assessment in any of the Central Zone Member States where authorisation of FF-075 is being applied for. An assessment of the "driver" of the risk assessment indicates that azoxystrobin drives the risk assessments for a number of FOCUS Step 1-4 scenarios. The risk to aquatic invertebrates, algae and fish from acute exposure to prothioconazole and azoxystrobin from applications of FF-075 is, therefore, covered by the calculation of the ETR_{mix-CA}/RQ_{mix} and the risk assessment for azoxystrobin for use in winter cereals.

The acute mixture toxicity assessment for uses of FF-075 in spring cereals (BBCH 30-69) concludes acceptable risk to algae and fish at FOCUS Step 2 and 3, respectively, for all scenarios. For the acute exposure to aquatic invertebrates, an acceptable risk was concluded for all FOCUS Step 1 – 3 PEC_{SW} scenarios, with the exception of FOCUS Step 3 scenario D1 Ditch. However, the scenario D1 is not a concern for the Central Zone Member States. An assessment of the "driver" of the risk assessment indicates that azoxystrobin drives the risk assessments for a number of FOCUS Step 1-4 scenarios for uses in spring cereals. The risk to aquatic invertebrates, algae and fish from acute exposure to prothioconazole and azoxystrobin from applications of FF-075 in spring cereals is, therefore, covered by the calculation of the ETR_{mix-CA}/RQ_{mix} and the risk assessment for azoxystrobin.

The chronic mixture toxicity assessment for uses of FF-075 in winter oilseed rape (BBCH 55-69) concludes acceptable risk to fish and aquatic invertebrates at FOCUS Step 4 when a 10 m vegetative strip and a 10 m no spray buffer is applied. An assessment of the "driver" of the risk assessment indicates that prothioconazole-desmethio (M04) drives the chronic fish risk assessments for all FOCUS Step 1-3 scenarios for uses in winter oilseed rape. The assessment of chronic risk to fish is covered by the risk assessment

for prothioconazole-desthio (M04). A “driver” was not identified for the entire chronic risk assessment for aquatic invertebrates. The risk to aquatic invertebrates from chronic exposure to prothioconazole-desthio (M04) and azoxystrobin from applications of FF-075 in winter oilseed rape is, therefore, covered by the calculation of the RQ_{mix} and the risk assessment for prothioconazole-desthio (M04).

The chronic mixture toxicity assessment for uses of FF-075 in winter cereals (BBCH30-69) concludes acceptable risk aquatic invertebrates at Focus Step 2, and acceptable risk to fish at FOCUS Step 4 when a 20 m vegetative strip and a 20 m no spray buffer is applied. The assessment of chronic risk to fish is also covered by the risk assessment for prothioconazole-desthio (M04). A driver was not identified for the chronic aquatic invertebrate risk assessment for applications of FF-075 to winter cereals. The assessment is, therefore, covered by the calculation of the RQ_{mix} .

The chronic mixture toxicity assessment for uses of FF-075 in spring cereals (BBCH 30-69) concludes acceptable risk to aquatic invertebrates at FOCUS Step 2 and acceptable chronic risk to fish FOCUS Step 4 when a 10 m vegetative strip and a 10 m no spray buffer is applied with the exception of scenario R4 stream in the assessment of chronic effects on fish. The peak concentration in the R4 Stream scenario is expected to exceed the RAC on a single occasion lasting for only 0.583 days (EPAT analysis – Section B8). Furthermore, R4 is not of concern for the Member States in the Central Zone.

~~Acute and chronic mixture toxicity assessment has been conducted. The acute mixture toxicity assessment for uses in winter oilseed rape (BBCH 55-69) concludes acceptable risk to fish, aquatic invertebrates and algae when risk mitigation is applied as a 10m vegetative strip and a 10m no spray.~~

~~The acute mixture toxicity assessment for uses of FF 075 in winter cereals (BBCH 30-69) concludes acceptable risk to algae at FOCUS Step 2 for all scenarios. An acceptable risk to fish and aquatic invertebrates from applications of FF-075 were concluded at FOCUS Step 4 when a 10 m vegetative buffer and a 10 m no spray buffer were applied. Outstanding risks for D1 ditch, D2 ditch and D2 stream scenarios were identified for fish and aquatic invertebrates. However, with the exception of the D2 scenario in France, the D1 and D2 scenarios are not considered relevant for national assessment in any of the Central Zone Member States where authorisation of FF 075 is being applied for.~~

~~The acute mixture toxicity assessment for uses of FF 075 in spring cereals (BBCH 30-69) concludes acceptable risk to algae and fish at FOCUS Step 2 and 3, respectively, for all scenarios. An acceptable risk to aquatic invertebrates from applications of FF 075 were concluded at FOCUS Step 4 when a 10 m vegetative buffer and a 10 m no spray buffer were applied. Outstanding risk for the D1 ditch scenario was identified for aquatic invertebrates. However, the D1 scenarios are not considered relevant for national assessment in any of the Central Zone Member States where authorisation of FF-075 is being applied for. Therefore, an acceptable risk to aquatic invertebrates is concluded.~~

~~The chronic mixture toxicity assessment for uses of FF 075 in winter oilseed rape (BBCH 55-69) concludes acceptable risk to fish and aquatic invertebrates at FOCUS Step 4 when a 10 m vegetative strip and a 10 m no spray buffer is applied.~~

~~The chronic mixture toxicity assessment for uses of FF 075 in winter cereals (BBCH30-69) concludes acceptable risk aquatic invertebrates at Focus Step 2, and acceptable risk to fish at FOCUS Step 4 when a 20 m vegetative strip and a 20 m no spray buffer is applied to accommodate fails in the risk assessment with a 10 m vegetative strip and 10 m no spray buffer in scenarios R3 stream and R4 stream.~~

~~The chronic mixture toxicity assessment for uses of FF 075 in spring cereals (BBCH 30-69) concludes acceptable risk to aquatic invertebrates at FOCUS Step 2 and acceptable chronic risk to fish FOCUS Step 4 when a 10 m vegetative strip and a 10 m no spray buffer is applied with the exception of scenario R4 stream in the assessment of chronic effects on fish.~~

~~For prothioconazole, and the metabolites prothioconazole S methyl (M01), prothioconazole-triazolylketone (M42) and 1,2,4 triazole (M13), acceptable risk to aquatic organisms was identified for~~

~~uses in oilseed rape, and winter and spring cereals from spray drift and drainage at Tier 1.~~

For prothioconazole, and the metabolites prothioconazole-S-methyl (M01), prothioconazole-triazolylketone (M42) and 1,2,4-triazole (M13), acceptable risk to aquatic organisms was identified for uses in oilseed rape, and winter and spring cereals from spray drift and drainage at Tier 1.

For prothioconazole, and the metabolites prothioconazole-S-methyl (M01), prothioconazole-triazolylketone (M42) and 1,2,4-triazole (M13), acceptable risk to aquatic organisms was identified for uses in oilseed rape, and winter and spring cereals at Tier 1.

For prothioconazole, and the metabolites prothioconazole-S-methyl (M01), prothioconazole-triazolylketone (M42) and 1,2,4-triazole (M13), acceptable risk to aquatic organisms was identified for uses in oilseed rape, and winter and spring cereals at Tier 1.

For prothioconazole-desthio (M04), potential risks were identified at Tier 1 from chronic exposure of fish following winter and spring applications to winter and spring cereals (BBCH 30-69) and applications made to oilseed rape (BBCH 55-69). However, an acceptable risk to fish from chronic exposure to prothioconazole-desthio was concluded when a 20 m vegetative strip and 20 m no spray buffer was applied for uses in winter oilseed rape and spring cereals. Potential risks are identified at FOCUS Step 4 from long-term exposure to fish for FOCUS SW scenario R4 stream following winter applications of FF-075 to winter cereals. The peak concentration in the R4 Stream scenario is expected to exceed the RAC on a single occasion lasting for only 0.583 days (EPAT analysis – Section B8). Therefore, an acceptable risk is concluded from prothioconazole-desthio resulting from applications of FF-075 to winter cereals in accordance with the GAP.

For azoxystrobin, potential risks were identified at FOCUS Steps 1-3 from acute aquatic invertebrates and algae and chronic exposure of aquatic invertebrates for uses in winter oilseed rape (BBCH 55-69), and winter and spring cereals (BBCH 30-69). However, when the RAC applied to the acute and chronic aquatic invertebrate risk assessments was refined to 3.3 µg/L and the endpoint for algae was refined to 262 µg/L by means of calculating a geometric mean, in line with the agreed endpoints outlined in EFSA Journal (2010); 8(4):1542, safe use to aquatic organisms was concluded at FOCUS Step 3 for uses in winter oilseed rape (BBCH 55-69).

For the intended uses in winter and spring cereals (BBCH 30-60) calculated PEC/RAC ratios for azoxystrobin did not indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for acute and long term exposure to aquatic invertebrates) in FOCUS Step 3 scenario R4 stream when the RAC was refined in accordance with the agreed approach (EFSA Journal 2010; 8(4):1542). The risk was resolved at FOCUS Step 4 when a 10 m vegetative strip and a 10 m no spray buffer was applied.

For the metabolites of azoxystrobin, R234886, R401553 and R402173, an acceptable risk to aquatic organisms was identified for uses in winter oilseed rape and winter and spring cereals at FOCUS Step 1.

An acceptable risk to aquatic organisms was concluded from uses of FF-075 in winter oilseed rape (BBCH 55-69) and winter and spring cereals (BBCH 30-69) when applied 1 m from the from the water body.

9.1.1.3 **Effects** on bees (KCP 10.3.1)

Regulatory testing with honeybees (*Apis mellifera*) and bumblebees (*Bombus terrestris*) has been conducted with prothioconazole, azoxystrobin and the formulated product, FF-075, in accordance with EU requirements for bees. An acceptable acute and long-term risk to bees is expected from the proposed uses of FF-075 in winter and spring cereals, and winter oilseed rape in accordance with the proposed GAP.

9.1.1.4 Effects on arthropods other than bees (KCP 10.3.2)

Regulatory testing with the non-target arthropod indicator species *Typhlodromus pyri*, *Aphidius rhopalosiphi*, *Coccinella septempunctata* and *Chrysoperla carnea* has been conducted with FF-075 in accordance with EU requirements. Results from these studies, show that FF-075 has low toxicity to non-target arthropods. At Tier II the PER in-field or off-field values for both GAP scenarios are below the 50% effect rate for all species, indicating a low risk to non-target arthropods within the treated fields, and adjacent untreated habitat.

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

Regulatory testing with *Folsomia candida* and *Hypoaspis aculeifer* has been conducted with azoxystrobin, prothioconazole and the relevant soil metabolites. Regulatory testing with earthworms (*Eisenia fetida*), has been conducted with azoxystrobin, the relevant metabolites of azoxystrobin, and the relevant metabolites of prothioconazole, prothioconazole-desthio (M04) and prothioconazole-S-methyl (M01) in accordance with EU requirements for soil macrofauna. Results from the studies with prothioconazole, azoxystrobin and the relevant metabolites, summarised in the EFSA Review Reports, show that the active substance and the studies with the relevant metabolites have low toxicity to soil macrofauna. Regulatory testing with earthworms, *Folsomia candida* and *Hypoaspis aculeifer* has also been conducted with FF-075. Results from these studies show that FF-075 has low toxicity to non-target soil organism.

All acute and long-term TER values were calculated to be in excess of the accepted trigger values and a low risk for non-target soil organisms was concluded.

Regulatory testing with soil microorganisms has been conducted with FF-075, prothioconazole, azoxystrobin and the relevant metabolites in accordance with EU requirements. Results from these studies, summarised in the EFSA Review Reports for prothioconazole, and Appendix 2 (FF-075), show that the formulated product, active substance and relevant metabolites have low toxicity to soil microorganisms.

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

The TER values considering the data gathered from the seedling emergence and vegetative vigour studies, for FF-075 are greater than the predicted exposure rates derived from the treatment of winter oilseed rape (BBCH 55-69) and winter and spring cereals (BBCH 30-69) at a distance of 1m from the treated field. Acceptable risk to non-target higher plants is concluded from uses of FF-075 in accordance with the GAP.

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

An assessment of the effects of prothioconazole and azoxystrobin on the inhibition of the respiration rate of aerobic wastewater micro-organisms resulted in EC₅₀ values of >10,000 mg a.s./L and >3.2 mg a.s./L, respectively. Therefore, it can be assumed that adverse effects on methods of sewage treatment are unlikely when FF-075 is applied according to GAP.

9.1.2 Grouping of intended uses for risk assessment

In the Central Zone the maximum proposed use rates for FF-075 are 0.8 L/ha for winter oilseed rape and 1.0 L/ha for cereals. The formulated product has a relative density of 1.100 g/mL, which corresponds to a formulation application rate of 880 g/ha for winter oilseed rape and 1100 g/ha for cereals.

9.1.3 Consideration of metabolites

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

Table 9.11-2 Metabolites of prothioconazole

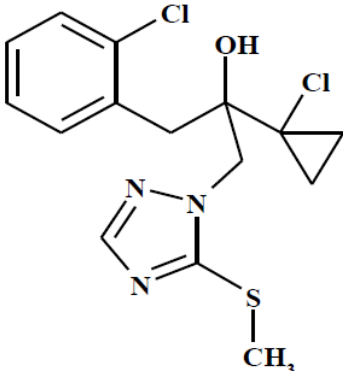
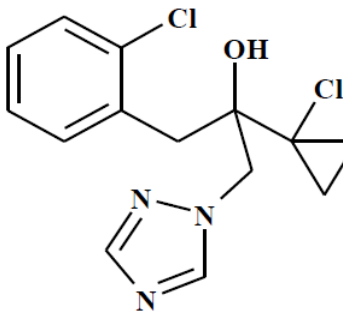
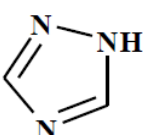
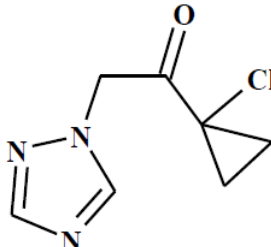
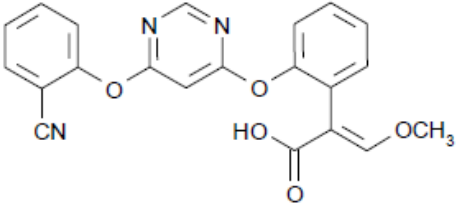
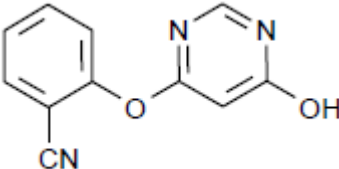
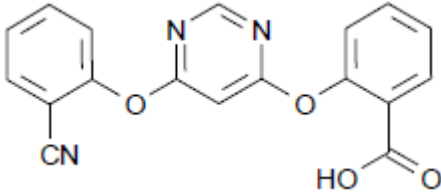
Metabolite	Molar mass	Chemical structure	Maximum observed occurrence in compartments	Exposure assessment required due to
Prothioconazole-S-methyl (M01) (JAU 6476-S-methyl)	358.3		Soil:- 14.6% Water/Sediment:- 12.7%	PEC _{gw} PEC _{soil} PEC _{sw/sed}
Prothioconazole-desthio (M04) (JAU 6476-desthio)	312.2		Soil:- 57.1% Water/Sediment:- 54.6%	PEC _{gw} PEC _{soil} PEC _{sw/sed}
1,2,4-triazole (M13)	69.1		Soil:- Not formed Water/Sediment:- 41.8%	PEC _{sw/sed}
Prothioconazole-triazolylketone (M42) (JAU 6476-triazolylketone)	185.7		Soil:- Not formed Water/Sediment:- 9.1%	PEC _{sw/sed}

Table 9.11-3 Metabolites of azoxystrobin

Metabolite	Molar mass	Chemical structure	Maximum observed occurrence in compartments	Exposure assessment required due to:
R234886	389.4		Soil: 28.8% Water/Sediment:- 18.1%	PEC _{gw} : >10% of a.s. in soil, leaching assessment required PEC _{soil} : >10% of a.s. in soil, assessment required as not covered by EU assessment PEC _{sw/seq} : >10% of a.s. in soil and aquatic systems, assessment required as not covered by EU assessment
R401553	213.2		Soil: 17% Water/Sediment:- 8.9% (aqueous photolysis)	PEC _{gw} : >10% of a.s. in soil, leaching assessment required PEC _{soil} : >10% of a.s. in soil, assessment required as not covered by EU assessment PEC _{sw/seq} : >10% of a.s. in soil, assessment required as not covered by EU assessment
R402173	333.3		Soil: 17% Water/Sediment:- 2.4% (aqueous photolysis)	PEC _{gw} : >10% of a.s. in soil, leaching assessment required PEC _{soil} : >10% of a.s. in soil, assessment required as not covered by EU assessment PEC _{sw/seq} : >10% of a.s. in soil, assessment required as not covered by EU assessment

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

Avian toxicity studies have been carried out with prothioconazole, prothioconazole-destio (M04), and azoxystrobin. Full details of these studies are provided in the respective prothioconazole EU DAR, (2005), the EFSA Conclusion, (2007) for prothioconazole, and the EFSA Journal (2010) for azoxystrobin.

Effects on birds of FF-075 were not evaluated as part of the EU assessment of prothioconazole. An assessment has been provided in Section 9.2.2.1.

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

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

Species	Substance	Exposure System	Results	Reference
<i>Colinus virginianus</i>	Prothioconazole	Oral	LD ₅₀ = >2000 mg a.s./kg bw	EFSA Scientific

Species	Substance	Exposure System	Results	Reference
		1 d Acute	(Extrapolated value– 3776 mg a.s./kg bw¹) NOEL = 200 mg a.s./kg bw.	Report (2007) 106, 1-98. DAR, V.3, Annex B, B.9, (2005). xxxxxxx BAR LD 028
<i>Colinus virginianus</i>	Prothioconazole-desthio (M04)	Oral 1 d Acute	LD ₅₀ = >2000 mg metabolite/kg bw NOEL = 500 mg metabolite./kg bw.	EFSA Scientific Report (2007) 106, 1-98. DAR, V.3, Annex B, B.9, (2005). xxxxxxx VB 009
<i>Colinus virginianus</i>	Azoxystrobin	Oral 1 d Acute	LD ₅₀ = >2000 mg a.s./kg bw	EFSA Journal (2010); 8(4):1542 xxxxxxx 1992.AVS95-00132
<i>Colinus virginianus</i>	Prothioconazole	Dietary 5-d short term	LC ₅₀ = >5000 mg a.s./kg diet (calc. LD ₅₀ = >1413 mg a.s./kg bw/day)	EFSA Scientific Report (2007) 106, 1-98. DAR, V.3, Annex B, B.9, (2005). xxxxxxx BAR/LC 005
<i>Colinus virginianus</i>	Prothioconazole-desthio (M04)	Dietary 5-d short term	LC ₅₀ = 4090 mg metabolite/kg diet (calc. LD ₅₀ = >297 mg metabolite/kg bw/day²)	EFSA Scientific Report (2007) 106, 1-98. DAR, V.3, Annex B, B.9, (2005). xxxxxxx BAR/LC 011
<i>Colinus virginianus</i>	Azoxystrobin	Dietary 5-d short term	LC ₅₀ = >5200 mg/kg diet LD₅₀ = >1179 mg a.s./kg bw/day	EFSA Journal (2010); 8(4):1542 xxxxxxx 1992. AVS95-00135
<i>Anas platyrhynchos</i>	Prothioconazole	21-week Dietary reproductive toxicity	NOEC = 700 mg a.s./kg diet NOEL = 78 mg a.s./kg bw/d	EFSA Scientific Report (2007) 106, 1-98. DAR, V.3, Annex B, B.9, (2005). xxxxxxx 2000b. 259919

Species	Substance	Exposure System	Results	Reference
<i>Colinus virginianus</i>	Prothioconazole-desthio (M04)	20-week Dietary reproductive	NOEC = 173 mg metabolite/kg diet (calc. NOEL = 14.8 mg metabolite/kg bw/d)	EFSA Scientific Report (2007) 106, 1-98. DAR, V.3, Annex B, B.9, (2005). xxxxxxx BAR REP006.
<i>Colinus virginianus</i>	Azoxystrobin	20-week Dietary reproductive toxicity	NOEL = >1200 mg/kg diet NOEC = 117 mg a.s./kg bw/day	EFSA Journal (2010); 8(4):1542 xxxxxxx AVS95-00137

Values highlighted in bold are used in the risk assessment.

¹. Extrapolation according to EFSA (2009) Chapter 2.1.2. has been applied to the acute endpoint LD₅₀ >2000 mg a.s./kg bw 1999) since 10 animals were tested and there were no mortalities at the limit dose (extrapolation factor = 1.888). Risk assessment will include both the extrapolated and non-extrapolated LD₅₀ for completeness.

². Value represents the dose converted from the test group in which No Effect on mortality or food consumption was reported (1243 mg/kg diet/day multiplied by the mean daily food consumption (6.4 g/day for the first 5 days exposure period) divided by the mean bodyweight (26.75 g for the 5-day exposure period)). Taken from EFSA, 2007.

9.2.1.1 Justification for new endpoints

The study of the acute oral toxicity of prothioconazole to bobwhite quail is considered suitable for use in regulatory risk assessment. As no mortality was observed in this study, EFSA (2009) guidance (section 2.1.2) permits the extrapolation of a definitive acute oral LD₅₀ endpoint from the greater-than value of > 2000 mg a.s./kg bw provided by the limit test. Ten individuals were used per dose group in this study, so an extrapolation factor of 1.888 is appropriate and the resulting estimated acute LD₅₀ is 3776 mg a.s./kg bw. This is considered to be the appropriate avian acute oral toxicity endpoint for use in regulatory risk assessment. For completeness, both acute oral toxicity endpoints are included in the regulatory risk assessment.

According to EFSA (2009) guidance, avian dietary (short term) risk assessments are only necessary on occasions when a dietary LD₅₀ (expressed in terms of a daily dose) is lower than the corresponding acute oral LD₅₀. In the case of both prothioconazole and azoxystrobin, the short-term LDD₅₀ values appear to be lower than the corresponding acute oral LD₅₀, but this is an artefact that has arisen because both endpoints are greater-than values which exceed the highest dose administered in the respective studies. The short-term dietary LDD₅₀ values, therefore, considered solely for the purpose of completeness.

Similarly, in the case of prothioconazole-desthio (M04), the short-term LDD₅₀ (>297 mg metabolite/kg bw/d), is lower than the corresponding acute oral LD₅₀ (>2000 mg a.s./kg bw/d) but this is an artefact of the unbound endpoints for both studies. The short-term dietary LDD₅₀ values has, however, been included for completeness.

No new chronic bird toxicity studies were submitted for approval of FF-075. The active substance endpoint used in the risk assessment for the renewal of prothioconazole (EFSA, 2007) was NOEL = 78 mg a.s./kg bw/d. It should be noted that the LD₅₀/10 is >200 mg a.s./kg bw which is higher than the lower NOEL endpoint. It is, therefore, considered appropriate to retain the NOEL used in the approval of prothioconazole (78 mg a.s./kg bw/d) as cited in the EFSA Scientific report (2007).

The active substance endpoint used in the risk assessment for the renewal of azoxystrobin (EFSA, 2010)

was NOEL = 117 mg a.s./kg bw/d. It should be noted that the acute LD₅₀/10 for azoxystrobin is >200 mg a.s./kg bw which is higher than the lower NOEL endpoint. It is, therefore, considered appropriate to retain the NOEL used in the approval of prothioconazole (117 mg a.s./kg bw/d) as cited in the EFSA Scientific report (2010).

The chronic avian endpoint for prothioconazole-desthio (M04) (NOEL = 14.8 mg metabolite/kg bw/d), is unchanged from the renewal of the approval for active substance (EFSA, 2007). The LD₅₀/10 is >200 mg metabolite/kg bw/d when considering the acute oral endpoint, and 29.7 mg metabolite/kg bw/d when considering the short-term dietary endpoint. Both values are higher than the NOEL endpoint of 14.8 mg metabolite/kg bw/d and it is, therefore, appropriate to consider the lower value in the risk assessment.

Avian data for the preparation, FF-075, is not available. Although acute avian data for the formulation is a data requirement under EU Regulations 284/2013, implementing EU Reg. (EC) 1107/2009, the experimentally-derived data for prothioconazole, prothioconazole-desthio and azoxystrobin (>2000 mg/kg bw/d for each substance), indicates low toxicity to birds at the screening and first-tier step. Therefore, in order to avoid unnecessary testing of vertebrates, further testing of the formulation has been waived in favour of conducting the risk assessment with the estimated value (LD₅₀ mix) (See Section 9.2.2).

9.2.2 Toxicity data

Mammalian toxicity studies have been carried out with prothioconazole, its relevant metabolite, prothioconazole-desthio (M04), and azoxystrobin. Full details of these studies are provided in the respective prothioconazole EU DAR, (2005) and the EFSA Conclusions (2007).

Effects on mammals of FF-075 were not evaluated as part of the EU assessment of prothioconazole. An assessment has been provided in Section 9.3.2.1.

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

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

Species	Substance	Exposure System	Results	Reference
Rat	Prothioconazole	Oral 1 d Acute	LD ₅₀ = > 6200 mg a.s./kg bw	EFSA Scientific Report (2007) 106, 1-98. DAR, V.3, Annex B, B.6, (2005). xxxxxxx M-012312-01-1
Mouse	Prothioconazole-desthio (M04)	Oral 1 d Acute	LD ₅₀ male = 2235 mg metabolite/kg bw LD ₅₀ female = 3459 mg metabolite/kg bw	EFSA Scientific Report (2007) 106, 1-98. DAR, V.3, Annex B, B.6, (2005). xxxxxxx M-292722-01-1

Species	Substance	Exposure System	Results	Reference
Rat	Azoxystrobin	Oral 1 d Acute	LD₅₀ = >5000 mg a.s./kg bw/day	EFSA Journal (2010); 8(4):1542
Rat	Prothioconazole	Dietary Reproductive toxicity Two-generation study	NOAEC _{parent} = 9.7 mg/kg bw/day NOAEC _{repro.} = 95.6 mg a.s./kg bw NOAEL _{offspring} = 95.6 mg a.s./kg bw/day	EFSA Scientific Report (2007) 106, 1-98. DAR, V.3, Annex B, B.6, (2005). xxxxxxx Report No. 110500
Rat	Prothioconazole-desthio (M04)	Dietary Reproductive toxicity Two-generation study	NOEC _{repro.} = 10.0 mg metabolite/kg bw/day NOEC _{parent} = 2.5 mg metabolite/kg bw/day	EFSA Scientific Report (2007) 106, 1-98. Author name redacted, 2001.
Rat	Azoxystrobin	Dietary Reproductive toxicity Two-generation study	NOEC repro = 32 mg a.s./kg bw/day NOEL = 300 mg/kg feed	EFSA Journal (2010); 8(4):1542

Values highlighted in bold are used in the risk assessment.

9.2.2.1 Justification for new endpoints

The acute and long-term endpoints selected for inclusion in the regulatory risk assessment are in agreement with the endpoints selected in the prothioconazole DAR (2005), Prothioconazole EFSA Scientific Review (2007) and the azoxystrobin EFSA Journal (2010).

Mammalian data for the preparation, FF-075, is not available. Although acute mammalian data for the formulation is a data requirement under EU Regulations 284/2013, implementing EU Reg. (EC) 1107/2009, the experimentally-derived data for prothioconazole, prothioconazole-desthio and azoxystrobin (>6200, 2235 and >5000 mg/kg bw/d for prothioconazole, prothioconazole-desthio and azoxystrobin, respectively), indicates acceptable risk to mammals. Therefore, in order to avoid unnecessary testing of vertebrates, further testing of the formulation has been waived in favour of conducting the risk assessment with the estimated value (LD₅₀ mix) (See Section 9.3.2).

9.2.3 Risk assessment for spray applications

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

The acute toxicity to birds has been estimated assuming dose additivity of the single active substances in the formulation with the following equation (EFSA Journal 2009; 7(12): 1438):

where: $X(a.s.i)$ is the fraction of the active substance i in the mixture (the sum $\Sigma(a.s.i)$ must be 1)
 $LD_{50}(a.s.i)$ is the acute toxicity for the active substance i .

Considering the lowest oral LD_{50} values determined for prothioconazole and azoxystrobin of > 2000 mg/kg bw, for both active substances, and their nominal concentrations in FF-075 (18.18% w/w and 13.64 % w/w for prothioconazole and azoxystrobin, respectively¹), the calculated LD_{50} value for FF-075 is equivalent to **>2000 mg/kg bw**.

	Prothioconazole	Azoxystrobin
Content in the formulation FF-075 (% w/w)	18.18%	13.64%
Fraction in the a.s. mixture	$18.18 / 31.82 = 0.57$	$13.64 / 31.82 = 0.43$
LD_{50} of a.s. [mg/kg bw]	2000	2000
Fraction / LD_{50}	$0.57 / 2000$	$0.43 / 2000$
	0.000285	0.000215
Sum		
$1 / \text{sum} = \text{predicted } LD_{50} \text{ (mix)}$	2000 mg mix/kg bw	

Experimentally derived data for the formulation, FF-075, is not available to perform a comparison with the estimated value LD_{50} (mix) of 2000 mg/kg bw. Therefore, the estimated value (LD_{50} mix) of >2000 mg/kg bw is used in the screening assessment.

In accordance with the guidance (EFSA Journal 2009; 7(12): 1438) a chronic mixture toxicity assessment is not required.

Risk Assessment for combined exposure

According to the EFSA Journal (2009)², the simultaneous exposure of animals to residues of two or more potential toxic substances should be considered in the risk assessment. Therefore, for the assessment of acute effects, a surrogate LD_{50} for the mixture of active substances with known toxicity was derived assuming dose additivity of toxicity. For the calculation, the following equation was used:

¹ Certificate of Analysis. Prothioconazole 200 g/L + Azoxystrobin 150 g/L Suspension Concentrate. Study Number 2879. 2020.

² European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12): 1438. [139 pp.].

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

With:

$X(a.s._i)$ = fraction of each a.s. in the mixture

$LD_{50}(a.s._i)$ = acute toxicity value for each a.s.

Acute risks from combined exposure

Azoxystrobin + prothioconazole

The active substance content of the formulation Euskatel Pro (Azoxystrobin 150 g/L + Prothioconazole 200 g/L) addressed in this dossier is 15 % azoxystrobin and 20 % prothioconazole, making up a total of 350 g a.s./L product.

Tables below shows the calculation of the predicted LD_{50} (mix) of azoxystrobin and prothioconazole when mixed in these proportions (step 1 in Appendix 2 to the EFSA GD 2009).

Avian LD_{50} (mix) for azoxystrobin and prothioconazole when combined (step 1 in EFSA GD 2009, Appendix B)

	Azoxystrobin	Prothioconazole
Content in the formulation g/L	150 53.3*	200 70.4*
Fraction in the a.s. mixture	0.4285 0.41	0.5715 0.59
LD_{50} of a.s. [mg/kg bw]	1179	>1413
Fraction / LD_{50}	0.000363 0.000364	0.000404 0.000404
Sum	0.0007674 0.000768	
1/ sum = predicted LD_{50} (mix)	1303.10 mg mix/kg bw 1302.1 mg mix/kg bw	

*d=1.669 g/l according to information given in PART C

Avian “tox per fraction” for the Euskatel Pro (step 1 in EFSA GD 2009, Appendix B)

	Azoxystrobin	Prothioconazole	“mix”
Content in the formulation 5.4	450 153.8*	200 102*	350 157.3*
Fraction in mixture	0.4285 0.43	0.5715 0.57	
LD ₅₀ (mg/kg bw)	1179	>1413	LD _{mix} =1303.10 LD _{mix} ~1302
Tox per fraction	2751.45 2741.86	2472.44 2478.94	
Contribution to predicted toxicity	52.64% 52.52%	47.36 47.48%	

*d=1.1669 g/l according to information given in PART C

Azoxystrobin contributes to 52.52 % to mixture toxicity, while the prothioconazole have an impact on the predicted risk of 47.48 %, therefore, surrogate LD₅₀ of 1302.10 mg/kg bw should be used in the acute risk assessment.

Reproductive risks from combined exposure

Azoxystrobin + prothioconazole

Avian NOEL (mix) for azoxystrobin and prothioconazole when combined (step 1 in EFSA GD 2009, Appendix B)

	Azoxystrobin	Prothioconazole
Content in the formulation	150 55.3%	200 20%
Fraction in the a.s. mixture	0.4285 0.43	0.5715 0.57
NOEL ₅₀ of a.s. [mg/kg bw]	117	78
Fraction / NOEL	0.00366234 0.00367	0.0073269 0.0073
Sum	0.0011 0.0109%	
1/ sum = predicted NOEL (mix)	90.90 mg mix/kg bw 91.15 mg mix/kg bw	

Avian “tox per fraction” for the Euskatel Pro (step 1 in EFSA GD 2009, Appendix B)

	Azoxystrobin	Prothioconazole	“mix”
Content in the formulation	150 55.3%	200 20%	350 35.3%
Fraction in mixture	0.4285 0.43	0.5715 0.57	
NOEL (mg/kg bw)	117	78	NOEL _{mix} =90.90 91.15
Tox per fraction	273.04 272.09	136.48 136.84	
Contribution to predicted toxicity	33.30 66.62 %	70.70 33.38 %	

Azoxystrobin contributes to 66.62 % to mixture toxicity, while the prothioconazole have an impact on the predicted risk of 33.38%, therefore, surrogate NOEL of 91.15 mg /kg bw was used in the chronic risk assessment.

Azoxystrobin + JAU-desthio (metabolite of prothioconazole)

The combined risk to birds should also be evaluated for the metabolite JAU-desthio prothioconazole. Because it is not possible to determine the exact application dose for the metabolite, combitox was not evaluated according to Finney's equation, but using the combined toxicity risk (TER_{MIX}) with concentration-addition is estimated based on the following equation:

$$TER_{mix} = 1 / \left(\frac{1}{TER_{a.s.1}} + \frac{1}{TER_{a.s.2}} + \dots + \frac{1}{TER_{a.s.i}} \right)$$

An acceptable risk is expected when TER_{combi} > trigger.

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

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

Table 9.2-3: Screening assessment of the acute, short-term dietary and long-term/reproductive risk for birds due to the use of FF-075 in winter and spring cereals prothioconazole.

Intended use		Winter and spring cereals			
Active substance		Prothioconazole			
Application rate (g/ha)		2 x 200 g a.s./ha (minimum 14-d interval)			
Acute toxicity (mg/kg bw)		>2000			
TER criterion		10			
Crop scenario	Indicator species for screening	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a
N/A	Small omnivorous bird	158.8	1.2	38.112	52
Acute toxicity (mg/kg bw)		3776 (extrapolated value)			
TER criterion		10			
Crop scenario	Indicator species for screening	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a
N/A	Small omnivorous bird	158.8	1.2	38.112	99
Dietary toxicity (mg/kg bw)		>1413*			
TER criterion		10			
Crop scenario	Indicator species for screening	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a
Growth stage					
N/A	Small omnivorous bird	158.8	1.2	38.112	37
Reprod. toxicity (mg/kg bw/d)		78			
TER criterion		5			

Crop scenario Growth stage	Indicator species for screening	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{lt}
N/A	Small omnivorous bird	64.8	0.742	9.61632	8.11

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

*The lowest endpoint was reported from the short term dietary toxicity. This endpoint should be rather used in the risk assessment.

Table 9.2-3: Screening assessment of the acute, short-term dietary and long-term/reproductive risk for birds due to the use of FF-075 in winter oilseed rape - prothioconazole.

Intended use		Winter oilseed rape			
Active substance		Prothioconazole			
Application rate (g/ha)		2 x 160 g a.s./ha (minimum 14-day interval)			
Acute toxicity (mg/kg bw)		>2000			
TER criterion		10			
Crop scenario Growth stage	Indicator species for screening	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a
N/A	Small omnivorous bird	158.8	1.2	30.4896	66
Acute toxicity (mg/kg bw)		3776 (extrapolated value)			
TER criterion		10			
Crop scenario Growth stage	Indicator species for screening	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a
N/A	Small omnivorous bird	158.8	1.2	30.4896	124
Dietary toxicity (mg/kg bw)		>1413*			
TER criterion		10			
Crop scenario Growth stage	Indicator species for screening	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a

N/A	Small omnivorous bird	158.8	1.2	30.4896	46
Reprod. toxicity (mg/kg bw/d)		78			
TER criterion		5			
Crop scenario	Indicator species for screening	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{lt}
N/A	Small omnivorous bird	64.8	0.742	7.693056	10.14

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

*The lowest endpoint was reported from the short term dietary toxicity. This endpoint should be rather used in the risk assessment.

Table 9.2-4: Screening assessment of the acute, short-term dietary and long-term/reproductive risk for birds due to the use of FF-075 in winter and spring cereals - prothioconazole-desthio (M04).

Intended use		Winter and spring cereals			
Metabolite		Prothioconazole-desthio (M04)			
Application rate (g/ha)		2 x 200 g a.s./ha (minimum 14-day interval)			
Acute toxicity (mg/kg bw)		>2000			
TER criterion		10			
Crop scenario	Indicator species for screening	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a
N/A	Small omnivorous bird	158.8	1.2	38.112	52
Dietary toxicity (mg/kg bw)		>297**			
TER criterion		10			
Crop scenario	Indicator species for screening	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a
Growth stage					
N/A	Small omnivorous bird	158.8	1.2	38.112	8
Reprod. toxicity (mg/kg bw/d)		14.8			
TER criterion		5			

Crop scenario Growth stage	Indicator species for screening	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{lt}
N/A	Small omnivorous bird	64.8	0.742	9.61632	1.54

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

****The lowest endpoint was reported from the short term dietary toxicity. This endpoint should be rather used in the risk assessment.**

Table 9.2-5: Screening assessment of the acute, short-term dietary and long-term/reproductive risk for birds due to the use of FF-075 in winter oilseed rape - prothioconazole-desthio (M04).

Intended use	Winter oilseed rape				
Metabolite	Prothioconazole-desthio (M04)				
Application rate (g/ha)	2 x 160 g a.s./ha (minimum 14-day interval)				
Acute toxicity (mg/kg bw)	>2000				
TER criterion	10				
Crop scenario Growth stage	Indicator species for screening	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a
N/A	Small omnivorous bird	158.8	1.2	30.4896	66
Dietary toxicity (mg/kg bw)	>297**				
TER criterion	10				
Crop scenario Growth stage	Indicator species for screening	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a
N/A	Small omnivorous bird	158.8	1.2	30.4896	10
Reprod. toxicity (mg/kg bw/d)	14.8				
TER criterion	5				
Crop scenario Growth stage	Indicator species for screening	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{lt}
N/A	Small omnivorous bird	64.8	0.742	7.693056	1.92

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

****The lowest endpoint was reported from the short term dietary toxicity. This endpoint should be rather used in the risk assessment.**

The TER_a values for both prothioconazole and prothioconazole-desthio (M04) are greater than the trigger of 10 for both the acute and dietary endpoints, indicating low acute risks to birds from exposure to FF-075 to winter and spring cereals and winter oilseed rape at the proposed label rates.

The TER_{lt} values are greater than the trigger of 5 for prothioconazole, indicating low long-term risks to birds from prothioconazole from applications of FF-075 at the proposed label rates for all uses.

The TER_{lt} values are lower than the trigger of 5 for prothioconazole-desthio (M04) for applications to winter and spring cereals and winter oilseed rape at the proposed label rates, indicating potential risk to birds. Tier 1 long-term risk assessment of prothioconazole-desthio (M04) is, therefore, required.

As the acute and chronic risks are acceptable at the screening step, which already takes into account worst case assumptions, no restriction on BBCH is required for this aspect of the risk assessment.

Table 9.2-6: Screening assessment of the acute, short-term dietary and long-term/reproductive risk for birds due to the use of FF-075 in winter and spring cereals azoxystrobin.

Intended use		Winter and spring cereals				
Active substance		Azoxystrobin				
Application rate (g/ha)		2 x 150 g a.s./ha (minimum 14-d interval)				
Acute toxicity (mg/kg bw)		>2000				
TER criterion		10				
Crop scenario	Indicator species for screening	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a	
Growth stage						
N/A	Small omnivorous bird	158.8	1.2	28.584	70	
Dietary toxicity (mg/kg bw)		>1179***				
TER criterion		10				
Crop scenario	Indicator species for screening	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a	
Growth stage						
N/A	Small omnivorous bird	158.8	1.2	28.584	41	
Cereals , BBCH30-39	Indicator species for Tier 1	SV ₉₀	MAF ₉₀	DDD ₉₀	TER _a	

				(mg/kg bw/d)	
	Small mnivorous lark Woodlark (Lilla arb.)	12	1.2	2.16	545.83
Reprod. toxicity (mg/kg bw/d)		117			
TER criterion		5			
Crop scenario Growth stage	Indicator species for screening	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{lt}
N/A	Small omnivorous bird	64.8	0.742	7.21224	16.22

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

***According to the Guidance EFSA/2009/1438, where the dietary LC₅₀ is lower than the acute LD₅₀, the dietary value should be used in the acute risk assessment. Therefore, LC₅₀ >1179 mg azoxystrobin/kg bw/day should be used in the risk assessment.

Table 9.2-7: Screening and Tier 1 assessment of the acute, short-term dietary and long-term/reproductive risk for birds due to the use of FF-075 in winter oilseed rape azoxystrobin.

Intended use		Winter oilseed rape			
Active substance		Azoxystrobin			
Application rate (g/ha)		2 x 120 g a.s./ha (minimum 14-d interval)			
Acute toxicity (mg/kg bw)		>2000			
TER criterion		10			
Crop scenario Growth stage	Indicator species for screening	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a
N/A	Small omnivorous bird	158.8	1.2	22.8672	87
Dietary toxicity (mg/kg bw)		>1179***			
TER criterion		10			
Crop scenario Growth stage	Indicator species for screening	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a
N/A	Small omnivorous bird	158.8	1.2	22.8672	52

Reprod. toxicity (mg/kg bw/d)		117			
TER criterion		5			
Crop scenario	Indicator species for screening	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{lt}
N/A	Small omnivorous bird	64.8	0.742	5.769792	20.28

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

***According to the Guidance EFSA/2009/1438, where the dietary LC₅₀ is lower than the acute LD₅₀, the dietary value should be used in the acute risk assessment. Therefore, LC₅₀ >1179 mg azoxystrobin/kg bw/day should be used in the risk assessment.

The TER_a and TER_{lt} values for azoxystrobin are greater than the trigger of 10 and 5, respectively, for the acute and dietary endpoints, and the long-term reproductive endpoint. This indicates low acute and chronic risks to birds from exposure to FF-075 to winter and spring cereals (BBCH 30-69) and winter oilseed rape (BBCH 55-69) at the proposed label rates.

Table 9.2-8: Screening assessment of the acute, short-term dietary and long-term/reproductive risk for birds due to the use of FF-075 in winter and spring cereals – FF-075

Intended use		Winter and spring cereals			
Active substance		FF-075			
Application rate (g/ha)		2 x 1100 g formulation/ha (minimum 14 d interval)			
Acute toxicity (mg/kg bw)		>2000 (estimated value (LD ₅₀ -mix))			
TER criterion		10			
Crop scenario	Indicator species for screening	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a
N/A	Small omnivorous bird	158.8	1.2	209.616	>10

Table 9.2-9: Screening assessment of the acute, short-term dietary and long-term/reproductive risk for birds due to the use of FF-075 in winter oilseed rape – FF-075.

Intended use		Winter oilseed rape			
Active substance		FF-075			

Application rate (g/ha)		2 x 880 g formulation/ha (minimum 14 d interval)			
Acute toxicity (mg/kg bw)		>2000 (estimated value (LD ₅₀ mix)			
TER criterion		10			
Crop scenario Growth stage	Indicator species for screening	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a
N/A	Small omnivorous bird	158.8	1.2	167.693	>12

The TER_a values for FF-075 are greater than or equal to the trigger of 10 for uses in both winter and spring cereals, and winter oilseed rape. This indicates low acute and chronic risks to birds from exposure to FF-075 to winter and spring cereals (BBCH 30-69) and winter oilseed rape (BBCH 55-69) at the proposed label rates.

Tier 1 Prothioconazole-desthio (M04) acute and long risk assessment

Table 9.2-10: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of FF-075 in winter and spring cereals (BBCH-30-69) – prothioconazole-desthio

Intended use		Winter and spring cereals			
Metabolite		Prothioconazole-desthio (M04)			
Application rate (g/ha)		2 x 200 g a.s./ha (minimum 14-day interval)			
Reprod. toxicity (mg/kg bw/d)		14.8			
TER criterion		5			
Crop scenario Growth stage	Indicator/generic focal species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{lt}
Cereals, BBCH 30-39	Small omnivorous bird “lark” Woodlark (<i>Lullula arborea</i>)	5.4	0.742	0.801	18.47
Cereals, BBCH ≥ 40	Small omnivorous bird “lark” Woodlark (<i>Lullula arborea</i>)	3.3	0.742	0.490	30.22
Intended use		Winter and spring cereals			
Metabolite		Prothioconazole-desthio (M04)			
Application rate (g/ha)		2 x 200 g a.s./ha (minimum 14-day interval)			

Acute toxicity (mg/kg bw/d)		>297			
TER criterion		10			
Crop scenario Growth stage	Indicator/generic focal species	SV_{90th}	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_A
Cereals, BBCH 30-39	Small omnivorous bird "lark" Woodlark (<i>Lullula arborea</i>)	12	1.2	2.88	103.125

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

Table 9.2-11: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of FF-075 in winter oilseed rape (BBCH-55-69) – prothioconazole-desthio (M04)

Intended use		Winter oilseed rape			
Metabolite		Prothioconazole-desthio (M04)			
Application rate (g/ha)		2 x 160 g a.s./ha (minimum 14-day interval)			
Reprod. toxicity (mg/kg bw/d)		14.8			
TER criterion		5			
Crop scenario Growth stage	Indicator/generic focal species	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{lt}
Oilseed rape BBCH 30-99	Small insectivorous bird "dunnock" Dunnock (<i>Prunella modularis</i>)	2.7	0.742	0.321	46.17
Oilseed rape BBCH ≥40	Small omnivorous bird "lark" Woodlark (<i>Lullula arborea</i>)	2.7	0.742	0.321	46.17
Oilseed rape BBCH ≥40	Medium herbivorous/granivorous bird "pigeon" Wood pigeon (<i>Columba palumbus</i>)	0.9	0.742	0.107	138.51

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

The first-tier TER_A and TER_{lt} values are greater than the trigger value of 5, respectively, indicating low acute and long-term risks to birds from prothioconazole-desthio (M04) following applications of FF-075 at the proposed label rates.

Table 9.2-12: First-tier assessment of long-term/reproductive risk for birds due to the use of Euskatel Pro in winter and spring cereals – azoksystrobin.

Intended use	Winter and spring cereals				
a.s.	Azoksystrobin				
Application rate (g/ha)	2 x 150 g a.s./ha (minimum 14-day interval)				
Reprod. toxicity (mg/kg bw/d)	14.8				
TER criterion	5				
Crop scenario Growth stage	Indicator/generic focal species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{lt}
Cereals, BBCH 30-39	Small omnivorous bird "lark" Woodlark (<i>Lullula arborea</i>)	5.4	0.742	0.601	24.62
Cereals, BBCH ≥ 40	Small omnivorous bird "lark" Woodlark (<i>Lullula arborea</i>)	3.3	0.742	0.367	40.32

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

Table 9.2-13: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of Euskatel Pro in winter oilseed rape – azoksystrobin.

Intended use	Winter oilseed rape				
a.s.	Azoksystrobin				
Application rate (g/ha)	2 x 120 g a.s./ha (minimum 14-day interval)				
Reprod. toxicity (mg/kg bw/d)	14.8				
TER criterion	5				
Crop scenario Growth stage	Indicator/generic focal species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{lt}
Oilseed rape BBCH 30-99	Small insectivorous bird "dun-nock" Dunnock (<i>Prunella modularis</i>)	2.7	0.742	0.24	61.66
Oilseed rape BBCH ≥40	Small omnivorous bird "lark" Woodlark (<i>Lullula arborea</i>)	2.7	0.742	0.24	61.66
Oilseed rape BBCH ≥40	Medium herbivorous/granivorous bird "pigeon" Wood pigeon (<i>Columba palumbus</i>)	0.9	0.742	0.08	185

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

The first-tier TER_a and TER_{lt} values are greater than the trigger value of 5, respectively, indicating low acute and long-term risks to birds from prothioconazole-desithio (M04) following applications of FF-075 at the proposed label rates.

Combitox risk assessment:

zRMS provided the own risk assessment based on LD₅₀ and NOELmix calculations.

Screening assessment of the acute risk for birds due to the use of Euskatel Pro in cereals.

Intended use	Cereals				
Active substance/product	Euskatel Pro				
Application rate (g/ha)	2 x 350				
LD ₅₀ (mix) (mg/kg bw)	130 10				
TER criterion	10				
Crop scenario Growth stage	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a
Cereals	Small omnivorous birds	158.8	1.2	66.7	19.52

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

Screening assessment of the acute risk for birds due to the use of Euskatel Pro in oilseed rape.

Intended use	Oilseed rape				
Active substance/product	Euskatel Pro				
Application rate (g/ha)	2 x 280				
LD ₅₀ (mix) (mg/kg bw)	130 10				
TER criterion	10				
Crop scenario Growth stage	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a
Oilseed rape	Small omnivorous birds	158.8	1.2	53.35	2.30

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

According to results, no unacceptable acute risk is obtained in all crops according to the proposed GAP.

Reproductive risks from combined exposure

Screening assessment of the chronic risk for birds due to the use of Euskatel Pro in cereals.

Intended use	Cereals				
Active substance/product	Euskatel Pro				
Application rate (g/ha)	2 x 350				
NOEL (mix) (mg/kg bw)	91.3				
TER criterion	5				
Crop scenario Growth stage	Indicator/generic focal species	SV	MAF _m x TWA	DDD (mg/kg bw/d)	TER _{lt}
Cereals	Small omnivorous birds	64.8	1.4 x 0.53	16.82	5.42

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER:

toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Screening assessment of the acute risk for birds due to the use of Euskatel Pro in oilseed rape.

Intended use	Oilseed rape				
Active substance/product	Euskatel Pro				
Application rate (g/ha)	2 x 280				
NOEL (mix) (mg/kg bw)	11.5				
TER criterion	5				
Crop scenario Growth stage	Indicator/generic focal species	SV	MAF _m x TWA	DDD (mg/kg bw/d)	TER _{it}
Oilseed rape	Small omnivorous birds	64.8	1.4 x0.53	13.46	6.77

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

According to results, no unacceptable acute risk is obtained in all crops according to the proposed GAP.

Azoxystrobin + JAU-desthio (metabolite of prothioconazole)

Screening step assessment of the acute risk for birds due to the use of Euskatel Pro in all crops.

Intended use	Cereals, oilseed rape				
Application rate (g/ha)	2 × 350 for cereals, 2 x 280 for oilseed rape				
Crop scenario	Indicator species	TER _{azoxystrobin}	TER _{prothioconazole- desthio}	TER criterion	TER _{combi}
Cereals	Small omnivorous bird	41	8	10	6.71
Oilseed rape		52	14.8		11.68

TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Screening step assessment of the chronic risk for birds exposed to Euskatel Pro in all crops

Intended use	Cereals, oilseed rape				
Application rate (g/ha)	Euskatel Pro				
Crop scenario	Indicator species	TER _{azoxystrobin}	TER _{prothioconazole- desthio}	TER criterion	TER _{combi}
Cereals	Small omnivorous bird	16.22	1.54	5	1.40
Oilseed rape		20.28	1.92		1.75

Tier 1

First tier risk assessment of the acute risk for birds exposed to Euskatel Pro in all crops

Intended use	Cereals
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Application rate (g/ha)		Euskatel Pro			
Crop scenario	Indicator species	TER _{azoxystrobin}	TER _{prothioconazole-desthio}	TER _{criterion}	TER _{combi}
Cereals BBCH 30-39	Small omnivorous bird “lark”	545.83	103.125	10	86.74

First tier risk assessment of the chronic risk for birds exposed to Euskatel Pro in all crops

Intended use		Cereals, oilseed rape			
Application rate (g/ha)		Euskatel Pro			
Crop scenario	Indicator species	TER _{azoxystrobin}	TER _{prothioconazole-desthio}	TER _{criterion}	TER _{combi}
Cereals BBCH 30-39	Small omnivorous bird “lark”	24.62	18.47	5	10.56
Oilseed rape, BBCH 30-99	Small insectivorous bird, “dunnock”	61.66	46.17		27.027

TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

*the risk assessment is performed for the worst case

The acute and reproductive risk assessment for birds for combined exposure demonstrated acceptable risk for all required scenarios.

zRMS comment:

The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438; hereafter referred to as EFSA/2009/1438). Safe use of azoxystrobin, prothioconazole and prothioconazole-desthio (M04) birds were confirmed based on TER_A and TER_{LT} above the trigger values of 10 and 5, respectively, indicating the acute and long-term risk is acceptable.

In addition, the combitox risk assessment is considered as acceptable to birds.

9.2.3.1 Higher-tier risk assessment

Since acceptable acute and long-term risks have been concluded for birds exposed to prothioconazole, prothioconazole-desthio (M04) and azoxystrobin at the screening level and first-tier, higher-tier risk assessment is not required for the proposed uses of FF-075.

9.2.3.2 Drinking water exposure

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

Leaf scenario

Since FF-075 is not intended to be applied on leafy vegetables forming heads or crop plants with compa-

rable water collecting structures at principal growth stage 4 or later, the leaf scenario does not have to be considered.

Puddle scenario

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

With a $K(f)_{oc}$ of 1765 L/kg, prothioconazole belongs to the group of more sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use in cereals also covers the risk for birds from all other intended uses.

Effective application rate (g/ha)=	2 x 200		
Acute toxicity (mg/kg bw) =	3776	Quotient =	0.1
Dietary toxicity (mg/kg bw) =	>1413	Quotient =	<0.28
Reprod. toxicity (mg/kg bw/d) =	78	Quotient =	5.13

Since the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed the critical value of 3000, a quantitative risk assessment (calculation of TER values) for prothioconazole is not necessary.

With a $K(f)_{oc}$ of 574 L/kg, prothioconazole-desthio (M04) belongs to the group of more sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use in cereals also covers the risk for birds from all other intended uses (see 0).

Effective application rate (g/ha)=	2 x 200		
Acute toxicity (mg/kg bw) =	>2000	Quotient =	<0.2
Dietary toxicity (mg/kg bw) =	>297	Quotient =	<1.35
Reprod. toxicity (mg/kg bw/d) =	14.8	Quotient =	27.03

Since the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed the critical value of 3000, a quantitative risk assessment (calculation of TER values) for prothioconazole-desthio (M04) is not necessary.

With a $K(f)_{oc}$ of 207-594 L/kg, azoxystrobin belongs to the group of less sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use in cereals also covers the risk for birds from all other intended uses.

Effective application rate (g/ha)=	2 x 150		
Acute toxicity (mg/kg bw) =	2000	Quotient =	0.15
Dietary toxicity (mg/kg bw) =	>1179	Quotient =	<0.25
Reprod. toxicity (mg/kg bw/d) =	117	Quotient =	2.56

Since the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed the critical value of 50, a quantitative risk assessment (calculation of TER values) for azoxystrobin is not necessary.

zRMS comment:

Since is not a for spray applications / not intended to be applied on leafy vegetables forming heads or crop plants with comparable water collecting structures at principal growth stage 4 or later.

Therefore, the leaf scenario does not have to be considered taking onto account the proposed uses (cere-

als). Evaluation of exposing for birds through the drinking water Puddle scenario for the active substances, demonstrate that the acceptable risk for birds for proposed use pattern of **Euskatel Pro**.

9.2.3.3 Effects of secondary poisoning

The log P_{ow} of prothioconazole amounts to 3.4 at pH4 and thus exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is required.

The log P_{ow} of prothioconazole-desthio (M04) amounts to 3.04 at pH 4 and thus exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is required.

The log P_{ow} of prothioconazole-S-methyl (M01) is predicted to be 4.19 at pH 7, (EFSA, 2007), and thus exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is required.

The log P_{ow} of azoxystrobin is predicted to be 2.5 at 20°C (without pH dependence), (EFSA, 2010), and thus, does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is, therefore, not required.

Risk assessment for earthworm-eating birds via secondary poisoning

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

Assessment of the risk for earthworm-eating birds due to exposure to Prothioconazole and prothioconazole metabolites via bioaccumulation in earthworms (secondary poisoning)

Parameter	Prothioconazole	Prothioconazole-desthio (M04)	Prothioconazole-S-methyl (M01)	comments
log P_{ow} / P_{ow}	3.4 / 2511.9	3.04 / 1096.5	4.19 / 15448.2	EFSA Scientific Report (2007) 106, 1-98
Koc	1765	575.4	2556.3	Geomean (M04 and M01) EFSA Scientific Report (2007) 106, 1-98
foc	0.02	0.02	0.02	Default

Assessment of the risk for fish-eating birds due to exposure to Prothioconazole and Prothioconazole-desthio (M04) via bioaccumulation in fish (secondary poisoning)

Parameter	Prothioconazole	Prothioconazole-desthio (M04)	comments
BCF _{fish}	19.7	65	EFSA Scientific report (2007) 106, 1-98

Table 9.2-12: Assessment of the risk for earthworm-eating birds due to exposure to prothioconazole via bioaccumulation in earthworms (secondary poisoning) for the intended use in winter and spring cereals (BBCH 30-69)

Parameter	Prothioconazole	comments
PEC _{soil} (mg/kg soil)	0.0550	
log P _{ow} / P _{ow}	3.4 (at pH 4)/2512	EFSA Conclusion (EFSA Scientific Report (2007) 106, 1-98)
Koc	1765	
foc	0.02	Default
BCF _{worm}	0.878	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + 0.12 \times P_{ow}) / foc \times Koc$
PEC _{worm}	0.048	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.050689	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	78.0	
TER _{lt}	1 539	

TER values shown in bold fall below the relevant trigger.

Table 9.2-13: Assessment of the risk for earthworm-eating birds due to exposure to prothioconazole via bioaccumulation in earthworms (secondary poisoning) for the intended use in winter oilseed rape (BBCH 55-69).

Parameter	Prothioconazole	comments
PEC _{soil} (mg/kg soil)	0.0440	
log P _{ow} / P _{ow}	3.4 (at pH 4)/2512	EFSA Conclusion (EFSA Scientific Report (2007) 106, 1-98)
Koc	1765	
foc	0.02	Default
BCF _{worm}	0.878	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + 0.12 \times P_{ow}) / foc \times Koc$
PEC _{worm}	0.039	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.040551	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	78.0	
TER _{lt}	1 923	

TER values shown in bold fall below the relevant trigger.

The TER_{lt} for the assessment of the risk for earthworm-eating birds due to exposure to prothioconazole via bioaccumulation in earthworms does not fall below the relevant trigger TER value of 5, indicating low risk to birds following applications of FF-075 to cereals (BBCH 30-69) and winter oilseed rape (BBCH 55-69).

Table 9.2-14: Assessment of the risk for earthworm-eating birds due to exposure to prothioconazole-desthio (M04) via bioaccumulation in earthworms (secondary poisoning) for the intended use in winter and spring cereals (BBCH 30-69)

Parameter	Prothioconazole-desthio (M04)	comments
PEC_{soil} (mg/kg soil)	0.0569	
$\log P_{ow} / P_{ow}$	3.04/1100	EFSA Conclusion (EFSA Scientific Report (2007) 106, 1-98)
Koc	575.4	
foc	0.02	Default
BCF_{worm}	1.22	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.12 \times P_{ow}) / foc \times Koc$
PEC_{worm}	0.069	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.07289	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	14.8	
TER_{lt}	203	

TER values shown in bold fall below the relevant trigger.

Table 9.2.2-15: Assessment of the risk for earthworm-eating birds due to exposure to prothioconazole-desthio (M04) via bioaccumulation in earthworms (secondary poisoning) for the intended use in winter oilseed rape (BBCH 55-69).

Parameter	Prothioconazole-desthio (M04)	comments
PEC_{soil} (mg/kg soil)	0.0456	
$\log P_{ow} / P_{ow}$	3.04/1100	EFSA Conclusion (EFSA Scientific Report (2007) 106, 1-98)
Koc	575.4	
foc	0.02	Default
BCF_{worm}	1.22	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.12 \times P_{ow}) / foc \times Koc$
PEC_{worm}	0.056	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.058415	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	14.8	
TER_{lt}	253	

TER values shown in bold fall below the relevant trigger.

The TER_{lt} for the assessment of the risk for earthworm-eating birds due to exposure to prothioconazole-desthio (M04) via bioaccumulation in earthworms does not fall below the relevant trigger TER value of 5, indicating low risk to birds following applications of FF-075 to winter and spring cereals (BBCH 30-69)

and winter oilseed rape (BBCH 55-69).

Table 9.2-16: Assessment of the risk for earthworm-eating birds due to exposure to prothioconazole-S-methyl (M01) via bioaccumulation in earthworms (secondary poisoning) for the intended use in winter and spring cereals (BBCH 30-69).

Parameter	Prothioconazole-S- methyl (M01)	Comments
PEC _{soil} (mg/kg soil)	0.0163	
log P _{ow} / P _{ow}	4.19/20,000	EFSA Conclusion (EFSA Scientific Report (2007) 106, 1-98)
Koc	2256.3	
foc	0.02	Default
BCF _{worm}	5.337	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + 0.12 \times P_{ow}) / foc \times Koc$
PEC _{worm}	0.087	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.091344	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	7.8*	Derived endpoint
TER _{lt}	85	

TER values shown in bold fall below the relevant trigger.

*Endpoint estimated as parent NOEL/10.

Table 9.2-17: Assessment of the risk for earthworm-eating birds due to exposure to prothioconazole-S-methyl (M01) via bioaccumulation in earthworms (secondary poisoning) for the intended use in winter oil seed rape (BBCH 30-80).

Parameter	Prothioconazole-S- methyl (M01)	Comments
PEC _{soil} (mg/kg soil)	0.0130	
log P _{ow} / P _{ow}	4.19/20,000	EFSA Conclusion (EFSA Scientific Report (2007) 106, 1-98)
Koc	2256.3	
foc	0.02	Default
BCF _{worm}	5.337	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + 0.12 \times P_{ow}) / foc \times Koc$
PEC _{worm}	0.069	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.072851	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	7.8*	Derived endpoint
TER _{lt}	107	

TER values shown in bold fall below the relevant trigger.

*Endpoint estimated as parent NOEL/10.

The TER_{lt} for the assessment of the risk for earthworm-eating birds due to exposure to prothioconazole-S-methyl (M01) via bioaccumulation in earthworms does not fall below the relevant trigger TER value of 5, indicating low risk to birds following applications of FF-075 to winter and spring cereals (BBCH 30-69) and winter oilseed rape (BBCH 55-69).

Risk assessment for fish-eating birds via secondary poisoning

According to EFSA/2009/1438, the risk for piscivorous birds is assessed for a bird of 1000 g body weight with a daily food consumption of 159 g. Bioaccumulation in fish is estimated based on predicted concentrations in surface water.

Table: 9.2-18: Assessment of the risk for fish-eating birds due to exposure to prothioconazole via bioaccumulation in fish (secondary poisoning) for the intended use in winter oilseed rape (BBCH 55-69. 2 x 160 g a.s./ha, applied 14-day interval)

Parameter	Prothioconazole	Comments
PEC _{sw} (mg/L)	0.0347521	Section 8 – Step 1
BCF _{fish}	18.8 19.7	EFSA Conclusion (EFSA Scientific Report (2007) 106, 1-98)
BMF	N/A	biomagnification factor (relevant for BCF ≥ 2000)
PEC _{fish}	0.6533395 0.6846	PEC _{fish} = PEC _{water} × BCF _{fish}
Daily dietary dose (mg/kg bw/d)	0.103881 0.109	DDD = PEC _{fish} × 0.159
NOEL (mg/kg bw/d)	78	EFSA Conclusion, 2007
TER _{lt}	750.86 715.6	

TER values shown in bold fall below the relevant trigger.

Table 9.2-19: Assessment of the risk for fish-eating birds due to exposure to prothioconazole via bioaccumulation in fish (secondary poisoning) for the intended use in winter and spring cereals (BBCH 30-69. 2 x 200 g a.s./ha, applied 14-day interval)

Parameter	Prothioconazole	Comments
PEC _{sw} (mg/L)	0.0434401	Section 8 – Step 1
BCF _{fish}	18.8 19.7	EFSA Conclusion (EFSA Scientific Report (2007) 106, 1-98)
BMF	N/A	biomagnification factor (relevant for BCF ≥ 2000)
PEC _{fish}	0.82 0.86	PEC _{fish} = PEC _{water} × BCF _{fish}
Daily dietary dose (mg/kg bw/d)	0.1304 0.1360	DDD = PEC _{fish} × 0.159
NOEL (mg/kg bw/d)	78	EFSA Conclusion, 2007
TER _{lt}	598.25 573.53	

TER values shown in bold fall below the relevant trigger.

The TER_{lt} for the assessment of the risk for fish-eating birds due to exposure to prothioconazole via bioaccumulation in fish does not fall below the relevant trigger TER value of 5, indicating low risk to birds following applications of FF-075 to cereals (BBCH 30-69) and winter oilseed rape (BBCH 55-69).

Table 9.2-20: Assessment of the risk for fish-eating birds due to exposure to prothioconazole-desthio (M04) via bioaccumulation in fish (secondary poisoning) for the intended use in winter oilseed rape (BBCH 55-69. 2 x 160 g a.s./ha, applied 14-day interval)

Parameter	Prothioconazole-desthio (M04)	comments
PEC _{sw} (mg/L)	0.0626570	Section 8 – Step 1
BCF _{fish}	45 65	EFSA Conclusion (EFSA Scientific Report (2007) 106, 1-98)
BMF	N/A	biomagnification factor (relevant for BCF ≥ 2000)
PEC _{fish}	2.819565 4.07	PEC _{fish} = PEC _{water} × BCF _{fish}
Daily dietary dose (mg/kg bw/d)	0.4483108 0.64	DDD = PEC _{fish} × 0.159
NOEL (mg/kg bw/d)	14.8	EFSA Conclusion (EFSA Scientific Report (2007) 106, 1-98)
TER _{lt}	33.01 23.13	

TER values shown in bold fall below the relevant trigger.

Table 9.2-21: Assessment of the risk for fish-eating birds due to exposure to prothioconazole-desthio (M04) via bioaccumulation in fish (secondary poisoning) for the intended use in winter and spring cereals (BBCH 30-69. 2 x 200 g a.s./ha, applied 14-day interval)

Parameter	Prothioconazole-desthio (M04)	comments
PEC _{sw} (mg/L)	0.0783212	Section 8 – Step 1
BCF _{fish}	45 65	EFSA Conclusion (EFSA Scientific Report (2007) 106, 1-98)
BMF	N/A	biomagnification factor (relevant for BCF ≥ 2000)
PEC _{fish}	3.52 5.09	PEC _{fish} = PEC _{water} × BCF _{fish}
Daily dietary dose (mg/kg bw/d)	0.5604 0.809	DDD = PEC _{fish} × 0.159
NOEL (mg/kg bw/d)	14.8	EFSA Conclusion (EFSA Scientific Report (2007) 106, 1-98)
TER _{lt}	26.41 18.29	

TER values shown in bold fall below the relevant trigger.

The TER_{lt} for the assessment of the risk for fish-eating birds due to exposure to prothioconazole-desthio (M04) via bioaccumulation in fish does not fall below the relevant trigger TER value of 5, indicating low risk to birds following applications of FF-075 to cereals (BBCH 30-69) and winter oilseed rape (BBCH 55-69).

The assessment of the risk for fish-eating birds due to exposure to prothioconazole-S-methyl (M01) via bioaccumulation in fish is considered a conservative assessment as the long-term NOEL for the metabolite is assumed to be 10 x more toxic than the parent (7.8 mg /kg bw/d). Risk has been assessed against both the best (319 L/kg) and worse-case (1995 L/kg) whole fish BCF values for prothioconazole-S-methyl (M01).

Table 9.2-22: Assessment of the risk for fish-eating birds due to exposure to prothioconazole-S- methyl (M01) via bioaccumulation in fish (secondary poisoning) for the intended use in winter oilseed rape (BBCH 55-69. 2 x 160 g a.s./ha, applied 14-day interval)) assuming the best-case whole fish BCF value of 319 L/kg.

Parameter	Prothioconazole-S-methyl (M01)	Comments
PEC _{sw} (mg/L)	0.0073267	FOCUS Step 1 (Section 8)
BCF _{fish}	319	EFSA Conclusion (EFSA Scientific Report (2007) 106, 1-98)
BMF	N/A	biomagnification factor (relevant for BCF ≥ 2000)
PEC _{fish}	2.3372173	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.3716176	$DDD = PEC_{fish} \times 0.159$
NOEL (mg/kg bw/d)	7.8*	Derived endpoint
TER _{lt}	20.99	

TER values shown in bold fall below the relevant trigger.

*Endpoint estimated as parent NOEL/10.

Table 9.2-23: Assessment of the risk for fish-eating birds due to exposure to prothioconazole-S- methyl (M01) via bioaccumulation in fish (secondary poisoning) for the intended use in winter oilseed rape (BBCH 55-69. 2 x 160 g a.s./ha, applied 14-day interval)) assuming the worse-case whole fish BCF value of 1995 L/kg.

Parameter	Prothioconazole-S-methyl (M01)	Comments
PEC _{sw} (mg/L)	0.0073267	FOCUS Step 1 (Section 8)
BCF _{fish}	1995	EFSA Conclusion (EFSA Scientific Report (2007) 106, 1-98)
BMF	N/A	biomagnification factor (relevant for BCF ≥ 2000)
PEC _{fish}	2.3372173	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	2.3240659	$DDD = PEC_{fish} \times 0.159$
NOEL (mg/kg bw/d)	7.8*	Derived endpoint
TER _{lt}	3.36	

TER values shown in bold fall below the relevant trigger.

*Endpoint estimated as parent NOEL/10.

The TER_{lt} for the assessment of the risk for fish-eating birds due to exposure to prothioconazole-S-methyl (M01) via bioaccumulation in fish does not fall below the relevant trigger TER value of 5 when considering the best-case whole fish BCF value of 319 L/kg, indicating low risk to birds following applications of FF-075 to winter oilseed rape (BBCH 55-69).

When the worse-case whole fish BCF value of 1995 kg/L is considered in combination with the conservative assumption of toxicity for the metabolite (10 x more toxic than prothioconazole), the TER_{lt} falls below the relevant trigger TER value of 5. Further refinement of the risk assessment is required and, therefore, the risk to fish-eating birds exposed to prothioconazole-S-methyl (M01) via bioaccumulation in fish has been assessed considering the worse-case FOCUS Step 2 PEC_{sw} values as a more realistic scenario.

Table 9.2-24: Assessment of the risk for fish-eating birds due to exposure to prothioconazole-S- methyl (M01) via bioaccumulation in fish (secondary poisoning) for the intended use in winter oilseed rape (BBCH 55-69. 2 x 160 g a.s./ha, applied 14-day interval)) assuming the worse-case whole fish BCF value of 1995 L/kg and the worse-case FOCUS Step 2 PEC_{sw} value.

Parameter	Prothioconazole-S-methyl (M01)	Comments
PEC_{sw} (mg/L)	0.0003609	Southern Zone FOCUS Step 2
BCF_{fish}	1995	EFSA Conclusion (EFSA Scientific Report (2007) 106, 1-98)
BMF	N/A	biomagnification factor (relevant for $BCF \geq 2000$)
PEC_{fish}	0.7199955	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.1144793	$DDD = PEC_{fish} \times 0.159$
NOEL (mg/kg bw/d)	7.8*	Derived endpoint
TER_{lt}	68.13	

TER values shown in bold fall below the relevant trigger.

*Endpoint estimated as parent NOEL/10.

The TER_{lt} for the assessment of the risk for fish-eating birds due to exposure to prothioconazole-S-methyl (M01) via bioaccumulation in fish does not fall below the relevant trigger TER value of 5 when considering the worse-case whole fish BCF value of 1995 L/kg, and the worse-case FOCUS Step 2 PEC_{sw} value (Southern Zone PEC_{sw} value), indicating low risk to birds following applications of FF-075 to winter oilseed rape (BBCH 55-69).

Table 9.2-25: Assessment of the risk for fish-eating birds due to exposure to prothioconazole-S- methyl (M01) via bioaccumulation in fish (secondary poisoning) for the intended use in winter and spring cereals (BBCH 30-69. 2 x 200 g a.s./ha, applied 14-day interval)) assuming the best-case whole fish BCF value of 319 L/kg.

Parameter	Prothioconazole-S-methyl (M01)	Comments
PEC_{sw} (mg/L)	0.0091584	FOCUS Step 1 (Section 8)
BCF_{fish}	319	EFSA Conclusion, 2007
BMF	N/A	biomagnification factor (relevant for $BCF \geq 2000$)
PEC_{fish}	2.92	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.4645	$DDD = PEC_{fish} \times 0.159$
NOEL (mg/kg bw/d)	7.8*	Derived endpoint
TER_{lt}	16.79	

TER values shown in bold fall below the relevant trigger.

*Endpoint estimated as parent NOEL/10.

Table 9.2-26: Assessment of the risk for fish-eating birds due to exposure to prothioconazole-S- methyl (M01) via bioaccumulation in fish (secondary poisoning) for the intended use in winter and spring cereals (BBCH 30-69. 2 x 200 g a.s./ha, applied 14-day interval)) assuming the best-case whole fish BCF value of 1995 L/kg.

Parameter	Prothioconazole-S-methyl (M01)	Comments
PEC _{sw} (mg/L)	0.0091584	FOCUS Step 1 (Section 8)
BCF _{fish}	1995	EFSA Conclusion, 2007
BMF	N/A	biomagnification factor (relevant for BCF ≥ 2000)
PEC _{fish}	18.27	PEC _{fish} = PEC _{water} × BCF _{fish}
Daily dietary dose (mg/kg bw/d)	2.9051	DDD = PEC _{fish} × 0.159
NOEL (mg/kg bw/d)	7.8*	Derived endpoint
TER _{lt}	2.68	

TER values shown in bold fall below the relevant trigger.

*Endpoint estimated as parent NOEL/10.

The TER_{lt} for the assessment of the risk for fish-eating birds due to exposure to prothioconazole-S-methyl (M01) via bioaccumulation in fish does not fall below the relevant trigger TER value of 5 when considering the best-case whole fish BCF value of 319 L/kg, indicating low risk to birds following applications of FF-075 to winter and spring cereals (BBCH 30-69). However, when the worse-case whole fish BCF value of 1995 kg/L is considered in combination with the conservative assumption of toxicity for the metabolite (10 x more toxic than prothioconazole), the TER_{lt} falls below the relevant trigger TER value of 5. Further refinement of the risk assessment is required and, therefore, the risk to fish-eating birds exposed to prothioconazole-S-methyl (M01) via bioaccumulation in fish has been assessed considering the worse-case FOCUS Step 2 PEC_{sw} values as more realistic scenarios.

Table 9.2-27: Assessment of the risk for fish-eating birds due to exposure to prothioconazole-S-methyl (M01) via bioaccumulation in fish (secondary poisoning) for the intended use in winter and spring cereals (BBCH 30-69. 2 x 200 g a.s./ha, applied 14-day interval), assuming the worse-case whole fish BCF value of 1995 L/kg and the worse-case FOCUS Step 2 PEC_{sw} values.

Parameter	Prothioconazole-S-methyl (M01)	Comments
PEC _{sw} (mg/L)	0.0011532	Southern Zone FOCUS Step 2
BCF _{fish}	1995	EFSA Conclusion, 2007
BMF	N/A	biomagnification factor (relevant for BCF ≥ 2000)
PEC _{fish}	2.300634	PEC _{fish} = PEC _{water} × BCF _{fish}
Daily dietary dose (mg/kg bw/d)	0.3658008	DDD = PEC _{fish} × 0.159
NOEL (mg/kg bw/d)	7.8*	Derived endpoint
TER _{lt}	21.32308	

TER values shown in bold fall below the relevant trigger.

*Endpoint estimated as parent NOEL/10.

The TER_{lt} for the assessment of the risk for fish-eating birds due to exposure to prothioconazole-S-methyl

(M01) via bioaccumulation in fish does not fall below the relevant trigger TER value of 5 when considering the worse-case whole fish BCF value of 1995 L/kg, and FOCUS Step 2 PEC_{sw} values, indicating low risk to birds following applications of FF-075 to winter and spring cereals (BBCH 30-69).

zRMS comments:

The risk for fish-eating birds and earthworms-eating birds due to exposure to prothioconazole and its metabolites such as: (M01), (M04) is considered as acceptable.

9.2.3.4 Biomagnification in terrestrial food chains

Not relevant.

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

Not relevant.

9.2.5 Overall conclusions

Regulatory testing with birds has been conducted with prothioconazole, azoxystrobin and the relevant metabolite, prothioconazole-desthio (M04) in accordance with EU requirements for quail (*Colinus virginianus*) and the mallard duck (*Anas platyrhynchos*). Results from these studies, summarised in the EFSA Review Reports for prothioconazole and azoxystrobin show that the active substances and prothioconazole-desthio (M04) have low toxicity to birds.

The estimated toxicity for FF-075, derived from the combined assessment of the active ingredients, demonstrates low risk to birds from applications of the formulated product.

The assessment of risk from secondary poisoning of earthworm-eating birds and fish-eating birds via exposure to prothioconazole, prothioconazole-desthio (M04) and prothioconazole-S-methyl (M01) via bioaccumulation in earthworms and fish indicates low risk to birds following applications of FF-075 in accordance with the proposed GAP.

An acceptable acute and long-term risk to birds is expected from the proposed uses of FF-075 in winter oilseed rape (BBCH 55-69) and winter and spring cereals (BBCH 30-69).

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

9.3.1 Risk assessment for spray applications

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

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

The acute toxicity to mammals is estimated assuming dose additivity of the single active substances in the formulation with the following equation (EFSA Journal 2009; 7(12): 1438):

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

where: $X(a.s._i)$ is the fraction of the active substance i in the mixture (the sum $\Sigma(a.s._i)$ must be 1)
 $LD_{50}(a.s._i)$ is the acute toxicity for the active substance i .

Considering the lowest LD_{50} values determined for prothioconazole and azoxystrobin of >6200 and >5000 mg/kg bw, respectively, and their nominal concentrations in FF-075 (18.18% and 13.64 %w/w, respectively), the resulting $LD_{50}(mix)$ value is **>5621 mg/kg bw**.

	Prothioconazole	Azoxystrobin
Content in the formulation FF-075 (%w/w)	18.18%	13.64%
Fraction in the a.s. mixture	18.18/ 31.82 = 0.57	13.64/ 31.82 = 0.43
LD_{50} of a.s. [mg/kg bw]	6200	5000
Fraction / LD_{50}	0.57 / 6200 0.0000919	0.43 / 5000 0.000086
Sum	0.0001779	
1/ sum = pre- dicted LD_{50} (mix)	>5621 mg mix/kg bw	

Experimentally derived data for the formulation, FF-075, is not available to perform a comparison with the estimated value $LD_{50}(mix)$ of >5621 mg/kg bw. Therefore, the estimated value ($LD_{50}(mix)$) of >5621 mg/kg bw is used in the screening assessment.

In accordance with the guidance (EFSA Journal 2009; 7(12): 1438) a chronic mixture toxicity assessment is not required.

Risk Assessment for combined exposure

According to the EFSA Journal (2009)³, the simultaneous exposure of animals to residues of two or more potential toxic substances should be considered in the risk assessment. Therefore, for the assessment of acute effects, a surrogate LD₅₀ for the mixture of active substances with known toxicity was derived assuming dose additivity of toxicity. For the calculation, the following equation was used:

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

With:

X (a.s._i) = fraction of each a.s. in the mixture

LD₅₀ (a.s._i) = acute toxicity value for each a.s.

Acute risks from combined exposure

Azoxystrobin + prothioconazole

The active substance content of the formulation Euskatel Pro addressed in this dossier is 153.8 g/L azoxystrobin and 204 g/L prothioconazole, making up a total of 357.8 g a.s./L.

Tables below shows the calculation of the predicted LD₅₀ (mix) of azoxystrobin and prothioconazole when mixed in these proportions (step 1 in Appendix 2 to the EFSA GD 2009).

Avian LD₅₀ (mix) for azoxystrobin and prothioconazole when combined as Eustakel Pro (step 1 in EFSA GD 2009, Appendix B)

	Azoxystrobin	Prothioconazole
Content in the formulation	150 153.8	200 204
Fraction in the a.s. mixture	0.4285 0.43	0.5715 0.57
LD ₅₀ of a.s. [mg/kg bw]	>5000	>6200
Fraction / LD ₅₀	0.0000857 0.000085	0.00009217 0.0000915
Sum	0.000177 0.0001775	
1/ sum = predicted LD ₅₀ (mix)	5649.72 5621.15	

³ European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12): 1438. [139 pp.].

Avian “tox per fraction” for the Euskatel Pro ((step 1 in EFSA GD 2009, Appendix B)

	Azoxystrobin	Prothioconazole	“mix”
Content in the formulation	150 55.8	200 204	350 357.8
Fraction in mixture	0.4285 0.43	0.5715 0.57	1.0
LD ₅₀ (mg/kg bw)	>5000	>6200	LD _{mix} =5649.72 5621.13
Tox per fraction	11 668.6 11 627.90	10 848.64 10 877.20	
Contribution to predicted toxicity	48.42 51.66%	51.58 48.33%	

Azoxystrobin contributes to 51.66%% to mixture toxicity, while the prothioconazole have an impact on the predicted risk of 48.33%, therefore, surrogate LD₅₀ of 5621.13 mg/kg bw should be used in the acute risk assessment.

Chronic risks from combined exposure

Azoxystrobin + prothioconazole

Avian NOEL (mix) for azoxystrobin and prothioconazole when combined as of Eustakel Pro (step 1 in EFSA GD 2009, Appendix B)

	Azoxystrobin	Prothioconazole
Content in the formulation	150 153.8	200 33.4
Fraction in the a.s. mixture	0.4285 0.43	0.5715 0.57
NOEL ₅₀ of a.s. [mg/kg bw]	32	95.6
Fraction / NOEL	0.0134	0.00597
Sum	0.01937	
1/ sum = predicted NOEL (mix)	51.62 mg mix/kg bw	

Avian “tox per fraction” for the Euskatel Pro (step 1 in EFSA GD 2009, Appendix B)

	Azoxystrobin	Prothioconazole	“mix”
Content in the formulation	150 153.8	200 33.4	350 357.3
Fraction in mixture	0.4285 0.43	0.5715 0.57	
NOEL (mg/kg bw)	32	95.6	NOEL _{mix} = 51.62
Tox per fraction	74.679 34.418	167.28 167.23	
Contribution to predicted toxicity	69.12 30.64%	30.88 59.23%	

Azoxystrobin contributes to 30.64 % to mixture toxicity, while the prothioconazole have an impact on the predicted risk of 59.23%, therefore, surrogate NOEL of 51.62 mg /kg bw was used in the chronic risk assessment.

Azoxystrobin + JAU-desthio (metabolite of prothioconazole)

The combined risk to mammals should also be evaluated for the metabolite JAU-desthio prothioconazole. Because it is not possible to determine the exact application dose for the metabolite, combitox was not evaluated according to Finney's equation, but using the combined toxicity risk (TER_{MIX}) with concentration-addition is estimated based on the following equation:

$$TER_{mix} = 1 / \left(\frac{1}{TER_{a.s.1}} + \frac{1}{TER_{a.s.2}} + \dots + \frac{1}{TER_{a.s.i}} \right)$$

An acceptable risk is expected when TER_{combi} > trigger.

9.3.1.1 First tier assessment (screening/generic focal species)

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

Table 9.3-1: Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of FF-075 in winter and spring cereals – prothioconazole.

Intended use		Winter and spring cereals			
Active substance		Prothioconazole			
Application rate (g/ha)		2 x 200 g a.s./ha (minimum 14-day interval)			
Acute toxicity (mg/kg bw)		>6200			
TER criterion		10			
Crop scenario	Indicator species for screening	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a
Growth stage					
N/A	Small herbivorous mammal	118.4	1.2	28.416	218
Reprod. toxicity (mg/kg bw/d)		95.6			
TER criterion		5			
Crop scenario	Indicator species for screening	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{lt}
Growth stage					
N/A	Small omnivorous mammal	48.3	0.742	7.16772	13.34

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

Table 9.3-3: Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of FF-075 in winter oilseed rape – prothioconazole.

Intended use		Winter oilseed rape			
Active substance		Prothioconazole			
Application rate (g/ha)		2 x 160 g a.s./ha (minimum 14-day interval)			
Acute toxicity (mg/kg bw)		>6200			
TER criterion		10			
Crop scenario	Indicator species for screening	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a
Growth stage					
N/A	Small herbivorous mammal	118.4	1.2	22.7328	273

Reprod. toxicity (mg/kg bw/d)		95.6			
TER criterion		5			
Crop scenario	Indicator species for screening	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{It}
Growth stage					
N/A	Small omnivorous mammal	48.3	0.742	5.734176	16.67

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

Table 9.3-4: Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of FF-075 in winter and spring cereals – prothioconazole-desthio (M04).

Intended use		Winter and spring Cereals			
Active substance		Prothioconazole-desthio (M04)			
Application rate (g/ha)		2 x 200 g a.s./ha (14-day minimum interval)			
Acute toxicity (mg/kg bw)		2235			
TER criterion		10			
Crop scenario	Indicator species for screening	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a
Growth stage					
N/A	Small herbivorous mammal	118.4	1.2	28.416	79
Reprod. toxicity (mg/kg bw/d)		10			
TER criterion		5			
Crop scenario	Indicator species for screening	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{It}
Growth stage					
N/A	Small omnivorous mammal	48.3	0.742	7.16772	1.40

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

Table 9.3-5: Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of FF-075 in winter oilseed rape – prothioconazole-desthio (M04).

Intended use	Winter oilseed rape
Active substance	Prothioconazole-desthio (M04)

Application rate (g/ha)		2 x 160 g a.s./ha (minimum 14-day interval)			
Acute toxicity (mg/kg bw)		2235			
TER criterion		10			
Crop scenario Growth stage	Indicator species for screening	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a
N/A	Small herbivorous mammal	118.4	1.2	22.7328	98
Reprod. toxicity (mg/kg bw/d)		10			
TER criterion		5			
Crop scenario Growth stage	Indicator species for screening	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{lt}
N/A	Small omnivorous mammal	48.3	0.742	5.734176	1.74

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

The TER_a values for both prothioconazole and prothioconazole-desthio (M04) are greater than the trigger of 10, indicating low acute risks to mammals from exposure to FF-075 to winter and spring cereals (BBCH 30-69) and winter oilseed rape (BBCH 55-69), at the proposed label rates.

The TER_{lt} values are greater than the trigger of 5 for prothioconazole, indicating low long-term risks to mammals from prothioconazole from applications of FF-075 at the proposed label rates.

The TER_{lt} values are lower than the trigger of 5 for prothioconazole-desthio (M04) for applications to winter and spring cereals and winter oilseed rape at the proposed label rates, indicating potential risk to mammals. Tier 1 long-term risk assessment of prothioconazole-desthio (M04) is, therefore, required.

Table 9.3-6: Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of FF-075 in winter and spring cereals – azoxystrobin.

Intended use		Winter and spring cereals			
Active substance		Azoxystrobin			
Application rate (g/ha)		2 x 150 g a.s./ha (minimum 14-day interval)			
Acute toxicity (mg/kg bw)		>5000			
TER criterion		10			
Crop scenario Growth stage	Indicator species for screening	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a

N/A	Small herbivorous mammal	118.4	1.2	21.312	235
Reprod. toxicity (mg/kg bw/d)		32			
TER criterion		5			
Crop scenario	Indicator species for screening	SV_m	MAF_m × TWA	DDD_m	TER_{lt}
Growth stage				(mg/kg bw/d)	
N/A	Small omnivorous mammal	48.3	0.742	5.37579	5.95

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

Table 9.3-7: Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of FF-075 in winter oilseed rape – azoxystrobin.

Intended use		Winter oilseed rape			
Active substance		Azoxystrobin			
Application rate (g/ha)		2 x 120 g a.s./ha (minimum 14-day interval)			
Acute toxicity (mg/kg bw)		>5000			
TER criterion		10			
Crop scenario	Indicator species for screening	SV₉₀	MAF₉₀	DDD₉₀	TER_a
Growth stage				(mg/kg bw/d)	
N/A	Small herbivorous mammal	118.4	1.2	17.0496	293
Reprod. toxicity (mg/kg bw/d)		32			
TER criterion		5			
Crop scenario	Indicator species for screening	SV_m	MAF_m × TWA	DDD_m	TER_{lt}
Growth stage				(mg/kg bw/d)	
N/A	Small omnivorous mammal	48.3	0.742	4.300632	7.44

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

The TER_a and TER_{lt} values for azoxystrobin are greater than the trigger of 10 and 5, respectively, indicating low acute and chronic risks to mammals from exposure to FF-075 to winter and spring cereals (BBCH 30-69) and winter oilseed rape (BBCH 55-69), at the proposed label rates.

Table 9.3-8: Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of FF-075 in winter and spring cereals – FF-075.

Intended use		Winter and spring cereals			
Active substance		FF-075			
Application rate (g/ha)		2 x 1100 g formulation/ha (minimum 14-day interval)			
Acute toxicity (mg/kg bw)		>5621			
TER criterion		10			
Crop scenario	Indicator species for screening	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a
Growth stage					
N/A	Small herbivorous mammal	118.4	1.2	156.288	>36

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

Table 9.3-9: Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of FF-075 in winter oilseed rape – FF-075.

Intended use		Winter oilseed rape			
Active substance		FF-075			
Application rate (g/ha)		2 x 880 g formulation/ha (minimum 14-day interval)			
Acute toxicity (mg/kg bw)		>5621			
TER criterion		10			
Crop scenario	Indicator species for screening	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a
Growth stage					
N/A	Small herbivorous mammal	118.4	1.2	125.0304	>45

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

The TER_a and TER_{lt} values for azoxystrobin are greater than the trigger of 10 and 5, respectively, indicating low acute and chronic risks to mammals from exposure to FF-075 to winter and spring cereals and winter oilseed rape, at the proposed label rates.

As the acute and chronic risks are acceptable at the screening step, which already takes into account worst case assumptions, no restriction on BBCH is required for this aspect of the risk assessment.

Table 9.3-10: First-tier assessment of the long-term/reproductive risk for mammals due to the use of FF-075 in winter and spring cereals (BBCH 30-69) – prothioconazole-desthio (M04).

Intended use		Winter and spring cereals			
Metabolite		Prothioconazole-desthio (M04)			
Application rate (g/ha)		2 x 200 g a.s./ha (14-day minimum interval)			
Reprod. toxicity (mg/kg bw/d)		10			
TER criterion		5			
Crop scenario	Indicator/generic focal species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{it}
Growth stage					
Cereals, BBCH >20	Small insectivorous mammal “shrew” Common shrew (<i>Sorex araneus</i>)	1.9	0.742	0.282	35.47
Cereals, BBCH ≥ 40	Small herbivorous mammal “vole” Common vole (<i>Microtus arvalis</i>)	21.7	0.742	3.220	3.11
Cereals, BBCH 30-39	Small omnivorous mammal “mouse” Wood mouse (<i>Apodemus sylvaticus</i>)	3.9	0.742	0.579	17.28
Cereals, BBCH ≥ 40	Small omnivorous mammal “mouse” Wood mouse (<i>Apodemus sylvaticus</i>)	2.3	0.742	0.341	29.30

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

Table 9.3-11: First-tier assessment of the long-term/reproductive risk for mammals due to the use of FF-075 in winter oilseed rape (BBCH 55-69) – prothioconazole-desthio (M04).

Intended use		Winter oilseed rape
Metabolite		Prothioconazole-desthio (M04)
Application rate (g/ha)		2 x 160 g a.s./ha (14-day minimum interval)

Reprod. toxicity (mg/kg bw/d)		10			
TER criterion		5			
Crop scenario Growth stage	Indicator/generic focal species	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{tt}
Oilseed rape BBCH ≥20	Small insectivorous mammal “shrew” Common shrew (<i>Sorex araneus</i>)	1.9	0.742	0.226	44.33
Oilseed rape BBCH ≥40	Small herbivorous mammal “vole” Common vole (<i>Microtus arvalis</i>)	18.1	0.742	2.149	4.65
Oilseed rape All Season	Large herbivorous mammal “lagomorph” Rabbit (<i>Oryctolagus cuniculus</i>)	14.3	0.742	1.698	5.89
Oilseed rape BBCH ≥40	Small omnivorous mammal “mouse” Wood mouse (<i>Apodemus sylvaticus</i>)	1.9	0.742	0.226	44.33

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

The Tier 1 TER_{tt} values, calculated in accordance with EFSA/2009/1438, for the winter and spring cereals (BBCH 30-69), and winter oilseed rape (BBCH 55-69), are greater than the Annex VI trigger of 5 for prothioconazole-dethio (M04), with the exception of cereals (BBCH ≥ 40), and winter oilseed rape (BBCH ≥40), indicating potential risk to small herbivorous mammal “vole”, Common vole (*Microtus arvalis*). Higher-tier long-term risk assessment of prothioconazole-dethio (M04) to small herbivorous mammal “vole” Common vole (*Microtus arvalis*) is, therefore, required.

The following refined higher-tier risk assessment for prothioconazole-dethio (M04) has been conducted in accordance with the methods presented in the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438.

9.3.1.1 Higher-tier risk assessment

The following refined risk assessment for prothioconazole-dethio (M04) has been conducted in accordance with the methods presented in the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438.

In winter and spring cereals and winter oilseed rape, the EFSA (2009) guidance considers that the small, exclusive herbivores represented by voles, are relevant at growth stage BBCH ≥ 40. According to EFSA, (2009), the interception rate remains constant at 70% in cereals and 75% in oilseed rape, corresponding to 30% and 25% deposition, respectively, on the ground and low-growing vegetation accessible to voles at the base of the crop throughout BBCH GS ≥ 40.

The interception rates assigned by the EFSA (2009) guidance document are taken from those applied at FOCUS Step 2 for surface water modelling and are considered to be conservative, *i.e.* they err on the side of under-estimating interception and over-estimating ground deposition. A more refined set of interception values is used for groundwater modelling (FOCUS, 2014), which provides an expanded range of interception rates corresponding to different crop growth stages. An interception rate of 90% is assigned to the flowering stages of cereal crops, which span BBCH 40-89, an interception rate of 80% is assigned to oilseed rape crops spanning BBCH 40-89. The refined assessment, presented below, considers long-term exposure to prothioconazole-desthio (M04) following the use of FF-075 according to the GAP and during the crop growth stage window BBCH 30-69 in winter and spring cereals and BBCH 55-69 in winter oilseed rape. The EFSA (2009) default interception rate of 70% is substituted with the FOCUS (2014) 90% interception rate (deposition factor = 0.1) that applies to cereal crops at BBCH 40-89, and 80% interception rate (deposition factor = 0.2) that applies to oilseed rape crops at BBCH 40-89.

Table 9.3-12: Higher tier assessment of the long-term risk for mammals due to the use of FF-075 in winter and spring cereals (2 x 200 g a.s./ha (minimum 14-day interval) BBCH 30-69) – prothioconazole-desthio (M04).

Cereals and Oilseed Rape	Generic focal species	NOEL (long-term) (mg a.s./kg bw/d)	Refined SV (long-term)	Rate applied (kg a.s./ha)	MAF _m ^b	f _{twa} ^c	DDD ^d	TER _{LT} ^e	Annex VI trigger
Cereals BBCH ≥ 40	Small herbivore “vole”	10	7.2 ^a	0.2	1.4	0.53	1.07	9.3	5

^a Refined short-cut value ($RUD \times FIR/bw \times \text{refined deposition rate}$) based on mean residues (zero-intercepted RUD and FIR/bw from EFSA, 2009, Annex I, Table I.2, deposition rate from FOCUS, 2000): $54.2 \times 1.33 \times 0.1$.

^b Multiple application factor for mean residue concentration, based on two applications separated by an interval of 14 days and an assumed DT₅₀ value of 10 days (from EFSA (2009), Table 13).

^c Time-weighted average factor for residue decline. EFSA, 2009 default value is 0.53, based on an assumed DT₅₀ value of 10 days.

^d Daily dietary dose ($DDD = SV \times \text{Rate} \times MAF \times f_{twa}$).

^e Long-term toxicity/exposure ratio ($TER_{LT} = NOEL \text{ (mg a.s./kg bw/d)}/DDD$).

Table 9.3-13: Higher tier assessment of the long-term risk for mammals due to the use of FF-075 in winter oilseed rape (2 x 160 g a.s./ha (minimum 14-day interval) BBCH 55-69) – prothioconazole-desthio (M04).

Cereals and Oilseed Rape	Generic focal species	NOEL (long-term) (mg a.s./kg bw/d)	Refined SV (long-term)	Rate applied (kg a.s./ha)	MAF _m ^b	f _{twa} ^c	DDD ^d	TER _{LT} ^e	Annex VI trigger
Oilseed Rape BBCH ≥ 40	Small herbivore “vole”	10	14.4 ^a	0.160	1.4	0.53	1.71	5.85	5

^a Refined short-cut value ($RUD \times FIR/bw \times \text{refined deposition rate}$) based on mean residues (zero-intercepted RUD and FIR/bw from EFSA, 2009, Annex I, Table I.2, deposition rate from FOCUS, 2000): $54.2 \times 1.33 \times 0.2$.

^b Multiple application factor for mean residue concentration, based on two applications separated by an interval of 14 days and an assumed DT_{50} value of 10 days (from EFSA (2009), Table 13).

^c Time-weighted average factor for residue decline. EFSA, 2009 default value is 0.53, based on an assumed DT_{50} value of 10 days.

^d Daily dietary dose ($DDD = SV \times \text{Rate} \times (MAF \times f_{TWA})$).

^e Long-term toxicity/exposure ratio ($TER_{LT} = NOEL \text{ (mg a.s./kg bw/d)}/DDD$).

The TER_{lt} values exceed the Annex VI trigger of 5 for prothioconazole-desthio (M04) when considering both relevant mammalian species in cereals and oilseed rape, and when deposition factors in cereals are refined, according to the guidance in EFSA, (2009) in accordance with Focus (2014). This indicates low risk to mammalian species from applications of FF-075 in accordance with the GAP.

Combined risk assessment:

Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of FF-075 in winter and spring cereals

Intended use		Winter and spring cereals			
Active substance		Combined a.s.			
Application rate (g/ha)		350			
Acute toxicity (mg/kg bw)		5649.72			
TER criterion		10			
Crop scenario	Indicator species for screening	SV_{90}	MAF_{90}	DDD_{90} (mg/kg bw/d)	TER_a
Growth stage					
N/A	Small herbivorous mammal	118.4	1.2	49.72	113.63
Reprod. toxicity (mg/kg bw/d)		51.62			
TER criterion		5			
Crop scenario	Indicator species for screening	SV_m	$MAF_m \times TWA$	DDD_m (mg/kg bw/d)	TER_{lt}
Growth stage					
N/A	Small omnivorous mammal	48.3	0.742	12.54	4.11

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

Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of FF-075 in winter oilseed rape

Intended use		Winter oilseed rape
Active substance		Combined
Application rate (g/ha)		280

Acute toxicity (mg/kg bw)		5649.72			
TER criterion		10			
Crop scenario	Indicator species for screening	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a
Growth stage					
N/A	Small herbivorous mammal	118.4	1.2	39.80	141.95
Reprod. toxicity (mg/kg bw/d)		51.62 mg mix/kg bw			
TER criterion		5			
Crop scenario	Indicator species for screening	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{it}
Growth stage					
N/A	Small omnivorous mammal	48.3	0.742	10.03	5.14

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

Tier 1

Intended use		Winter and spring cereals			
A.s.		Combined risk assessment			
Application rate (g/ha)		2 x 350			
Reprod. toxicity (mg/kg bw/d)		51.62			
TER criterion		5			
Crop scenario	Indicator/generic focal species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{it}
Growth stage					
Cereals, BBCH >20	Small insectivorous mammal "shrew" Common shrew (<i>Sorex araneus</i>)	1.9	0.742	0.493	123.3
Cereals, BBCH ≥ 40	Small herbivorous mammal "vole" Common vole (<i>Microtus arvalis</i>)	21.7	0.742	5.63	9.16
Cereals, BBCH 30-39	Small omnivorous mammal "mouse" Wood mouse (<i>Apodemus sylvaticus</i>)	3.9	0.742	1.01	51.10
Cereals, BBCH ≥ 40	Small omnivorous mammal "mouse"	2.3	0.742	0.597	86.46

	Wood mouse (<i>Apodemus sylvaticus</i>)				
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SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER

Metabolite (M04)

Screening step assessment of the acute risk for mammals due to the use of Euskatel Pro in all crops

Intended use		Cereals, oilseed rape			
Application rate (g/ha)		2 × 350 for cereals, 2 x 280 for oilseed rape			
Crop scenario	Indicator species	TER _{azoxystrobin}	TER _{prothioconazole-desthio}	TER criterion	TER _{combi}
Cereals	Small herbivorous mammals	235	79	10	62.5
Oilseed rape		293	98		73.53

TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Screening step assessment of the reproductive risk for mammals exposed to in Euskatel Pro all-crops

Intended use		Cereals, oilseed rape			
Application rate (g/ha)		2 × 350 for cereals, 2 x 280 for oilseed rape			
Crop scenario	Indicator species	TER _{azoxystrobin}	TER _{prothioconazole-desthio}	TER criterion	TER _{combi}
Cereals	Small herbivorous mammals	5.95	1.40	5	1.14
Oilseed rape		7.44	1.74		1.42

TIER 1

First tier risk assessment of the reproductive risk for mammals exposed to Euskatel Pro in all crops

Intended use		Cereals, Oilseed rape			
Application rate (g/ha)		2 × 350 for cereals, 2 x 280 for oilseed rape			
Crop scenario	Indicator species	TER _{azoxystrobin}	TER _{prothioconazole-desthio}	TER criterion	TER _{combi}
Cereals, BBCH ≥ 40	Small herbivorous mammal “vole”	79	3.11	5	3.03
	Common vole (<i>Microtus arvalis</i>)	98	4.65		4.44

In the case of the herbivorous mammal – vole, based on the lowest TER_{LT} values among the species, the long-term risk in cereals and oilseed rape were not met. Therefore, a calculations were performed using refined parameter of DF for M04 metabolite such as: 0.1 for cereals and 0.2 for oilseed rape BBCH 30-89 according to GD FOCUS 2014 .

Higher tier risk assessment of the reproductive risk for mammals exposed to Euskatel Pro in all crops

Intended use		Cereals, oilseed rape			
Application rate (g/ha)		2 × 350 for cereals, 2 x 280 for oilseed rape			
Crop scenario	Indicator species	TER _{azoxystrobin}	TER _{prothioconazole-desthio}	TER _{criterion}	TER _{combi}
Cereals, BBCH ≥ 40 Oilseed rape , BBCH ≥ 40	Small herbivorous mammal “vole”	79 98	9.3 5.85		8.3 5.55

The reproductive risk assessment for mammals for combined exposure demonstrated acceptable risk for cereals and oilseed rape.

9.3.1.2 Drinking water exposure

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

Puddle scenario

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

With a K(f)_{oc} of 1765 L/kg, prothioconazole belongs to the group of more sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use in cereals also covers the risk for birds from all other intended uses.

Effective application rate (g/ha)=	2 x 200		
Acute toxicity (mg/kg bw) =	>6200	Quotient =	<0.06
Reprod. toxicity (mg/kg bw/d) =	95.6	Quotient =	4.18

Since the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed the critical value of 3000, a quantitative risk assessment (calculation of TER values) for prothioconazole is not necessary.

With a K(f)_{oc} of 574 L/kg, prothioconazole-desthio (M04) belongs to the group of more sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use in cereals also covers the risk for birds from all other intended uses.

Effective application rate (g/ha)=	2 x 200		
Acute toxicity (mg/kg bw) =	2235	Quotient =	0.18

Reprod. toxicity (mg/kg bw/d) =	10.0	Quotient =	40.0
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Since the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed the critical value of 3000, a quantitative risk assessment (calculation of TER values) for prothioconazole-desthio (M04) is not necessary.

With a K(f)oc of 207-594 L/kg, azoxystrobin belongs to the group of less sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use in cereals also covers the risk for birds from all other intended uses.

Effective application rate (g/ha)=	2 x 150		
Acute toxicity (mg/kg bw) =	5000	Quotient =	0.06
Reprod. toxicity (mg/kg bw/d) =	32	Quotient =	9.375

Since the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed the critical value of 50, a quantitative risk assessment (calculation of TER values) for azoxystrobin is not necessary

9.3.1.3 Effects of secondary poisoning

The log P_{ow} of prothioconazole amounts to 3.4 at pH 4 and thus exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is required.

The log P_{ow} of prothioconazole-desthio (M04) amounts to 3.04 at pH 4 and thus exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is required.

The log P_{ow} of prothioconazole-S-methyl (M01) is predicted to be 4.19 at pH 7, (EFSA, 2007), and thus exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is required.

The log P_{ow} of azoxystrobin is predicted to be 2.5 at 20°C (not pH dependent) (EFSA, 2010), and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is, therefore, not required.

Risk assessment for earthworm-eating mammals via secondary poisoning

According to EFSA/2009/1438, the risk for vermivorous mammals is assessed for a small mammal of 10 g body weight with a daily food consumption of 12.8 g. Bioaccumulation in earthworms is estimated based on predicted concentrations in soil.

Table 9.3-14: Assessment of the risk for earthworm-eating mammals due to exposure to prothioconazole via bioaccumulation in earthworms (secondary poisoning) for the intended use in winter and spring cereals (BBCH 30-69)

Parameter	Prothioconazole	Comments
PEC _{soil} (mg/kg soil)	0.0550	
log P_{ow} / P_{ow}	3.4 (at pH 4)/2512	EFSA Conclusion (EFSA Scientific Report (2007) 106, 1-98)
Koc	1765	
foc	0.02	Default
BCF _{worm}	0.878	BCF _{worm/soil} = (PEC _{worm,ww} /PEC _{soil,dw})

Parameter	Prothioconazole	Comments
		$= (0.84 + 0.12 \times P_{ow}) / foc \times Koc$
PEC _{worm}	0.048	PEC _{worm} = PEC _{soil} × BCF _{worm/soil}
Daily dietary dose (mg/kg bw/d)	0.061792	DDD = PEC _{worm} × 1.28
NOEAL (mg/kg bw/d)	95.6	
TER _{lt}	1,547	

TER values shown in bold fall below the relevant trigger.

Table 9.3-15: Assessment of the risk for earthworm-eating mammals due to exposure to prothioconazole via bioaccumulation in earthworms (secondary poisoning) for the intended use in oilseed rape (BBCH 55-69).

Parameter	Prothioconazole	Comments
PEC _{soil} (mg/kg soil)	0.0440	
log P _{ow} / P _{ow}	3.4 (at pH 4)/2512	EFSA Conclusion (EFSA Scientific Report (2007) 106, 1-98)
Koc	1765	
foc	0.02	Default
BCF _{worm}	0.878	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.12 \times P_{ow}) / foc \times Koc$
PEC _{worm}	0.039	PEC _{worm} = PEC _{soil} × BCF _{worm/soil}
Daily dietary dose (mg/kg bw/d)	0.049434	DDD = PEC _{worm} × 1.28
NOEL (mg/kg bw/d)	95.6	
TER _{lt}	1,934	

TER values shown in bold fall below the relevant trigger.

The TER_{lt} for the assessment of the risk for earthworm-eating mammals due to exposure to prothioconazole via bioaccumulation in earthworms does not fall below the relevant trigger TER value of 5, indicating low risk to mammals following applications of FF-075 to winter and spring cereals (BBCH 30-69) and winter oilseed rape (BBCH 55-69).

Table 9.3-16: Assessment of the risk for earthworm-eating mammals due to exposure to prothioconazole-desthio (M04) via bioaccumulation in earthworms (secondary poisoning) for the intended use in winter and spring cereals (BBCH 30-69)

Parameter	Prothioconazole-desthio (M04)	Comments
PEC _{soil} (mg/kg soil)	0.0569	
log P _{ow} / P _{ow}	3.04/1100	EFSA Conclusion (EFSA Scientific Report (2007) 106, 1-98)
Koc	575.4	
foc	0.02	Default
BCF _{worm}	1.22	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.12 \times P_{ow}) / foc \times Koc$

Parameter	Prothioconazole-desthio (M04)	Comments
PEC _{worm}	0.069	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.088857	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	10	
TER _{It}	113	

TER values shown in bold fall below the relevant trigger.

Table 9.3-17: Assessment of the risk for earthworm-eating mammals due to exposure to prothioconazole-desthio (M04) via bioaccumulation in earthworms (secondary poisoning) for the intended use in winter oilseed rape (BBCH 55-69).

Parameter	Prothioconazole-desthio (M04)	Comments
PEC _{soil} (mg/kg soil)	0.0456	
log P _{ow} / P _{ow}	3.04/ 1100	EFSA Conclusion (EFSA Scientific Report (2007) 106, 1-98)
Koc	575.4	
foc	0.02	Default
BCF _{worm}	1.22	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.12 \times P_{ow}) / foc \times Koc$
PEC _{worm}	0.056	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.071210	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	10	
TER _{It}	140	

TER values shown in bold fall below the relevant trigger.

The TER_{It} for the assessment of the risk for earthworm-eating mammals due to exposure to prothioconazole-desthio (M04) via bioaccumulation in earthworms does not fall below the relevant trigger TER value of 5, indicating low risk to mammals following applications of FF-075 to winter and spring cereals (BBCH 30-69) and winter oilseed rape (BBCH 55-69).

Table 9.3-18: Assessment of the risk for earthworm-eating mammals due to exposure to prothioconazole-S-methyl (M01) via bioaccumulation in earthworms (secondary poisoning) for the intended use in winter and spring cereals (BBCH 30-69).

Parameter	Prothioconazole-S-methyl (M01)	Comments
PEC _{soil} (mg/kg soil)	0.0569	
log P _{ow} / P _{ow}	4.19/20,000	EFSA Conclusion (EFSA Scientific Report (2007) 106, 1-98)
Koc	2256.3	
foc	0.02	Default
BCF _{worm}	5.337	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$

Parameter	Prothioconazole-S-methyl (M01)	Comments
		$= (0.84 + 0.12 \times P_{ow}) / foc \times Koc$
PEC _{worm}	0.304	PEC _{worm} = PEC _{soil} × BCF _{worm/soil}
Daily dietary dose (mg/kg bw/d)	0.388708	DDD = PEC _{worm} × 1.28
NOEL (mg/kg bw/d)	9.56*	
TER _{lt}	25	

TER values shown in bold fall below the relevant trigger.

*Endpoint estimated as parent NOEL/10.

Table 9.3-19: Assessment of the risk for earthworm-eating mammals due to exposure to prothioconazole-S-methyl (M01) via bioaccumulation in earthworms (secondary poisoning) for the intended use in winter oilseed rape (BBCH 55-69).

Parameter	Prothioconazole-S-methyl (M01)	Comments
PEC _{soil} (mg/kg soil)	0.0456	
log P _{ow} / P _{ow}	4.19/20,000	EFSA Conclusion (EFSA Scientific Report (2007) 106, 1-98)
Koc	2256.3	
foc	0.02	Default
BCF _{worm}	5.337	BCF _{worm/soil} = (PEC _{worm,ww} /PEC _{soil,dw}) $= (0.84 + 0.12 \times P_{ow}) / foc \times Koc$
PEC _{worm}	0.243	PEC _{worm} = PEC _{soil} × BCF _{worm/soil}
Daily dietary dose (mg/kg bw/d)	0.311513	DDD = PEC _{worm} × 1.28
NOEL (mg/kg bw/d)	9.56*	
TER _{lt}	31	

TER values shown in bold fall below the relevant trigger.

*Endpoint estimated as parent NOEL/10.

The TER_{lt} for the assessment of the risk for earthworm-eating mammals due to exposure to prothioconazole-S-methyl (M01) via bioaccumulation in earthworms does not fall below the relevant trigger TER value of 5, indicating low risk to mammals following applications of FF-075 to winter and spring cereals (BBCH 30-69) and winter oilseed rape (BBCH 55-69).

Risk assessment for fish-eating mammals via secondary poisoning

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

Table 9.3-20: Assessment of the risk for fish-eating mammals due to exposure to prothioconazole via bioaccumulation in fish (secondary poisoning) for the intended use in winter oilseed rape (BBCH 55-69)

Parameter	Prothioconazole	Comments
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PEC _{sw} (mg/L)	0.0347521	Section 8 – Step 1
BCF _{fish}	18.8 19.7	EFSA Conclusion (EFSA Scientific Report (2007) 106, 1-98)
BMF	N/A	biomagnification factor (relevant for BCF ≥ 2000)
PEC _{fish}	0.6533395 0.684	PEC _{fish} = PEC _{water} × BCF _{fish}
Daily dietary dose (mg/kg bw/d)	0.0928 0.0972	DDD = PEC _{fish} × 0.142
NOEL (mg/kg bw/d)	95.6	EFSA Conclusion, 2007
TER _{lt}	1030.46 983.54	

TER values shown in bold fall below the relevant trigger.

Table 9.3-21: Assessment of the risk for fish-eating mammals due to exposure to prothioconazole via bioaccumulation in fish (secondary poisoning) for the intended use in winter and spring cereals (BBCH 30-69)

Parameter	Prothioconazole	Comments
PEC _{sw} (mg/L)	0.0434401	Section 8 – Step 1
BCF _{fish}	18.8 19.7	EFSA Conclusion (EFSA Scientific Report (2007) 106, 1-98)
BMF	N/A	biomagnification factor (relevant for BCF ≥ 2000)
PEC _{fish}	0.82 0.855	PEC _{fish} = PEC _{water} × BCF _{fish}
Daily dietary dose (mg/kg bw/d)	0.1160 0.1215	DDD = PEC _{fish} × 0.142
NOEL (mg/kg bw/d)	95.6	EFSA Conclusion (EFSA Scientific Report (2007) 106, 1-98)
TER _{lt}	824.37 786.83	

TER values shown in bold fall below the relevant trigger.

The TER_{lt} for the assessment of the risk for fish-eating mammals due to exposure to prothioconazole via bioaccumulation in fish does not fall below the relevant trigger TER value of 5, indicating low risk to mammals following applications of FF-075 to winter and spring cereals (BBCH 30-69) and winter oilseed rape (BBCH 55-69).

Table 9.3-22: Assessment of the risk for fish-eating mammals due to exposure to prothioconazole-desthio (M04) via bioaccumulation in fish (secondary poisoning) for the intended use in winter oilseed rape (BBCH 55-69)

Parameter	Prothioconazole-desthio (M04)	Comments
PEC _{sw} (mg/L)	0.0626570	Section 8 – Step 1
BCF _{fish}	45 65	EFSA Conclusion (EFSA Scientific Report (2007) 106, 1-98)
BMF	N/A	biomagnification factor (relevant for BCF ≥ 2000)
PEC _{fish}	2.819565 4.072	PEC _{fish} = PEC _{water} × BCF _{fish}

Daily dietary dose (mg/kg bw/d)	0.4004 0.578	DDD = PEC _{fish} × 0.142
NOEL (mg/kg bw/d)	10	EFSA Conclusion (EFSA Scientific Report (2007) 106, 1-98)
TER _{lt}	24.98 17.30	

TER values shown in bold fall below the relevant trigger.

Table 9.3-23: Assessment of the risk for fish-eating mammals due to exposure to prothioconazole-desthio (M04) via bioaccumulation in fish (secondary poisoning) for the intended use in winter and spring cereals (BBCH 30-69).

Parameter	Prothioconazole-desthio (M04)	Comments
PEC _{sw} (mg/L)	0.0783212	Section 8– Step 1
BCF _{fish}	45 65	EFSA Conclusion (EFSA Scientific Report (2007) 106, 1-98)
BMF	N/A	biomagnification factor (relevant for BCF ≥ 2000)
PEC _{fish}	3.52 5.09	PEC _{fish} = PEC _{water} × BCF _{fish}
Daily dietary dose (mg/kg bw/d)	0.50047 0.723	DDD = PEC _{fish} × 0.142
NOEL (mg/kg bw/d)	10	EFSA Conclusion (EFSA Scientific Report (2007) 106, 1-98)
TER _{lt}	19.98 13.83	

TER values shown in bold fall below the relevant trigger.

The TER_{lt} for the assessment of the risk for fish-eating mammals due to exposure to prothioconazole-desthio (M04) via bioaccumulation in fish does not fall below the relevant trigger TER value of 5, indicating low risk to mammals following applications of FF-075 to winter and spring cereals (BBCH 30-69) and winter oilseed rape (BBCH 55-69).

The assessment of the risk for fish-eating birds due to exposure to prothioconazole-S-methyl (M01) via bioaccumulation in fish is considered a conservative assessment as the long-term NOEL for the metabolite is assumed to be 10 x more toxic than the parent (9.56 mg /kg bw/d). Risk has been assessed against both the best (319 L/kg) and worse-case (19995 L/kg) whole fish BCF values for prothioconazole-S-methyl (M01).

Table 9.3-24: Assessment of the risk for fish-eating mammals due to exposure to prothioconazole-S-methyl (M01) via bioaccumulation in fish (secondary poisoning) for the intended use in winter oilseed rape (BBCH 55-69. 2 x 160 g a.s./ha, applied 14-day interval) assuming the best-case whole fish BCF value of 319 L/kg.

Parameter	Prothioconazole-S -methyl (M01)	Comments
PEC _{sw} (mg/L)	0.0073267	FOCUS Step 1 (Section 8)
BCF _{fish}	319	EFSA Conclusion (EFSA Scientific Report (2007) 106, 1-98)
BMF	N/A	biomagnification factor (relevant for BCF ≥ 2000)
PEC _{fish}	2.3372173	PEC _{fish} = PEC _{water} × BCF _{fish}

Daily dietary dose (mg/kg bw/d)	0.3319	DDD = $PEC_{fish} \times 0.142$
NOEL (mg/kg bw/d)	9.56*	Derived endpoint
TER _{lt}	28.81	

TER values shown in bold fall below the relevant trigger.

*Endpoint estimated as parent NOEL/10.

Table 9.3-25: Assessment of the risk for fish-eating mammals due to exposure to prothioconazole-S-methyl (M01) via bioaccumulation in fish (secondary poisoning) for the intended use in winter oilseed rape (BBCH 55-69. 2 x 160 g a.s./ha, applied 14-day interval) assuming the worst-case whole fish BCF value of 1995 L/kg.

Parameter	Prothioconazole-S - methyl (M01)	Comments
PEC _{sw} (mg/L)	0.0073267	FOCUS Step 1 (Section 8)
BCF _{fish}	1995	EFSA Conclusion (EFSA Scientific Report (2007) 106, 1-98)
BMF	N/A	biomagnification factor (relevant for BCF ≥ 2000)
PEC _{fish}	14.616767	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	2.0756	DDD = $PEC_{fish} \times 0.142$
NOEL (mg/kg bw/d)	9.56*	Derived endpoint
TER _{lt}	4.61	

TER values shown in bold fall below the relevant trigger.

*Endpoint estimated as parent NOEL/10.

The TER_{lt} for the assessment of the risk for fish-eating mammals due to exposure to prothioconazole-S-methyl (M01) via bioaccumulation in fish does not fall below the relevant trigger TER value of 5 when considering the best-case whole fish BCF value of 319 L/kg, indicating low risk to mammals following applications of FF-075 to winter oilseed rape (BBCH 55-69). However, when the worse-case whole fish BCF value of 1995 kg/L is considered in combination with the conservative assumption of toxicity for the metabolite (10 x more toxic than prothioconazole), the TER_{lt} falls below the relevant trigger TER value of 5. Further refinement of the risk assessment is required and, therefore, the risk to fish-eating mammals exposed to prothioconazole-S-methyl (M01) via bioaccumulation in fish has been assessed considering the worse-case FOCUS Step 2 PEC_{sw} value as a more realistic scenario.

Table 9.3-26: Assessment of the risk for fish-eating mammals due to exposure to prothioconazole-S-methyl (M01) via bioaccumulation in fish (secondary poisoning) for the intended use in winter oilseed rape (BBCH 55-69. 2 x 160 g a.s./ha, applied 14-day interval), assuming the worse-case whole fish BCF value of 1995 L/kg and the worse-case FOCUS Step 2 PEC_{sw} value.

Parameter	Prothioconazole-S - methyl (M01)	Comments
PEC _{sw} (mg/L)	0.0003609	Southern Zone Step 2
BCF _{fish}	1995	EFSA Conclusion (EFSA Scientific Report (2007) 106, 1-98)
BMF	N/A	biomagnification factor (relevant for BCF ≥ 2000)
PEC _{fish}	0.7199955	$PEC_{fish} = PEC_{water} \times BCF_{fish}$

Daily dietary dose (mg/kg bw/d)	0.1022	$DDD = PEC_{fish} \times 0.142$
NOEL (mg/kg bw/d)	9.56*	Derived endpoint
TER_{It}	93.51	

TER values shown in bold fall below the relevant trigger.

*Endpoint estimated as parent NOEL/10.

The TER_{It} for the assessment of the risk for fish-eating mammals due to exposure to prothioconazole-S-methyl (M01) via bioaccumulation in fish does not fall below the relevant trigger TER value of 5 when considering the worse-case whole fish BCF value of 1995 L/kg, and the worse-case FOCUS Step 2 PEC_{sw} value (Southern Zone PEC_{sw} value), indicating low risk to mammals following applications of FF-075 to oilseed rape (BBCH 55-69).

Table 9.3-27: Assessment of the risk for fish-eating mammals due to exposure to prothioconazole-S-methyl (M01) via bioaccumulation in fish (secondary poisoning) for the intended use in winter and spring cereals (BBCH 30-69, 2 x 200 g a.s./ha, applied 14-day interval), assuming the best-case whole fish BCF value of 319 L/kg

Parameter	Prothioconazole-S - methyl (M01)	Comments
PEC _{sw} (mg/L)	0.0091584	FOCUS Step 1 (Section 8)
BCF _{fish}	319	EFSA Conclusion (EFSA Scientific Report (2007) 106, 1-98)
BMF	N/A	biomagnification factor (relevant for $BCF \geq 2000$)
PEC _{fish}	2.92	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.4148557203	$DDD = PEC_{fish} \times 0.142$
NOEL (mg/kg bw/d)	9.56*	Derived endpoint
TER_{It}	23.04	

TER values shown in bold fall below the relevant trigger.

*Endpoint estimated as parent NOEL/10.

Table 9.3-28: Assessment of the risk for fish-eating mammals due to exposure to prothioconazole-S-methyl (M01) via bioaccumulation in fish (secondary poisoning) for the intended use in winter and spring cereals (BBCH 30-69, 2 x 200 g a.s./ha, applied 14-day interval), assuming the worst-case whole fish BCF value of 1995 L/kg.

Parameter	Prothioconazole-S - methyl (M01)	Comments
PEC _{sw} (mg/L)	0.0091584	FOCUS Step 1 (Section 8)
BCF _{fish}	1995	EFSA Conclusion (EFSA Scientific Report (2007) 106, 1-98)
BMF	N/A	biomagnification factor (relevant for $BCF \geq 2000$)
PEC _{fish}	18.27	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	2.594483136	$DDD = PEC_{fish} \times 0.142$
NOEL (mg/kg bw/d)	9.56*	Derived endpoint
TER_{It}	3.68	

TER values shown in bold fall below the relevant trigger.

*Endpoint estimated as parent NOEL/10.

The TER_{lt} for the assessment of the risk for fish-eating mammals due to exposure to prothioconazole-S-methyl (M01) via bioaccumulation in fish does not fall below the relevant trigger TER value of 5 when considering the best-case whole fish BCF value of 319 L/kg, and FOCUS Step 1 PEC_{sw} values, indicating low risk to mammals following applications of FF-075 to winter cereals (BBCH 30-69). However, when the worse-case whole fish BCF value of 1995 kg/L is considered in combination with the conservative assumption of toxicity for the metabolite (10 x more toxic than prothioconazole), the TER_{lt} falls below the relevant trigger TER value of 5. Further refinement of the risk assessment is required and, therefore, the risk to fish-eating mammals exposed to prothioconazole-S-methyl (M01) via bioaccumulation in fish has been assessed considering the worse-case FOCUS Step 2 PEC_{sw} value as a more realistic scenario.

Table 9.3-29: Assessment of the risk for fish-eating mammals due to exposure to prothioconazole-S-methyl (M01) via bioaccumulation in fish (secondary poisoning) for the intended use in winter and spring cereals (BBCH 30-69. 2 x 200 g a.s./ha, applied 14-day interval), assuming the worse-case whole fish BCF value of 1995 L/kg and the Northern Zone FOCUS Step 2 PEC_{sw} value.

Parameter	Prothioconazole-S - methyl (M01)	Comments
PEC_{sw} (mg/L)	0.0011532	Southern Zone Step 2
BCF_{fish}	1995	EFSA Conclusion (EFSA Scientific Report (2007) 106, 1-98)
BMF	N/A	biomagnification factor (relevant for $BCF \geq 2000$)
PEC_{fish}	2.300634	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.3267	$DDD = PEC_{fish} \times 0.142$
NOEL (mg/kg bw/d)	9.56*	Derived endpoint
TER_{lt}	29.26	

TER values shown in bold fall below the relevant trigger.

*Endpoint estimated as parent NOEL/10.

The TER_{lt} for the assessment of the risk for fish-eating mammals due to exposure to prothioconazole-S-methyl (M01) via bioaccumulation in fish does not fall below the relevant trigger TER value of 5 when considering the worse-case whole fish BCF value of 1995 L/kg, and the worse-case FOCUS Step 2 PEC_{sw} value (Southern zone PEC_{sw} value), indicating low risk to mammals following applications of FF-075 to winter and spring cereals (BBCH 30-69).

zRMS comment:

The risk for fish-eating mammals and earthworms-eating mammals due to exposure to prothioconazole and its metabolites such as: (M01), (M04) is considered as acceptable.

9.3.1.4 Biomagnification in terrestrial food chains

Not relevant.

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

Not relevant.

9.3.3 Overall conclusions

Regulatory testing with mammals has been conducted with azoxystrobin, prothioconazole and the relevant metabolite, prothioconazole-desthio (M04), in accordance with EU requirements for rat and mouse. Results from these studies, summarised in the EFSA Review Reports for azoxystrobin and prothioconazole, show that the active substances and metabolite, prothioconazole-desthio (M04), have low toxicity to mammals.

The TER_{it} values do not exceed the Annex VI trigger of 5 for prothioconazole-desthio (M04) when considering both relevant mammalian species in winter and spring cereals and winter oilseed rape, and when deposition factors in cereals are refined, according to the guidance in EFSA, (2009).

The estimated toxicity for FF-075, derived from the combined assessment of the active ingredients, demonstrates low risk to mammals from applications of the formulated product in accordance with the GAP.

The assessment of risk from secondary poisoning of earthworm-eating mammals and fish-eating mammals via exposure to prothioconazole, prothioconazole-desthio (M04) and prothioconazole-S-methyl (M01) via bioaccumulation in earthworms and fish indicates low risk to mammals following applications of FF-075 in accordance with the proposed GAP.

Low risk to mammalian species is expected from applications of FF-075 in accordance with the GAP.

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

No data on reptiles and terrestrial amphibians are available for FF-075. In the absence of a specific framework, the data and risk assessment for birds and mammals are considered an adequate surrogate for other terrestrial vertebrates. No overt toxicity has been observed in any of the avian and mammalian studies relevant for the ecotoxicological risk assessment. In addition, low acute and long-term risks were concluded for birds and mammals. Since terrestrial amphibians and reptiles' diets have generally a lower vegetation content than those of the focal bird and mammal species considered in the previous risk assessment, it is expected that exposure to feed items possibly contaminated with FF-075 will be lower for terrestrial amphibians and reptiles than birds and mammals. As such, no adverse effects or risks are expected for reptiles and terrestrial amphibians exposed to prothioconazole, azoxystrobin or prothioconazole-desthio (M04) following applications of FF-075.

9.5 Effects on aquatic organisms (KCP 10.2)

9.5.1 Toxicity data

Studies on the toxicity to aquatic organisms have been conducted with FF-075, prothioconazole, azoxystrobin and the relevant metabolites. Full details of these studies are provided in the respective prothioconazole EU DAR, (2005), EFSA Conclusions, (2007) for prothioconazole and the EFSA Conclusions (FF-075) for azoxystrobin.

Effects on aquatic organisms of FF-075 were not evaluated as part of the EU assessment of prothioconazole.

zole. New data submitted with this application are summarised in Appendix 2.

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

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

Species	Substance	Exposure System	Results	Reference
<i>Oncorhynchus mykiss</i>	Prothioconazole	96 h, s	LC ₅₀ = 1.83 mg a.s./L_{mm} NOEC = 0.99 mg a.s./L	EFSA Scientific Report (2007) 106, 1-98. DAR, V.3, Annex B, B.9, (2005). xxxxxxx DOM 99076
<i>Oncorhynchus mykiss</i>	Prothioconazole-desthio (M04)	96 h, s	LC ₅₀ = 6.63 mg metabolite./L_{nom} NOEC = 2.34 mg metabolite/L	EFSA Scientific Report (2007) 106, 1-98. DAR, V.3, Annex B, B.9, (2005). xxxxxxx FF-298
<i>Oncorhynchus mykiss</i>	Prothioconazole-S-methyl (M01)	96 h, ss	LC ₅₀ = 1.8 mg metabolite/L_{mm} NOEC = 0.265 mg metabolite/L	EFSA Scientific Report (2007) 106, 1-98. DAR, V.3, Annex B, B.9, (2005). xxxxxxx, 2001d. DOM 21047.
<i>Oncorhynchus mykiss</i>	Prothioconazole-triazolylketone (M42)	96-h	LC ₅₀ = 0.183 mg metabolite/L¹	
<i>Oncorhynchus mykiss</i>	1,2,4-triazole (M13)	96 h, s	LC ₅₀ = 498 mg metabolite/L_{mm} NOEC = 51.5 mg metabolite/L	EFSA J 2014: 12(1):3485 DAR, V.3, Annex B, B.9, (2005). xxxxxxx Project No. 821418
<i>Oncorhynchus mykiss</i>	Azoxystrobin	96 h, ft	EC ₅₀ = 0.47 mg a.s./L (n)	EFSA Journal (2010); 8(4):1542 xxxxxxx 1993 WAT95-50541

Species	Substance	Exposure System	Results	Reference
<i>Lepomis macrochirus</i>	Azoxyst robin	96 h, ft	EC ₅₀ = 1.1 mg a.s./L (n)	EFSA Journal (2010); 8(4):1542 xxxxxx WAT95-50736
<i>Oncorhynchus mykiss</i>	R234886	96 h, ft	EC ₅₀ = >150 mg metabolite/L (m)	EFSA Journal (2010); 8(4):1542 xxxxxxx 1993. WAT95-50546
<i>Oncorhynchus mykiss</i>	R401553	96 h, ft	EC ₅₀ = >120 mg metabolite/L (n)	EFSA Journal (2010); 8(4):1542 xxxxxxx 2002. SYN501657/0002
<i>Oncorhynchus mykiss</i>	R402173	96 h, ft	EC ₅₀ = 62 mg metabolite/L (n)	EFSA Journal (2010); 8(4):1542 xxxxxxx 2002. SYN511114/0001 7338/B, 2013671
<i>Oncorhynchus mykiss</i>	Prothioconazole	97-day ELS	NOEC (Reduction in swim-up and increase in time to swim-up) = 0.308 mg a.s./L _{mm}	EFSA Scientific Report (2007) 106, 1-98. DAR, V.3, Annex B, B.9, (2005). xxxxxxx 2001e. DOM 20028
<i>Oncorhynchus mykiss</i>	Prothioconazole-desthio (M04)	96-day ELS	NOEC (deformities) = 0.00334 mg metabolite/L _{mm}	EFSA Scientific Report (2007) 106, 1-98. DAR, V.3, Annex B, B.9, (2005). xxxxxxx 2002. 1022.013.321
<i>Oncorhynchus mykiss</i>	Prothioconazole-S-methyl (M01)	96-day ELS	NOEC = 0.0308 mg metabolite/L ¹	
<i>Oncorhynchus mykiss</i>	Prothioconazole-triazolylketone (M42)	96-day ELS	NOEC = 0.0308 mg metabolite/L ¹	
<i>Oncorhynchus mykiss</i>	1,2,4-triazole (M13)	97-day ELS	NOEC = 3.2 mg metabolite/L	EFSA J 2014; 12(1):3485

Species	Substance	Exposure System	Results	Reference
				DAR, V.3, Annex B, B.9, (2005). xxxxxx, 2002. DOM 21060
<i>Pimephales promelas</i>	Azoxystrobin	33-day ELS ft	NOEC growth = 0.147 mg a.s./L (m)	EFSA Journal (2010); 8(4):1542 xxxxxx 1994. WAT95-50584
<i>Lepomis macrochirus</i>	Prothioconazole	Bioconcentration	<ul style="list-style-type: none"> Whole fish BCF value for all labelled compounds = 18.8 (normalised to 6% lipid content) Half-life (CT₅₀ days) = 0.80 (whole fish) Level of residues (%) after 14-day depuration phase: 9 %	EFSA Scientific Report (2007) 106, 1-98. DAR, V.3, Annex B, B.9, (2005). xxxxxx 2001. DOM 21003
<i>Lepomis macrochirus</i>	Prothioconazole-desthio (M04)	Bioconcentration	<ul style="list-style-type: none"> Whole fish BCF value for all labelled compounds = 45 (normalised to 6% lipid content) Half-life (CT₅₀) = 0.4 – 0.5 d (whole fish) Level of residues (%) after 14-day depuration phase: 4 % 	EFSA Scientific Report (2007) 106, 1-98. DAR, V.3, Annex B, B.9, (2005). xxxxxx 2001. DOM 20006
<i>Lepomis macrochirus</i>	Prothioconazole-S methyl (M01)	Bioconcentration	<ul style="list-style-type: none"> Regression-based: BCF = 319 Arnot-Gobas BCF = 764.8 – 844.5 (lower – upper trophic; including biotransformation rate estimates) Arnot-Gobas BCF= 1995 (upper trophic; assuming biotransformation rate of 0)	Rapporteur Assessment Report (2018, UK). Endpoints recalculated by RMS, (UK), 2018. xxxxxxx. (2013)
<i>Daphnia magna</i>	Prothioconazole	48 h, s	EC ₅₀ = 1.3 mg a.s./L_{nom}	EFSA Scientific Report (2007) 106, 1-98. DAR, V.3, Annex B, B.9, (2005). Heimbach, 1999c.

Species	Substance	Exposure System	Results	Reference
				HBF/DM 212
<i>Daphnia magna</i>	Prothioconazole-desthio (M04)	48 h, s	EC ₅₀ = >10 mg metabolite/L (n)	EFSA Scientific Report (2007) 106, 1-98. DAR, V.3, Annex B, B.9, (2005). Heimbach, 1990a. HBF DM 95
<i>Daphnia magna</i>	Prothioconazole-S-methyl (M01)	48-h, s	EC ₅₀ = 2.8 mg metabolite/L(n)	EFSA Scientific Report (2007) 106, 1-98. DAR, V.3, Annex B, B.9, (2005). Dorgerloh and Sommer, 2001b. DOM 21055
<i>Daphnia magna</i>	Prothioconazole-triazolylketone (M42)	48-h	EC ₅₀ = 0.13 mg metabolite/L ¹	
<i>Daphnia magna</i>	1,2,4-triazole (M13)	48-h	EC ₅₀ = 900 mg metabolite/L(n)	EFSA Journal 2014;12(1):3485 DAR, V.3, Annex B, B.9, (2005). Rufli, 1983b Project No. 821416
<i>Daphnia magna</i>	Azoxystrobin 250 SC	48-h, s	EC ₅₀ = 0.11 mg a.s./L(n)	EFSA Journal (2010); 8(4):1542 Rapley, J.H., Kearson, L.L., and Hamer, M.J., 1995. WAT95-50585
<i>Daphnia magna</i>	Azoxystrobin	48-h	EC ₅₀ = 0.23 mg a.s./L(m)	EFSA Journal (2010); 8(4):1542 Farrelly, E., and Hamer, M.J., 1995. WAT95-50597
<i>Macrocyclopsis fuscus</i>	Azoxystrobin	48-h	EC ₅₀ = 0.13 mg a.s./L(n)	EFSA Journal (2010); 8(4):1542 Farrelly, E., Kearson, L.L., Rapley, J.H., and Hamer, M.J., 1995. WAT95-50586

Species	Substance	Exposure System	Results	Reference
<i>Mysidopsis bahia</i>	Azoxystrobin	96-h	48-h EC ₅₀ = 0.055 mg a.s./L(n) 96-h EC ₅₀ = 0.068 mg a.s./L(n)	EFSA Journal (2010); 8(4):1542 Kent SJ, Sankey SA, Grinell AJ, 1993. ICI5504/0925
<i>Crassostrea gigas</i>	Azoxystrobin	48-h	EC ₅₀ = 1.3 mg a.s./L(n)	EFSA Journal (2010); 8(4):1542 Kent SJ, Sankey SA, Grinell AJ, 1994. ICI5504/0927
<i>Daphnia pulex</i>	Azoxystrobin	48-h	EC ₅₀ = 2 mg a.s./L(n)	Azoxystrobin AR 11 Vol 3, B9 (May 2009) Rapley, J.H., Kearson, L.L., and Hamer, M.J.1995a, WAT95-50596
<i>Chironomus riparius</i>	Azoxystrobin	48-h	EC ₅₀ = 2.1 mg a.s./L(n)	Azoxystrobin AR 11 Vol 3, B9 (May 2009) Farrelly, E., Kearson, L.L., Rapley, J.H., and Hamer, M.J.1995d, WAT95-50589
<i>Gammarus pulex</i>	Azoxystrobin	48-h	EC ₅₀ = 3.5 mg a.s./L(n)	Azoxystrobin AR 11 Vol 3, B9 (May 2009) Farrelly, E., Kearson, L.L., Rapley, J.H., and Hamer, M.J.1995b, WAT95-50587
<i>Choaborus crystallinus</i>	Azoxystrobin	48-h	EC ₅₀ = 160 mg a.s./L(n)	Azoxystrobin AR 11 Vol 3, B9 (May 2009) Farrelly, E., Kearson, L.L., Rapley, J.H., and Hamer, M.J.1995e, WAT95-50590
<i>Cloen dipterum</i>	Azoxystrobin	48-h	EC ₅₀ = 320 mg a.s./L(n)	Azoxystrobin AR 11 Vol 3, B9 (May 2009) Farrelly, E., Kearson, L.L., Rapley, J.H., and Hamer, M.J.1995g,

Species	Substance	Exposure System	Results	Reference
				WAT95-50592
<i>Asellus aquaticus</i>	Azoxystrobin	48-h	EC ₅₀ = >400 mg a.s./L(n)	Azoxystrobin AR 11 Vol 3, B9 (May 2009) Farrelly, E., Kearson, L.L., Rapley, J.H., and Hamer, M.J.1995c, WAT95-50588
<i>Ischnura elegans</i>	Azoxystrobin	48-h	EC ₅₀ = >400 mg a.s./L(n)	Azoxystrobin AR 11 Vol 3, B9 (May 2009) Farrelly, E., Kearson, L.L., Rapley, J.H., and Hamer, M.J.1995f, WAT95-50591
<i>Notonecta glauca</i>	Azoxystrobin	48-h	EC ₅₀ = >400 mg a.s./L(n)	Azoxystrobin AR 11 Vol 3, B9 (May 2009) Farrelly, E., Kearson, L.L., Rapley, J.H., and Hamer, M.J.1995c, WAT95-50593
<i>Brachyonus calyciflorus</i>	Azoxystrobin	48-h	EC ₅₀ = >400 mg a.s./L(n)	Azoxystrobin AR 11 Vol 3, B9 (May 2009) Farrelly, E., Kearson, L.L., Rapley, J.H., and Hamer, M.J.1995h, WAT95-50594
<i>Lymnaea stagnalis</i>	Azoxystrobin	48-h	EC ₅₀ = >400 mg a.s./L(n)	Azoxystrobin AR 11 Vol 3, B9 (May 2009) Farrelly, E., Kearson, L.L., Rapley, J.H., and Hamer, M.J.1995i, WAT95-50595
<i>Daphnia magna</i>	R234886	48-h	EC ₅₀ = >180 mg metabolite/L(n)	EFSA Journal (2010); 8(4):1542 Kent, S.J., Sankey, S.A., Banner, A.J., and Johnson, P.A., 1993. WAT95-50545
<i>Daphnia magna</i>	R402173	48-h	EC ₅₀ = >100 mg metabolite/L(n)	EFSA Journal (2010); 8(4):1542

Species	Substance	Exposure System	Results	Reference
				Wallace SJ, 2002a SYN11114/0002
<i>Daphnia magna</i>	R401553	48-h	EC ₅₀ = >120 mg metabolite/L(n)	EFSA Journal (2010); 8(4):1542 Bowles AJ, Wallace SJ, 2002a. SYN5016579003
<i>Daphnia magna</i>	Prothioconazole	21-d, ss	NOEC (reproduction of offspring)= 0.56 mg a.s./L (n)	EFSA Scientific Report (2007) 106, 1-98. DAR, V.3, Annex B, B.9, (2005). Hendel and Sommer, 2001. HDB/RDM 67
<i>Daphnia magna</i>	Prothioconazole-desthio (M04)	21-d, ss	NOEC (reduction of offspring) = 0.1 mg metabolite/L (m)	EFSA Scientific Report (2007) 106, 1-98. DAR, V.3, Annex B, B.9, (2005). Dorgerloh and Sommer, 2001c. DOM 21036
<i>Daphnia magna</i>	Prothioconazole-S methyl (M01)	21-d, ss	NOEC = 0.056 mg metabolite/L ¹	
<i>Daphnia magna</i>	Prothioconazole-triazolylketone (M42)	21-d, ss	NOEC = 0.056 mg metabolite/L ¹	
<i>Daphnia magna</i>	1,2,4-triazole (M13)	21-d, ss	NOEC = 0.056 mg metabolite/L ¹	
<i>Daphnia magna</i>	Azoxystrobin	21-d, s	NOEC (reproduction) = 0.0044 mg a.s./L (n)	EFSA Journal (2010); 8(4):1542 Rapley, J.H., Farrelly, E., and Hamer, M.J., 1994. WAT95-50540
<i>Mysidopsis bahia</i>	Azoxystrobin	28-d	NOEC (adult mortality) = 0.00954 mg a.s./L (mm)	EFSA Journal (2010); 8(4):1542 Boeri RL, Magazu JP, Ward TJ, 1997. ICI5504/0952
<i>Chironomus riparius</i>	Prothioconazole	28 d, static spiked water	NOEC (emergence and development rate) = 9.14 mg a.s./L _{nom}	EFSA Scientific Report (2007) 106, 1-98.

Species	Substance	Exposure System	Results	Reference
				DAR, V.3, Annex B, B.9, (2005). Hendel, 2000a HDB/CH 42
<i>Chironomus riparius</i>	Prothioconazole-desthio (M04)	28 d, static spiked water	NOEC (emergence) = 2.0 mg metabolite/L_{nom}	EFSA Scientific Report (2007) 106, 1-98. DAR, V.3, Annex B, B.9, (2005). Hendel, 2000b. HDB/CH 43
<i>Chironomus riparius</i>	Prothioconazole-S methyl (M01)	28 d, static spiked water	NOEC (emergence and development rate) = 0.914 mg metabolite/L¹	
<i>Chironomus riparius</i>	Prothioconazole-triazolylketone (M42)	28 d, static spiked water	NOEC (emergence and development rate) = 0.914 mg metabolite/L¹	
<i>Chironomus riparius</i>	1,2,4-triazole (M13)	28 d, static spiked water	NOEC (emergence and development rate) = 0.914 mg metabolite/L¹	
<i>Chironomus riparius</i>	Azoxystrobin	28 d, s	NOEC = 0.8 mg a.s./L_{nom}	EFSA Journal (2010); 8(4):1542 Farrelly, E., Kearson, L.L., Rapley, J.H., and Hamer, M.J., 1995d. WAT95-50589
<i>Pseudokirchneriella subcapitata</i>	Prothioconazole	72-h, s	E _r C ₅₀ = 2.18 mg a.s./L_{i.m} E _b C ₅₀ = 1.10 mg a.s./L _{i.m} .	EFSA Scientific Report (2007) 106, 1-98. DAR, V.3, Annex B, B.9, (2005). Dorgerloh, 2000b. DOM 99107
<i>Scendesmus subspicatus</i>	Prothioconazole-desthio (M04)	72-h	E _r C ₅₀ = 0.55 mg metabolite/L E _b C ₅₀ = 0.073 mg metabolite/L	EFSA Scientific Report (2007) 106, 1-98. DAR, V.3, Annex B, B.9, (2005). Heimbach, 1990b

Species	Substance	Exposure System	Results	Reference
				HBF AL 78
<i>Pseudokirchneriella subcapitata</i>	Prothioconazole-S methyl (M01)	72-h,s	ErC ₅₀ = 47.4 mg metabolite/L i.m EbC ₅₀ = 3.77 mg metabolite/L i.m.	EFSA Scientific Report (2007) 106, 1-98. DAR, V.3, Annex B, B.9, (2005). Dorgerloh and Sommer, 2001a. DOM 21028
<i>Pseudokirchneriella subcapitata</i>	Prothioconazole-triazolyl ketone (M42)	72-h,s	ErC ₅₀ = 0.218 mg metabolite/L ¹	
<i>Pseudokirchneriella subcapitata</i>	1,2,4-triazole (M13)	72-h,s	ErC ₅₀ = >22.5 mg metabolite/L i.m	EFSA Journal 2014; 12(1):3485 DAR, V.3, Annex B, B.9, (2005). Palmer, Kendall and Krueger (2001). Report No. 528A 101
<i>Selenastrum capricornutum</i>	Azoxystrobin	72-h, s	ErC ₅₀ = 0.36 mg a.s./L (m)	EFSA Journal (2010); 8(4):1542 Smyth, D.D., Kent, S.J., Craig, N.C.D., Sankey, S.S., and Grinell, A.J., 1993. WAT95-50538
<i>Selenastrum capricornutum</i>	Azoxystrobin	72-h, s	ErC ₅₀ = 0.16 mg a.s./L (n)	EFSA Journal (2010); 8(4):1542. Smyth, D.D., Kent, S.J., Craig, N.C.D., Sankey, S.S., and Grinell, A.J., 1993. WAT95-50547
<i>Skeletonema costatum</i>	Azoxystrobin	72-h, s	ErC ₅₀ = 0.3 mg a.s./L (n) EbC ₅₀ = 0.098 mg a.s./L (n)	EFSA Journal (2010); 8(4):1542 Smyth DV, Kent SJ, Sankey SA, Johnson PA, 1994. ICI5504/0966
<i>Navicula pelliculosa</i>	Azoxystrobin	120-h, s	ErC ₅₀ = 0.146 mg a.s./L (n) EbC ₅₀ = 0.014 mg a.s./L (n)	EFSA Journal (2010); 8(4):1542 Smyth DV, Sankey SA, Kent SJ, Sytanley RD, 1994. ICI5504/0965

Species	Substance	Exposure System	Results	Reference
	Azoxystrobin		Geomean of algae endpoints = 0.262 mg a.s./L	EFSA Journal (2010); 8(4):1542
<i>Anabaena flos-aquae</i>	Azoxystrobin	120-h, s	E _r C ₅₀ = 13.9 mg a.s./L (m) E _b C ₅₀ = 9.5 mg a.s./L (m)	EFSA Journal (2010); 8(4):1542 Smyth DV, Kent SI, Sankey, S.A, Shearing JM, 1994. ICI5504/0967
<i>Selenastrum capricornutum</i>	R234886	72-h, s	EC ₅₀ = 47.0 mg a.s./L (m)	EFSA Journal (2010); 8(4):1542 Smyth, D.V., Kent, S.J., Craig, N.C.D., Sankey, S.S., and Johnson, P.A., 1993. WAT95-50544
<i>Selenastrum capricornutum</i>	R402173	72-h, s	E _r C ₅₀ = 67 mg a.s./L (n) E _b C ₅₀ = 67 mg a.s./L (n)	EFSA Journal (2010); 8(4):1542 Wallace SJ, Woodyer JM, 2002. SYN511114/0003
<i>Selenastrum capricornutum</i>	R401553	72-h, s	E _r C ₅₀ = >120 mg a.s./L (n) E _b C ₅₀ = >120 mg a.s./L (n)	EFSA Journal (2010); 8(4):1542 Bowler AJ, Wallace SJ, 2002b. SYN501657/0004
<i>Lemna gibba</i>	Azoxystrobin	14-d, s	EC ₅₀ (fronds) = >6.4 EC ₅₀ (dry weight) = 3.2	EFSA Journal (2010); 8(4):1542 Smyth, D.V., Kent, S.J., and Sankey, R.D., 1994a. ICI5504/0963
Higher-tier studies (micro- or mesocosm studies)				

A mesocosm study is available for Azoxystrobin which was evaluated during the previous inclusion of the active substance (EFSA Journal (2010); 8(4):1542, Cole JFH, Everett CJ, Gentle W, 2000, Report No. SYNICI5504/0976).

The Notifier proposed that the no observed ecologically adverse effects concentration (NOEAEC) is 10 µg/L. No uncertainty or assessment factor was proposed. There were effects at all concentrations, hence it is not possible to establish a NOEC. The treatment related effects at 10 µg/L were considered to be relatively short-lived and restricted to decreases in the following parameters:

Daphnia spp – effects at 10 µg/L were noted at 3, 7 and 14 days

Total cladocera – effects at 10 µg/L were noted at 3, 7 and 14 days

Copepoda nauplii – effects at day 35

Copepoda Cyclopoid copepodites – effects at 10 µg/L were noted at days 7 and 10,

Copepoda Cyclopoid adults – effects were noted on day 3 only

Sphaeriidae – significantly fewer on days 72 and 93 for samples collected via nets, there were significantly fewer on days 22, 30 44 and 72.

Species	Substance	Exposure System	Results	Reference
<p>Total mollusc – in samples collected via nets were lower on days 22 and 72 Total macroinvertebrates – in sample collected via nets were lower on day 30. The following groups increased and were probably the result of indirect effects: <i>Chydorus</i> – significantly greater numbers on study day 10 and 28 <i>Pompholyx</i> sp – significantly greater numbers than the control on day 14 only <i>Testudinella</i> sp – there were significantly greater numbers than the control on days 42 and 35. Total rotifer – there were significantly greater numbers than the control on days 3, 35, 42 and 56. It should however be noted that there was only one application and there was only chemical analysis 21 hours after application; due to this it is proposed that the effect concentrations should be based on the initial nominal concentrations.</p>				

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

Values highlighted in bold are used in the risk assessment.

¹Metabolite endpoint assumes toxicity is x10 more toxic than the parent endpoint (EFSA Journal 2007).

Table 9.5-1: Endpoints and effect values relevant for the risk assessment for aquatic organisms – FF-075

Species	Substance	Exposure System	Results	Reference
<i>Daphnia magna</i>	FF-075	48-h	EC ₅₀ = 2.97 mg product/L_{nom} (2.34 – 3.6 mg/L 95 % CL)	New study KCP 10.2.1/01 Li, (2021). Study No. 2859.
<i>Pseudokirchneriella subcapitata</i>	FF-075	96 h, s	ErC ₅₀ = 4.65 mg/L_{nom} EyC ₅₀ = 1.39 mg/L _{nom}	New study KCP 10.2.1/02 Li, (2021). Study No. 2858.
<i>Lemna gibba</i>	FF-075	7-day	ErC ₅₀ (fronds) = 16.52 mg/L EyC ₅₀ (frond area) = 2.75	New study KCP 10.2.1/03 Li, (2021). Study No. 2867.
Higher-tier studies (micro- or mesocosm studies)				
N/A				

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

9.5.1.1 Justification for new endpoints

New studies with the formulation, FF-075, which have not previously been evaluated, are available and are summarised in Appendix 2. An additional acute toxicity study with fish for the formulated product was not deemed necessary due to the available data for the active substance, and, additionally, the applicant aimed to minimise the testing of vertebrates, in-line with the EFSA supporting publication 2019:EN-

1673.⁴

9.5.2 Risk assessment

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

Mixture Toxicity Assessment

The mixture toxicity assessment, presented, follows the EFSA Aquatic Guidance 2013 and uses the Central Zone Harmonised tool (AGD_Aquamix_v1.15 (10.5281/zenodo.4593676)). Each use (applications to winter oilseed rape, winter cereals and spring cereals) has been assessed and presented separately. Please refer to the corresponding AGD_Aquamix_v 1.15 spreadsheets which are embedded in Appendix 3 for the detailed assessment. In the acute and chronic mixture toxicity assessments, where different species have been tested in the standard systems, endpoints have been selected to allow species to correspond between substances. Refined endpoints for algae and aquatic invertebrates have been entered as additional/Tier 2AB data in the tool where appropriate. Due to limited vertebrate testing, only one chronic fish early life stage study is available for both prothioconazole-desithio and azoxystrobin and different species, *Oncorhynchus mykiss* and *Pimephales promelas* respectively, were tested. In the absence of additional available data, the mixture toxicity assessment has been conducted using the endpoints from the available studies.

The EFSA guidance (Section 10.3) states that when using concentration-addition the calculations should be made using only “specific endpoints and to defined taxonomic groups” i.e. those which can be additive and are from comparable species. The endpoints used in the mixture assessment are set out in the table, below.

In accordance with the Birds and Mammals risk guidance (EFSA/2009/1438), EC₁₀ values would, preferably be used for the chronic assessment. However, in the case of both prothioconazole-desithio (M04) and azoxystrobin, the endpoints have been taken from the respective EFSA Conclusion reports where only the NOEC values are provided.

The overview of the relevant toxicological endpoints:

	Relevant active substance/metabolite data			Formulation data
Active substances	Prothioconazole	Prothioconazole-desithio (M04)	Azoxystrobin	FF-075
Concentration in Product (g a.s./L)	200	200 (worse case)	150	-
LC ₅₀ fish (mg a.s./L)	1.83	6.63	0.47	-
NOEC fish (mg a.s./L)	0.308	0.00334	0.147	-
EC ₅₀ invertebrates (mg a.s./L)	1.3	≥10	0.23 ³ 0.33 ¹	2.97
NOEC invertebrates (mg a.s./L)	0.56	0.1	0.0044 ³ 0.33 ¹	-
EC ₅₀ algae (mg a.s./L)	2.18	0.55	0.36 ⁴ 0.262 ²	4.65

¹ Agreed derived endpoint for azoxystrobin, taken from derived RAC outlined in EFSA Journal (2010); 8(4):1542

² Agreed geomean outlined in EFSA Journal (2010); 8(4):1542

⁴ EFSA (European Food Safety Authority), 2019. Technical report on the outcome of the Pesticides Review Meeting on general recurring issues in ecotoxicology. EFSA supporting publication 2019:EN-1673. 117 pp. doi:10.2903/sp.efsa.2019.EN-1673.

³ *Daphnia magna* endpoint used in the first tier assessment as a standard species

⁴ *Pseudokirchneriella subcapitata* endpoint used in the first tier

An overview of the relevant species and toxicological endpoints:

Organism group	Active substances/metabolite/formulation	Species	Relevant active substance/metabolite data
Fish acute (LC ₅₀ (mg a.s./L))	Prothioconazole	<i>Oncorhynchus mykiss</i>	1.83
	Prothioconazole-desthio (M04)		6.63
	Azoxystrobin		0.47
	FF-075	-	-
Aquatic invertebrates (EC ₅₀ (mg a.s./L))	Prothioconazole	<i>Daphnia magna</i>	1.3
	Prothioconazole-desthio (M04)		>10
	Azoxystrobin		0.23 ³
			0.33 ¹
	FF-075		2.97
Algae (EC ₅₀ (mg a.s./L))	Prothioconazole	<i>Pseudokirchneriella subcapitata</i>	2.18
	Prothioconazole-desthio (M04)	<i>Scenedesmus subspicatus</i>	0.55
	Azoxystrobin	<i>Selenastrum capricornutum</i>	0.36 ⁴
			0.262 ²
	FF-075	<i>Pseudokirchneriella subcapitata</i>	4.65
Chronic fish (NOEC (mg a.s./L))	Prothioconazole	<i>Oncorhynchus mykiss</i>	0.308
	Prothioconazole-desthio (M04)		0.00334
	Azoxystrobin	<i>Pimephales promelas</i>	0.147
	FF-075		
Aquatic invertebrates (NOEC (mg a.s./L))	Prothioconazole	<i>Daphnia magna</i>	0.56
	Prothioconazole-desthio (M04)		0.1
			0.0044 ³
	Azoxystrobin		0.33 ¹
	FF-075	-	-

¹ Agreed derived endpoint for azoxystrobin, taken from derived RAC outlined in EFSA Journal (2010); 8(4):1542

² Agreed geomean outlined in EFSA Journal (2010); 8(4):1542

³ *Daphnia magna* endpoint used in the first-tier assessment as a standard species

⁴ *Pseudokirchneriella subcapitata* endpoint used in the first tier

Concentration of the active substance/metabolite used in the mixture toxicity assessment

	Relevant active substance/metabolite		
Active substance/metabolite	Prothioconazole	Prothioconazole-desthio (M04)	Azoxystrobin
Concentration in Product (g a.s./L)	200	200 (worse-case based on parent)	150

An overview of the worse-case PEC_{sw} values for winter oilseed rape:

FOCUS Step & Scenario		Prothioconazole (µg a.i./L)	Azoxystrobin (µg a.i./L)
Step 1		34.7521	54.7696
Step 2	N Europe	1.6962	3.8143
	S Europe	1.6962	6.2023
Step 3	D2 ditch	1.431	2.538
	D2 stream	0.8869	1.587
	D3 ditch	1.014	0.7612
	D4 pond	0.0515	0.2223
	D4 stream	0.8535	0.6404
	D5 pond	0.0518	0.1228
	D5 stream	0.9029	0.6779
	R1 pond	0.0849	0.3225
	R1 stream	0.6688	1.825
	R3 stream	0.9415	1.973
Step 4		10 m vegetative strip + 10m no spray buffer	10 m vegetative strip + 10m no spray buffer
	D2 ditch	0.1472	2.538
	D2 stream	0.1718	1.587
	D3 ditch	0.1458	0.1094
	D4 pond	0.0217	0.2189

	D4 stream	0.1654	0.2584
	D5 pond	0.0217	0.1228
	D5 stream	0.1749	0.1849
	R1 pond	0.0217	0.1363
	R1 stream	0.2418	0.8307
	R3 stream	0.2323	0.8834

An overview of the worse-case PEC_{sw} values for winter cereals (across early and late uses):

FOCUS Step & Scenario		Prothioconazole (µg a.i./L)		Azoxystrobin (µg a.i./L)	
Step 1		43.4401		68.462	
Step 2	N Europe	2.1202		11.3349	
	S Europe	2.1202		20.8868	
Step 3	D1 ditch	1.281		5.082	
	D1 stream	1.12		3.179	
	D2 ditch	1.278		7.364	
	D2 stream	1.085		4.667	
	D3 Ditch	1.268		0.9513	
	D4 Pond	0.0612		0.5513	
	D4 Stream	1.065		0.7989	
	D5 Pond	0.0652		0.1803	
	D5 Stream	1.181		0.8865	
	D6 Ditch	1.277		0.9585	
	R1 Pond	0.104		0.3926	
	R1 Stream	0.8338		2.94	
	R3 Stream	1.178		3.219	
	R4 Stream	0.8374		4.196	
Step 4		10 m vegeta- tive strip + 10m no spray buffer	20 m vegeta- tive strip + 20m no spray buffer	10 m vegeta- tive strip + 10m no spray buffer	20 m vegetative strip + 20m no spray buffer
	D1 ditch	0.1842	0.0957	5.082	5.082
	D1 stream	0.2171	0.1128	3.179	3.179
	D2 ditch	0.1838	0.0955	7.364	7.364
	D2 stream	0.2103	0.1093	4.667	4.667
	D3 ditch	0.1823	0.0947	0.1367	0.0569
	D4 pond	0.0272	0.0182	0.5484	0.5469
	D4 stream	0.2063	0.1072	0.5819	0.5819
	D5 pond	0.0272	0.0182	0.1803	0.1784
	D5 stream	0.2289	0.1189	0.2763	0.2763
	D6 ditch	0.1836	0.0954	0.4351	0.4351
	R1 pond	0.0272	0.0181	0.1661	0.0675

	R1 stream	0.2956	0.1548	1.335	0.6994
	R3 stream	0.3021	0.1585	1.469	0.7704
	R4 stream	0.3401	0.1776	1.909	1

An overview of the worse-case PEC_{sw} values for spring cereals (across early and late uses):

FOCUS Step & Scenario		Prothioconazole (µg a.i./L)		Azoxystrobin (µg a.i./L)
Step 1		43.4401		68.462
Step 2	N Europe	2.1202		11.3349
	S Europe	2.1202		20.8868
Step 3	D1 ditch	1.803		3.885
	D1 stream	1.12		2.43
	D3 Ditch	1.269		0.9525
	D4 Pond	0.0651		0.792
	D4 Stream	1.091		0.8192
	D5 Pond	0.0648		0.1845
	D5 Stream	1.105		0.83
	R4 Stream	0.8775		3.069
Step 4		10 m vegeta- tive strip + 10m no spray buffer	20 m vegeta- tive strip + 20m no spray buffer	10 m vegetative strip + 10m no spray buffer
	D1 ditch	0.1842	0.0957	3.885
	D1 stream	0.2171	0.1128	2.43
	D3 ditch	0.1825	0.0946	0.1369
	D4 pond	0.0272	0.0182	0.7875
	D4 stream	0.2115	0.1043	0.7818
	D5 pond	0.0272	0.0182	0.1845
	D5 stream	0.2141	0.1071	0.2733
	R4 stream	0.3938	0.2057	1.379

Consideration of the relevance of metabolites in the combined assessment

Acute combined assessment

In accordance with the FAQ Aquatic MixTox Tool, v1, 2021, an assessment of the relevance of toxic metabolites to the mixture toxicity assessment was performed and is outlined, below.

1- Is the metabolite clearly toxic (i.e. of equal or higher toxicity compared to the parent)?

Prothioconazole-desthio has comparable acute toxicity to fish, is less toxic to aquatic invertebrates and is more toxic to algae than the parent active substance, prothioconazole. Therefore, based on the worse-case assessment of toxicity (the algal endpoint) it is assumed that prothioconazole-desthio is considered to be

of equal or higher toxicity to the parent.

- Yes, go to 2

2- Is the metabolite much more contributing to the risk* than the a.s.? (i.e. > 90% of risk due to PEC-metab/RACmetab + PECA/RACA attributed to PECmetab/ RACmetab at the most critical/ worst-case(s) FOCUS step and scenario(s))?)

Considering the worse-case endpoint (algal endpoint of 0.55 mg metabolite/L), the corresponding algal endpoint for the parent (2.18 mg a.s./L), and the FOCUS Step 1 PEC values (Section 8), the following calculations have been conducted:

Use	PEC _{metabolite} (µg/L)	RAC _{metabolite} (µg/L)	PEC _{active sub-} stance (µg/L)	RAC _{active sub-} stance (µg/L)	Metabolite contribution to risk (%)
Winter oilseed rape	62.657	55	34.7521	218	87.75
Winter and spring cereals	78.3212	55	43.4401	218	87.7

For uses in both winter oilseed rape, and winter and spring cereals, in accordance with the proposed GAP, the metabolite does not contribute >90% of the risk.

- No or unknown: go to 3

3- Is the maximum formation rate of the metabolite within the test duration of the a.s.? (based on information available from water-sediment degradation tests)

The maximum formation rate of the metabolite, prothioconazole-desthio, within the water/sediment system occurred after 7 days (please refer to Section 8), which was within the study time scale for the active substance.

- Yes: see below, section M2

- The toxic metabolite is of equal or higher toxicity compared to the parent:

- Option1: The test endpoint is expressed in mg parent/L. The test endpoint is derived considering that the toxicity in the test is only attributed to the concentration of the parent in the test (instead of the sum parent + metab, (option 2)). This approach is considered as suitable and conservative if metabolites have not been measured in the parent test (please note that however generally, especially toxic metabolites should be measured).

The metabolite was not measured in the parent test and, therefore, the test endpoint will be expressed as mg parent/L.

Conduct a mixture toxicity risk assessment based on e.g. A (forming a metabolite) and B (has no relevant metabolite). Use the mixture tox tool to enter either the endpoint of A expressed in mg sum parent A/L (Option 1).

For Option 1, use PEC_{max} of substance A only, since only the a.s. is considered for EP derivation;

The Applicant assumes that the guidance is requesting that the parent endpoint is used in the mixture toxicity assessment and is compared to the maximum parent PEC_{max} values in each FOCUS scenario (FOCUS Step 1-4).

The decision scheme presented in the EFSA Guidance document (2013) is used to assess the mixture toxicity, step by step. The following conclusions for each proposed use have been taken from the accompanying AGD_AquaMix_v1.15 tool located in Appendix 3.

Chronic combined assessment

In accordance with the FAQ Aquatic MixTox Tool, v1, 2021, an assessment of the relevance of toxic metabolites to the mixture toxicity assessment was performed and is outlined, below.

1- Is the metabolite clearly toxic (i.e. of equal or higher toxicity compared to the parent)?

Prothioconazole-desthio (M04), is equal to or more toxic than the parent substance , prothioconazole.

- Yes, go to 2

2- Is the metabolite much more contributing to the risk than the a.s.? (i.e. > 90% of risk due to PEC_{metab}/RAC_{metab} + PECA/RACA attributed to PEC_{metab}/ RAC_{metab} at the most critical/ worst-case(s) FOCUS step and scenario(s))?*

Considering the worse-case chronic endpoint (chronic fish endpoint of 0.00334 mg metabolite/L), the corresponding chronic fish endpoint for the parent (0.308 mg a.s./L), and the FOCUS Step 1 PEC values (Section 8), the following calculations have been conducted:

Use	PEC _{metabolite} (µg/L)	RAC _{metabolite} (µg/L)	PEC _{active substance} (µg/L)	RAC _{active substance} (µg/L)	Metabolite contribution to risk (%)
Winter oilseed rape	62.657	0.334	34.7521	30.8	99.4
Winter and spring cereals	78.3212	0.334	43.4401	30.8	99.4

Therefore, for uses in both winter oilseed rape, and winter and spring cereals, in accordance with the proposed GAP, the metabolite does contribute >90% of the risk.

- Yes: conduct the MixTox assessment based on the metabolite and B.

In accordance with the decision scheme, the chronic mixture toxicity assessment will be conducted with prothioconazole-desthio (M04) and azoxystrobin. As a worse-case assumption, the proportion of the metabolite in the formulation will be assumed to be equal to that of the parent, prothioconazole (200 g /L).

Consideration of the mixture toxicity of FF-075

According to the EFSA Aquatic Guidance (2013) it is recommended to compare the measured endpoints of the formulation derived from experimental testing (EC_{xPPP}) and the calculated mixture toxicity by concentration addition (EC_{x mix-CA}). This is to determine whether there is any synergism or antagonism between the active substances. This comparison may also indicate that relevant toxicity contributions of co-formulants not included in the calculation do occur.

The deviation between calculated and measured toxicity is termed model deviation ratio (MDR). The observed and calculated mixture toxicity are considered in agreement if the model deviation ratio (MDR) is between 0.2 and 5.

Equation 13 of the EFSA Aquatic Guidance (page 148) details the calculated mixture toxicity by concentration addition.

$$ECx_{mix-CA} = \left(\sum_i^n \frac{p_i}{ECx_i} \right)^{-1}$$

where:

n = number of mixture components

i = index from 1...n mixture components

p_i = the ith component as a relative fraction of the mixture composition (note Σ p_i must be 1)

ECx_i = concentration of component i provoking x% effect (pragmatically, NOEC_i may be inserted, too)

The model deviation ratio (MDR) is then calculated using Equation 15 of the EFSA Aquatic guidance (page 149).

$$MDR = \frac{ECx_{mix-CA} \text{ (calculated mixture toxicity)}}{ECx_{PPP} \text{ (measured mixture toxicity)}}$$

The EFSA guidance (Section 10.3) states that when using concentration-addition the calculations should be made using only “specific endpoints and to defined taxonomic groups” i.e. those which can be additive and are from comparable species.

An acute fish toxicity test has not been conducted with the formulation FF-075 for the reasons set out in Section 9.5.1.1. Additionally, there is no chronic data available for the formulated product. Therefore, calculation of the acute MDR for fish and chronic MDR values have not been conducted.

Table 9.5-3: Mixture toxicity of FF-075 to fish, aquatic invertebrates, and algae calculated according to assumption of concentration addition and MDR analysis.

Test Group	Test substance	Concentration of active substance in formulation FF-075 (g/L)	Fraction of active substance in the formulation mixture	L/EC ₅₀ for active substance (mg/L)	Fraction of active substance / L/EC ₅₀ for the active substance	Calculated L/EC ₅₀ mix-CA (mg a.s./L) ^a	Measured L/EC ₅₀ PPP (mg form./L)	MDR ^b L/EC ₅₀ mix-CA / L/EC ₅₀ PPP
Acute Fish (<i>Oncorhynchus mykiss</i>)	Prothioconazole	200	0.57	1.83	0.311	0.817	-	-
	Azoxystrobin	150	0.43	0.47	0.915			
	Total	350	1	-	1.226	-	-	-
Acute Aquatic invertebrates (<i>Daphnia magna</i>)	Prothioconazole	200	0.57	1.3	0.438	0.575	2.97	0.46
	Azoxystrobin	150	0.43	0.33	1.303			
	Total	350	1	-	1.741	-	-	-

Algae (Green algae)	Prothioconazole	200	0.57	2.18	0.261	0.527	4.65	0.47
	Azoxystrobin	150	0.43	0.262	0.0011			
Total	-	350	1	-	1.641	-	-	-
Chronic Fish (<i>Onchorhynchus mykiss</i> and <i>Pimephales promelas</i>)	Prothioconazole-desthio (M04) ^c	200 (worse case assumption)	0.57	0.00334	170.66	0.0057	-	-
	Azoxystrobin	150	0.43	0.147	2.93		-	-
Total	-	350	1	-	173.59	-	-	-
Chronic Aquatic invertebrates (<i>Daphnia magna</i>)	Prothioconazole-desthio (M04) ^c	200 (worse case assumption)	0.57	0.1	5.7	0.143	-	-
	Azoxystrobin	150	0.43	0.33	1.30		-	-
Total	-	350	1	-	7.00	-	-	-

Note: Calculations have been undertaken using unrounded values consequently outcomes may not be reproducible when using the input figures given in the table.

^a Predicted mixture toxicity under assumption of concentration-addition.

^b In accordance with EFSA Aquatic Guidance Document, mixture toxicity conforms to assumptions of concentration addition when model deviation ratio (MDR; $EC_{X_{mix-CA}}/EC_{X_{PPP}}$) is between 0.2 and 5.

^c Assessment based on endpoints with the metabolite, prothioconazole-desthio (M04), in accordance with the assessment outlined under “Combined chronic risk assessment” of this submission.

The model deviation ratio (MDR) values in the table above indicate that toxicity of FF-075 to aquatic invertebrates and algae is as predicted on the assumption of concentration addition ($0.2 < MDR < 5$). This indicates that the measured toxicity of the formulation does not deviate from the expectations of additive toxicity. Therefore, combination risk assessment, if required, can be performed using active substance data.

The decision scheme in Section 10.3.11 of EFSA’s Aquatic Guidance Document should be followed using the AquaMix Tool. The Tool clearly states that “if different assessment factors or additional data (e.g. sensitive species) are available, it is recommended to go directly to Step 8b (given synergism is excluded)”.

For aquatic invertebrates and algae additional acute data on sensitive species are available for azoxystrobin and as synergism can be excluded, the mixture assessment should start at Step 8b.

Step 7 in the AquaMix Tool requires an assessment of synergistic effects where data with the formulated product is not available in order to calculate an MDR value, as is the case for acute effects in fish and the chronic effects to fish and aquatic invertebrates.

The AquaMix Tool asks “Is there evidence of synergistic interactions between mixture components might occur (e.g. based on toxicological knowledge from literature or from counter-checking measured and calculated mixture toxicity in other species)”

The decision scheme in Section 10.3.4 of EFSA's Aquatic Guidance Document which outlines the process of counter-checking calculated and measured mixture toxicity refers to *Equation 15* of EFSA's Aquatic Guidance Document and the MDR calculations presented in Table 9.5-3.

In the absence of chronic aquatic formulation data for FF-075 an assessment of synergy has been conducted using the available acute aquatic invertebrate and algal data for the metabolite, prothioconazole-desthio (M04), azoxystrobin and FF-075. Prothioconazole-desthio (M04) and azoxystrobin have maximum formation in water/sediment systems at day 7 and 0, respectively, indicating acute modes of action. Therefore, the use of acute ecotoxicological data to assess synergy is justified. The MDR ratios for aquatic invertebrates and algae indicate no synergism between prothioconazole and azoxystrobin when applied as FF-075, supporting the assumption that there are no synergistic effects to consider for acute and chronic effects on fish or chronic effects to aquatic invertebrates.

On this basis, the decision scheme in the AquaMix Tool directs the risk assessment for acute effects on fish to start at Step 8a. As the AF values in the AquaMix Tool have been adjusted to accommodate the chronic assessment, the decision scheme directs the risk assessment for chronic effects on fish and aquatic invertebrates to start at Step 8b.

Combined acute risk assessment based on calculated mixture toxicity (ETR_{mix} or RQ_{mix}) (prothioconazole-azoxystrobin)

Winter Oilseed Rape

Following EFSA's decision scheme the use of the calculated mixture toxicity (RQ_{mix}) is appropriate for the combined risk assessment for aquatic invertebrates and algae. The risk assessment has been conducted using the AquaMix Tool v 1.15.

$$RQ_{mix} = \sum_{i=1}^n \frac{PEC_i}{RAC_i}$$

If $RQ < 1$: Low risk

If $RQ > 1$: Low risk not demonstrated/check exposure refinement options

Table 9.5-4 Aquatic organisms: acceptability of risk ($RQ < 1$) for the mixture for each organism group based on the Aquatic Guidance MixTox Step 8b and FOCUS Step 1-3 scenarios – winter oilseed rape.

Group	Inverteb. acute	Algae
FOCUS Scenario	RQ _{mix}	
Step 1		
I	19.27	2.25
Step 2		
N-Europe	1.29	0.15
S-Europe	2.01	0.24
Step3		
D2 Ditch	0.88	0.10
D2 Stream	0.55	0.06

Group	Inverteb. acute	Algae
FOCUS Scenario	RQ _{mix}	
Step 1		
D3 Ditch	0.31	0.03
D4 Pond	39.68	2.37
D4 Stream	0.26	0.03
D5 Pond	0.04	0.001
D5 Stream	0.27	0.03
R1 Pond	0.10	0.01
R1 Stream	0.11	0.01
R3 Stream	0.13	0.01

RQ_{mix}: Risk quotient for the mixture
RQ_{mix} above the relevant trigger of 1 are shown in bold
Calculations conducted using AGD_AquaMix_v1.15 spreadsheet

Table 9.5-5: Aquatic organisms: acceptability of risk (RQ < 1) for the mixture for each organism group based on the Aquatic Guidance MixTox Step 8b and FOCUS Step 4 scenarios – winter oilseed rape.

Group	Inverteb. acute	Algae
FOCUS Scenario	RQ _{mix}	
	10 m vegetative strip + 10 m no spray buffer	
D4 Pond	0.07	0.01

RQ_{mix}: Risk quotient for the mixture
RQ_{mix} above the relevant trigger of 1 are shown in bold
Calculations conducted using AGD_AquaMix_v1.15 spreadsheet

Using the RQ_{mix} approach an acceptable risk is demonstrated for aquatic invertebrates and algae for all FOCUS Step 1 – 3 PEC_{SW} scenarios, with the exception of FOCUS Step 3 scenario D4 Pond, which is resolved at FOCUS Step 4 with the use of a 10 m vegetative buffer strip and a 10 m no spray buffer zone.

Step 5 in the AquaMix Tool - calculation of individual toxicity units (TUs) also need to be taken into consideration.

$$\sum_{i=1}^n TU_i = \sum_{i=1}^n \frac{c_i}{ECx_i}$$

Table 9.5-6: Aquatic organisms: calculation of Toxicity Units (TU) for aquatic invertebrates based on the Aquatic Guidance MixTox Step 5- winter oilseed rape.

FOCUS Scenario	Prothioconazole	Azoxystrobin	Sum
----------------	-----------------	--------------	-----

Proportion of active at PEC _{mix}			
Step 1	0.39	0.61	1.00
Step 2			
N-Europe	0.31	0.69	1.00
S-Europe	0.21	0.79	1.00
Step 3			
D2 Ditch	0.36	0.64	1.00
D2 Stream	0.36	0.64	1.00
D3 Ditch	0.57	0.43	1.00
D4 Pond	1.00	0.00	1.00
D4 Stream	0.57	0.43	1.00
D5 Pond	0.30	0.70	1.00
D5 Stream	0.57	0.43	1.00
R1 Pond	0.21	0.79	1.00
R1 Stream	0.79	0.21	1.00
R3 Stream	0.83	0.17	1.00
Individual TU (EC ₅₀ aquatic invertebrate a.s. based [mg sum of a.s/L])			
Step 1	0.027	0.166	0.193
Step 2			
N-Europe	0.001	0.012	0.013
S-Europe	0.001	0.019	0.020
Step 3			
D2 Ditch	0.001	0.008	0.009
D2 Stream	0.001	0.005	0.005
D3 Ditch	0.001	0.002	0.003
D4 Pond	0.396	0.001	0.397
D4 Stream	0.001	0.002	0.003
D5 Pond	0.000	0.000	0.000
D5 Stream	0.001	0.002	0.003
R1 Pond	0.000	0.001	0.001
R1 Stream	0.001	0.001	0.001
R3 Stream	0.001	0.001	0.001
% TU			
Step 1	13.9	86.1	100.0
Step 2			
N-Europe	10.1	89.9	100.0
S-Europe	6.5	93.5	100.0
Step 3			

D2 Ditch	12.5	87.5	100.0
D2 Stream	12.4	87.6	100.0
D3 Ditch	25.3	74.7	100.0
D4 Pond	99.8	0.2	100.0
D4 Stream	25.3	74.7	100.0
D5 Pond	9.7	90.3	100.0
D5 Stream	25.3	74.7	100.0
R1 Pond	6.3	93.7	100.0
R1 Stream	48.2	51.8	100.0
R3 Stream	54.8	45.2	100.0

Calculations conducted using AGD AquaMix_v1.15 spreadsheet

Calculations are based on the additional data (e.g. sensitive species) where entered

Values highlighted in bold demonstrate that one active substance is driving the risk assessment for that scenario.

The results indicate that there is no clear driver of the risk assessment for uses of FF-075 in winter oilseed rape, and the risk to aquatic invertebrates is covered by the calculation of the RQ_{mix} , presented in Tables 9.5-4 and 9.5-5, and the risk assessment for the individual active substances for uses in winter oilseed rape.

Table 9.5-7: Aquatic organisms: calculation of Toxicity Units (TU) for algae based on the Aquatic Guidance MixTox Step 5- winter oilseed rape.

FOCUS Scenario	Prothioconazole	Azoxystrobin	Sum
Proportion of active at PEC _{mix}			
Step 1	0.39	0.61	1.00
Step 2			
N-Europe	0.31	0.69	1.00
S-Europe	0.21	0.79	1.00
Step 3			
D2 Ditch	0.36	0.64	1.00
D2 Stream	0.36	0.64	1.00
D3 Ditch	0.57	0.43	1.00
D4 Pond	1.00	0.00	1.00
D4 Stream	0.57	0.43	1.00
D5 Pond	0.30	0.70	1.00
D5 Stream	0.57	0.43	1.00
R1 Pond	0.21	0.79	1.00
R1 Stream	0.79	0.21	1.00
R3 Stream	0.83	0.17	1.00

Individual TU (EC ₅₀ algae a.s. based [mg sum of a.s/L])			
Step 1	0.016	0.209	0.225
Step 2			
N-Europe	0.001	0.015	0.015
S-Europe	0.001	0.024	0.024
Step 3			
D2 Ditch	0.001	0.010	0.010
D2 Stream	0.000	0.006	0.006
D3 Ditch	0.000	0.003	0.003
D4 Pond	0.236	0.001	0.237
D4 Stream	0.000	0.002	0.003
D5 Pond	0.000	0.000	0.000
D5 Stream	0.000	0.003	0.003
R1 Pond	0.000	0.001	0.001
R1 Stream	0.000	0.001	0.001
R3 Stream	0.000	0.001	0.001
% TU			
Step 1	7.1	92.9	100.0
Step 2			
N-Europe	5.1	94.9	100.0
S-Europe	3.2	96.8	100.0
Step 3			
D2 Ditch	6.3	93.7	100.0
D2 Stream	6.3	93.7	100.0
D3 Ditch	13.8	86.2	100.0
D4 Pond	99.6	0.4	100.0
D4 Stream	13.8	86.2	100.0
D5 Pond	4.8	95.2	100.0
D5 Stream	13.8	86.2	100.0
R1 Pond	3.1	96.9	100.0
R1 Stream	30.6	69.4	100.0
R3 Stream	36.5	63.5	100.0

Calculations conducted using AGD AquaMix_v1.15 spreadsheet

Calculations are based on the additional data (e.g. sensitive species) where entered

Values highlighted in bold demonstrate that one active substance is driving the risk assessment for that scenario.

The results indicate that there is no clear driver of the risk assessment for uses of FF-075 in winter oilseed rape, and the risk to algae is covered by the calculation of the RQ_{mix}, presented in Tables 9.5-4 and 9.5-5 and the risk assessment for the individual active substances for use in winter oilseed rape.

Following EFSA's decision scheme the use of the calculated mixture toxicity (ETR_{mix}) is appropriate for the combined risk assessment for the combined acute risk to fish. The risk assessment has been conducted using the AquaMix Tool v 1.15.

$$\text{Equation 18: } ETR_{mix-CA} = \frac{PEC_{mix}}{ECx_{mix-CA}}$$

Table 9.5-8: Aquatic organisms: acceptability of risk ($ETR_{mix-CA} < 1/AF(0.01)$) for the mixture for the acute effect on fish based on the Aquatic Guidance MixTox Step 8a and FOCUS Step 1-3 scenarios – winter oilseed rape.

Group	Fish acute
FOCUS Scenario	ETR_{mix-CA}
Step 1	
	0.14
Step 2	
N-Europe	0.01
S-Europe	0.01
Step3	
D2 Ditch	0.01
D2 Stream	0.00
D3 Ditch	0.00
D4 Pond	0.28
D4 Stream	0.00
D5 Pond	0.00
D5 Stream	0.00
R1 Pond	0.00
R1 Stream	0.00
R3 Stream	0.00

ETR_{mix} : Risk quotient for the mixture
 ETR_{mix-CA} above the relevant trigger of 0.01 are shown in bold in accordance with the conclusions in the GD_AquaMix_v1.15 spreadsheet.
Calculations conducted using AGD_AquaMix_v1.15 spreadsheet

Table 9.5-9: Aquatic organisms: acceptability of risk ($ETR_{mix-CA} < 1/AF(0.01)$) for the mixture for acute effect on fish based on the Aquatic Guidance MixTox Step 8a and FOCUS Step 4 scenarios – winter oilseed rape.

Group	Fish acute
FOCUS Scenario	ETR_{mix-CA}
	10 m vegetative strip + 10 m no spray buffer

Group	Fish acute
FOCUS Scenario	ETR _{mix-CA}
	10 m vegetative strip + 10 m no spray buffer
D4 Pond	0.00

ETR_{mix}: Risk quotient for the mixture
ETR_{mix} above the relevant trigger of 1 are shown in bold
Calculations conducted using AGD_AquaMix_v1.15 spreadsheet

Using the ETR_{mix-CA} approach an acceptable risk is demonstrated for fish from acute exposure to the mixture for all FOCUS Step 1 – 3 PEC_{SW} scenarios, with the exception of FOCUS Step 3 scenario D4 Pond, which is resolved at FOCUS Step 4 with the use of a 10 m vegetative buffer strip and a 10 m no spray buffer zone.

However, Step 5 in the AquaMix Tool - calculation of individual toxicity units (TUs) also needs to be taken into consideration.

TUs refer to the ratio between the concentration (i.e. c_i) of a mixture component and its toxicological endpoint (e.g. EC₅₀). In addition, the TU of a mixture has been defined as the sum of TU of each individual chemical of that mixture. The following equation is used to calculate TUs:

$$\sum_{i=1}^n TU_i = \sum_{i=1}^n \frac{c_i}{ECx_i}$$

Table 9.5-10: Aquatic organisms: calculation of Toxicity Units (TU) for acute exposure of fish based on the Aquatic Guidance MixTox Step 5- winter oilseed rape.

FOCUS Scenario	Prothioconazole	Azoxystrobin	Sum
Proportion of active at PEC_{mix}			
Step 1	0.39	0.61	1.00
Step 2			
N-Europe	0.31	0.69	1.00
S-Europe	0.21	0.79	1.00
Step 3			
D2 Ditch	0.36	0.64	1.00
D2 Stream	0.36	0.64	1.00
D3 Ditch	0.57	0.43	1.00
D4 Pond	1.00	0.00	1.00
D4 Stream	0.57	0.43	1.00
D5 Pond	0.30	0.70	1.00
D5 Stream	0.57	0.43	1.00
R1 Pond	0.21	0.79	1.00
R1 Stream	0.79	0.21	1.00

R3 Stream	0.83	0.17	1.00
Individual TU (LC₅₀ fish a.s. based [mg sum of a.s/L])			
Step 1	0.019	0.117	0.193
Step 2			
N-Europe	0.001	0.008	0.013
S-Europe	0.001	0.013	0.020
Step 3			
D2 Ditch	0.001	0.005	0.009
D2 Stream	0.000	0.003	0.005
D3 Ditch	0.001	0.002	0.003
D4 Pond	0.281	0.000	0.397
D4 Stream	0.000	0.001	0.003
D5 Pond	0.000	0.000	0.000
D5 Stream	0.000	0.001	0.003
R1 Pond	0.000	0.001	0.001
R1 Stream	0.000	0.000	0.001
R3 Stream	0.001	0.000	0.001
% TU			
Step 1	14.0	86.0	100.0
Step 2			
N-Europe	10.3	89.7	100.0
S-Europe	6.6	93.4	100.0
Step 3			
D2 Ditch	12.6	87.4	100.0
D2 Stream	12.6	87.4	100.0
D3 Ditch	25.5	74.5	100.0
D4 Pond	99.8	0.2	100.0
D4 Stream	25.5	74.5	100.0
D5 Pond	9.8	90.2	100.0
D5 Stream	25.5	74.5	100.0
R1 Pond	6.3	93.7	100.0
R1 Stream	48.5	51.5	100.0
R3 Stream	55.1	44.9	100.0

Calculations conducted using AGD AquaMix_v1.15 spreadsheet

Calculations are based on the additional data (e.g. sensitive species) where entered

Values highlighted in bold demonstrate that one active substance is driving the risk assessment for that scenario.

The results indicate that there is no clear driver of the risk assessment for uses of FF-075 in winter oilseed rape, and the risk to fish from acute exposure to the combined active substances is covered by the calculation of the ETR_{mix-CA}, presented in Tables 9.5-8 and 9.5-9 and the risk assessments for the individual active substances for uses in winter oilseed rape.

Conclusion

The acute mixture toxicity assessment for uses in winter oilseed rape (BBCH 55-69) concludes acceptable risk in the ETR_{mix-CA}/RQ_{mix} assessments for fish, aquatic invertebrates and algae when risk mitigation is applied as a 10m vegetative strip and a 10m no spray. An assessment of the “driver” of the risk assessment indicates that both active substances drive the risk assessments for different FOCUS scenarios. The risk to aquatic invertebrates, algae and fish from acute exposure to prothioconazole and azoxystrobin from applications of FF-075 is, therefore, covered by the calculation of the ETR_{mix-CA}/RQ_{mix} and the risk assessment for the individual active substances for use in winter oilseed rape.

Winter Cereals

Following EFSA’s decision scheme the use of the calculated mixture toxicity (RQ_{mix}) is appropriate for the combined risk assessment for aquatic invertebrates and algae. The risk assessment has been conducted using the AquaMix Tool v 1.15.

$$RQ_{mix} = \sum_{i=1}^n \frac{PEC_i}{RAC_i}$$

If $RQ < 1$: Low risk

If $RQ > 1$: Low risk not demonstrated/check exposure refinement options

Table 9.5-11: Aquatic organisms: acceptability of risk ($RQ < 1$) for the mixture for each organism group based on the Aquatic Guidance MixTox Step 8b and FOCUS Step 1-3 scenarios – winter cereals.

Group	Inverteb. acute	Algae
FOCUS Scenario	RQ_{mix}	
Step 1		
	24.09	2.81
Step 2		
N-Europe	3.60	0.44
S-Europe	6.49	0.81
Step3		
D1 Ditch	1.64	0.20
D1 Stream	1.05	0.13
D2 Ditch	2.33	0.29
D2 Stream	1.50	0.18
D3 Ditch	0.39	0.04
D4 Pond	0.17	0.02
D4 Stream	0.32	0.04
D5 Pond	0.06	0.01
D5 Stream	0.36	0.04

Group	Inverteb. acute	Algae
FOCUS Scenario	RQ _{mix}	
D6 Ditch	0.39	0.04
R1 Pond	0.13	0.02
R1 Stream	0.96	0.12
R3 Stream	1.07	0.13
R4 Stream	1.34	0.16

RQ_{mix}: Risk quotient for the mixture
RQ_{mix} above the relevant trigger of 1 are shown in bold
Calculations conducted using AGD_AquaMix_v1.15 spreadsheet

Table 9.5-12: Aquatic organisms: acceptability of risk (RQ < 1) for the mixture for each organism group based on the Aquatic Guidance MixTox Step 8b and FOCUS Step 4 scenarios – winter cereals.

Group	Inverteb. acute	
FOCUS Scenario	RQ _{mix}	
	10 m vegetative strip + 10 m no spray buffer	20 m vegetative strip + 20 m no spray buffer
D1 Ditch	1.09	1.09
D1 Stream	0.69	0.68
D2 Ditch	1.58	1.57
D2 Stream	1.00	1.00
R3 Stream	0.33	0.17
R4 Stream	0.42	0.22

RQ_{mix}: Risk quotient for the mixture
RQ_{mix} above the relevant trigger of 1 are shown in bold
Calculations conducted using AGD_AquaMix_v1.15 spreadsheet

Using the RQ_{mix} approach an acceptable risk is demonstrated for acute exposure to aquatic invertebrates and algae for all FOCUS Step 1 – 3 PEC_{sw} scenarios, with the exception of FOCUS Step 3 scenarios D1 Ditch, D1 Stream, D2 Ditch, D2 Stream, R3 Stream and R4 Stream for aquatic invertebrates. An acceptable risk in the FOCUS Step 4 D1 Stream, R3 Stream and R4 Stream scenarios was concluded with the inclusion of a 10 m buffer zone and a 10 m no spray buffer. Outstanding risks were identified at FOCUS Step 4 for D1 and D2 scenarios. However, the D1 and D2 scenarios are not a concern for the Central Zone Member States.

However, Step 5 in the AquaMix Tool - calculation of individual toxicity units (TUs) also need to be taken into consideration.

$$\sum_{i=1}^n TU_i = \sum_{i=1}^n \frac{c_i}{ECx_i}$$

Table 9.5-13: Aquatic organisms: calculation of Toxicity Units (TU) for aquatic invertebrates

based on the Aquatic Guidance MixTox Step 5- winter cereals.			
FOCUS Scenario	Prothioconazole	Azoxystrobin	Sum
Proportion of active at PEC _{mix}			
Step 1	0.39	0.61	1.00
Step 2			
N-Europe	0.16	0.84	1.00
S-Europe	0.09	0.91	1.00
Step 3			
D1 Ditch	0.20	0.80	1.00
D1 Stream	0.26	0.74	1.00
D2 Ditch	0.15	0.85	1.00
D2 Stream	0.19	0.81	1.00
D3 Ditch	0.57	0.43	1.00
D4 Pond	0.10	0.90	1.00
D4 Stream	0.57	0.43	1.00
D5 Pond	0.27	0.73	1.00
D5 Stream	0.57	0.43	1.00
D6 Ditch	0.57	0.43	1.00
R1 Pond	0.21	0.79	1.00
R1 Stream	0.22	0.78	1.00
R3 Stream	0.27	0.73	1.00
R4 Stream	0.17	0.83	1.00

Individual TU (EC ₅₀ aquatic invertebrate a.s. based [mg sum of a.s/L])			
Step 1	0.033	0.207	0.241
Step 2			
N-Europe	0.002	0.034	0.036
S-Europe	0.002	0.063	0.065
Step 3			
D1 Ditch	0.001	0.015	0.016
D1 Stream	0.001	0.010	0.010
D2 Ditch	0.001	0.022	0.023
D2 Stream	0.001	0.014	0.015
D3 Ditch	0.001	0.003	0.004
D4 Pond	0.000	0.002	0.002
D4 Stream	0.001	0.002	0.003
D5 Pond	0.000	0.001	0.001
D5 Stream	0.001	0.003	0.004
D6 Ditch	0.001	0.003	0.004
R1 Pond	0.000	0.001	0.001
R1 Stream	0.001	0.009	0.010
R3 Stream	0.001	0.010	0.011
R4 Stream	0.001	0.013	0.013
% TU			
Step 1	13.9	86.1	100.0
Step 2			
N-Europe	4.5	95.5	100.0
S-Europe	2.5	97.5	100.0
Step 3			
D1 Ditch	6.0	94.0	100.0
D1 Stream	8.2	91.8	100.0
D2 Ditch	4.2	95.8	100.0
D2 Stream	5.6	94.4	100.0
D3 Ditch	25.3	74.7	100.0
D4 Pond	2.7	97.3	100.0
D4 Stream	25.3	74.7	100.0
D5 Pond	8.4	91.6	100.0
D5 Stream	25.3	74.7	100.0
D6 Ditch	25.3	74.7	100.0
R1 Pond	6.3	93.7	100.0
R1 Stream	6.7	93.3	100.0

R3 Stream	8.5	91.5	100.0
R4 Stream	4.8	95.2	100.0

Calculations conducted using AGD AquaMix_v1.15 spreadsheet

Calculations are based on the additional data (e.g. sensitive species) where entered

Values highlighted in bold demonstrate that one active substance is driving the risk assessment for that scenario.

Although azoxystrobin is the driver of the risk assessment for 10 out of the 14 FOCUS Step 3 scenarios, there is no clear driver of the entire risk assessment for aquatic invertebrates for uses in winter cereals. The risk to aquatic invertebrates is covered by the calculation of the RQ_{mix} , presented in Tables 9.5-11 and 9.5-12, and the risk assessment for azoxystrobin for uses in winter cereals.

Table 9.5-14: Aquatic organisms: calculation of Toxicity Units (TU) for algae based on the Aquatic Guidance MixTox Step 5- winter cereals.

FOCUS Scenario	Prothioconazole	Azoxystrobin	Sum
Proportion of active at PEC_{mix}			
Step 1	0.39	0.61	1.00
Step 2			
N-Europe	0.16	0.84	1.00
S-Europe	0.09	0.91	1.00
Step 3			
D1 Ditch	0.20	0.80	1.00
D1 Stream	0.26	0.74	1.00
D2 Ditch	0.15	0.85	1.00
D2 Stream	0.19	0.81	1.00
D3 Ditch	0.57	0.43	1.00
D4 Pond	0.10	0.90	1.00
D4 Stream	0.57	0.43	1.00
D5 Pond	0.27	0.73	1.00
D5 Stream	0.57	0.43	1.00
D6 Ditch	0.57	0.43	1.00
R1 Pond	0.21	0.79	1.00
R1 Stream	0.22	0.78	1.00
R3 Stream	0.27	0.73	1.00
R4 Stream	0.17	0.83	1.00

Individual TU (EC ₅₀ algae a.s. based [mg sum of a.s/L])			
Step 1	0.020	0.261	0.281
Step 2			
N-Europe	0.001	0.043	0.044
S-Europe	0.001	0.080	0.081
Step 3			
D1 Ditch	0.001	0.019	0.020
D1 Stream	0.001	0.012	0.013
D2 Ditch	0.001	0.028	0.029
D2 Stream	0.000	0.018	0.018
D3 Ditch	0.001	0.004	0.004
D4 Pond	0.000	0.002	0.002
D4 Stream	0.000	0.003	0.004
D5 Pond	0.000	0.001	0.001
D5 Stream	0.001	0.003	0.004
D6 Ditch	0.001	0.004	0.004
R1 Pond	0.000	0.001	0.002
R1 Stream	0.000	0.011	0.012
R3 Stream	0.001	0.012	0.013
R4 Stream	0.000	0.016	0.016
% TU			
Step 1	7.1	92.9	100.0
Step 2			
N-Europe	2.2	97.8	100.0
S-Europe	1.2	98.8	100.0
Step 3			
D1 Ditch	2.9	97.1	100.0
D1 Stream	4.1	95.9	100.0
D2 Ditch	2.0	98.0	100.0
D2 Stream	2.7	97.3	100.0
D3 Ditch	13.8	86.2	100.0
D4 Pond	1.3	98.7	100.0
D4 Stream	13.8	86.2	100.0
D5 Pond	4.2	95.8	100.0
D5 Stream	13.8	86.2	100.0
D6 Ditch	13.8	86.2	100.0
R1 Pond	3.1	96.9	100.0
R1 Stream	3.3	96.7	100.0

R3 Stream	4.2	95.8	100.0
R4 Stream	2.3	97.7	100.0

Calculations conducted using AGD AquaMix_v1.15 spreadsheet

Calculations are based on the additional data (e.g. sensitive species) where entered

Values highlighted in bold demonstrate that one active substance is driving the risk assessment for that scenario.

Although azoxystrobin is the driver of the risk assessment for 10 out of the 14 FOCUS Step 3 scenarios, there is no clear driver of the entire risk assessment for algae for uses in winter cereals. The risk to algae is covered both by the calculation of the RQ_{mix} , presented in Tables 9.5-11 and 9.5-12, and the risk assessment for azoxystrobin for uses winter cereals.

Following EFSA's decision scheme the use of the calculated mixture toxicity (ETR_{mix}) is appropriate for the acute combined risk assessment for fish. The risk assessment has been conducted using the AquaMix Tool v 1.15.

$$\text{Equation 18: } ETR_{mix-CA} = \frac{PEC_{mix}}{ECx_{mix-CA}}$$

Table 9.5-15: Aquatic organisms: acceptability of risk ($ETR_{mix-CA} < 1/AF$ (0.01)) for the mixture for acute affects to fish based on the Aquatic Guidance MixTox Step 8a and FOCUS Step 1-3 scenarios – winter cereals

Group	Fish acute
FOCUS Scenario	ETR_{mix-CA}
Step 1	
	0.17
Step 2	
N-Europe	0.03
S-Europe	0.05
Step3	
D1 Ditch	0.01
D1 Stream	0.01
D2 Ditch	0.02
D2 Stream	0.01
D3 Ditch	0.00
D4 Pond	0.00
D4 Stream	0.00
D5 Pond	0.00
D5 Stream	0.00
D6 Ditch	0.00
R1 Pond	0.00
R1 Stream	0.01
R3 Stream	0.01

Group	Fish acute
FOCUS Scenario	ETR_{mix-CA}
R4 Stream	0.01

RQ_{mix}: Risk quotient for the mixture

ETR_{mix-CA} above the relevant trigger of 0.01 are shown in bold (Note, the values are rounded to decimal places in accordance with the AGD_AquaMix_v1.15 spreadsheet. Indications of values above the trigger are in accordance with the tool.)

Calculations conducted using AGD_AquaMix_v1.15 spreadsheet

Using the ETR_{mix-CA} approach an acceptable acute risk is demonstrated for fish for all FOCUS Step 1 – 3 PEC_{SW} scenarios, with the exception of FOCUS Step 3 scenarios D1 Ditch, D2 Ditch and D2 Stream. Outstanding risks were identified at FOCUS Step 4 for D1 and D2 scenarios. However, the D1 and D2 scenarios are not a concern for the Central Zone Member States.

However, Step 5 in the AquaMix Tool - calculation of individual toxicity units (TUs) also need to be taken into consideration.

Table 9.5-16: Aquatic organisms: calculation of Toxicity Units (TU) for acute exposure of fish based on the Aquatic Guidance MixTox Step 5- winter cereals.

FOCUS Scenario	Prothioconazole	Azoxystrobin	Sum
Proportion of active at PEC_{mix}			
Step 1	0.39	0.61	1.00
Step 2			
N-Europe	0.16	0.84	1.00
S-Europe	0.09	0.91	1.00
Step 3			
D1 Ditch	0.20	0.80	1.00
D1 Stream	0.26	0.74	1.00
D2 Ditch	0.15	0.85	1.00
D2 Stream	0.19	0.81	1.00
D3 Ditch	0.57	0.43	1.00
D4 Pond	0.10	0.90	1.00
D4 Stream	0.57	0.43	1.00
D5 Pond	0.27	0.73	1.00
D5 Stream	0.57	0.43	1.00
D6 Ditch	0.57	0.43	1.00
R1 Pond	0.21	0.79	1.00
R1 Stream	0.22	0.78	1.00
R3 Stream	0.27	0.73	1.00
R4 Stream	0.17	0.83	1.00
Individual TU (LC₅₀ fish a.s. based [mg sum of a.s/L])			
Step 1	0.024	0.146	0.169

Step 2			
N-Europe	0.001	0.024	0.025
S-Europe	0.001	0.044	0.046
Step 3			
D1 Ditch	0.001	0.011	0.012
D1 Stream	0.001	0.007	0.007
D2 Ditch	0.001	0.016	0.016
D2 Stream	0.001	0.010	0.011
D3 Ditch	0.001	0.002	0.003
D4 Pond	0.000	0.001	0.001
D4 Stream	0.001	0.002	0.002
D5 Pond	0.000	0.000	0.000
D5 Stream	0.001	0.002	0.003
D6 Ditch	0.001	0.002	0.003
R1 Pond	0.000	0.001	0.001
R1 Stream	0.000	0.06	0.007
R3 Stream	0.001	0.007	0.007
R4 Stream	0.000	0.009	0.009
% TU			
Step 1	14.0	86.0	100.0
Step 2			
N-Europe	4.6	95.4	100.0
S-Europe	2.5	87.5	100.0
Step 3			
D1 Ditch	6.1	93.9	100.0
D1 Stream	8.3	91.7	100.0
D2 Ditch	4.3	95.7	100.0
D2 Stream	5.6	94.4	100.0
D3 Ditch	25.5	74.5	100.0
D4 Pond	2.8	97.2	100.0
D4 Stream	25.5	74.5	100.0
D5 Pond	8.5	91.5	100.0
D5 Stream	25.5	74.5	100.0
D6 Ditch	25.5	74.5	100.0
R1 Pond	6.4	93.6	100.0
R1 Stream	6.8	93.2	100.0
R3 Stream	8.6	91.4	100.0
R4 Stream	4.9	95.1	100.0

Calculations conducted using AGD AquaMix_v1.15 spreadsheet
Calculations are based on the additional data (e.g. sensitive species) where entered
Values highlighted in bold demonstrate that one active substance is driving the risk assessment for that scenario.

Although azoxystrobin is the driver of the risk assessment for 10 out of the 14 FOCUS Step 3 scenarios, there is no clear driver of the entire risk assessment for fish for uses in winter cereals. The risk to fish is covered both by the calculation of the ETR_{mix-CA} , presented in Table 9.5-15, and the risk assessment for azoxystrobin for uses of FF-075 in winter cereals.

Conclusion

The acute mixture toxicity assessment for uses of FF-075 in winter cereals (BBCH 30-69) concludes acceptable risk to algae at FOCUS Step 2 for all scenarios. An acceptable risk to fish from applications of FF-075 were concluded at FOCUS Step3 for all relevant scenarios. An acceptable risk to aquatic invertebrates was concluded at FOCUS Step 4 for all relevant scenarios when a 10 m vegetative buffer and a 10 m no spray buffer were applied. Outstanding risks for D1 ditch, D2 ditch and D2 stream scenarios were identified for aquatic invertebrates. However, D1 and D2 scenarios are not considered relevant for national assessment in any of the Central Zone Member States where authorisation of FF-075 is being applied for. An assessment of the “driver” of the risk assessment indicates that azoxystrobin drives the risk assessments for a number of FOCUS Step 1-4 scenarios. The risk to aquatic invertebrates, algae and fish from acute exposure to prothioconazole and azoxystrobin from applications of FF-075 is, therefore, covered by the calculation of the ETR_{mix-CA}/RQ_{mix} and the risk assessment for azoxystrobin for use in winter cereals.

Spring Cereals

Following EFSA’s decision scheme the use of the calculated mixture toxicity (RQ_{mix}) is appropriate for the combined risk assessment for aquatic invertebrates and algae. The risk assessment has been conducted using the AquaMix Tool v 1.15.

$$RQ_{mix} = \sum_{i=1}^n \frac{PEC_i}{RAC_i}$$

If $RQ < 1$: Low risk

If $RQ > 1$: Low risk not demonstrated/check exposure refinement options

Table 9.5-17: Aquatic organisms: acceptability of risk ($RQ < 1$) for the mixture for each or organism group based on the Aquatic Guidance MixTox Step 8b and FOCUS Step 1-3 scenarios – spring cereals.

Group	Inverteb. acute	Algae
FOCUS Scenario	RQ_{mix}	
Step 1		
I	24.09	2.81
Step 2		
N-Europe	3.60	0.44
S-Europe	6.49	0.81
Step3		

Group	Inverteb. acute	Algae
FOCUS Scenario	RQ _{mix}	
D1 Ditch	1.32	0.16
D1 Stream	0.82	0.10
D3 Ditch	0.39	0.04
D4 Pond	0.25	0.03
D4 Stream	0.33	0.04
D5 Pond	0.06	0.01
D5 Stream	0.34	0.04
R4 Stream	1.00	0.12

RQ_{mix}: Risk quotient for the mixture

RQ_{mix} above the relevant trigger of 1 are shown in bold

Calculations conducted using AGD_AquaMix_v1.15 spreadsheet

Using the RQ_{mix} approach an acceptable risk is demonstrated for algae at FOCUS Step 2. For the acute exposure to aquatic invertebrates, an acceptable risk was concluded for all FOCUS Step 1 – 3 PEC_{sw} scenarios, with the exception of FOCUS Step 3 scenario D1 Ditch. However, the scenario D1 is not a concern for the Central Zone Member States.

Step 5 in the AquaMix Tool - calculation of individual toxicity units (TUs) also need to be taken into consideration.

$$\sum_{i=1}^n TU_i = \sum_{i=1}^n \frac{c_i}{ECx_i}$$

Table 9.5-18: Aquatic organisms: calculation of Toxicity Units (TU) for aquatic invertebrates based on the Aquatic Guidance MixTox Step 5- spring cereals.

FOCUS Scenario	Prothioconazole	Azoxystrobin	Sum
Proportion of active at PEC_{mix}			
Step 1	0.39	0.61	1.00
Step 2			
N-Europe	0.16	0.84	1.00
S-Europe	0.09	0.91	1.00
Step 3			
D1 Ditch	0.32	0.68	1.00
D1 Stream	0.32	0.68	1.00
D3 Ditch	0.57	0.43	1.00
D4 Pond	0.08	0.92	1.00
D4 Stream	0.57	0.43	1.00
D5 Pond	0.26	0.74	1.00
D5 Stream	0.57	0.43	1.00

R4 Stream	0.22	0.78	1.00
Individual TU (EC₅₀ aquatic invertebrate a.s. based [mg sum of a.s/L])			
Step 1	0.033	0.207	0.241
Step 2			
N-Europe	0.002	0.034	0.036
S-Europe	0.002	0.063	0.065
Step 3			
D1 Ditch	0.001	0.012	0.013
D1 Stream	0.001	0.007	0.008
D3 Ditch	0.001	0.003	0.004
D4 Pond	0.000	0.002	0.002
D4 Stream	0.001	0.002	0.003
D5 Pond	0.000	0.001	0.001
D5 Stream	0.001	0.003	0.003
R4 Stream	0.001	0.009	0.010
% TU			
Step 1	13.9	86.1	100.0
Step 2			
N-Europe	4.5	95.5	100.0
S-Europe	2.5	97.5	100.0
Step 3			
D1 Ditch	10.5	89.5	100.0
D1 Stream	10.5	89.5	100.0
D3 Ditch	25.3	74.7	100.0
D4 Pond	2.0	98.0	100.0
D4 Stream	25.3	74.7	100.0
D5 Pond	8.2	91.8	100.0
D5 Stream	25.3	74.7	100.0
R4 Stream	6.8	93.2	100.0

Calculations conducted using AGD AquaMix_v1.15 spreadsheet

Calculations are based on the additional data (e.g. sensitive species) where entered

Values highlighted in bold demonstrate that one active substance is driving the risk assessment for that scenario.

Although azoxystrobin is the driver of the risk assessment for 3 out of the 8 FOCUS Step 3 scenarios, there is no clear driver of the entire risk assessment for aquatic invertebrates for uses in spring cereals. The risk to aquatic invertebrates is covered by the calculation of the RQ_{mix}, presented in Table 9.5-17, and the risk assessment for azoxystrobin.

Table 9.5-19: Aquatic organisms: calculation of Toxicity Units (TU) for algae based on the Aquatic Guidance MixTox Step 5- spring cereals.

FOCUS Scenario	Prothioconazole	Azoxystrobin	Sum
Proportion of active at PEC_{mix}			
Step 1	0.39	0.61	1.00
Step 2			
N-Europe	0.16	0.84	1.00
S-Europe	0.09	0.91	1.00
Step 3			
D1 Ditch	0.32	0.68	1.00
D1 Stream	0.32	0.68	1.00
D3 Ditch	0.57	0.43	1.00
D4 Pond	0.08	0.92	1.00
D4 Stream	0.57	0.43	1.00
D5 Pond	0.26	0.74	1.00
D5 Stream	0.57	0.43	1.00
R4 Stream	0.22	0.78	1.00

Individual TU (EC ₅₀ algae a.s. based [mg sum of a.s/L])			
Step 1	0.020	0.261	0.281
Step 2			
N-Europe	0.001	0.043	0.044
S-Europe	0.001	0.080	0.081
Step 3			
D1 Ditch	0.001	0.015	0.016
D1 Stream	0.001	0.009	0.010
D3 Ditch	0.001	0.004	0.004
D4 Pond	0.000	0.003	0.003
D4 Stream	0.001	0.003	0.004
D5 Pond	0.000	0.001	0.001
D5 Stream	0.001	0.003	0.004
R4 Stream	0.000	0.012	0.012
% TU			
Step 1	7.1	92.9	100.0
Step 2			
N-Europe	22	97.8	100.0
S-Europe	1.2	98.8	100.0
Step 3			
D1 Ditch	5.3	94.7	100.0
D1 Stream	5.2	94.8	100.0
D3 Ditch	13.8	86.2	100.0
D4 Pond	1.0	99.0	100.0
D4 Stream	13.8	86.2	100.0
D5 Pond	4.1	95.9	100.0
D5 Stream	13.8	86.2	100.0
R4 Stream	3.3	96.7	100.0

Calculations conducted using AGD AquaMix_v1.15 spreadsheet

Calculations are based on the additional data (e.g. sensitive species) where entered

Values highlighted in bold demonstrate that one active substance is driving the risk assessment for that scenario.

Although azoxystrobin is the driver of the risk assessment for the majority of the FOCUS Step 1-3 scenarios, this active substance does not drive the entire risk assessment. The risk to algae is covered both by the calculation of the RQ_{mix}, presented in Table 9.5-17, and the risk assessment for azoxystrobin for uses in spring cereals.

Following EFSA's decision scheme the use of the calculated mixture toxicity (ETR_{mix}) is appropriate for the acute combined risk assessment for fish. The risk assessment has been conducted using the AquaMix Tool v 1.15.

Equation 18:
$$ETR_{mix-CA} = \frac{PEC_{mix}}{ECx_{mix-CA}}$$

Table 9.5-20: Aquatic organisms: acceptability of risk ($ETR_{mix-CA} < 1/AF$ (0.01)) for the acute effect on fish based on the Aquatic Guidance MixTox Step 8a and FOCUS Step 1-3 scenarios – spring cereals

Group	Fish acute
FOCUS Scenario	ETR_{mix-CA}
Step 1	
	0.17
Step 2	
N-Europe	0.03
S-Europe	0.05
Step3	
D1 Ditch	0.01
D1 Stream	0.01
D3 Ditch	0.00
D4 Pond	0.00
D4 Stream	0.00
D5 Pond	0.00
D5 Stream	0.00
R4 Stream	0.01

RQ_{mix}: Risk quotient for the mixture
 ETR_{mix-CA} above the relevant trigger of 0.01 are shown in bold (Note, the values are rounded to decimal places in accordance with the AGD_AquaMix_v1.15 spreadsheet. Indications of values above the trigger are in accordance with the tool.)
Calculations conducted using AGD_AquaMix_v1.15 spreadsheet

Using the ETR_{mix-CA} approach an acceptable acute risk is demonstrated for fish for all FOCUS Step 3 PEC_{SW} scenarios,

Step 5 in the AquaMix Tool - calculation of individual toxicity units (TUs) also need to be taken into consideration.

Table 9.5-21: Aquatic organisms: calculation of Toxicity Units (TU) for acute exposure of fish based on the Aquatic Guidance MixTox Step 5- spring cereals.

FOCUS Scenario	Prothioconazole	Azoxystrobin	Sum
Proportion of active at PEC_{mix}			
Step 1	0.39	0.61	1.00
Step 2			
N-Europe	0.16	0.84	1.00
S-Europe	0.09	0.91	1.00
Step 3			
D1 Ditch	0.32	0.68	1.00
D1 Stream	0.32	0.68	1.00
D3 Ditch	0.57	0.43	1.00
D4 Pond	0.08	0.92	1.00
D4 Stream	0.57	0.43	1.00
D5 Pond	0.26	0.74	1.00
D5 Stream	0.57	0.43	1.00
R4 Stream	0.22	0.78	1.00
Individual TU (LC₅₀ fish a.s. based [mg sum of a.s/L])			
Step 1	0.024	0.146	0.169
Step 2			
N-Europe	0.001	0.024	0.025
S-Europe	0.001	0.044	0.046
Step 3			
D1 Ditch	0.001	0.008	0.009
D1 Stream	0.001	0.005	0.006
D3 Ditch	0.001	0.002	0.003
D4 Pond	0.000	0.002	0.002
D4 Stream	0.001	0.002	0.002
D5 Pond	0.000	0.000	0.000
D5 Stream	0.001	0.002	0.002
R4 Stream	0.000	0.007	0.007
% TU			
Step 1	14.0	86.0	100.0
Step 2			
N-Europe	4.6	95.4	100.0
S-Europe	2.5	97.5	100.0
Step 3			
D1 Ditch	10.6	89.4	100.0
D1 Stream	10.6	89.4	100.0
D3 Ditch	25.5	74.5	100.0

FOCUS Scenario	Prothioconazole	Azoxystrobin	Sum
D4 Pond	2.1	97.9	100.0
D4 Stream	25.5	74.5	100.0
D5 Pond	8.3	91.7	100.0
D5 Stream	25.5	74.5	100.0
R4 Stream	6.8	93.2	100.0

Calculations conducted using AGD AquaMix_v1.15 spreadsheet

Calculations are based on the additional data (e.g. sensitive species) where entered

Values highlighted in bold demonstrate that one active substance is driving the risk assessment for that scenario.

Azoxystrobin is the driver of the risk assessment for the FOCUS Step 2 and some FOCUS Step 3 scenarios. However, there is no clear driver of the entire risk assessment for fish for uses in spring cereals. The acute risk to fish is covered both by the calculation of the ETR_{mix-CA} , presented in Table 9.5-20, and the risk assessment for azoxystrobin for uses in spring cereals.

Conclusion

The acute mixture toxicity assessment for uses of FF-075 in spring cereals (BBCH 30-69) concludes acceptable risk to algae and fish at FOCUS Step 2 and 3, respectively, for all scenarios. For the acute exposure to aquatic invertebrates, an acceptable risk was concluded for all FOCUS Step 1 – 3 PEC_{SW} scenarios, with the exception of FOCUS Step 3 scenario D1 Ditch. However, the scenario D1 is not a concern for the Central Zone Member States. An assessment of the “driver” of the risk assessment indicates that azoxystrobin drives the risk assessments for a number of FOCUS Step 1-4 scenarios for uses in spring cereals. The risk to aquatic invertebrates, algae and fish from acute exposure to prothioconazole and azoxystrobin from applications of FF-075 in spring cereals is, therefore, covered by the calculation of the ETR_{mix-CA}/RQ_{mix} and the risk assessment for azoxystrobin.

Combined chronic risk assessment based on calculated mixture toxicity (ETR_{mix} or RQ_{mix}) (prothioconazole-desithio (M04)/azoxystrobin)

Winter Oilseed Rape

Following EFSA’s decision scheme the use of the calculated mixture toxicity (RQ_{mix}) is appropriate for the combined chronic risk assessment for fish and aquatic invertebrates. The risk assessment has been conducted using the AquaMix Tool v 1.15.

$$RQ_{mix} = \sum_{i=1}^n \frac{PEC_i}{RAC_i}$$

If $RQ < 1$: Low risk

If $RQ > 1$: Low risk not demonstrated/check exposure refinement options

Table 9.5-22: Aquatic organisms: acceptability of risk ($RQ < 1$) for the mixture for each organism group based on the Aquatic Guidance MixTox Step 8b and FOCUS Step 1-3 scenarios – winter oilseed rape.

Group	Fish chronic	Inverteb. chronic
FOCUS Scenario	RQ _{mix}	
Step 1		
	107.77	5.13
Step 2		
N-Europe	5.34	0.29
S-Europe	5.50	0.36
Step3		
D2 Ditch	4.46	0.22
D2 Stream	2.76	0.14
D3 Ditch	3.09	0.12
D4 Pond	1541.93	51.51
D4 Stream	2.60	0.10
D5 Pond	0.16	0.01
D5 Stream	2.75	0.11
R1 Pond	0.28	0.02
R1 Stream	2.01	0.07
R3 Stream	2.83	0.10

RQ_{mix} : Risk quotient for the mixture

RQ_{mix} above the relevant trigger of 1 are shown in bold

Calculations conducted using AGD_AquaMix_v1.15 spreadsheet

Table 9.5-23: Aquatic organisms: acceptability of risk ($RQ < 1$) for the mixture for each organism group based on the Aquatic Guidance MixTox Step 8b and FOCUS Step 4 scenarios – winter oilseed rape.

Group	Fish chronic	Inverteb. chronic
FOCUS Scenario	RQ_{mix}	
	10 m vegetative strip + 10 m no spray buffer	
D2 Ditch	0.61	-
D2 Stream	0.62	-
D3 Ditch	0.44	-
D4 Pond	0.08	0.01
D4 Stream	0.51	-
D5 Stream	0.54	-

Group	Fish chronic	Inverteb. chronic
FOCUS Scenario	RQ _{mix}	
	10 m vegetative strip + 10 m no spray buffer	
R1 Stream	0.78	-
R3 Stream	0.76	-

RQ_{mix}: Risk quotient for the mixture

RQ_{mix} above the relevant trigger of 1 are shown in bold

Calculations conducted using AGD_AquaMix_v1.15 spreadsheet

Using the RQ_{mix} approach an acceptable chronic risk is demonstrated for fish and aquatic invertebrates for all FOCUS Step 1 – 3 PEC_{SW} scenarios, with the exception of FOCUS Step 3 D2, D3, D4, D5 Stream, R1 Stream and R3 Stream scenarios. The outstanding risk in these scenarios is resolved at FOCUS Step 4 with the use of a 10 m vegetative buffer strip and a 10 m no spray buffer zone.

Step 5 in the AquaMix Tool - calculation of individual toxicity units (TUs) also need to be taken into consideration.

$$\sum_{i=1}^n TU_i = \sum_{i=1}^n \frac{c_i}{ECx_i}$$

Table 9.5-24: Aquatic organisms: calculation of Toxicity Units (TU) for fish based on the Aquatic Guidance MixTox Step 5- winter oilseed rape.

FOCUS Scenario	Prothioconazole-desthio (M04)	Azoxystrobin	Sum
Proportion of active at PEC _{mix}			
Step 1	0.39	0.61	1.00
Step 2			
N-Europe	0.31	0.69	1.00
S-Europe	0.21	0.79	1.00

Step 3			
D2 Ditch	0.36	0.64	1.00
D2 Stream	0.36	0.64	1.00
D3 Ditch	0.57	0.43	1.00
D4 Pond	1.00	0.00	1.00
D4 Stream	0.57	0.43	1.00
D5 Pond	0.30	0.70	1.00
D5 Stream	0.57	0.43	1.00
R1 Pond	0.21	0.79	1.00
R1 Stream	0.79	0.21	1.00
R3 Stream	0.83	0.17	1.00
Individual TU (NOEC Fish a.s. based [mg sum of a.s/L])			
Step 1	10.405	0.373	10.777
Step 2			
N-Europe	0.508	0.026	0.534
S-Europe	0.508	0.042	0.550
Step 3			
D2 Ditch	0.428	0.017	0.446
D2 Stream	0.266	0.011	0.276
D3 Ditch	0.304	0.005	0.309
D4 Pond	154.192	0.002	154.193
D4 Stream	0.256	0.004	0.260
D5 Pond	0.016	0.001	0.016
D5 Stream	0.270	0.005	0.275
R1 Pond	0.025	0.002	0.028
R1 Stream	0.200	0.001	0.201
R3 Stream	0.282	0.001	0.283
% TU			
Step 1	96.5	3.5	100.0
Step 2			
N-Europe	95.1	4.9	100.0
S-Europe	92.3	7.7	100.0
Step 3			
D2 Ditch	96.1	3.9	100.0
D2 Stream	96.1	3.9	100.0
D3 Ditch	98.3	1.7	100.0
D4 Pond	100.00	0.0	100.0
D4 Stream	98.3	1.7	100.0

D5 Pond	94.9	5.1	100.0
D5 Stream	98.3	1.7	100.0
R1 Pond	92.1	7.9	100.0
R1 Stream	99.4	0.6	100.0
R3 Stream	99.5	0.5	100.0

Calculations conducted using AGD AquaMix_v1.15 spreadsheet

Calculations are based on the additional data (e.g. sensitive species) where entered

Values highlighted in bold demonstrate that one active substance is driving the risk assessment for that scenario.

Based on these results it is clear that prothioconazole-desthio (M04) drives the chronic risk assessment for fish and thus the risk to this test group is covered by the assessment for the metabolite.

Table 9.5-25: Aquatic organisms: calculation of Toxicity Units (TU) for aquatic invertebrates based on the Aquatic Guidance MixTox Step 5- winter oilseed rape.

FOCUS Scenario	Prothioconazole-desthio (M04)	Azoxystrobin	Sum
Proportion of active at PEC_{mix}			
Step 1	0.39	0.61	1.00
Step 2			
N-Europe	0.31	0.69	1.00
S-Europe	0.21	0.79	1.00
Step 3			
D2 Ditch	0.36	0.64	1.00
D2 Stream	0.36	0.64	1.00
D3 Ditch	0.57	0.43	1.00
D4 Pond	1.00	0.00	1.00
D4 Stream	0.57	0.43	1.00
D5 Pond	0.30	0.70	1.00
D5 Stream	0.57	0.43	1.00
R1 Pond	0.21	0.79	1.00
R1 Stream	0.79	0.21	1.00
R3 Stream	0.83	0.17	1.00

Individual TU (NOEC aquatic invertebrates a.s. based [mg sum of a.s/L])			
Step 1	0.348	0.166	0.513
Step 2			
N-Europe	0.017	0.012	0.029
S-Europe	0.017	0.019	0.036
Step 3			
D2 Ditch	0.014	0.008	0.022
D2 Stream	0.009	0.005	0.014
D3 Ditch	0.010	0.002	0.012
D4 Pond	5.150	0.001	5.151
D4 Stream	0.009	0.002	0.010
D5 Pond	0.001	0.000	0.001
D5 Stream	0.009	0.002	0.011
R1 Pond	0.001	0.001	0.002
R1 Stream	0.007	0.001	0.007
R3 Stream	0.009	0.001	0.010
% TU			
Step 1	67.7	32.3	100.0
Step 2			
N-Europe	59.5	40.5	100.0
S-Europe	47.4	52.6	100.0
Step 3			
D2 Ditch	65.0	35.0	100.0
D2 Stream	64.8	35.2	100.0
D3 Ditch	81.5	18.5	100.0
D4 Pond	100.0	0.0	100.0
D4 Stream	81.5	18.5	100.0
D5 Pond	58.2	41.8	100.0
D5 Stream	81.5	18.5	100.0
R1 Pond	46.5	53.5	100.0
R1 Stream	92.4	7.6	100.0
R3 Stream	94.0	6.0	100.0

Calculations conducted using AGD AquaMix_v1.15 spreadsheet

Calculations are based on the additional data (e.g. sensitive species) where entered

Values highlighted in bold demonstrate that one active substance is driving the risk assessment for that scenario.

The results indicate that there is no clear driver of the risk assessment for uses of FF-075 in winter oilseed rape, and the risk to aquatic invertebrates is covered by the calculation of the RQ_{mix} , presented in Tables 9.5-22 and 9.5-23 and the risk assessment for prothioconazole-desithio (M04) for use in winter oilseed rape.

Conclusion

The chronic mixture toxicity assessment for uses of FF-075 in winter oilseed rape (BBCH 55-69) concludes acceptable risk to fish and aquatic invertebrates at FOCUS Step 4 when a 10 m vegetative strip and a 10 m no spray buffer is applied. An assessment of the “driver” of the risk assessment indicates that prothioconazole-desthio (M04) drives the chronic fish risk assessments for all FOCUS Step 1-3 scenarios for uses in winter oilseed rape. The assessment of chronic risk to fish is covered by the risk assessment for prothioconazole-desthio (M04). A “driver” was not identified for the entire chronic risk assessment for aquatic invertebrates. The risk to aquatic invertebrates from chronic exposure to prothioconazole-desthio (M04) and azoxystrobin from applications of FF-075 in winter oilseed rape is, therefore, covered by the calculation of the RQ_{mix} and the risk assessment for prothioconazole-desthio (M04).

Winter cereals

Following EFSA’s decision scheme the use of the calculated mixture toxicity (RQ_{mix}) is appropriate for the combined chronic risk assessment for fish and aquatic invertebrates. The risk assessment has been conducted using the AquaMix Tool v 1.15.

$$RQ_{mix} = \sum_{i=1}^n \frac{PEC_i}{RAC_i}$$

If $RQ < 1$: Low risk

If $RQ > 1$: Low risk not demonstrated/check exposure refinement options

Table 9.5-26: Aquatic organisms: acceptability of risk ($RQ < 1$) for the mixture for each organism group based on the Aquatic Guidance MixTox Step 8b and FOCUS Step 1-3 scenarios – winter cereals.

Group	Fish chronic	Inverteb. chronic
FOCUS Scenario	RQ _{mix}	
Step 1		
	134.72	6.42
Step 2		
N-Europe	7.12	0.56
S-Europe	7.77	0.84
Step3		
D1 Ditch	4.18	0.28
D1 Stream	3.57	0.21
D2 Ditch	4.33	0.35
D2 Stream	3.57	0.25
D3 Ditch	3.86	0.16
D4 Pond	0.22	0.02
D4 Stream	3.24	0.13
D5 Pond	0.21	0.01

Group	Fish chronic	Inverteb. chronic
FOCUS Scenario	RQ _{mix}	
D5 Stream	3.60	0.14
D6 Ditch	3.89	0.16
R1 Pond	0.34	0.02
R1 Stream	2.70	0.17
R3 Stream	3.75	0.22
R4 Stream	2.79	0.21

RQ_{mix}: Risk quotient for the mixture

RQ_{mix} above the relevant trigger of 1 are shown in bold

Calculations conducted using AGD_AquaMix_v1.15 spreadsheet

Table 9.5-27: Aquatic organisms: acceptability of risk (RQ < 1) for the mixture for each organism group based on the Aquatic Guidance MixTox Step 8b and FOCUS Step 4 scenarios – winter cereals

Group	Fish chronic	
FOCUS Scenario	RQ _{mix}	
	10 m vegetative strip + 10 m no spray buffer	20 m vegetative strip + 20 m no spray buffer
D1 Ditch	0.90	!
D1 Stream	0.87	!
D2 Ditch	1.05	0.79
D2 Stream	0.95	!
D3 Ditch	0.56	!
D4 Stream	0.66	!
D5 Stream	0.70	!
D6 Ditch	0.58	
R1 Stream	0.98	!
R3 Stream	1.00	0.53
R4 Stream	1.15	0.60

RQ_{mix}: Risk quotient for the mixture

RQ_{mix} above the relevant trigger of 1 are shown in bold

Calculations conducted using AGD_AquaMix_v1.15 spreadsheet

Using the RQ_{mix} approach an acceptable chronic risk is demonstrated aquatic invertebrates at FOCUS Step 2. An acceptable chronic risk to fish was demonstrated at FOCUS Step 3 with the exception of scenarios D1, D2, D3 Ditch, D4 Stream, D5 Stream, R1 Stream, R3 Stream and R4 Stream. Risk for these scenarios is resolved at FOCUS Step 4 with the use of a 20 m vegetative buffer strip and a 20 m no spray buffer zone.

Step 5 in the AquaMix Tool - calculation of individual toxicity units (TUs) also need to be taken into

consideration.

$$\sum_{i=1}^n TU_i = \sum_{i=1}^n \frac{c_i}{ECx_i}$$

Table 9.5-28: Aquatic organisms: calculation of Toxicity Units (TU) for fish based on the Aquatic Guidance MixTox Step 5- winter cereals

FOCUS Scenario	Prothioconazole-desthio (M04)	Azoxystrobin	Sum
Proportion of active at PEC_{mix}			
Step 1	0.39	0.61	1.00
Step 2			
N-Europe	0.16	0.84	1.00
S-Europe	0.09	0.91	1.00
Step 3			
D1 Ditch	0.20	0.80	1.00
D1 Stream	0.26	0.74	1.00
D2 Ditch	0.15	0.85	1.00
D2 Stream	0.19	0.81	1.00
D3 Ditch	0.57	0.43	1.00
D4 Pond	0.10	0.90	1.00
D4 Stream	0.57	0.43	1.00
D5 Pond	0.27	0.73	1.00
D5 Stream	0.57	0.43	1.00
D6 Ditch	0.57	0.43	1.00
R1 Pond	0.21	0.79	1.00
R1 Stream	0.22	0.78	1.00
R3 Stream	0.27	0.73	1.00
R4 Stream	0.17	0.83	1.00

Individual TU (NOEC Fish a.s. based [mg sum of a.s/L])			
Step 1	13.006	0.466	13.472
Step 2			
N-Europe	0.635	0.077	0.712
S-Europe	0.635	0.142	0.777
Step 3			
D1 Ditch	0.384	0.035	0.418
D1 Stream	0.335	0.022	0.357
D2 Ditch	0.383	0.050	0.433
D2 Stream	0.325	0.032	0.357
D3 Ditch	0.380	0.006	0.386
D4 Pond	0.018	0.004	0.022
D4 Stream	0.319	0.005	0.324
D5 Pond	0.020	0.001	0.021
D5 Stream	0.354	0.006	0.360
D6 Ditch	0.382	0.007	0.389
R1 Pond	0.031	0.003	0.034
R1 Stream	0.250	0.020	0.270
R3 Stream	0.353	0.022	0.375
R4 Stream	0.251	0.029	0.279
% TU			
Step 1	96.5	3.5	100.0
Step 2			
N-Europe	89.2	10.8	100.0
S-Europe	81.7	18.3	100.0
Step 3			
D1 Ditch	91.7	8.3	100.0
D1 Stream	93.9	6.1	100.0
D2 Ditch	88.4	11.6	100.0
D2 Stream	91.1	8.9	100.0
D3 Ditch	98.3	1.7	100.0
D4 Pond	83.0	17.0	100.0
D4 Stream	98.3	1.7	100.0
D5 Pond	94.1	5.9	100.0
D5 Stream	98.3	1.7	100.0
D6 Ditch	98.3	1.7	100.0
R1 Pond	92.1	7.9	100.0
R1 Stream	92.6	7.4	100.0

R3 Stream	94.2	5.8	100.0
R4 Stream	89.8	10.2	100.0

Calculations conducted using AGD AquaMix_v1.15 spreadsheet

Calculations are based on the additional data (e.g. sensitive species) where entered

Values highlighted in bold demonstrate that one active substance is driving the risk assessment for that scenario.

Prothioconazole-desthio (M04) is the driver of the risk assessment for the FOCUS Step 1 and some FOCUS Step 3 scenarios. However, there is no clear driver of the entire risk assessment for fish for uses in winter cereals. The acute risk to fish is covered both by the calculation of the RQ_{mix} , presented in Tables 9.5-26 and 9.5-27, and the risk assessment for prothioconazole-desthio (M04) for uses in winter cereals.

Table 9.5-29: Aquatic organisms: calculation of Toxicity Units (TU) for aquatic invertebrates based on the Aquatic Guidance MixTox Step 5- winter cereals

FOCUS Scenario	Prothioconazole-desthio (M04)	Azoxystrobin	Sum
Proportion of active at PEC_{mix}			
Step 1	0.39	0.61	1.00
Step 2			
N-Europe	0.16	0.84	1.00
S-Europe	0.09	0.91	1.00
Step 3			
D1 Ditch	0.20	0.80	1.00
D1 Stream	0.26	0.74	1.00
D2 Ditch	0.15	0.85	1.00
D2 Stream	0.19	0.81	1.00
D3 Ditch	0.57	0.43	1.00
D4 Pond	0.10	0.90	1.00
D4 Stream	0.57	0.43	1.00
D5 Pond	0.27	0.73	1.00
D5 Stream	0.57	0.43	1.00
D6 Ditch	0.57	0.43	1.00
R1 Pond	0.21	0.79	1.00
R1 Stream	0.22	0.78	1.00
R3 Stream	0.27	0.73	1.00
R4 Stream	0.17	0.83	1.00

Individual TU (NOEC Aquatic invertebrates a.s. based [mg sum of a.s/L])			
Step 1	0.434	0.207	0.642
Step 2			
N-Europe	0.021	0.034	0.056
S-Europe	0.021	0.063	0.084
Step 3			
D1 Ditch	0.013	0.015	0.028
D1 Stream	0.011	0.010	0.021
D2 Ditch	0.013	0.022	0.035
D2 Stream	0.011	0.014	0.025
D3 Ditch	0.013	0.003	0.016
D4 Pond	0.001	0.002	0.002
D4 Stream	0.011	0.002	0.013
D5 Pond	0.001	0.001	0.001
D5 Stream	0.012	0.003	0.014
D6 Ditch	0.013	0.003	0.016
R1 Pond	0.001	0.001	0.002
R1 Stream	0.008	0.009	0.017
R3 Stream	0.012	0.010	0.022
R4 Stream	0.008	0.013	0.021
% TU			
Step 1	67.7	32.3	100.0
Step 2			
N-Europe	38.2	61.8	100.0
S-Europe	25.1	74.9	100.0
Step 3			
D1 Ditch	45.4	54.6	100.0
D1 Stream	53.8	46.2	100.0
D2 Ditch	36.4	63.6	100.0
D2 Stream	43.4	56.6	100.0
D3 Ditch	81.5	18.5	100.0
D4 Pond	26.8	73.2	100.0
D4 Stream	81.5	18.5	100.0
D5 Pond	54.4	45.6	100.0
D5 Stream	81.5	18.5	100.0
D6 Ditch	81.5	18.5	100.0
R1 Pond	46.6	53.4	100.0
R1 Stream	48.3	51.7	100.0

R3 Stream	54.7	45.3	100.0
R4 Stream	39.7	60.3	100.0

Calculations conducted using AGD AquaMix_v1.15 spreadsheet

Calculations are based on the additional data (e.g. sensitive species) where entered

Values highlighted in bold demonstrate that one active substance is driving the risk assessment for that scenario.

The results indicate that there is no driver of the chronic risk assessment for uses of FF-075 in winter cereals. The risk to aquatic invertebrates is covered by the calculation of the RQ_{mix} , presented in Table 9.5-26.

Conclusion

The chronic mixture toxicity assessment for uses of FF-075 in winter cereals (BBCH 30-69) concludes acceptable risk aquatic invertebrates at Focus Step 2, and acceptable risk to fish at FOCUS Step 4 when a 20 m vegetative strip and a 20 m no spray buffer is applied. The assessment of chronic risk to fish is also covered by the risk assessment for prothioconazole-desthio (M04). A driver was not identified for the chronic aquatic invertebrate risk assessment for applications of FF-075 to winter cereals. The assessment is, therefore, covered by the calculation of the RQ_{mix} .

Spring cereals

Following EFSA's decision scheme the use of the calculated mixture toxicity (RQ_{mix}) is appropriate for the combined chronic risk assessment for fish and aquatic invertebrates. The risk assessment has been conducted using the AquaMix Tool v 1.15.

$$RQ_{mix} = \sum_{i=1}^n \frac{PEC_i}{RAC_i}$$

If $RQ < 1$: Low risk

If $RQ > 1$: Low risk not demonstrated/check exposure refinement options

Table 9.5-30: Aquatic organisms: acceptability of risk ($RQ < 1$) for the mixture for each organism group based on the Aquatic Guidance MixTox Step 8b and FOCUS Step 1-3 scenarios – spring cereals.

Group	Fish chronic	Inverteb. chronic
FOCUS Scenario	RQ _{mix}	
Step 1		
I	134.72	6.42
Step 2		
N-Europe	7.12	0.56
S-Europe	7.77	0.84
Step3		
D1 Ditch	5.66	0.30
D1 Stream	3.52	0.19
D3 Ditch	3.86	0.16

Group	Fish chronic	Inverteb. chronic
FOCUS Scenario	RQ_{mix}	
D4 Pond	0.25	0.03
D4 Stream	3.32	0.13
D5 Pond	0.21	0.01
D5 Stream	3.36	0.14
R4 Stream	2.84	0.18

RQ_{mix}: Risk quotient for the mixture

RQ_{mix} above the relevant trigger of 1 are shown in bold

Calculations conducted using AGD_AquaMix_v1.15 spreadsheet

Table 9.5-31: Aquatic organisms: acceptability of risk (RQ < 1) for the mixture for each organism group based on the Aquatic Guidance MixTox Step 8b and FOCUS Step 4 scenarios – winter cereals

Group	Fish chronic
FOCUS Scenario	RQ_{mix}
	10 m vegetative strip + 10 m no spray buffer
D1 Ditch	0.82
D1 Stream	0.82
D3 Ditch	0.56
D4 Stream	0.69
D5 Stream	0.66
R4 Stream	1.27

RQ_{mix}: Risk quotient for the mixture

RQ_{mix} above the relevant trigger of 1 are shown in bold

Calculations conducted using AGD_AquaMix_v1.15 spreadsheet

Using the RQ_{mix} approach an acceptable chronic risk is demonstrated aquatic invertebrates at FOCUS Step 2. An acceptable chronic risk to fish was demonstrated at FOCUS Step 3 with the exception of D1, D3 Ditch, D4 Stream, D5 Stream and R4 Stream. Risk for these scenarios is resolved at FOCUS Step 4 with the use of a 10 m vegetative buffer strip and a 10 m no spray buffer zone with the exception for scenario R4 Stream. The peak concentration in the R4 Stream scenario is expected to exceed the RAC on a single occasion lasting for only 0.583 days (EPAT analysis – Section B8). Furthermore, R4 is not of concern for the Member States in the Central Zone.

Step 5 in the AquaMix Tool - calculation of individual toxicity units (TUs) also need to be taken into consideration.

$$\sum_{i=1}^n TU_i = \sum_{i=1}^n \frac{c_i}{ECx_i}$$

Table 9.5-32: Aquatic organisms: calculation of Toxicity Units (TU) for fish based on the

Aquatic Guidance MixTox Step 5- spring cereals			
FOCUS Scenario	Prothioconazole-desthio (M04)	Azoxystrobin	Sum
Proportion of active at PEC _{mix}			
Step 1	0.39	0.61	1.00
Step 2			
N-Europe	0.16	0.84	1.00
S-Europe	0.09	0.91	1.00
Step 3			
D1 Ditch	0.32	0.68	1.00
D1 Stream	0.32	0.68	1.00
D3 Ditch	0.57	0.43	1.00
D4 Pond	0.08	0.92	1.00
D4 Stream	0.57	0.43	1.00
D5 Pond	0.26	0.74	1.00
D5 Stream	0.57	0.43	1.00
R4 Stream	0.22	0.78	1.00
Individual TU (NOEC Fish a.s. based [mg sum of a.s/L])			
Step 1	13.006	0.466	13.472
Step 2			
N-Europe	0.635	0.077	0.712
S-Europe	0.635	0.142	0.777
Step 3			
D1 Ditch	0.540	0.026	0.566
D1 Stream	0.335	0.017	0.352
D3 Ditch	0.380	0.006	0.386
D4 Pond	0.019	0.005	0.025
D4 Stream	0.327	0.006	0.332
D5 Pond	0.019	0.001	0.021
D5 Stream	0.331	0.006	0.336
R4 Stream	0.263	0.021	0.284

% TU			
Step 1	96.5	3.5	100.0
Step 2			
N-Europe	89.2	10.8	100.0
S-Europe	81.7	18.3	100.0
Step 3			
D1 Ditch	95.3	4.7	100.0
D1 Stream	95.3	4.7	100.0
D3 Ditch	98.3	1.7	100.0
D4 Pond	78.3	21.7	100.0
D4 Stream	98.3	1.7	100.0
D5 Pond	93.9	6.1	100.0
D5 Stream	98.3	1.7	100.0
R4 Stream	82.6	7.4	100.0

Calculations conducted using AGD AquaMix_v1.15 spreadsheet

Calculations are based on the additional data (e.g. sensitive species) where entered

Values highlighted in bold demonstrate that one active substance is driving the risk assessment for that scenario.

Prothioconazole-desthio (M04) is the driver of the risk assessment for the FOCUS Step 1 and some FOCUS Step 3 scenarios. However, there is no clear driver of the entire risk assessment for fish for uses in spring cereals. The acute risk to fish is covered both by the calculation of the RQ_{mix} , presented in Tables 9.5-30 and 9.5-31, and the risk assessment for prothioconazole-desthio (M04) for uses in spring cereals.

Table 9.5-33: Aquatic organisms: calculation of Toxicity Units (TU) for aquatic invertebrates based on the Aquatic Guidance MixTox Step 5- winter cereals

FOCUS Scenario	Prothioconazole-desthio (M04)	Azoxystrobin	Sum
Proportion of active at PEC_{mix}			
Step 1	0.39	0.61	1.00
Step 2			
N-Europe	0.16	0.84	1.00
S-Europe	0.09	0.91	1.00

Step 3			
D1 Ditch	0.32	0.68	1.00
D1 Stream	0.32	0.68	1.00
D3 Ditch	0.57	0.43	1.00
D4 Pond	0.08	0.92	1.00
D4 Stream	0.57	0.43	1.00
D5 Pond	0.26	0.74	1.00
D5 Stream	0.57	0.43	1.00
R4 Stream	0.22	0.78	1.00
Individual TU (NOEC Aquatic invertebrates a.s. based [mg sum of a.s/L])			
Step 1	0.434	0.207	0.642
Step 2			
N-Europe	0.021	0.034	0.056
S-Europe	0.021	0.063	0.084
Step 3			
D1 Ditch	0.018	0.012	0.030
D1 Stream	0.011	0.007	0.019
D3 Ditch	0.013	0.003	0.016
D4 Pond	0.001	0.002	0.003
D4 Stream	0.011	0.002	0.013
D5 Pond	0.001	0.001	0.001
D5 Stream	0.011	0.003	0.014
R4 Stream	0.009	0.009	0.018
% TU			
Step 1	67.7	32.3	100.0
Step 2			
N-Europe	38.2	61.8	100.0
S-Europe	25.1	74.9	100.0
Step 3			
D1 Ditch	60.5	39.5	100.0
D1 Stream	60.3	39.7	100.0
D3 Ditch	81.5	18.5	100.0
D4 Pond	21.3	78.7	100.0
D4 Stream	81.5	18.5	100.0
D5 Pond	53.7	46.3	100.0
D5 Stream	81.5	18.5	100.0
R4 Stream	48.5	51.5	100.0

Calculations conducted using AGD AquaMix_v1.15 spreadsheet
Calculations are based on the additional data (e.g. sensitive species) where entered

Values highlighted in bold demonstrate that one active substance is driving the risk assessment for that scenario.

The results indicate that there is no driver of the chronic risk assessment for uses of FF-075 in spring cereals. The risk to aquatic invertebrates is covered by the calculation of the RQ_{mix} , presented in Table 9.5-30.

Conclusion

The chronic mixture toxicity assessment for uses of FF-075 in spring cereals (BBCH 30-69) concludes acceptable risk to aquatic invertebrates at FOCUS Step 2 and acceptable chronic risk to fish FOCUS Step 4 when a 10 m vegetative strip and a 10 m no spray buffer is applied with the exception of scenario R4 stream in the assessment of chronic effects on fish. The peak concentration in the R4 Stream scenario is expected to exceed the RAC on a single occasion lasting for only 0.583 days (EPAT analysis – Section B8). Furthermore, R4 is not of concern for the Member States in the Central Zone.

zRMS comment:

We agree with calculation for mixture toxicity assessment provided by the applicant. It should be noted the chronic mixture toxicity assessment is required only at national level according to recommendation given I Central Zone during Harmonisation meeting in Desau, 2019.

In addition, the risk assessment for R4 scenario was not evaluated by zRMS with EPAT analysis – Section B8).

9.5.2.1 Prothiconazole and its metabolites

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

Table 9.5-2: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prothioconazole for each organism group based on FOCUS Steps 1 and 2 calculations for the use of FF-075 in winter oilseed rape (2 x 160 g a.s./ha, BBCH 55-69. Applied with a minimum 14-day interval)

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged
Test species		<i>Oncorhynchus mykiss</i>	<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Daphnia magna</i>	<i>Pseudokirchn. subcapitata</i>	<i>Chironomus riparius</i>
Endpoint		LC ₅₀	NOEC	EC ₅₀	NOEC	E _r C ₅₀	NOEC _{water}
(µg/L)		1830	308	1300	560	2180	9140
AF		100	10	100	10	10	10
RAC (µg/L)		18.3	30.8	13	56	218	914
FOCUS Scenario	PEC _{max} (µg/L)	PEC/RAC Ratio					
Step 1							
	34.7521	1.90	1.13	2.67	0.621	0.159	0.04
Step2							
N-Europe	1.6962	0.09	0.06	0.13	0.03	0.008	0.002
S-Europe	1.6962	0.09	0.06	0.13	0.03	0.008	0.002

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

Table 9.5-4: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prothioconazole for each organism group based on FOCUS Steps 1 and 2 calculations for the use of FF-075 in winter and spring cereals (2 x 200 g a.s./ha, BBCH 30-69. Applied with a minimum 14-day interval)

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged
Test species		<i>Oncorhynchus mykiss</i>	<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Daphnia magna</i>	<i>Pseudokirchn. subcapitata</i>	<i>Chironomus riparius</i>
Endpoint		LC ₅₀	NOEC	EC ₅₀	NOEC	E _r C ₅₀	NOEC _{water}
(µg/L)		1830	308	1300	560	2180	9140
AF		100	10	100	10	10	10
RAC (µg/L)		18.3	30.8	13	56	218	914
FOCUS Scenario	PEC _{max} (µg/L)	PEC/RAC Ratio					
Step 1							
	43.4401	2.37	1.41	3.34	0.78	0.20	0.05
Step2							
N-Europe	2.1202	0.12	0.07	0.16	0.04	0.01	0.002
S-Europe	2.1202	0.12	0.07	0.16	0.04	0.01	0.002

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

For winter applications to oilseed rape and cereals, and spring applications to cereals, acceptable risk to aquatic organisms from prothioconazole is observed at FOCUS Step 2 following the application of FF-075.

Table 9.5-5: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prothioconazole-desthio (M04) for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of FF-075 in winter oilseed rape (2 x 160 g a.s./ha, BBCH 55-69. Applied with a minimum 14-day interval)

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged
Test species		<i>Oncorhynchus mykiss</i>	<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Daphnia magna</i>	<i>Pseudokirchn. subcapitata</i>	<i>Chironomus riparius</i>
Endpoint		LC ₅₀	NOEC	EC ₅₀	NOEC	E _r C ₅₀	EC ₁₀ Water
(µg/L)		6630	3.34	10000	100	550	2000
AF		100	10	100	10	10	10
RAC (µg/L)		66.3	0.334	100	10	55	200
FOCUS Scenario	PEC _{max} (µg/L)	PEC/RAC Ratio					
Step 1							
	62.657	0.95	187.60	0.63	6.27	1.14	0.31
Step 2							
N-Europe	2.064	0.03	6.18	0.02	0.21	0.04	0.010
S-Europe	3.2818	0.05	9.83	0.03	0.003	0.06	0.016
Step 3							
D3 Ditch	0.0985	0.001	0.295	0.0010	0.010	0.002	0.0005
D4 Pond	0.0207	0.0003	0.062	0.0002	0.002	0.0004	0.0001
D4 Stream	0.0593	0.001	0.178	0.001	0.006	0.001	0.0003
D5 Pond	0.021	0.0003	0.063	0.0002	0.002	0.0004	0.0001
D5 Stream	0.0789	0.001	0.236	0.001	0.008	0.001	0.0004

R1 Pond	0.133	0.002	0.398	0.001	0.013	0.0024	0.001
R1 Stream	0.7872	0.012	2.357	0.008	0.079	0.014	0.004
R3 Stream	0.7569	0.011	2.266	0.0076	0.076	0.0138	0.004

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

For prothioconazole-desthio (M04), potential risks are identified at FOCUS Step 3 from long-term exposure to fish for FOCUS SW scenarios R1 Stream and R3 stream following winter applications of FF-075 to winter oilseed rape. Further refinement of the risk assessment is required.

Table 9.5-6: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prothioconazole-desthio (M04) for each organism group based on FOCUS Steps 1 and 2, and worse-case early and late application FOCUS Step 3 calculations for the use of FF-075 in winter cereals (2 x 200 g a.s./ha, BBCH 30-69. Applied with a minimum 14-day interval)

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged
Test species		<i>Oncorhynchus mykiss</i>	<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Daphnia magna</i>	<i>Pseudokirchn. subcapitata</i>	<i>Chironomus riparius</i>
Endpoint		LC ₅₀	NOEC	EC ₅₀	NOEC	E _r C ₅₀	NOEC _{water}
(µg/L)		6630	3.34	≥10000	100	550	2000
AF		100	10	100	10	10	10
RAC (µg/L)		66.3	0.334	≥100	10	55	200
FOCUS Scenario	PEC _{max} (µg/L)	PEC/RAC Ratio					
Step 1							
	78.3212	1.18	234.49	0.78	7.83	1.42	0.39
Step 2							
N-Europe	5.929	0.09	17.75	0.06	0.59	0.11	0.030
S-Europe	10.8003	0.16	32.34	0.11	0.011	0.20	0.054
Step 3							

D3 Ditch	0.1226	0.002	0.37	0.001	0.01	0.002	0.0006
D4 Pond	0.0251	0.000	0.08	0.000	0.003	0.0005	0.0001
D4 Stream	0.0791	0.001	0.24	0.001	0.008	0.001	0.0004
D5 Pond	0.0267	0.000	0.08	0.0003	0.003	0.0005	0.0001
D5 Stream	0.114	0.002	0.34	0.001	0.011	0.002	0.0006
R1 Pond	0.1651	0.002	0.49	0.002	0.02	0.003	0.0008
R1 Stream	1.027	0.015	3.07	0.01	0.103	0.02	0.005
R3 Stream	0.9933	0.015	2.97	0.01	0.10	0.02	0.005
R4 Stream	1.475	0.022	4.42	0.01	0.15	0.027	0.007

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

For prothioconazole-desthio (M04), potential risks are identified at FOCUS Step 3 from long-term exposure to fish for FOCUS SW scenarios R1 Stream, R3 stream and R4 stream following winter applications of FF-075 to cereals and winter oilseed rape. R3 and R4 scenarios are not a concern for the Central Zone Member States included in the GAP. However, further refinement of the risk assessment is required for the R1 stream scenario.

Table 9.5-7: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prothioconazole-desthio (M04) for each organism group based on FOCUS Steps 1 and 2, and worse-case early and late application FOCUS Step 3 calculations for the use of FF-075 in spring cereals (2 x 200 g a.s./ha, BBCH 30-69. Applied with a minimum 14-day interval)

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. pro-longed	Algae	Sed. dwell. prolonged
Test species		<i>Oncorhynchus mykiss</i>	<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Daphnia magna</i>	<i>Pseudokirchn. subcapitata</i>	<i>Chironomus riparius</i>
Endpoint		LC ₅₀	NOEC	EC ₅₀	NOEC	E _r C ₅₀	NOEC _{water}
(µg/L)		6630	3.34	10000	100	550	2000
AF		100	10	100	10	10	10

RAC (µg/L)		66.3	0.334	100	10	55	200
FOCUS Scenario	PEC _{max} (µg/L)	PEC/RAC Ratio					
Step 1							
	78.3212	1.18	234.49	0.78	7.83	1.42	0.39
Step 2							
N-Europe	5.929	0.09	17.75	0.06	0.59	0.11	0.030
S-Europe	10.8003	0.16	32.34	0.11	0.011	0.20	0.054
Step 3							
D3 Ditch	0.1517	0.002	0.45	0.002	0.02	0.003	0.0008
D4 Pond	0.0269	0.000	0.08	0.000	0.003	0.0005	0.0001
D4 Stream	0.0892	0.001	0.27	0.001	0.009	0.002	0.0004
D5 Pond	0.0265	0.000	0.08	0.0003	0.003	0.0005	0.0001
D5 Stream	0.0977	0.001	0.29	0.001	0.010	0.002	0.0005
R4 Stream	1.208	0.018	3.62	0.01	0.12	0.022	0.006

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

For prothioconazole-desthio (M04), potential risks are identified at FOCUS Step 3 from long-term exposure to fish for the FOCUS SW scenario R4 stream following spring applications of FF-075 to cereals. The R4 scenario is not a concern for the Central Zone Member States. However, FOCUS Step 4 refinement of the risk assessment is included for completeness.

Table 9.5-8: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prothioconazole-S-methyl (M01) for each organism group based on FOCUS Steps 1 and 2 calculations for the use of FF-075 in winter oilseed rape (2 x 160 g a.s./ha, BBCH 55-69. Applied with a minimum 14-day interval)

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged
Test species		<i>Oncorhynchus mykiss</i>	<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Daphnia magna</i>	<i>Pseudokirchn. subcapitata</i>	<i>Chironomus riparius</i>

Endpoint		LC ₅₀	NOEC	EC ₅₀	NOEC	E _r C ₅₀	NOEC _{Water}
(µg/L)		1800	30.8	2800	56	47400	914
AF		100	10	100	10	10	10
RAC (µg/L)		18.0	3.08	28	5.6	4740	91.4
FOCUS Scenario	PEC _{max} (µg/L)	PEC/RAC Ratio					
Step 1							
	7.3267	0.41	2.38	0.26	1.31	0.0015	0.08
Step 2							
N-Europe	0.2333	0.01	0.08	0.01	0.04	0.0000	0.003
S-Europe	0.3609	0.02	0.12	0.01	0.002	0.0001	0.004

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

For winter applications to oilseed rape, acceptable risk to aquatic organisms from prothioconazole-S-methyl (M01) is observed at FOCUS Step 2 following the application of FF-075.

Table 9.5-9: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prothioconazole-S-methyl (M01) for each organism group based on FOCUS Steps 1 and 2 calculations for the use of FF-075 in winter and spring cereals (2 x 200 g a.s./ha, BBCH 30-69. Applied with a minimum 14-day interval)

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged
Test species		<i>Oncorhynchus mykiss</i>	<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Daphnia magna</i>	<i>Pseudokirchn. subcapitata</i>	<i>Chironomus riparius</i>
Endpoint		LC ₅₀	NOEC	EC ₅₀	NOEC	E _r C ₅₀	NOEC _{Water}
(µg/L)		1800	30.8	2800	56	47400	914
AF		100	10	100	10	10	10

RAC (µg/L)		18.0	3.08	28	5.6	4740	91.4
FOCUS Scenario	PEC_{max} (µg/L)	PEC/RAC Ratio					
Step 1							
	9.1584	0.51	2.97	0.33	1.64	0.0019	0.10
Step 2							
N-Europe	0.6426	0.04	0.21	0.02	0.11	0.0001	0.007
S-Europe	1.1532	0.06	0.37	0.04	0.007	0.0002	0.013

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

For winter and spring applications to cereals (BBCH 30-69), acceptable risk to aquatic organisms from prothioconazole-S-methyl (M01) is observed at FOCUS Step 2 following the application of FF-075.

Table 9.5-10: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prothioconazole-triazolylketone (M42) each organism group based on FOCUS Steps 1 and 2 calculations for the use of FF-075 in winter oilseed rape (2 x 160 g a.s./ha, BBCH 5-69. Applied with a minimum 14-day interval)

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged
Test species		<i>Oncorhynchus mykiss</i>	<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Daphnia magna</i>	<i>Pseudokirchn. subcapitata</i>	<i>Chironomus riparius</i>
Endpoint		LC ₅₀	NOEC	EC ₅₀	NOEC	E _r C ₅₀	NOEC _{Water}
(µg/L)		183	30.8	130	56	218	914
AF		100	10	100	10	10	10
RAC (µg/L)		1.83	3.08	1.3	5.6	21.8	91.4
FOCUS Scenario	PEC_{max} (µg/L)	PEC/RAC Ratio					

Step 1							
	5.3728	2.94	1.74	4.13	0.96	0.25	0.06
Step 2							
N-Europe	0.1395	0.08	0.05	0.11	0.02	0.01	0.002
S-Europe	0.1525	0.08	0.05	0.12	0.021	0.01	0.002

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

For winter oilseed rape (BBCH 55-69), acceptable risk to aquatic organisms from prothioconazole-triazolylketone (M42) is observed at FOCUS Step 2 following the application of FF-075 in accordance with the GAP.

Table 9.55-11: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prothioconazole-triazolylketone (M42) each organism group based on FOCUS Steps 1 and 2 calculations for the use of FF-075 in winter and spring cereals (2 x 200 g a.s./ha, BBCH 30-69. Applied with a minimum 14-day interval)

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged
Test species		<i>Oncorhynchus mykiss</i>	<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Daphnia magna</i>	<i>Pseudokirchn. subcapitata</i>	<i>Chironomus riparius</i>
Endpoint		LC ₅₀	NOEC	EC ₅₀	NOEC	E _r C ₅₀	NOEC _{water}
(µg/L)		183	30.8	130	56	218	914
AF		100	10	100	10	10	10
RAC (µg/L)		1.83	3.08	1.3	5.6	21.8	91.4
FOCUS Scenario	PEC _{max} (µg/L)	PEC/RAC Ratio					
Step 1							
	6.716	3.67	2.18	5.17	1.20	0.31	0.07
Step 2							
N-Europe	0.2101	0.11	0.07	0.16	0.04	0.01	0.002

S-Europe	0.262	0.14	0.09	0.20	0.036	0.01	0.003
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AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

For winter and spring cereals (BBCH 30-69), acceptable risk to aquatic organisms from prothioconazole-triazolylketone (M42) is observed at FOCUS Step 2 following the application of FF-075 in accordance with the GAP.

Table 9.5-12: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for 1,2,4-triazole (M13) for each organism group based on FOCUS Steps 1 and 2 calculations for the use of FF-075 in winter oilseed rape (2 x 160 g a.s./ha, BBCH 55-69. Applied with a minimum 14-day interval)

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged
Test species		<i>Oncorhynchus mykiss</i>	<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Daphnia magna</i>	<i>Pseudokirchn. subcapitata</i>	<i>Chironomus riparius</i>
Endpoint		LC ₅₀	NOEC	EC ₅₀	NOEC	E _r C ₅₀	NOEC _{Water}
(µg/L)		498000	3200	900000	56	22500	914
AF		100	10	100	10	10	10
RAC (µg/L)		4980	320	9000	5.6	2250	91.4
FOCUS Scenario	PEC _{max} (µg/L)	PEC/RAC Ratio					
Step 1							
	8.3037	0.0017	0.026	0.001	1.48	0.0037	0.09
Step 2							
N-Europe	0.2216	0.00004	0.0007	0.00002	0.04	0.0001	0.002
S-Europe	0.2416	0.000049	0.0008	0.00003	0.000005	0.0001	0.003

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

For winter oilseed rape (BBCH 55-69), acceptable risk to aquatic organisms from 1,2,4-triazole (M13) is observed at FOCUS Step 2 following the application of FF-

075 in accordance with the GAP.

Table 9.5-13: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for 1,2,4-triazole (M13) for each organism group based on FOCUS Steps 1 and 2 calculations for the use of FF-075 in winter and spring cereals (2 x 200 g a.s./ha, BBCH 30-69. Applied with a minimum 14-day interval)

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged
Test species		<i>Oncorhynchus mykiss</i>	<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Daphnia magna</i>	<i>Pseudokirchn. subcapitata</i>	<i>Chironomus riparius</i>
Endpoint		LC ₅₀	NOEC	EC ₅₀	NOEC	E _r C ₅₀	NOEC _{Water}
(µg/L)		498000	3200	900000	56	22500	914
AF		100	10	100	10	10	10
RAC (µg/L)		4980	320	9000	5.6	2250	91.4
FOCUS Scenario	PEC _{max} (µg/L)	PEC/RAC Ratio					
Step 1							
	10.3796	0.0021	0.0324	0.001	1.85	0.005	0.11
Step 2							
N-Europe	0.332	0.0001	0.001	0.00004	0.06	0.0001	0.004
S-Europe	0.412	0.0001	0.0013	0.00005	0.00001	0.0002	0.005

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

For winter and spring cereals (BBCH 30-69), acceptable risk to aquatic organisms from 1,2,4-triazole (M13) is observed at FOCUS Step 2 following the application of FF-075 in accordance with the GAP.

For the intended uses in winter oilseed rape (BBCH 55-69) and winter and spring cereals (BBCH 30-69), calculated PEC/RAC ratios for prothioconazole-desthio (M04) did not indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for long term exposure to fish as characterised by a NOEC for *Oncorhynchus mykiss* of 0.00334 mg metabolite/L in connection with an assessment factor of 10) in several FOCUS Steps 1-3 scenarios. Therefore, further PEC/RAC ratios were calculated based on FOCUS Step 4 PEC_{sw} considering reduced exposure of surface water bodies.

FOCUS Step 4 calculations were conducted for metabolite prothioconazole-desthio (M04). In accordance with the permissible mitigation measures for field crops in the Central Zone Member States, the calculations were performed by reducing spray drift deposition and runoff inputs to simulate the use of 10 and 20 metre spray drift and runoff reduction buffer zones (vegetated filter strips). The Step 4 calculations were performed using the SWAN tool (version 5.0.1).

Maximum PEC_{sw} values for prothioconazole-desthio (M04) at FOCUS Step 4 for each crop group are shown in Table 8.9-20 to Table 8.9-24. In accordance with FOCUS guidance, both multiple and single applications were simulated for each crop group and the highest resulting PEC_{sw} and PEC_{sed} values are selected for input into the risk assessment.

Table 9.5-14: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for prothioconazole-desthio (M04) based on FOCUS Step 4 calculations and toxicity data for prolonged exposure to fish with mitigation for the use of FF-075 in winter oilseed rape (2 x 160 g a.s./ha, BBCH 55-69. Applied with a minimum 14-day interval))

Group		Fish prolonged
Test species		<i>Oncorhynchus mykiss</i>
Endpoint		NOEC
(µg/L)		3.34
AF		10
RAC (µg/L)		0.334
FOCUS Scenario	PEC _{max} (µg/L)	
Step 4 (10 m vegetation strip + 10m spray buffer)		
	0.3582	1.07
	0.3388	1.01
Step 4 (20 m vegetation strip + 20 m spray buffer)		
R1 Stream	0.1876	0.56
R3 Stream	0.1764	0.53

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

For winter oilseed rape (BBCH 55-69), acceptable risk to fish from prolonged exposure to prothioconazole-desthio (M04) was observed when applying a 20 m no spray buffer zone and a 20 m vegetative strip at FOCUS Step 4 following applications of FF-075 in accordance with the GAP.

Table 9.5-15: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for prothioconazole-desthio (M04) based on FOCUS Step 4 calculations and toxicity data for prolonged exposure to fish with mitigation for the use of FF-075 in winter cereals (2 x 200 g a.s./ha, BBCH 30-69. Applied with a minimum 14-day interval).

Group		Fish prolonged
Test species		<i>Oncorhynchus mykiss</i>
Endpoint		NOEC
(µg/L)		3.34
AF		10
RAC (µg/L)		0.334
FOCUS Scenario	PEC _{max} (µg/L)	
Step 4 (10 m vegetation strip + 10m spray buffer)		
R1 Stream	0.4671	1.40
R3 Stream	0.4532	1.36
R4 Stream	0.6706	2.01
Step 4 (20 m vegetation strip + 20 m spray buffer)		
R1 Stream	0.2447	0.73
R3 Stream	0.2378	0.71
R4 Stream	0.3513	1.05

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

Potential risks are identified at FOCUS Step 4 from long-term exposure to fish for FOCUS SW scenario R4 stream following winter applications of FF-075 to cereals. The peak concentration in the R4 Stream scenario is expected to exceed the RAC on a single occasion lasting for only 0.583 days (EPAT analysis – Section B8). Furthermore, R4 is not of concern for the Member States in the Central Zone.

Table 9.5-16: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for prothioconazole-desthio (M04) based on FOCUS Step 4 calculations and toxicity data for prolonged exposure to fish with mitigation for the use of FF-075 in spring cereals (2 x 200 g a.s./ha, BBCH 30-69. Applied with a minimum 14-day interval)

Group		Fish prolonged
Test species		<i>Oncorhynchus mykiss</i>
Endpoint		NOEC
(µg/L)		3.34
AF		10
RAC (µg/L)		0.334

FOCUS Scenario	PEC _{max} (µg/L)	
Step 4 (10 m vegetation strip + 10m spray buffer)		
R4 Stream	0.5439	1.63
Step 4 (20 m vegetation strip + 20 m spray buffer)		
R4 Stream	0.2837	0.85

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

For spring cereals (BBCH 30-69), acceptable risk to fish from prolonged exposure to prothioconazole-desthio (M04) was observed when applying a 20 m no spray buffer zone and a 20 m vegetative strip at FOCUS Step 4 following applications of FF-075 in accordance with the GAP.

zRMS comment:

For the intended uses in winter oilseed rape (BBCH 55-69) and winter and spring cereals (BBCH 30-69), calculated PEC/RAC ratios for prothioconazole-desthio (M04) did not indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for long term exposure to fish as characterised by a NOEC for *Oncorhynchus mykiss* of 0.00334 mg metabolite/L in connection with an assessment factor of 10) in several FOCUS Steps 1-3 scenarios. Therefore, further PEC/RAC ratios were calculated based on FOCUS Step 4 PEC_{sw} considering reduced exposure of surface water bodies.

FOCUS Step 4 calculations were conducted for metabolite prothioconazole-desthio (M04).

We agree with the risk assessment for a.s.-prothioconazole and its metabolite prothioconazole-desthio (M04).

-For winter oilseed rape (BBCH 55-69), acceptable risk to fish from prolonged exposure to prothioconazole-desthio (M04) was observed for R1 and R3 scenarios when applying a 20 m no spray buffer zone and a 20 m vegetative strip at FOCUS Step 4 following applications of FF-075 in accordance with the GAP.

- For winter cereals (BBCH 30-69) acceptable risk to fish from prolonged exposure to prothioconazole-desthio (M04) was observed for R1 and R3 scenarios when applying a 20 m no spray buffer zone and a 20 m vegetative strip at FOCUS Step 4 following applications of FF-075 in accordance with the GAP.

-For spring cereals (BBCH 30-69), acceptable risk to fish from prolonged exposure to prothioconazole-desthio (M04) was observed when applying a 20 m no spray buffer zone and a 20 m vegetative strip at FOCUS Step 4 following applications of FF-075 in accordance with the GAP.

In addition, the potential risks are identified at FOCUS Step 4 from long-term exposure to fish for FOCUS SW scenario R4 stream following winter applications of FF-075 to cereals.

9.5.2.2 Azoxystrobin and its metabolites

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

Table 9.55-17: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for azoxystrobin for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of FF-075 in winter oilseed rape (2 x 120 g a.s./ha, BBCH 55-69. Applied with a minimum 14-day interval).

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged	Higher Plant
Test species		<i>Oncorhynchus mykiss</i>	<i>Pimephales promelas</i>	<i>Mysidopsis bahia</i>	<i>Mysidopsis bahia</i>	<i>Navicula pelliculosa</i>	<i>Chironomus riparius</i>	<i>Lemna gibba</i>
Endpoint		LC ₅₀	NOEC	EC ₅₀	NOEC	E _b C ₅₀	NOEC _{water}	ErC ₅₀
(µg/L)		470	147	55	9.54	14	800	3200
AF		100	10	100	10	10	10	10
RAC (µg/L)		4.7	14.7	0.55	0.954	1.4	80	320
FOCUS Scenario	PEC SW-max (µg/L)	PEC/RAC Ratio						
Step 1								
	54.7696	11.653	3.726	99.581	57.410	39.121	0.6846	0.1712
Step2								
N-Europe	3.8143	0.81	0.26	6.94	4.00	2.725	0.048	0.012
S-Europe	6.2023	1.32	0.42	11.28	6.50	4.430	0.078	0.019

Step 3								
D3 Ditch	0.7612	0.162	0.052	1.3840	0.798	0.544	0.0095	0.0024
D4 Pond	0.2223	0.0473	0.015	0.4042	0.233	0.1588	0.0028	0.0007
D4 Stream	0.6404	0.136	0.044	1.164	0.671	0.457	0.0080	0.0020
D5 Pond	0.1228	0.0261	0.008	0.2233	0.129	0.0877	0.0015	0.0004
D5 Stream	0.6779	0.144	0.046	1.233	0.711	0.484	0.0085	0.0021
R1 Pond	0.3225	0.069	0.022	0.586	0.338	0.2304	0.004	0.001
R1 Stream	1.825	0.388	0.124	3.318	1.913	1.304	0.023	0.006
R3 Stream	1.973	0.420	0.134	3.5873	2.068	1.4093	0.025	0.006

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

For applications to winter oilseed rape, outstanding concerns were observed at FOCUS Step 3 for scenarios D3 ditch, D4 stream, D5 stream, R1 stream and R3 stream following applications of FF-075 in accordance with the proposed GAP. Additional refinement of the risk assessment is required.

Table 9.55-18: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for azoxystrobin for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of FF-075 in winter cereals (2 x 150 g a.s./ha, BBCH 30-69. Applied with a minimum 14-day interval).

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged	Higher Plant
Test species		<i>Oncorhynchus mykiss</i>	<i>Pimephales promelas</i>	<i>Mysidopsis bahia</i>	<i>Mysidopsis bahia</i>	<i>Navicula pelliculosa</i>	<i>Chironomus riparius</i>	<i>Lemna gibba</i>
Endpoint		LC ₅₀	NOEC	EC ₅₀	NOEC	E _b C ₅₀	NOEC _{water}	ErC ₅₀
(µg/L)		470	147	55	9.54	14	800	3200
AF		100	10	100	10	10	10	10
RAC (µg/L)		4.7	14.7	0.55	0.954	1.4	80	320

FOCUS Scenario	PEC _{SW-max} (µg/L)	PEC/RAC Ratio						
Step 1								
	68.462	14.566	4.657	124.476	71.763	48.901	0.8558	0.0455
Step2								
N-Europe	11.3349	2.41	0.77	20.61	11.88	8.096	0.142	0.008
S-Europe	20.8868	4.44	1.42	37.98	21.89	14.919	0.261	0.014
Step 3								
D3 Ditch	0.9513	0.202	0.065	1.7296	0.997	0.680	0.0119	0.0030
D4 Pond	0.5513	0.1173	0.038	1.0024	0.578	0.3938	0.0069	0.0017
D4 Stream	0.7989	0.170	0.054	1.453	0.837	0.571	0.0100	0.0025
D5 Pond	0.1803	0.0384	0.012	0.3278	0.189	0.1288	0.0023	0.0006
D5 Stream	0.8865	0.189	0.060	1.612	0.929	0.633	0.0111	0.0028
R1 Pond	0.3926	0.084	0.027	0.714	0.412	0.2804	0.005	0.001
R1 Stream	2.94	0.626	0.200	5.345	3.082	2.100	0.037	0.009
R3 Stream	3.219	0.685	0.219	5.8527	3.374	2.2993	0.040	0.010
R4 Stream	4.196	0.893	0.285	7.6291	4.398	2.9971	0.052	0.013

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

For applications to winter cereals, outstanding concerns were observed at FOCUS Step 3 for scenarios D3 ditch, D4 pond, D4 stream, D5 stream, R1 stream, R3 stream and R4 stream following applications of FF-075 in accordance with the proposed GAP. Additional refinement of the risk assessment is required.

Table 9.55-19: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for azoxystrobin for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of FF-075 in spring cereals (2 x 150 g a.s./ha, BBCH 30-69. Applied with a minimum 14-day interval).

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged	Higher Plant
Test species		<i>Oncorhynchus mykiss</i>	<i>Pimephales promelas</i>	<i>Mysidopsis bahia</i>	<i>Mysidopsis bahia</i>	<i>Navicula pelliculosa</i>	<i>Chironomus riparius</i>	<i>Lemna gibba</i>
Endpoint		LC ₅₀	NOEC	EC ₅₀	NOEC	E _b C ₅₀	NOEC _{water}	ErC ₅₀
(µg/L)		470	147	55	9.54	14	800	3200
AF		100	10	100	10	10	10	10
RAC (µg/L)		4.7	14.7	0.55	0.954	1.4	80	320
FOCUS Scenario	PEC SW-max (µg/L)	PEC/RAC Ratio						
Step 1								
	68.462	14.566	4.657	124.476	71.763	48.901	0.8558	0.0455
Step2								
N-Europe	11.3349	2.41	0.77	20.61	11.88	8.096	0.142	0.008
S-Europe	20.8868	4.44	1.42	37.98	21.89	14.919	0.261	0.014
Step 3								
D3 Ditch	0.9525	0.203	0.065	1.7318	0.998	0.680	0.0119	0.0030
D4 Pond	0.792	0.1685	0.054	1.4400	0.830	0.5657	0.0099	0.0025
D4 Stream	0.8192	0.174	0.056	1.489	0.859	0.585	0.0102	0.0026
D5 Pond	0.1845	0.0393	0.013	0.3355	0.193	0.1318	0.0023	0.0006
D5 Stream	0.83	0.177	0.056	1.509	0.870	0.593	0.0104	0.0026
R4 Stream	3.069	0.653	0.209	5.5800	3.217	2.1921	0.038	0.010

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

bold

For applications to spring cereals, outstanding concerns were observed at FOCUS Step 3 for scenarios D3 ditch, D4 pond, D4 stream, D5 stream and R4 stream following applications of FF-075 in accordance with the proposed GAP. Additional refinement of the risk assessment is required.

The following refinement approach has been taken from the Azoxystrobin-addendum 2 to AIR DAR, Nov. 2009, and the agreed-upon endpoints listed in the EFSA Conclusions (2010).

Refinement of the azoxystrobin aquatic risk assessment

Acute aquatic organism risk assessment

In the case of azoxystrobin, there are data on a total of 14 species. According to the ‘Method 1’ of the PPR opinion (ref https://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620775612) it is possible to calculate a geometric mean from these endpoints and combine them with the standard assessment factor of 100.

Table 9.55-20: Available acute aquatic invertebrate endpoints.

Species	48-h EC/LC50 (µg a.s./L)	Reference; report number (a)
<i>Mysidopsis bahia</i> (marine shrimp)	68	Grinell et al, 1993; BL4785/B
<i>Mysidopsis bahia</i> (marine shrimp)	55* (96 hour LC ₅₀)	Grinell et al, 1993; BL4785/B
<i>Macrocyclops fuscus</i> (Cyclopoid copepod crustacean)	130	Farrelly et al, 1995a; RJ1793B
<i>Daphnia pulex</i> (Water flea; cladoceran crustacean)	200	Rapley et al, 1995b; RJ1798B
<i>Chironomus riparius</i> (Midge larva; dipteran insect)	210	Farrelly et al, 1995d; RJ1792B
<i>Daphnia magna</i> (Water flea; cladoceran crustacean)	280	Rapley et al, 1995a; RJ1797B
<i>Gammarus pulex</i> (Freshwater shrimp; amphipod crustacean)	350	Farrelly et al, 1995b; RJ1782B
<i>Crassostrea gigas</i> (Pacific oyster)	1300	Kent et al, 1994; BL4842/B
<i>Chaoborus crystallinus</i> (Phantom midge larva; dipteran insect)	1600	Farrelly et al, 1995e; RJ1792B
<i>Cloeon dipterum</i> (Mayfly nymph; ephemeropteran insect)	3200	Farrelly et al, 1995g; RJ1795B
<i>Asellus aquaticus</i> (Water-louse; isopod crustacean)	>4000	Farrelly et al, 1995c; RJ1789B
<i>Ischnura elegans</i> (Damselfly nymph; zygopteran insect)	>4000	Farrelly et al. 1995f; RJ1794B
<i>Notonecta glauca</i> (Water-boatman; hemipteran insect)	>4000	Rapley et al 1995c; RJ1799B
<i>Brachyonus calyciflorus</i> (Rotifer)	>4000	Farrelly et al 1995h; RJ1791B
<i>Lymnaea stagnalis</i> (Pond snail; gastropod mollusc)	>4000	Farrelly et al 1995i; RJ1796B
Geometric mean	892	RAC=8.9 µg a.s./L

In line with the requirements of an Article 33 submission, the approach assessed in the RAR (May, 2009) and accepted in the EFSA Conclusions (2010) has been applied to the current assessment. Accordingly, the more conservative, 96-hour endpoint for *Mysidopsis bahia* (LC₅₀ = 55µg a.s./L) has been used in the calculation. Additionally, it has been assumed that the ‘greater-than’ values for *Asellus aquaticus*, *Ischnura elegans*, *Notonecta glauca*, *Brachyonus calyciflorus* and *Lymnaea stagnalis* are equal to 4000 µg a.s./L. This is considered to be a worst-case assumption and was accepted during the previous inclusion of azoxystrobin (EFSA, 2010). It was also concluded that by removing the acute data for marine species, the regulatory data for the acute risk to aquatic invertebrates was not significantly affected. (RAR, 2009). Therefore, the data for *Mysidopsis bahia* is included in the calculation of the geomean and the proposed regulatory concentration, considering an assessment factor of 100, is 8.9 µg a.s./L.

The EFSA Conclusions (EFSA Journal (2010); 8(4):1542) concluded that the endpoint was at least equivalent to the level of protection afforded by the standard first tier dataset and the standard assessment factor, although concerns were raised about which species should and should not be included.

Chronic aquatic organism risk assessment

On the basis of the first-tier data presented in the Draft Assessment Report, the lowest aquatic endpoint is the chronic endpoint for *Mysidopsis bahia* of 9.54 µg a.s./L which gives a RAC of 0.954 µg a.s./L. The Peer Review Meeting considered the RAC to not take consideration of the information on the toxicity of azoxystrobin to other species, or under more realistic conditions.

Chronic data is available for two species, namely, *Daphnia magna* (NOEC = 44 µg a.s./L) and *Mysidopsis bahia* (NOEC = 9.54 µg a.s./L). According to the 'Method 1' of the PPR opinion (ref https://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620775612) it is possible to calculate a geometric mean from these endpoints and combine them with the standard assessment factor of 10. Using this approach, accepted during the original Annex 1 inclusion of azoxystrobin (EFSA, 2010), the geometric mean of the two endpoints is 20.5 µg a.s./L and, therefore, the regulatory concentration for the chronic risk to aquatic invertebrates is 2.1 µg a.s./L.

It should be noted that it is likely that this endpoint is overly protective as information from the Mysid shrimp study indicates that azoxystrobin is potentially an acute toxin and not a chronic toxin i.e. the effect is primarily an acute one and hence the NOEC is based on adult mortality, and not the reproductive parameter.

Risk to aquatic invertebrates and derivation of a regulatory concentration using an HC5 approach.

For the Annex 1 inclusion of azoxystrobin it was agreed that consideration of the lower limit HC5 (ie. the lower value of the 90% confidence interval around the median HC5 value) would be appropriate (RAR, 2009, EFSA Conclusion 2010). This is the concentration that, with 95% certainty, is lower than the EC50 values reported for 95% of the species. The use of this value in risk assessment with no additional uncertainty/assessment factor (AF) has been validated to some extent by comparing such values derived from laboratory studies conducted on a range of insecticides, herbicides and fungicides, with (at most) Class 2 effect in mesocosm studies conducted on the same compounds (RAR, 2009). It should be noted that as this approach has been validated against mesocosm studies, then it is considered to cover both acute and chronic effects. Due to this factor, and the lack of chronic data, no SSD was constructed for chronic effects.

Several approaches to the SSD calculation were proposed and the agreed-upon LLHC5 value was 7.15 µg a.s./L (Azoxystrobin-addendum 2 to AIR DAR, Nov 2009) which excluded all greater-than values from the calculation, but included marine species data (Please refer to Table 9.5-20 for the related endpoints).

Table 9.55-21: Summary of outputs of analysis of the addition of aquatic invertebrate data (RAR, 2009).

Combination of endpoints	HC5 µg a.s./L	LLHC5 µg a.s./L
All data (excluding the greater than values and the marine values)	54.2	Not required
All data (including marine data and > values presented as 4000 µg a.s./L)	65.1 (assuming the 96-hour endpoint for Mysid shrimp is used)	15.3
All data excluding 'greater than values' but including marine species	40.8 (assuming the 96-hour Mysid shrimp endpoint is used)	7.15

All data including greater than values but excluding marine species	94.4	20.8
All data excluding marine and greater than values	54.7	7.5

Values highlighted in bold were selected for consideration for the refinement of the risk assessment.

It was proposed to ignore those outputs where the ‘greater than’ values were converted to endpoints as a visual inspection of the graphical outputs indicated that the endpoints do not appear to fit the same distribution as the other endpoints (RAR Appendix, 2009). Additionally, when these endpoints were included, the goodness of fit assessment was rejected.

It was noted that the inclusion of marine data had little effect on the overall endpoint and, therefore, it was agreed that the lower value of 7.15 µg a.s./L should be considered when refining the RAC, as the lowest value.

The Peer Review Meeting concluded that the LLHC5 value was equivalent to a Class 1 or 2 effect in a mesocosm study. Concerns were raised about the limited data set and the fact this was not a standard refinement option. Despite this, the Meeting considered the LLHC5 value to be a useful endpoint to consider with other lines of evidence.

Consideration of the mesocosm study

The Peer Review Meeting considered that the mesocosm study was not of a high standard as there was a lack of analysis of azoxystrobin with respect to time; it was also considered that the study did not represent the proposed GAP. Finally, no NOEC could be determined due to the presence of impacts at the lowest concentration tested. The NOAEC was tentatively proposed as being 10 µg a.s./L. However, there was concern there was concern that this was not appropriate due to the duration and magnitude of the effects observed. The Meeting concluded that at 10 µg a.s./L there would be effects and, therefore, the RAC was <10 µg a.s./L for the mesocosm study.

The Meeting considered that each line of evidence was sufficient on its own to be used in regulatory risk assessments; however, they felt that it was possible to use information from all lines of evidence to determine a ‘regulatory acceptable concentration’ (RAC).

On the basis of all of the above information, the Meeting concluded that the RAC should be set at 3.3 µg a.s./L for the acute and chronic aquatic invertebrate risk assessments (Azoxystrobin-Addendum 2 to AIR DAR, 2009, and EFSA Conclusion, 2010). It should be noted that in selecting this endpoint the RAC is lower than the NEAC of 10 µg a.s./L, the lower limit of the HC5 of 7.15 µg a.s./L and the geometric mean of 892 µg a.s./L (RAC=8.9 µg a.s./L) but still higher than the value based on the tier 1 assessment.

The following refined aquatic risk assessment considers the agreed-upon refined acute and chronic aquatic invertebrate RAC of 3.3 µg/L and the agreed-upon geometric mean algal endpoint of 262 µg a.s./L (EFSA Journal 2010; 8(4):1542) in conjunction with the calculation FOCUS Step 1, 2 and 3.

zRMS comment:

We agree with refined acute and chronic aquatic invertebrate based on RAC of 3.3 µg/L value and the agreed-upon geometric mean algal endpoint of 262 µg a.s./L (EFSA Journal 2010; 8(4):1542).

Table 9.55-22: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for azoxystrobin for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of FF-075 in winter oilseed rape (2 x 120 g a.s./ha, BBCH 55-69. Applied with a minimum 14-day interval).

Test species		<i>Acute Aquatic Invertebrate</i>	<i>Chronic Aquatic Invertebrate</i>	<i>Algae (Geomean)</i>
Endpoint				EC ₅₀
(µg/L)				262
AF				10
RAC (µg/L)		3.3	3.3	26.2
FOCUS Scenario	PEC_{SW-max} (µg/L)	PEC/RAC Ratio		
Step 1				
	54.7696	16.597	16.597	2.090
Step2				
N-Europe	3.8143	1.16	1.16	0.146
S-Europe	6.2023	1.88	1.88	0.237
Step 3				
D3 Ditch	0.7612	0.2307	0.231	0.029
D4 Pond	0.2223	0.0674	0.067	0.0085
D4 Stream	0.6404	0.194	0.194	0.024
D5 Pond	0.1228	0.0372	0.037	0.0047
D5 Stream	0.6779	0.205	0.205	0.026
R1 Pond	0.3225	0.098	0.098	0.0123
R1 Stream	1.825	0.553	0.553	0.070
R3 Stream	1.973	0.5979	0.598	0.0753

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

For winter oilseed rape (BBCH 55-69), acceptable risk to aquatic organisms from azoxystrobin is observed at FOCUS Step 3 for all scenarios when the assessment is refined in accordance with the agreed algal endpoint and RAC for acute and chronic invertebrates.

Table 9.55-23: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for azoxystrobin for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of FF-075 in winter and spring cereals (2 x 150 g a.s./ha, BBCH 30-69. Applied with a minimum 14-day interval).

Test species		<i>Acute Aquatic Invertebrate</i>	<i>Chronic Aquatic Invertebrate</i>	<i>Algae (Geomean)</i>
Endpoint				EC ₅₀
(µg/L)				262
AF				10

RAC (µg/L)		3.3	3.3	26.2
FOCUS Scenario	PEC_{SW-max} (µg/L)	PEC/RAC Ratio		
Step 1				
	68.462	20.746	20.746	2.613
Step2				
N-Europe	11.3349	3.43	3.43	0.433
S-Europe	20.8868	6.33	6.33	0.797
Step 3				
D3 Ditch	0.9513	0.2883	0.288	0.036
D4 Pond	0.5513	0.1671	0.167	0.0210
D4 Stream	0.7989	0.242	0.242	0.030
D5 Pond	0.1803	0.0546	0.055	0.0069
D5 Stream	0.8865	0.269	0.269	0.034
R1 Pond	0.3926	0.119	0.119	0.0150
R1 Stream	2.94	0.891	0.891	0.112
R3 Stream	3.219	0.9755	0.975	0.1229
R4 Stream	4.196	1.271515	1.271515	0.1601527

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

For winter and spring cereals (BBCH 30-69), acceptable risk to aquatic organisms from azoxystrobin is observed at FOCUS Step 3 for all scenarios when the assessment is refined in accordance with the agreed algal endpoint and RAC for acute and chronic invertebrates, with the exception of scenario R4 stream. Further refinement is required.

For the intended uses in winter and spring cereals (BBCH 30-60) calculated PEC/RAC ratios for azoxystrobin did not indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for acute and long term exposure to aquatic invertebrates) in FOCUS Step 3 scenario R4 stream when the RAC was refined in accordance with the agreed approach (EFSA Journal 2010; 8(4):1542). Therefore, further PEC/RAC ratios were calculated based on FOCUS Step 4 PEC_{SW} considering reduced exposure of surface water bodies.

Table 9.5-24: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for azoxystrobin based on FOCUS Step 4 calculations and toxicity data for acute and prolonged exposure to aquatic invertebrates with mitigation for the use of FF-075 in winter and spring cereals (2 x 150 g a.s./ha, BBCH 30-69. Applied with a minimum 14-day interval))

Test species		<i>Acute Aquatic Invertebrate</i>	<i>Chronic Aquatic Invertebrate</i>
Endpoint			
(µg/L)			
AF			
RAC (µg/L)		3.3	3.3

FOCUS Scenario	PEC sw- max (µg/L)	PEC/RAC Ratio	
Step 4 (10 m vegetation strip + 10m spray buffer)			
R4 Stream	1.909	0.58	0.578

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

For winter and spring cereals (BBCH 30-69), acceptable risk to aquatic organisms from azoxystrobin is observed when applying a 10m vegetation strip and 10m spray buffer is employed at FOCUS Step 4 following applications of FF-075 in accordance with the GAP.

zRMS comment:

We agree with refined acute and chronic aquatic invertebrate based on RAC of 3.3 µg/L value and the agreed-upon geometric mean algal endpoint of 262 µg a.s./L (EFSA Journal 2010; 8(4):1542) in conjunction with the calculation FOCUS Step 1- 4.

For winter oilseed rape (BBCH 55-69), acceptable risk to aquatic organisms from azoxystrobin is observed at FOCUS Step 3 for all scenarios when the assessment is refined in accordance with the agreed algal endpoint and RAC for acute and chronic invertebrates.

For winter and spring cereals (BBCH 30-69), acceptable risk to aquatic organisms from azoxystrobin is observed at FOCUS Step 3 for all scenarios when the assessment is refined in accordance with the agreed algal endpoint and RAC for acute and chronic invertebrates, with the exception of scenario R4 stream. Further refinement is required.

Therefore, further PEC/RAC ratios were calculated based on FOCUS Step 4 PEC_{sw} considering reduced exposure of surface water bodies.

For winter and spring cereals (BBCH 30-69), acceptable risk to aquatic organisms from azoxystrobin is observed when applying a 10m vegetation strip and 10m spray buffer is employed at FOCUS Step 4 following applications of FF-075 in accordance with the GAP.

Table 9.55-25: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the metabolite of azoxystrobin, R234886 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of FF-075 in winter oilseed rape (2 x 120 g a.s./ha, BBCH 55-69. Applied with a minimum 14-day interval).

Group		Fish acute	Inverteb. acute	Algae
Test species		<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Selenastrum capricornutum</i>
Endpoint		LC ₅₀	EC ₅₀	E _r C ₅₀
(µg/L)		150000	180000	47000
AF		100	100	10
RAC (µg/L)		1500	1800	4700
FOCUS Scenario	PEC sw. max (µg/L)	PEC/RAC Ratio		
Step 1				

	35.617	0.024	0.020	0.008
Step2				
N-Europe	1.9602	0.001	0.001	0.0004
S-Europe	3.5885	0.002	0.002	0.001

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

For winter oilseed rape, an acceptable risk from R234886 to aquatic organisms is observed from applications of FF-075 in accordance with the proposed GAP.

Table 9.55-26: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the metabolite of azoxystrobin, R234886 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of FF-075 in winter and spring cereals (2 x 150 g a.s./ha, BBCH 30-69. Applied with a minimum 14-day interval).

Group		Fish acute	Inverteb. acute	Algae
Test species		<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Selenastrum capricornutum</i>
Endpoint		LC ₅₀	EC ₅₀	E _r C ₅₀
(µg/L)		150000	180000	47000
AF		100	100	10
RAC (µg/L)		1500	1800	4700
FOCUS Scenario	PEC sw. max (µg/L)	PEC/RAC Ratio		
Step 1				
	44.5213	0.030	0.025	0.009
Step2				
N-Europe	6.9279	0.005	0.004	0.0015
S-Europe	13.4409	0.009	0.007	0.003

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

For winter and spring cereals, an acceptable risk from R234886 to aquatic organisms is observed from applications of FF-075 in accordance with the proposed GAP.

Table 9.55-27: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the metabolite of azoxystrobin, R401553 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of FF-075 in winter oilseed rape (2 x 120 g a.s./ha, BBCH 55-69. Applied with a minimum 14-day interval).

Group		Fish acute	Inverteb. acute	Algae
Test species		<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Selenastrum capricornutum</i>
Endpoint		LC ₅₀	EC ₅₀	E _r C ₅₀

(µg/L)		120000	120000	120000
AF		100	100	10
RAC (µg/L)		1200	1200	12000
FOCUS Scenario	PEC sw. max (µg/L)	PEC/RAC Ratio		
Step 1				
	9.305	0.008	0.008	0.001
Step2				
N-Europe	0.2366	0.0002	0.0002	0.00002
S-Europe	0.3924	0.0003	0.0003	0.00003

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

For winter oilseed rape, an acceptable risk from R401553 to aquatic organisms is observed from applications of FF-075 in accordance with the proposed GAP.

Table 9.55-28: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the metabolite of azoxystrobin, R401553 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of FF-075 in winter and spring cereals (2 x 150 g a.s./ha, BBCH 30-69. Applied with a minimum 14-day interval).

Group		Fish acute	Inverteb. acute	Algae
Test species		<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Selenastrum capricornutum</i>
Endpoint		LC ₅₀	EC ₅₀	ErC ₅₀
(µg/L)		120000	120000	120000
AF		100	100	10
RAC (µg/L)		1200	1200	12000
FOCUS Scenario	PEC sw. max (µg/L)	PEC/RAC Ratio		
Step 1				
	11.6313	0.010	0.010	0.001
Step2				
N-Europe	0.7242	0.0006	0.0006	0.00006
S-Europe	1.3473	0.0011	0.0011	0.00011

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

For winter and spring cereals, an acceptable risk from R401553 to aquatic organisms is observed from applications of FF-075 in accordance with the proposed GAP.

Table 9.55-29: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the metabolite of azoxystrobin, R402173 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of FF-075 in winter oilseed rape (2 x 120 g a.s./ha, BBCH 55-69. Applied with a minimum 14-day interval).

Group		Fish acute	Inverteb. acute	Algae
Test species		<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Selenastrum capricornutum</i>
Endpoint		LC ₅₀	EC ₅₀	E _r C ₅₀
(µg/L)		62000	100000	67000
AF		100	100	10
RAC (µg/L)		620	1000	6700
FOCUS Scenario	PEC sw. max (µg/L)	PEC/RAC Ratio		
Step 1				
	12.4532	0.020	0.012	0.002
Step2				
N-Europe	0.2771	0.0004	0.0003	0.00004
S-Europe	0.5167	0.0008	0.0005	0.00008

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

For winter oilseed rape, an acceptable risk from R402173 to aquatic organisms is observed from applications of FF-075 in accordance with the proposed GAP.

Table 9.55-30: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the metabolite of azoxystrobin, R402173 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of FF-075 in winter and spring cereals (2 x 150 g a.s./ha, BBCH 30-69. Applied with a minimum 14-day interval).

Group		Fish acute	Inverteb. acute	Algae
Test species		<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Selenastrum capricornutum</i>
Endpoint		LC ₅₀	EC ₅₀	E _r C ₅₀
(µg/L)		62000	100000	67000
AF		100	100	10
RAC (µg/L)		620	1000	6700
FOCUS Scenario	PEC sw. max (µg/L)	PEC/RAC Ratio		
Step 1				
	15.5665	0.025	0.016	0.002
Step2				
N-Europe	1.0052	0.0016	0.0010	0.00015

S-Europe	1.9635	0.0032	0.0020	0.00029
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AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold.

For winter and spring cereals, an acceptable risk from R402173 to aquatic organisms is observed from applications of FF-075 in accordance with the proposed GAP.

zRMS comment:

For winter oilseed rape, winter and spring cereals an acceptable risk from metabolites (R402173, R401553 and R234886 to aquatic organisms is concluded from applications of FF-075 in accordance with the proposed GAP.

9.5.2.3 The aquatic risk assessment for the formulated product.

Table 9.5-31: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for FF-075 each organism group based on SWASH scenario calculations for the use of FF-075 in winter oilseed rape (2 x 880 g product/ha, BBCH 55-69, applied with a minimum 14-day interval)

Group			Inverteb. acute	Algae	Higher Plant
Test species			<i>Daphnia magna</i>	<i>Pseudokirchn. subcapitata</i>	<i>Lemna gibba</i>
Endpoint			EC ₅₀	ErC ₅₀	ErC ₅₀
(µg/L)			2970	4650	16520
AF			100	10	10
RAC (µg/L)			29.7	465	1652
SWASH Scenario	Distance (m)	PEC _{max} (µg/L)			
Ditch	Default	5.6537	0.19	0.0122	0.0034
	5	1.5325	0.05	0.0033	0.0009
	10	0.8128	0.03	0.0017	0.0005
	20	0.4223	0.01	0.0009	0.0003
Pond	Default	0.1928	0.01	0.0004	0.0001
	5	0.1668	0.01	0.0004	0.0001
	10	0.1199	0.00	0.0003	0.0001
	20	0.0801	0.00	0.0002	0.0000
Stream	Default	5.0348	0.17	0.0108	0.0030
	5	1.839	0.06	0.0040	0.0011
	10	0.9754	0.03	0.0021	0.0006
	20	0.5068	0.02	0.0011	0.0003

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

Table 9.5-32: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for FF-075 each organism group based on SWASH scenario calculations for the use of FF-075 in winter and spring cereals (2 x 1100 g product/ha, BBCH 30-69, applied with a minimum 14-day interval)

Group			Inverteb. acute	Algae	Higher Plant
Test species			<i>Daphnia magna</i>	<i>Pseudokirchn. subcapitata</i>	<i>Lemna gibba</i>
Endpoint			EC ₅₀	ErC ₅₀	ErC ₅₀
(µg/L)			2970	4650	16520
AF			100	10	10
RAC (µg/L)			29.7	465	1652
SWASH Scenario	Distance (m)	PEC _{gl-max} (µg/L)			
Ditch	Default	7.0671	0.24	0.0152	0.0043
	5	1.9156	0.06	0.0041	0.0012
	10	1.0159	0.03	0.0022	0.0006
	20	0.5279	0.02	0.0011	0.0003
Pond	Default	0.241	0.01	0.0005	0.0001
	5	0.2085	0.01	0.0004	0.0001
	10	0.1499	0.01	0.0003	0.0001
	20	0.1001	0.00	0.0002	0.0001
Stream	Default	6.2936	0.21	0.0135	0.0038
	5	2.2987	0.08	0.0049	0.0014
	10	1.2191	0.04	0.0026	0.0007
	20	0.6335	0.02	0.0014	0.0004

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

For uses in winter oilseed rape, and winter and spring cereals (BBCH 30-80, and BBCH 30-69, respectively), acceptable risk to aquatic organisms from FF-075 is observed following applications according to the GAP.

zRMS comment:

For uses in winter oilseed rape, and winter and spring cereals (BBCH 30-80, and BBCH 30-69, respectively), acceptable risk to aquatic organisms from FF-075 is concluded following applications according to the GAP.

9.5.3 Overall conclusions

Summary of Risk and mitigation measures

Use	Winter Oilseed Rape											
Assess- ment	Mixture Toxicity FF-075 (Acute)	Mixture Toxicity FF-075 (Chronic)	Prothiocona- zole	Prothiocona- zole-desthio (M04)	Prothiocona- zole -S- methyl (M01)	Prothiocona- zole tria- zolyketone	1.2.4- triazole	Azoxystro bin	R234886	R401553	R402173	FF-075
Mitiga- tion	Risk acceptable with 10 m VFS + 10 NSZ	Risk acceptable with 10 m VFS + 10 NSZ	Acceptable risk at FOCUS Step 2	Risk acceptable at FOCUS Step 4 with 20 m VFS + 20 NSZ	Acceptable risk at FOCUS Step 2	Acceptable risk at FOCUS Step 2	Acceptable risk at FOCUS Step 2	Acceptable risk at FOCUS Step 3	Acceptable risk at FOCUS Step 1	Acceptable risk at FOCUS Step 1	Acceptable risk at FOCUS Step 1	Acceptable risk with no mitigation

Use	Winter Cereals											
Assess- ment	Mixture Toxicity FF-075 (Acute)	Mixture Toxicity FF-075 (Chronic)	Prothiocona- zole	Prothioconazole- desthio (M04)	Prothioconazole -S- methyl (M01)	Prothiocona- zole tria- zolyketone	1.2.4- triazole	Azoxystro bin	R234886	R401553	R402173	FF-075

Mitigation	Risk acceptable with 10 m VFS + 10 NSZ	Risk acceptable with 20 m VFS + 20 NSZ	Acceptable risk at Focus Step 2	Risk acceptable at FOCUS Step 4 with 20 m VFS + 20 NSZ. Outstanding risk to fish from chronic exposure due to scenario R4 stream. However, minimal exceedance of RAC calculated (0m583 days)	Acceptable risk at Focus Step 2	Acceptable risk at Focus Step 2	Acceptable risk at Focus Step 2	Risk acceptable at FOCUS Step 4 with 10 m VFS + 10 NSZ.	Acceptable risk at Focus Step 1	Acceptable risk at Focus Step 1	Acceptable risk at Focus Step 1	Acceptable risk with no mitigation
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Use	Spring Cereals											
Assessment	Mixture Toxicity FF-075 (Acute)	Mixture Toxicity FF-075 (Chronic)	Prothioconazole	Prothioconazole-desthio (M04)	Prothioconazole -S-methyl (M01)	Prothioconazole triazoleketone	1,2,4-triazole	Azoxystrobin	R234886	R401553	R402173	FF-075
Mitigation	Acceptable risk at Focus Step 3.	Risk acceptable with 10 m VFS + 10 NSZ. Outstanding chronic risk to fish for R4 stream scenario.	Acceptable risk at Focus Step 2	Acceptable risk at Focus Step 3.	Acceptable risk at Focus Step 2	Acceptable risk at Focus Step 2	Acceptable risk at Focus Step 2	Risk acceptable at FOCUS Step 4 with 10 m VFS + 10 NSZ.	Acceptable risk at Focus Step 1	Acceptable risk at Focus Step 1	Acceptable risk at Focus Step 1	Acceptable risk with no mitigation

zRMS comment:

We agree with the risk assessment provided for the active substances and their metabolites and for formulation Euskatel Pro.
The mixture toxicity assessment was considered acceptable by zRMS.
The chronic mixture toxicity assessment was also included for fish and aquatic invertebrates.
The final risk mitigation measures for aquatic organism should be decided at MSs level.

Regulatory testing with fish, aquatic invertebrates, algae and aquatic macrophytes has been conducted with prothioconazole, azoxystrobin, the relevant metabolites and FF-075 in accordance with EU requirements.

Acute and chronic mixture toxicity assessment has been conducted. The acute mixture toxicity assessment for uses in winter oilseed rape (BBCH 55-69) concludes acceptable risk in the ETR_{mix-CA}/RQ_{mix} assessments for fish, aquatic invertebrates and algae when risk mitigation is applied as a 10m vegetative strip and a 10m no spray. An assessment of the “driver” of the risk assessment indicates that both active substances drive the risk assessments for different FOCUS scenarios. The risk to aquatic invertebrates, algae and fish from acute exposure to prothioconazole and azoxystrobin from applications of FF-075 is, therefore, covered by the calculation of the ETR_{mix-CA}/RQ_{mix} and the risk assessment for the individual active substances for use in winter oilseed rape.

The acute mixture toxicity assessment for uses of FF-075 in winter cereals (BBCH 30-69) concludes acceptable risk to algae at FOCUS Step 2 for all scenarios. An acceptable risk to fish from applications of FF-075 were concluded at FOCUS Step3 for all relevant scenarios. An acceptable risk to aquatic invertebrates was concluded at FOCUS Step 4 for all relevant scenarios when a 10 m vegetative buffer and a 10 m no spray buffer were applied. Outstanding risks for D1 ditch, D2 ditch and D2 stream scenarios were identified for aquatic invertebrates. However, D1 and D2 scenarios are not considered relevant for national assessment in any of the Central Zone Member States where authorisation of FF-075 is being applied for. An assessment of the “driver” of the risk assessment indicates that azoxystrobin drives the risk assessments for a number of FOCUS Step 1-4 scenarios. The risk to aquatic invertebrates, algae and fish from acute exposure to prothioconazole and azoxystrobin from applications of FF-075 is, therefore, covered by the calculation of the ETR_{mix-CA}/RQ_{mix} and the risk assessment for azoxystrobin for use in winter cereals.

The acute mixture toxicity assessment for uses of FF-075 in spring cereals (BBCH 30-69) concludes acceptable risk to algae and fish at FOCUS Step 2 and 3, respectively, for all scenarios. For the acute exposure to aquatic invertebrates, an acceptable risk was concluded for all FOCUS Step 1 – 3 PEC_{SW} scenarios, with the exception of FOCUS Step 3 scenario D1 Ditch. However, the scenario D1 is not a concern for the Central Zone Member States. An assessment of the “driver” of the risk assessment indicates that azoxystrobin drives the risk assessments for a number of FOCUS Step 1-4 scenarios for uses in spring cereals. The risk to aquatic invertebrates, algae and fish from acute exposure to prothioconazole and azoxystrobin from applications of FF-075 in spring cereals is, therefore, covered by the calculation of the ETR_{mix-CA}/RQ_{mix} and the risk assessment for azoxystrobin.

The chronic mixture toxicity assessment for uses of FF-075 in winter oilseed rape (BBCH 55-69) concludes acceptable risk to fish and aquatic invertebrates at FOCUS Step 4 when a 10 m vegetative strip and a 10 m no spray buffer is applied. An assessment of the “driver” of the risk assessment indicates that prothioconazole-desthio (M04) drives the chronic fish risk assessments for all FOCUS Step 1-3 scenarios for uses in winter oilseed rape. The assessment of chronic risk to fish is covered by the risk assessment for prothioconazole-desthio (M04). A “driver” was not identified for the entire chronic risk assessment for aquatic invertebrates. The risk to aquatic invertebrates from chronic exposure to prothioconazole-desthio (M04) and azoxystrobin from applications of FF-075 in winter oilseed rape is, therefore, covered by the calculation of the RQ_{mix} and the risk assessment for prothioconazole-desthio (M04).

The chronic mixture toxicity assessment for uses of FF-075 in winter cereals (BBCH 30-69) concludes acceptable risk aquatic invertebrates at Focus Step 2, and acceptable risk to fish at FOCUS Step 4 when a 20 m vegetative strip and a 20 m no spray buffer is applied. The assessment of chronic risk to fish is also covered by the risk assessment for prothioconazole-desthio (M04). A driver was not identified for the chronic aquatic invertebrate risk assessment for applications of FF-075 to winter cereals. The assessment is, therefore, covered by the calculation of the RQ_{mix} .

The chronic mixture toxicity assessment for uses of FF-075 in spring cereals (BBCH 30-69) concludes acceptable risk to aquatic invertebrates at FOCUS Step 2 and acceptable chronic risk to fish FOCUS Step

4 when a 10 m vegetative strip and a 10 m no spray buffer is applied with the exception of scenario R4 stream in the assessment of chronic effects on fish. The peak concentration in the R4 Stream scenario is expected to exceed the RAC on a single occasion lasting for only 0.583 days (EPAT analysis – Section B8). Furthermore, R4 is not of concern for the Member States in the Central Zone.

For prothioconazole, and the metabolites prothioconazole-S-methyl (M01), prothioconazole-triazolylketone (M42) and 1,2,4-triazole (M13), acceptable risk to aquatic organisms was identified for uses in oilseed rape, and winter and spring cereals from spray drift and drainage at Tier 1.

For prothioconazole, and the metabolites prothioconazole-S-methyl (M01), prothioconazole-triazolylketone (M42) and 1,2,4-triazole (M13), acceptable risk to aquatic organisms was identified for uses in oilseed rape, and winter and spring cereals from spray drift and drainage at Tier 1.

For prothioconazole, and the metabolites prothioconazole-S-methyl (M01), prothioconazole-triazolylketone (M42) and 1,2,4-triazole (M13), acceptable risk to aquatic organisms was identified for uses in oilseed rape, and winter and spring cereals at Tier 1.

For prothioconazole, and the metabolites prothioconazole-S-methyl (M01), prothioconazole-triazolylketone (M42) and 1,2,4-triazole (M13), acceptable risk to aquatic organisms was identified for uses in oilseed rape, and winter and spring cereals at Tier 1.

For prothioconazole-desthio (M04), potential risks were identified at Tier 1 from chronic exposure of fish following winter and spring applications to winter and spring cereals (BBCH 30-69) and applications made to oilseed rape (BBCH 55-69). However, an acceptable risk to fish from chronic exposure to prothioconazole-desthio was concluded when a 20 m vegetative strip and 20 m no spray buffer was applied for uses in winter oilseed rape and spring cereals. Potential risks are identified at FOCUS Step 4 from long-term exposure to fish for FOCUS SW scenario R4 stream following winter applications of FF-075 to winter cereals. The peak concentration in the R4 Stream scenario is expected to exceed the RAC on a single occasion lasting for only 0.583 days (EPAT analysis – Section B8). Therefore, an acceptable risk is concluded from prothioconazole-desthio resulting from applications of FF-075 to winter cereals in accordance with the GAP.

For azoxystrobin, potential risks were identified at FOCUS Steps 1-3 from acute aquatic invertebrates and algae and chronic exposure of aquatic invertebrates for uses in winter oilseed rape (BBCH 55-69), and winter and spring cereals (BBCH 30-69). However, when the RAC applied to the acute and chronic aquatic invertebrate risk assessments was refined to 3.3 µg/L and the endpoint for algae was refined to 262 µg/L by means of calculating a geometric mean, in line with the agreed endpoints outlined in EFSA Journal (2010); 8(4):1542, safe use to aquatic organisms was concluded at FOCUS Step 3 for uses in winter oilseed rape (BBCH 55-69).

For the intended uses in winter and spring cereals (BBCH 30-60) calculated PEC/RAC ratios for azoxystrobin did not indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for acute and long term exposure to aquatic invertebrates) in FOCUS Step 3 scenario R4 stream when the RAC was refined in accordance with the agreed approach (EFSA Journal 2010; 8(4):1542). The risk was resolved at FOCUS Step 4 when a 10 m vegetative strip and a 10 m no spray buffer was applied.

For the metabolites of azoxystrobin, R234886, R401553 and R402173, an acceptable risk to aquatic organisms was identified for uses in winter oilseed rape and winter and spring cereals at FOCUS Step 1.

An acceptable risk to aquatic organisms was concluded from uses of FF-075 in winter oilseed rape (BBCH 55-69) and winter and spring cereals (BBCH 30-69) when applied 1 m from the from the water body.

9.6 Effects on bees (KCP 10.3.1)

9.6.1 Toxicity data

Studies on the toxicity to bees have been carried out with active substance prothioconazole and azoxystrobin. Full details of these studies are provided in the respective prothioconazole EU DAR, (2005), prothioconazole EFSA Conclusions, (2007) and azoxystrobin EFSA Journal (2010).

Effects on honeybees and bumblebees of FF-075 were not evaluated as part of the EU assessment of prothioconazole. New data submitted with this application are summarised in Appendix 2.

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

Species	Substance	Exposure System	Results	Reference
<i>Apis mellifera</i>	Prothioconazole	48-hour, Oral	LD ₅₀ = >71 µg a.s./bee	EFSA Scientific Report (2007) 106, 1-98. DAR, V.3, Annex B, B.9, (2005). Wilhelmy, 1999.
<i>Apis mellifera</i>	Prothioconazole	48-hour, Contact	LD ₅₀ = >200 µg/bee	EFSA Scientific Report (2007) 106, 1-98. DAR, V.3, Annex B, B.9, (2005). Wilhelmy, 1999.
<i>Apis mellifera</i>	Azoxystrobin	48-hour, Oral	LD ₅₀ = >25 µg a.s./bee	EFSA Journal (2010); 8(4):1542 Gough, H.J., Jackson, D., Lewis, G.B., 1993.BIE 96-00077
<i>Apis mellifera</i>	Azoxystrobin	48-hour, Contact	LD ₅₀ = >200 µg/bee	EFSA Journal (2010); 8(4):1542 Gough, H.J., Jackson, D., Lewis, G.B., 1993.BIE 96-00077
<i>Apis mellifera</i>	FF-075	48-hour, Oral	LD ₅₀ = 526.41 µg product/bee (Equivalent to 156.61 µg total a.i./bee (89.60 µg Prothioconazole/bee and 67.01 µg Azoxystrobin /bee).	New Report KCP 10.3.1.1.1/01 Parker, T. (2020), Study report No.: 2862.

Species	Substance	Exposure System	Results	Reference
<i>Apis mellifera</i>	FF-075	48-hour, Contact	LD ₅₀ = >1161 µg product/bee	New Report KCP 10.3.1.1.2/01 Parker, T. (2020), Study report No.: 2863
<i>Bombus terrestris</i>	FF-075	96 and 48-hour, Oral	LD ₅₀ = >643 µg product/bee (Equivalent to 110 µg prothioconazole/bee and 81.9 µg azoxystrobin/bee)	New Report KCP 10.3.1.1.3/01 Wendling, K (2020) Report No. S19-03594
<i>Bombus terrestris</i>	FF-075	48-hour, Contact	LD ₅₀ = >800 µg product/bee (Equivalent to 136 µg prothioconazole/bee and >102 µg azoxystrobin/bee)	New Report KCP 10.3.1.1.3/01 Wendling, K (2020) Report No. S19-03594
<i>Apis mellifera</i>	FF-075	10-day feeding, Chronic Adult	10-day NOEDD = 6.10 µg product/bee/day (Equivalent to 1.04 µg prothionazole/bee/day and 0.78 µg azoxystrobin/bee/day) LDD ₁₀ = 9.13 µg product/bee/day	New Report KCP 10.3.1.2/01 Lozano, J. (2020), Study report No.: S20-00395
<i>Apis mellifera</i>	FF-075	22-day, Larval Repeated Exposure	NOED = 78.53 µg product/larva (measured concentration) (Equivalent to 13.38 µg prothionazole/bee/day and 10 µg azoxystrobin/bee/day)	New Report KCP 10.3.1.2/02 Lozano, J. (2020), Study report No.: S20-00396
Higher-tier studies (tunnel test, field studies)				
None Available				

Values highlighted in bold are used in the risk assessment.

9.6.1.1 Justification for new endpoints

A new chronic adult honeybee study, conducted to OECD 245 (2017), is submitted for the application. The study was conducted with the formulated product, FF-075, and the actual concentration of Prothioconazole in samples taken from test item feeding solutions was in the range from 80 to 120 % of the nominal concentrations, therefore the results are based on the nominal concentrations of the product. Consequently, for the purpose of the risk assessment, the endpoint will be compared to the application rate of the formulated product for each use.

A new 22-day repeated exposure study with honeybee larvae conducted to OECD 239 (2016) is submitted for the application. The study tested the effects of the formulated product, FF-075, on honeybee larvae. The measured concentrations of FF-075 in the diet were below the range of 80-120% of the nominal test concentration used in 13 of the 20 analysed samples. Since the correct dosage of nominal test item to diet could not be fully assured, the resulting endpoints used in the risk assessment are based on the mean analysed recovery (87.65 % of nominal).

9.6.2 Risk assessment

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

The Applicant recognizes the need to review the bee pollinator risk assessment based on scientific progress. However, the EFSA Bee Guidance Document issued in 2013⁵ has not been noted and therefore is not a realistically feasible way forward for assessing the chronic risk to honeybees. Therefore, the risk assessment, below, has been conducted following the EPPO 2010 scheme⁶ which provides a comparable level of protection to the EFSA approach and is based on the current scientific state of the art for bee pollinator risk assessment.

9.6.2.1 Hazard quotients for bees

Table 9.6-2: First-tier assessment of the acute risk for bees due to the use of FF-075 in winter and spring cereals and winter oilseed rape (BBCH 30-69 and BBCH 55-69, respectively)

Intended use		Winter and Spring Cereals (BBCH 30-69)	
Active substance		Prothioconazole	
Application rate (g/ha)		200	
Test design	LD₅₀ (lab.) (µg/bee)	Single application rate (g/ha)	Q_{HO}, Q_{HC} criterion: Q_H ≤ 50
Oral toxicity	> 71	200	<2.82
Contact toxicity	> 200		<1
Intended use		Winter oilseed Rape (BBCH 55-69)	
Active substance		Prothioconazole	
Application rate (g/ha)		160	
Test design	LD₅₀ (lab.) (µg/bee)	Single application rate (g/ha)	Q_{HO}, Q_{HC} criterion: Q_H ≤ 50
Oral toxicity	> 71	160	<2.25

⁵ European Food Safety Authority, 2013. EFSA Guidance Document on the risk assessment of plant protection products on bees (Apis mellifera, Bombus spp. and solitary bees). EFSA Journal 2013;11(7):3295, 268 pp.,

⁶ Environmental assessment scheme for plant protection products. OEPP/EPPO Bulletin Vol 40, Issue 3, December 2010, pp323-331

Contact toxicity	> 200		<0.8
Intended use		Winter and Spring Cereals (BBCH 30-69)	
Active substance		Azoxystrobin	
Application rate (g/ha)		150	
Test design	LD ₅₀ (lab.)	Single application rate	Q _{HO} , Q _{HC}
	(µg/bee)	(g/ha)	criterion: Q _H ≤ 50
Oral toxicity	> 25	150	<6
Contact toxicity	> 200		<0.75
Intended use		Winter oilseed Rape (BBCH 55-69)	
Active substance		Azoxystrobin	
Application rate (g/ha)		120	
Test design	LD ₅₀ (lab.)	Single application rate	Q _{HO} , Q _{HC}
	(µg/bee)	(g/ha)	criterion: Q _H ≤ 50
Oral toxicity	> 25	120	<4.8
Contact toxicity	> 200		<0.6
Intended use		Winter and Spring Cereals (BBCH 30-69)	
Formulated product		FF-075	
Application rate (g/ha)		2 x 1100 g/ha (14-day minimum interval)*	
Test design	LD ₅₀ (lab.)	Single application rate	Q _{HO} , Q _{HC}
	(µg/bee)	(g/ha)	criterion: Q _H ≤ 50
Oral toxicity	526.41	1100	2.09
Contact toxicity	>1161		<0.95
Intended use		Winter oilseed Rape (BBCH 355-69)	
Formulated product		FF-075	
Application rate (g/ha)		2 x 880 g/ha (14-day minimum interval)*	
Test design	LD ₅₀ (lab.)	Single application rate	Q _{HO} , Q _{HC}
	(µg/bee)	(g/ha)	criterion: Q _H ≤ 50
Oral toxicity	526.41	880	1.67
Contact toxicity	>1161		<0.75

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

* Application volume from GAP converted to mass based on density value 1.100 g/mL (KCP 2.6.1)

The HQ values for all scenarios are below the trigger value of 50, indicating low acute risk to honeybees from applications of FF-075 to winter and spring cereal and winter oilseed rape in accordance with the proposed GAP. Therefore, no further assessment is necessary.

The chronic adult risk assessment is based upon the EPPO 2010 risk assessment for systemic substances which is cited in the regulation as a current risk assessment scheme. It uses NOEDD values for the end-point so avoids the issues associated with the generation of LDD₅₀ values for substances of low toxicity and calculates exposure in a similar way to EFSA, 2013. The approach is also in line with other chronic risk assessments (e.g. birds and mammals). EPPO, 2010, recommended the calculation of a TER using the following equation: TER = NOEDD/daily dose. Daily dose (DD) is based on the worst-case sugar

requirement for a bee at 128 mg/bee/day (Rortais et al 2005)⁷ feeding exclusively from nectar containing 30% sugar. This value is compared to the adult NOEDD value for FF-075. The TER values are summarised, below.

Table 9.6-3: Chronic risk for adult honeybees due to the use of FF-075 in winter and spring cereals (BBCH 30-69, 2 x 1100 g FF-075/ha, 14-day application interval)

Intended use	Winter and spring cereals (BBCH 30-69) – 1100 g/ha (x 2 applied 14 days apart)					
Test design	Endpoint NOEDD µg product/bee/day	Single application rate (kg/ha)	Nectar consumption g	RUD Foliar spray mg/kg	Daily Dose µg a.s./bee ^a	TER
Lab Adult chronic oral (10d feeding – OECD)	6.10	1.10	0.427	2.9	1.36	4.49

^a Daily dose = App rate (0.128g/0.3) * RUD

Table 9.6-4: Chronic risk for adult honeybees due to the use of FF-075 in winter oilseed rape (BBCH 55-30-80), 2 x 880 g FF-075/ha, 14-day application interval)

Intended use	Oilseed rape (BBCH 30-80) - 880 g/ha (x 2 applied 14 days apart)					
Test design	Endpoint NOEDD µg product/bee/day	Single application rate (kg/ha)	Nectar consumption g	RUD Foliar spray mg/kg	Daily Dose µg a.s./bee ^a	TER
Lab Adult chronic oral (10d feeding – OECD)	6.10	0.880	0.427	2.9	1.08	5.65

^a Daily dose = App rate (0.128g/0.3) * RUD

The EPPO 2010 scheme proposes a trigger of 1 for assessment of the risk to honeybees. It is clear that with TER values >4 for FF-075 applied to winter and spring cereals, and winter oilseed rape in accordance with the GAP, that the proposed uses of FF-075 pose an acceptable chronic risk to adult bees.

Worst-case data from Rortais *et al.*, 2005⁷ as proposed in the EPPO scheme, have been used to estimate the consumption by bee larvae (198 mg in 5 days). In addition, worker larvae are considered to consume 2 mg pollen during their development phase (EFSA 2013). Thus, considering the mean RUD values for nectar and pollen in EFSA, 2013, (2.9 and 6.1, respectively), exposure can be estimated for the whole development period of 5 days. The concentration in the nectar and pollen can be calculated and compared with the NOED. These values have been compared to the adult NOED value for the active substance, prothioconazole according to the proposed uses and the TER values are summarised below.

Table 9.6-5: Chronic risk to honeybee larvae due to the use of FF-075 in winter and spring cereals (BBCH 30-69, (2 x 1100 g FF-075/ha, 14-day application interval)

⁷ Rortais A, Arnold G, Halm M-P, Touffet-Briens F (2005) Modes of honey bees exposure to systemic insecticides: estimated amounts of contaminated pollen and nectar consumed by different categories of bees. *Apidologie* 36: 71–83

Test design	Endpoint NOED µg prod- uct/larva/day	Single applica- tion rate (kg/ha)	Nectar dose ^a µg a.i./larvae	Pollen dose ^a µg a.i./larvae	Total dose µg a.i./larvae	TER
FF-075 Lab larval chronic (22d repeated exposure via diet)	78.53	1.10	0.63162	0.01342	0.64504	121.74

Assuming a foliar spray RUD of 2.9 for nectar and a RUD of 6.1 for pollen

^a Nectar dose = App rate * (0.198) * RUD, Pollen dose = App rate*(0.002)* RUD

Table 9.6-6: Chronic risk to honeybee larvae due to the use of FF-075 in winter oilseed rape (BBCH 30-69), (2 x 880 g FF-075/ha, 14-day application interval)

Test design	Endpoint NOED µg prod- uct/larva/day	Single applica- tion rate (kg/ha)	Nectar dose ^a µg a.i./larvae	Pollen dose ^a µg a.i./larvae	Total dose µg a.i./larvae	TER
FF-075 Lab larval chronic (22d repeated exposure via diet)	78.53	0.880	0.505296	0.010736	0.516032	152.18

Assuming a foliar spray RUD of 2.9 for nectar and a RUD of 6.1 for pollen

^a Nectar dose = App rate * (0.198) * RUD, Pollen dose = App rate*(0.002)* RUD

The EPPO 2010 scheme proposes a trigger of 1 for assessment of the risk to honeybees. It is clear that with TERs >100 for FF-075 there is a wide safety margin, indicating that the proposed uses pose an acceptable risk to bee larval development. Further assessment is not necessary.

zRMS comment:

The evaluation of the risk for bees was performed in accordance with the recommendations of the “Guidance Document on Terrestrial Ecotoxicology”, as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002) as according to conclusions of the Central Zone Steering Committee (CZSC), recommendations of EFSA (2013) should not be considered for the zonal evaluations until the guidance is noted at the EU level. Therefore, risk assessment based on indications of EFSA (2013) must be performed at the national level by CMS that do require such evaluation.

Based on the acute risk assessment with the consideration SANCO/10329/2002 rev.2 (final), October 17, 2002), HQ values for adult bees from exposure of Euskatel Pro are < 50, indicating an acceptable risk to adult bees.

Based on the chronic risk assessment with the consideration SANCO/10329/2002 rev.2 (final), October 17, 2002), HQ values from exposure of Euskatel Pro are >1, indicating an acceptable chronic risk to bees.

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

Since acceptable acute and chronic risks have been concluded for honeybees exposed to the active substance present in FF-075 at Tier 1 level, a higher-tier risk assessment is not required for the proposed uses of FF-075.

9.6.3 Effects on bumble bees

A new acute oral and contact study with the formulated product, FF-075, testing the effects against *Bombus terrestris*, has been submitted for the application. The requirement for acute studies with *Bombus terrestris* is outlined in the Guidance on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees), 2013, (EFSA Journal 2013;11(7):3295) and the data requirement is supported by a recognised standard method (OECD 247, 2017). The guidance document is yet to be noted and adopted and, therefore, there is currently no agreed risk assessment scheme by which the endpoints for this study should be assessed. However, the endpoints are unbound, high values, indicating low toxicity and suggesting that the proposed uses of FF-075 are unlikely to pose unacceptable acute risk to *Bombus terrestris*. The endpoints are reported in Table 9.6-1 for completeness.

9.6.4 Effects on solitary bees

Not relevant.

9.6.5 Overall conclusions

Regulatory testing with honeybees (*Apis mellifera*) and bumblebees (*Bombus terrestris*) has been conducted with prothioconazole, azoxystrobin and the formulated product, FF-075, in accordance with EU requirements for bees. An acceptable acute and long-term risk to bees is expected from the proposed uses of FF-075 in winter and spring cereals, and winter oilseed rape in accordance with the proposed GAP.

9.7 Effects on arthropods other than bees (KCP 10.3.2)

9.7.1 Toxicity data

Effects on non-target arthropods of FF-075 were not evaluated as part of the EU assessment of prothioconazole or azoxystrobin. New data submitted with this application are summarised in Appendix 2.

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

Species	Substance	Exposure System	Results	Reference
<i>Typhlodromus pyri</i> (protonymphs)	Azoxystrobin	Laboratory test glass plates (2D)	LR ₅₀ = >1500 g product/ha	EFSA Journal (2010); 8(4):1542 Taruza S., 2001. SYN-01-45
<i>Aphidius rhopalosiphi</i> (adults)	Azoxystrobin	Laboratory test glass plates (2D)	LR ₅₀ = >1000 g product/ha	EFSA Journal (2010); 8(4):1542 Stacey D., 2004. RJ3518B
<i>Typhlodromus pyri</i> (protonymphs)	FF-075	Laboratory test glass plates (2D)	LR ₅₀ = 2.189 L product/ha (Equivalent to 2407.9 g product/ha)*	New Study. KCP 10.3.2.1/01 Varela, S. (2021) Study report No.:

Species	Substance	Exposure System	Results	Reference
			ER ₅₀ = > 1300 L product/ha (Equivalent to 1430 g product/ha)*	S20-09657
<i>Aphidius rhopalosiphi</i> (adults)	FF-075	Laboratory test glass plates (2D)	LR ₅₀ = >12 L/ha product/ha (Equivalent to >13200 g product/ha)* ER ₅₀ = 1.495 L product/ha (Equivalent to 1644.5 g product/ha)*	New Study. KCP 10.3.2.1/02 Walter, C. & Stäbler, P. (2020) Study report No.: S19-04385
<i>Typhlodromus pyri</i> (protonymphs)	FF-075	Extended laboratory test detached apple leaves (3D)	LR ₅₀ = > 12000 g product/ha ER ₅₀ = >12000 g product/ha	New Study. KCP 10.3.2.1/03 Walter, C. and Stäbler, P (2020) Study report No.: S19-04389
<i>Aphidius rhopalosiphi</i> (adults)	FF-075	Extended laboratory test barley seedlings (3D)	LR ₅₀ = > 12000 g product/ha ER ₅₀ = >12000 g product/ha	New Study. KCP 10.3.2.1/04 Walter, C. and Stäbler, P (2020) Study report No.: S19-04386
<i>Coccinella septempunctata</i> (larvae)	FF-075	Extended laboratory test detached bean leaves (3D)	LR ₅₀ = >16 L product/ha (Equivalent to >17600 g product/ha)* ER ₅₀ = >16 L product/ha (Equivalent to >17600 g product/ha)*	New Study. KCP 10.3.2.1/05 Luna, F. (2020) Study report No.: S19- 04397
<i>Chrysoperla carnea</i> (larvae)	FF-075	Extended laboratory test detached bean leaves (3D)	LR ₅₀ = >12 L/ha product/ha (Equivalent to >13200 g product/ha)* ER ₅₀ = >12 L/ha product/ha (Equivalent to >13200 g product/ha)*	New Study. KCP 10.3.2.1/06 Walter, C. & Stäbler, P. (2019) Study report No.: S19-00968

Species	Substance	Exposure System	Results	Reference
Field or semi-field tests				
Not required, none performed.				

*Calculation based on a product density of 1.1 g/mL (KCP 2.6.1)

9.7.1.1 Justification for new endpoints

Data on the toxicity of the formulation to non-target arthropods is available and is used in the risk assessment.

9.7.2 Risk assessment

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

9.7.2.1 Risk assessment for in-field exposure

Table 9.7-2: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of FF-075 in winter and spring cereals (BBCH 30-69, 2 applications, 14-day application interval).

Intended use	Winter and spring cereals		
Product	Azoxystrobin		
Application rate (g/ha)	150		
MAF	1.7		
Test species	LR₅₀ (lab.)	PER_{in-field}	HQ_{in-field}
Tier I	(g/ha)	(g/ha)	criterion: HQ ≤ 2
<i>Typhlodromus pyri</i>	>1500	255	0.17
<i>Aphidius rhopalosiphi</i>	>1000		0.255
Intended use	Winter and spring cereals		
Product	FF-075		
Application rate (g/ha)	1100		
MAF	1.7		
Test species	LR₅₀ (lab.)	PER_{in-field}	HQ_{in-field}
Tier I	(g/ha)	(g/ha)	criterion: HQ ≤ 2
<i>Typhlodromus pyri</i>	2407.9	1870	0.78
<i>Aphidius rhopalosiphi</i>	>13200		0.14
Test species	LR₅₀ (lab.)	PER_{in-field}	PER_{in field} below rate with < 50% effect?

Tier II	(g/ha)	(g/ha)	
<i>Typhlodromus pyri</i>	>12000	1870	Yes
<i>Aphidius rhopalosiphi</i>	>12000		Yes
<i>Coccinella septempunctata</i>	>17600		Yes
<i>Chrysoperla carnea</i>	>13200		Yes

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

Table 9.7-3: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of FF-075 in winter oilseed rape (BBCH 55-69, 2 applications, 14-day application interval).

Intended use	Winter oilseed rape		
Product	Azoxystrobin		
Application rate (g/ha)	120		
MAF	1.7		
Test species	LR ₅₀ (lab.)	PER _{in-field}	HQ _{in-field}
Tier I	(g/ha)	(g/ha)	criterion: HQ ≤ 2
<i>Typhlodromus pyri</i>	>1500	204	0.136
<i>Aphidius rhopalosiphi</i>	>1000		0.204
Intended use	Winter oilseed rape		
Product	FF-075		
Application rate (g/ha)	880		
MAF	1.7		
Test species	LR ₅₀ (lab.)	PER _{in-field}	HQ _{in-field}
Tier I	(g/ha)	(g/ha)	criterion: HQ ≤ 2
<i>Typhlodromus pyri</i>	2407.9	1496	0.62
<i>Aphidius rhopalosiphi</i>	>13200		0.11
Test species	LR ₅₀ (lab.)	PER _{in-field}	PER _{in field} below rate with ≤ 50% effect?
Tier II	(g/ha)	(g/ha)	
<i>Typhlodromus pyri</i>	>12000	1496	Yes
<i>Aphidius rhopalosiphi</i>	>12000		Yes
<i>Coccinella septempunctata</i>	>17600		Yes
<i>Chrysoperla carnea</i>	>13200		Yes

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

9.7.2.2 Risk assessment for off-field exposure

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

Table 9.7-4: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of FF-075 in winter and spring cereals (BBCH 30-69, 2 applications, 14-day application interval).

Intended use	Winter and spring cereals				
Product	Azoxystrobin				
Application rate (g/ha)	150				
MAF	1.7				
vdf	40 5 for 2D				
Test species	LR ₅₀ (lab.)	Drift rate	PER _{off-field}	CF	HQ _{off-field}
Tier I	(g/ha)		(g/ha)		criterion: HQ ≤ 2
<i>Typhlodromus pyri</i>	>1500		6.069		≤0.04
<i>Aphidius rhopalosiphi</i>	>1000		12.138		≤0.08
Intended use	Winter and spring cereals				
Product	FF-075				
Application rate (g/ha)	1100				
MAF	1.7				
vdf	40 5 for 2D, 3D -none				
Test species	LR ₅₀ (lab.)	Drift rate	PER _{off-field}	CF	HQ _{off-field}
Tier I	(g/ha)		(g/ha)		criterion: HQ ≤ 2
<i>Typhlodromus pyri</i>	2407.9		44.506		0.18
<i>Aphidius rhopalosiphi</i>	>13200		89.012		0.36
Test species	LR ₅₀ (lab.)	Drift rate	PER _{off-field}	CF	PER _{off field} below rate with ≤ 50% effect?
Tier II	(g/ha)		(g/ha)		
<i>Typhlodromus pyri</i>	>12000		44.506 (2 D)		Yes
<i>Aphidius rhopalosiphi</i>	>12000		222.53 (3D)		Yes
<i>Coccinella septempunctata</i>	>17600			Yes	

<i>Chrysoperla carnea</i>	>13200			Yes
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MAF: Multiple application factor; vdf: Vegetation distribution factor; (corr.) PER: (corrected) Predicted environmental rate; CF: Correction factor; HQ: Hazard quotient. Criteria values shown in bold breach the relevant trigger.

Table 9.7-5: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of FF-075 in winter oilseed rape (BBCH 55-69, 2 applications, 14-day application interval).

Intended use	Winter oilseed rape				
Product	Azoxystrobin				
Application rate (g/ha)	120				
MAF	1.7				
vdf	10 5 for 2D, 3D -none				
Test species	LR₅₀ (lab.)	Drift rate	PER_{off-field}	CF	HQ_{off-field}
Tier I	(g/ha)		(g/ha)		criterion: HQ ≤ 2
<i>Typhlodromus pyri</i>	>1500	2.38%	4.8552	10	0.032
<i>Aphidius rhopalosiphi</i>	>1000		9.71		0.064
					0.049
					0.098
Intended use	Winter oilseed rape				
Product	FF-075				
Application rate (g/ha)	880				
MAF	1.7				
vdf	10 5 for 2D, 3D -none				
Test species	LR₅₀ (lab.)	Drift rate	PER_{off-field}	CF	HQ_{off-field}
Tier I	(g/ha)		(g/ha)		criterion: HQ ≤ 2
<i>Typhlodromus pyri</i>	2407.9	2.38%	35.6048	10	0.15
<i>Aphidius rhopalosiphi</i>	>13200		71.2096		0.30
					0.027
					0.054
Test species	LR₅₀ (lab.)	Drift rate	PER_{off-field}	CF	PER_{off-field} below rate with ≤ 50% effect?
Tier II	(g/ha)		(g/ha)		
<i>Typhlodromus pyri</i>	>12000	2.38%	35.6048 (2D) 178.024 (3D)	5	Yes
<i>Aphidius rhopalosiphi</i>	>12000				Yes
<i>Coccinella septempunctata</i>	>17600				Yes
<i>Chrysoperla carnea</i>	>13200				Yes

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

At Tier II the PER in-field or off-field values for both GAP scenarios are below the 50% effect rate for all species, indicating low risk to terrestrial non target arthropods in the in-field or off-field, from applications of FF-075 at rates up to 1100 g FF-075/ha. Therefore, no further assessment is necessary.

zRMS comments:

The calculations of the risk assessment for in – field for Euskatel Pro for two indicator species were verified by zRMS-PL.

HQ in -field and HQ-off field are below 2 based on laboratory studies (Tier1).

The PER-in and PER_{off}-field corrected for T.Pyri and A. rhopalosiphi and for additional species (based on the extended laboratory studies) are below the rate with ≤ 50 % effects. Therefore, this assessment indicates that Euskatel Pro poses low risk to in-field and off-field non-target arthropods following application according to the proposed use patterns.

9.7.2.3 Additional higher-tier risk assessment

Since acceptable acute risks have been concluded for non-target arthropods exposed to FF-075 at the Tier II level, an additional higher-tier risk assessment is not required for the proposed uses of FF-075.

9.7.2.4 Risk mitigation measures

No risk mitigation needed.

9.7.3 Overall conclusions

Regulatory testing with the non-target arthropod indicator species *Typhlodromus pyri*, *Aphidius rhopalosiphi*, *Coccinella septempunctata* and *Chrysoperla carnea* has been conducted with FF-075 in accordance with EU requirements. Results from these studies, show that FF-075 has low toxicity to non-target arthropods. At Tier II the PER in-field or off-field values for both GAP scenarios are below the 50% effect rate for all species, indicating a low risk to non-target arthropods within the treated fields, and adjacent untreated habitat.

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

9.8.1 Toxicity data

Studies on the toxicity to earthworms have been carried out with the azoxystrobin and the relevant metabolites, as well as the relevant metabolites of prothioconazole, prothioconazole-desthio (M04) and prothioconazole-S-methyl (M01). Full details of these studies are provided in the respective EFSA Journal (2010) for azoxystrobin, EU prothioconazole DAR, (2005), and EFSA Conclusions, (2007) for prothioconazole .

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of FF-075 were not evaluated as part of the EU assessment of prothioconazole. New data submitted with this application are summarised in Appendix 2.

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

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

Species	Substance	Exposure System	Results	Reference
<i>Eisenia fetida</i>	Prothioconazole-desthio (M04)	Mixed into substrate 56 d, chronic 10 % peat content	NOEC = 1.0 mg metabolite/kg dw EC ₁₀ = 1.05 mg metabolite/kg dw NOEC _{corr} = 0.5 mg metabolite/kg dw* 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.	EFSA Scientific Report (2007) 106, 1-98. DAR, V.3, Annex B, B.9, (2005). Meisner,2000d MPE/RG 332/00
<i>Eisenia fetida</i>	Prothioconazole-S-methyl (M01)	Mixed into substrate 56 d, chronic 10 % peat content	NOEC = 100 mg metabolite/kg dw EC ₁₀ = 342 mg metabolite/kg dw NOEC _{corr} = 50 mg metabolite/kg dw*	EFSA Scientific Report (2007) 106, 1-98. DAR, V.3, Annex B, B.9, (2005). Heimbach,2000b. HBF/RG 317
<i>Eisenia fetida</i>	YF 10537 (Azoxystrobin single-active formulation)	Mixed into substrate 56 d, chronic 10 % peat content	NOEC = 20 mg a.s./kg dw NOEC _{corr} = 10 mg metabolite/kg dw*	EFSA Journal (2010); 8(4):1542 Moser T, Rombke J, 2000. ICI5504/09
<i>Eisenia fetida</i>	FF-075	Mixed into substrate 56 d, 10 % peat content.	NOEC = 56 mg product/kg dw NOEC _{corr} = 28 mg product/kg dw* EC ₁₀ = 95.9 mg product/kg dw EC _{10corr} = 49.5 mg product/kg dw	New Report KCP 10.4.1.1/01 Parker, T. 2021 Study No. 2886
<i>Folsomia candida</i>	Prothioconazole	Mixed into substrate 28 d, chronic 10 % peat content	NOEC = 64 mg a.s./kg dw NOEC _{corr} = 32 mg a.s./kg dw*	EFSA Scientific Report (2007) 106, 1-98. DAR, V.3, Annex B, B.9, (2005).

Species	Substance	Exposure System	Results	Reference
				Nienstedt,2002. Report No. 1022.028.641.
<i>Folsomia candida</i>	Prothioconazole-desthio (M04)	Mixed into substrate 28 d, chronic 10 % peat content	NOEC = 62.5 mg metabolite/kg dw NOEC _{corr} = 31.25 mg metabolite/kg dw*	EFSA Scientific Report (2007) 106, 1-98. DAR, V.3, Annex B, B.9, (2005). Moser & Römbke, 2002. Report No. P1CR.
<i>Folsomia candida</i>	Prothioconazole-S-methyl (M01)	Mixed into substrate 28 d, chronic 10 % peat content	NOEC = 31.6 mg metabolite/kg dw NOEC _{corr} = 15.8 mg metabolite/kg dw*	EFSA Scientific Report (2007) 106, 1-98. DAR, V.3, Annex B, B.9, (2005). Moser & Scheffczyk, 2001. P35CR
<i>Hypoaspis aculeifer</i>	Prothioconazole	Mixed into substrate 14 d, chronic LUF 2.1 field soil	NOEC = >100 mg a.s./kg dw NOEC _{corr} = ≥50 mg a.s./kg dw*	EFSA Scientific Report (2007) 106, 1-98. DAR, V.3, Annex B, B.9, (2005). Hoogendoorn, 2000. Report No. B060HAE
<i>Folsomia candida</i>	YF 10537 (Azoxystrobin single-active formulation)	Mixed into substrate 28 d, chronic 10 % peat content	NOEC = 50 mg a.s./kg dw NOEC _{corr} = 25 mg a.s./kg dw*	EFSA Journal (2010); 8(4):1542 Barth M., 2001. ICI5504/1319
<i>Folsomia candida</i>	FF-075	Mixed into substrate 28 d, chronic 5% peat content	NOEC _{reproduction} = 100.0 mg product/kg dw soil NOEC _{corr} = 50 mg product/kg dw soil* . EC ₁₀ = 134.6 mg product/kg dw soil EC _{10corr} = 67.3 mg/kg dw soil*	New Report KCP 10.4.2/01 Senn, L (2021) Study report No.: 20AV6CR.
<i>Hypoaspis aculeifer</i>	FF-075	Mixed into substrate 14 d, chronic 5% peat content	NOEC = >1000 mg product/kg dw soil NOEC _{corr} = >500 mg product/kg dw soil*	New Report KCP 10.4.2.1/02 Senn, L (2021), Study report No.: 20AV3HR.

Species	Substance	Exposure System	Results	Reference
Field studies				
<i>Lumbricius terrestris</i> , <i>L. rubellus</i> , <i>L. castanea</i> , <i>Aporrectodea caliginosa</i> , <i>A. terrestris longa</i> . Prothioconazole EC 250 applied 3 × 200 g a.s./ha in a grass-land field site. 5 different species identified and assessed. 46% reduction in the number of <i>A. caliginosa</i> juveniles 7 weeks after first application (2 weeks after final application). No adverse effect 5 months after first application. (Maximum measured soil PEC 0.052 mg prothioconazole/kg based on soil sampling depth of 10 cm which is equivalent to a soil PEC of 0.104 mg prothioconazole/kg over the standard 5 cm depth). No further refinement needed to support A1 representative uses (0.2 kg a.s./ha in cereals, 0.175 kg a.s./ha in OSR). EFSA (2007) risk assessment trigger = 1			EFSA Scientific Report (2007) 106, 1-98 DAR, V.3, Annex B, B.9, (2005). Lechelt-Kuntze, 2002. .	
Litter bag test				
A12705A (a formulation containing azoxystrobin) was assessed in a litter bag study at a concentration equivalent to 0.5514 mg a.s./kg over a depth of 10 cm. The litter bag study was conducted at a rate of approximately 85% that of the peak plateau concentration. Ideally the study should have been carried out at the peak plateau concentration, however, given the lack of effects in this study, and the corresponding earthworm and <i>F. candida</i> results, it was felt that the risk to soil macroorganisms responsible for leaf litter breakdown has been addressed. 5.0% difference in the degradation of the leaf litter compared to the control after 188 days. Total degradation in the control was 64±10.9% compared to 60.7±10.6% in the azoxystrobin treatment.			EFSA Journal (2010); 8(4):1542 Kollmann S., 2004. ICI15504/2319	

Values highlighted are used in the risk assessment.

* The log Pow of prothioconazole, azoxystrobin and the –S-methyl (M01) and desthio (M04) metabolites is >2. Toxicity endpoints corrected by dividing by a factor of 2 in accordance with the EPPO earthworm scheme 2002. (EFSA Scientific Report (2007) 106, 1-98).

9.8.1.1 Justification for new endpoints

Additional chronic earthworm studies with the metabolites of azoxystrobin, R234886, R401553 and R402173 were not submitted for the purpose of the renewal of the active substance (2010). The risk from metabolite R234886 was considered addressed by the litter bag study (EFSA Journal (2010); 8(4):1542). Additionally, metabolites R401553 and R402173 are formed as a result of photolytic degradation and have half lives of 1.1 and 4.7 days, respectively, and do not trigger the need for additional information.

Commission Regulation 283/2013 requires testing with *Hypoaspis aculeifer* with either a single active formulation or the active substance. A study is not available for azoxystrobin. However, during the latest renewal of azoxystrobin (2010) it was concluded that the risk to soil macro-organisms responsible for leaf litter breakdown was sufficiently addressed with a leaf litter breakdown study.

The geometric mean of the DT₅₀ of azoxystrobin in soil is 10.7 days, whilst the DT_{90field} is 600.4 days. The normalised DT_{50field} and DT₉₀ field are 80.2 and 266.4 days. According to the Annex III point 10.6.2, if the DT_{90field} is greater than 365 days then a litter bag study is required.

The Notifier for the renewal for the active substance submitted a new litter bag study (Kollmann, S.L., 2004) testing the effects of 0.5514 mg a.s./kg. The study was considered acceptable. A 5% difference in the degradation of the leaf litter was found compared to the control at 188 days. Total degradation in the control was 64±10.9% compared to 60.7±10.6% in the azoxystrobin treatment.

9.8.2 Risk assessment

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

9.8.2.1 First-tier risk assessment

The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.

Table 9.8-2: First-tier assessment of the acute and chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of FF-075 in winter and spring cereals (BBCH 30-69).

Intended use	Winter and spring cereals (BBCH 30-69)		
Chronic effects on earthworms			
Substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{It} (criterion TER ≥ 5)
Prothioconazole-desthio (M04)	0.5 _{corr}	0.0569	8.79
Prothioconazole-S-methyl (M01)	50 _{corr}	0.0163	3067.48
Azoxystrobin	10 _{corr}	0.1292	77.40
FF-075	28 _{corr}	0.2933	95.47
Chronic effects on other soil macro- and mesofauna: <i>Folsomia candida</i>			
Substance	NOEC/EC ₁₀ (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{It} (criterion TER ≥ 5)
Prothioconazole	≥32 _{corr}	0.0550	≥581.82
Prothioconazole-desthio (M04)	31.25 _{corr}	0.0569	549.21
Prothioconazole-S-methyl (M01)	≥15.8 _{corr}	0.0163	≥969.33
Azoxystrobin	25 _{corr}	0.1292	193.50
FF-075	50 _{corr}	0.2933	170.47
Chronic effects on other soil macro- and mesofauna: <i>Hypaspis aculeifer</i>			
Substance	NOEC/EC ₁₀ (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{It} (criterion TER ≥ 5)
Prothioconazole	≥50 _{corr}	0.0550	≥909.09
FF-075	>500 _{c_{corr}}	0.2933	1,705

TER values shown in bold fall below the relevant trigger.

Table 9.8-3: First-tier assessment of the acute and chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of FF-075 in winter oilseed rape (BBCH 55-69).

Intended use	Winter oilseed rape (BBCH 55-69)		
Chronic effects on earthworms			
Metabolites and formualtion	NOEC	PEC _{soil}	TER _{It}

	(mg/kg dw)	(mg/kg dw)	(criterion TER ≥ 5)
Prothioconazole-desthio (M04)	0.5 _{corr}	0.0456	10.96
Prothioconazole-S-methyl (M01)	50 _{corr}	0.0130	3846.0
Azoxystrobin	10 _{corr}	0.1033	96,81
FF-075	28 _{corr}	0.2347	119.31

Chronic effects on other soil macro- and mesofauna: *Folsomia candida*

Active substance and metabolites	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{lt} (criterion TER ≥ 5)
Prothioconazole	≥32 _{corr}	0.0440	≥727.27
Prothioconazole-desthio (M04)	31.25 _{corr}	0.0456	685.31
Prothioconazole-S-methyl (M01)	≥15.8 _{corr}	0.0130	≥1215.40
Azoxystrobin	25 _{corr}	0.1033	242.01
FF-075	50 _{corr}	0.2347	213.04

Chronic effects on other soil macro- and mesofauna: *Hypoaspis aculeifer*

Active substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{lt} (criterion TER ≥ 5)
Prothioconazole	≥50 _{corr}	0.0440	≥1136.36
FF-075	>500 _{corr}	0.2347	2130.38

TER values shown in bold fall below the relevant trigger.

All TER_{lt} values calculated above are above the trigger of 5. Prothioconazole, azoxystrobin, FF-075, and the relevant metabolites of prothioconazole are not expected to pose a long-term risk to earthworms or other soil macro-organisms. Overall, use of FF-075 in winter and spring cereals (BBCH 30-69) and winter oilseed rape (BBCH 55-69) is not expected to pose a risk to earthworms and other soil macro-organisms. Therefore, no further assessment is necessary.

zRMS comment:

The acute and chronic TER values for active substances metabolites M01 and M04 and ppp Euskatel Pro and their metabolites for *Folsomia candida* were above the relevant Annex VI trigger of 5.

Therefore, it is concluded that Euskatel Pro and both active substans and metabolite M01 and M04 do not pose long-term risk to earthworms and other soil macro- and mesofauna when applied according to the proposed uses rates

9.8.2.2 Higher-tier risk assessment

Since acceptable chronic risks have been concluded for earthworms and non-target soil organisms exposed to FF-075 at the Tier 1 level, an additional higher-tier risk assessment is not required for the proposed uses of FF-075.

The geometric mean of the DT₅₀ of azoxystrobin in soil is 10.7 days, whilst the DT_{90field} is 600.4 days. The normalised DT_{50field} and DT₉₀ field are 80.2 and 266.4 days. According to the Annex III point 10.6.2, if the DT_{90field} is greater than 365 days then a litter bag study is required.

Table 9.8-4: Higher-tier assessment of the effects of applications of azoxystrobin on soil macro-organisms through a field litter bag study.

Test substance	Test System	Time scale	Soil PEC	TER	Trigger
A12705A	Straw degradation in soil	Max. 5% deviation from after 181 days control straw degradation @0.5514 mg.a.s./kg d. wt. soil	Winter and spring cereals (BBCH 30-69) = 0.1292 mg a.s./kg soil.	Less than 10% effect at maximum PEC	10% ¹
A12705A	Straw degradation in soil	Max. 5% deviation from after 181 days control straw degradation @0.5514 mg.a.s./kg d. wt. soil	Winter oilseed rape (BBCH 55-69) = 0.1033 mg a.s./kg soil	Less than 10% effect at maximum PEC	10% ¹

¹ Threshold proposed by EPFES guidance.

9.8.3 Overall conclusions

Regulatory testing with *Folsomia candida* and *Hypoaspis aculeifer* has been conducted with azoxystrobin, prothioconazole and the relevant soil metabolites. Regulatory testing with earthworms (*Eisenia fetida*), has been conducted with azoxystrobin, the relevant metabolites of azoxystrobin, and the relevant metabolites of prothioconazole, prothioconazole-desthio (M04) and prothioconazole-S-methyl (M01) in accordance with EU requirements for soil macrofauna. Results from the studies with prothioconazole, azoxystrobin and the relevant metabolites, summarised in the EFSA Review Reports, show that the active substance and the studies with the relevant metabolites have low toxicity to soil macrofauna. Regulatory testing with earthworms, *Folsomia candida* and *Hypoaspis aculeifer* has also been conducted with FF-075. Results from these studies show that FF-075 has low toxicity to non-target soil organism.

All acute and long-term TER values were calculated to be in excess of the accepted trigger values and a low risk for non-target soil organisms was concluded.

9.9 Effects on soil microbial activity (KCP 10.5)

9.9.1 Toxicity data

Studies of effects on soil microorganisms have been conducted with prothioconazole and the relevant metabolites of prothioconazole and azoxystrobin; prothioconazole-desthio (M04), prothioconazole-S-methyl (M01), R234886, R401553 and R402173. Full details of these studies are provided in the respec-

tive EFSA conclusions (2007 and 2010).

In the original DAR (1998) for azoxystrobin, two studies were submitted on soil microbial processes, one on nitrogen mineralisation and one on the carbon mineralisation. The endpoints for these studies showed no effect up to 2.5 kg a.s./ha (250 SC). For the purpose of the renewal of azoxystrobin (2010), data on the nitrogen transformation for the soil metabolites R234886, R401553 and R402173 were submitted and have been considered in the risk assessment, below.

Effects on soil microorganisms of FF-075 were not evaluated as part of the EU assessment of prothioconazole. New data submitted with this application are summarised in Appendix 2.

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

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

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	Prothioconazole	28 d, aerobic silty sand soil	NO ₃ formation rate NOEC = ≥2.71 mg a.s. /kg dw (2.0 kg a.s./ha equiv)	EFSA Scientific Report (2007) 106, 1-98. DAR, V.3, Annex B, B.9, (2005). Anderson, 1999b. AJO/203199
N-mineralisation	Prothioconazole-desthio (M04)	28 d, aerobic silty sand soil	NO ₃ formation rate NOEC = ≥0.27 mg /kg dw (0.2 kg/ha equiv)	EFSA Scientific Report (2007) 106, 1-98. DAR, V.3, Annex B, B.9, (2005). Anderson, 2000. AJO/209400
N-mineralisation	Prothioconazole - S-methyl (M01)	28 d, aerobic silty sand soil	NO ₃ formation rate NOEC = ≥2.69 mg /kg dw (2.0 kg/ha equiv)	EFSA Scientific Report (2007) 106, 1-98. DAR, V.3, Annex B, B.9, (2005). Anderson, 1999d. JO/203399
N-mineralisation	Azoxystrobin (250 SC)	28 d, aerobic silty sand soil	No effect up to 2.5 kg a.s./ha. (Equivalent to 3.33 mg a.s./kg dry wt. soil)	EFSA Journal (2010); 8(4):1542 Mason, G., Prevett, A., and Tarry, A.R., 1994. BMF 95-00030
N-mineralisation	R234886	28 d, aerobic silty sand soil	No effect at 1 and 10 mg/kg soil dry wt.	EFSA Journal (2010); 8(4):1542

Endpoint	Substance	Exposure System	Results	Reference
				Lemnitzer B, 2002 R234886/0002
N-mineralisation	R401553	28 d, aerobic silty sand soil	No effect at 0.528 and 2.63 mg test item/kg dry wt. soil.	EFSA Journal (2010); 8(4):1542 Schulz L. 2008. SYN501657/0007
N-mineralisation	R402173	28 d, aerobic silty sand soil	No effect at 0.826 and 4.131 mg test item/kg dry wt.	EFSA Journal (2010); 8(4):1542 Schulz L. 2008a SYN501114/0002
N-mineralisation	FF-075	28 d, aerobic silty sand soil	NO ₃ formation rate NOEC = ≥6.845 mg product/kg dw	New Report KCP 10.5/01 Li, 2021 2864

Values highlighted in bold are used in the risk assessment.

9.9.1.1 Justification for new endpoints

Data on the toxicity of the formulation to soil organisms is available and is used in the risk assessment.

9.9.2 Risk assessment

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

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

Table 9.9-2: Assessment of the risk for effects on soil micro-organisms due to the use of FF-075 in cereals (BBCH 30-69)

Intended use	Winter and spring cereals, BBCH 30-69		
N-mineralisation			
Product/active substance	Max. conc. with effects ≤ 25 % (mg/kg dw)	PEC _{soil} (mg/kg dw)	Risk acceptable?
Prothioconazole	≥2.71	0.0550	Yes
Prothioconazole-desthio (M04)	≥0.27	0.0569	Yes
Prothioconazole-S-methyl (M01)	≥2.69	0.0163	Yes
Azoxystrobin	≥3.33	0.1292	Yes

R234886	≥ 10	0.0222	Yes
R401553	≥ 2.643	0.0072	Yes
R402173	≥ 4.131	0.0112	Yes
FF-075	≥ 6.845	0.2933	Yes

Table 9.9-3: Assessment of the risk for effects on soil micro-organisms due to the use of FF-075 in winter oilseed rape (BBCH 55-69)

Intended use	Winter oilseed rape, BBCH 55-69		
N-mineralisation			
Product/active substance	Max. conc. with effects ≤ 25 % (mg/kg dw)	PEC _{soil} (mg/kg dw)	Risk acceptable?
Prothioconazole	≥2.71	0.0440	Yes
Prothioconazole-desthio (M04)	≥0.27	0.0456	Yes
Prothioconazole-S-methyl (M01)	≥2.69	0.0130	Yes
Azoxystrobin	≥3.33	0.1033	Yes
R234886	≥10	0.0178	Yes
R401553	≥2.643	0.0058	Yes
R402173	≥4.131	0.0090	Yes
FF-075	≥6.845	0.2347	Yes

zRMS comment:

The risk assessment for soil micro-organism after exposure of both active substances and their metabolites has accepted by the zRMS. The effects on the nitrogen transformations are acceptable (<25%) at concentration which is higher than the maximum relevant PEC_s for the maximum application rate of active substances and the product Euskatel Pro.

9.9.3 Overall conclusions

Regulatory testing with soil microorganisms has been conducted with FF-075, prothioconazole, azoxystrobin and the relevant metabolites in accordance with EU requirements. Results from these studies, summarised in the EFSA Review Reports for prothioconazole, and Appendix 2 (FF-075), show that

the formulated product, active substance and relevant metabolites have low toxicity to soil microorganisms.

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

9.10.1 Toxicity data

Studies on the toxicity to non-target terrestrial plants have been carried out with prothioconazole and azoxystrobin. Full details of these studies are provided in the respective EU DAR, (2005), and EFSA Conclusions, (2007).

Screening data for azoxystrobin was originally assessed during the original inclusion of azoxystrobin (1998). The data assessed the effects of azoxystrobin on 25 species in pre-emergence situations and 27 species in post-emergence situations with an application rate of 4 kg a.s./ha. Effects were below the trigger for further data for non-herbicides. However, the data is not included in the agreed list of endpoints in the recent EFSA Journals (EFSA Journal (2010); 8(4):1542) and has, therefore, not been considered in the risk assessment, below. Additional data for non-target plants was submitted for the purpose of the renewal of azoxystrobin in 2010 and is included in the agreed list of endpoints (EFSA Journal (2010); 8(4):1542). The data was reported in “Design of field experiments and the measurement and analysis of plant responses. Pages 15-23 in B. Truelove, ed. Research Methods in Weed Science, Frans et al. 1997 (Porch et al, 2002).”. The most sensitive species were lettuce, radish and wheat (ER₅₀ emergence >20 mg a.s./kg soil). This data has been considered in the following risk assessment.

Effects on non-target terrestrial plants of FF-075 were not evaluated as part of the EU assessment of prothioconazole. New data submitted with this application are summarised in Appendix 2.

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

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

Species	Substance	Exposure System	Results	Reference
<i>Amaranthus retroflexus</i>	Prothioconazole	21 d Seedling emergence	200 g a.s./ha (tested application rate) = 5% phytotoxic effects	EFSA Scientific Report (2007) 106, 1-98. DAR, V.3, Annex B, B.9, (2005). Meisner, P: Kolb, U, 2000. MPE NTP
<i>Amaranthus retroflexus</i> , <i>Beta vulgaris</i>	Prothioconazole	21 d Vegetative vigour	250 g a.s./ha (tested application rate) = 10 % phytotoxic effects	EFSA Scientific Report (2007) 106, 1-98. DAR, V.3, Annex B, B.9, (2005).

Species	Substance	Exposure System	Results	Reference
				Meisner, P: Kolb, U, 2000. MPE NTP
Lettuce, radish and wheat	Azoxystrobin	Seedling emergence	ER ₅₀ emergence = >20 mg a.s./kg soil (> 15000 g a.s./ha)	EFSA Journal (2010); 8(4):1542 Porch JR, Krueger H O. 2002. ICI5504/1376
1) <i>Allium cepa</i> (m) 2) <i>Avena sativa</i> (m) 3) <i>Brassica rapa</i> (d) 4) <i>Cucumis sativus</i> (d) 5) <i>Pisum sativum</i> (d) 1) <i>Solanum lycopersicum</i> (d)	FF-075	21 d Vegetative vigour	ER ₅₀ plant weight = > 8.52 L product /ha ER ₅₀ plant height = >8.52 L product /ha	New Study. KCP 10.6/02 Förster, B (2021) Study report No.: 20AV6PB
6) <i>Allium cepa</i> (m) 7) <i>Avena sativa</i> (m) 8) <i>Brassica rapa</i> (d) 9) <i>Cucumis sativus</i> (d) 10) <i>Pisum sativum</i> (d) 11) <i>Solanum lycopersicum</i> (d)	FF-075	21 d Seedling emergence	¹⁾ ER ₅₀ plant weight = > 8.5 L product /ha ²⁾ ER ₅₀ plant height = >8.5 L product /ha	New Study. KCP 10.6/01 Förster, B (2021) Study report No.: 20AV6PA

m: monocotyledonous; d: dicotyledonous

9.10.1.1 Justification for new endpoints

New GLP Tier 2 seedling emergence and vegetative vigour studies, performed with the dual-active formulated product, FF-075, have been submitted for evaluation to support the registration of FF-075.

9.10.2 Risk assessment

9.10.2.1 Tier-1 risk assessment (based screening data)

Tests at rates up to 200 g a.s./ha for seedling emergence and 250 g a.s./ha for vegetative vigour were conducted with prothioconazole a.s. technical material and effects were below the critical threshold as defined by the “Guidance Document on Terrestrial Ecotoxicology”, (SANCO/10329/2002 rev.2 final, 2002). Tested concentrations at which < 50% phytotoxic effects were observed exceed the highest field application rate in winter and spring cereals (BBCH-30-69) and winter oilseed rape (BBCH 30-80) and are thus considered an indicator for an acceptable risk.

Screening data for azoxystrobin was originally assessed during the original inclusion of azoxystrobin (1998). The data assessed the effects of azoxystrobin on 25 species in pre-emergence situations and 27 species in post-emergence situations with an application rate of 4 kg a.s./ha. Effects were below the trigger for further data for non-herbicides. However, the data is not included in the agreed list of endpoints in the recent EFSA Journals (EFSA Journal (2010); 8(4):1542) and has, therefore, not been considered in the risk assessment.

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

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

Table 9.10-2: Assessment of the risk for non-target plants due to the use of FF-075 in winter oilseed rape (BBCH 55-69).

Intended use		Winter Oilseed Rape (BBCH 55-69)		
Active substance/product		Azoxystrobin		
Application rate (g/ha)		2 x 120 g/ha (14-day interval)		
MAF		1.7		
Test species	ER₅₀ (g/ha)	Drift rate	PER_{off-field} (g/ha)	TER criterion: TER ≥ 5
Lettuce, radish and wheat Seedling emergence	>15000	2.77% (1m)	5.65	>2654.9

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

Table 9.10-3: Assessment of the risk for non-target plants due to the use of FF-075 in winter and spring cereals (BBCH 30-69).

Intended use		Winter and spring cereals (BBCH 30-69)		
Active substance/product		Azoxystrobin		
Application rate (g/ha)		2 x 150 g/ha (14-day interval)		
MAF		1.7		
Test species	ER₅₀ (g/ha)	Drift rate	PER_{off-field} (g/ha)	TER criterion: TER ≥ 5
2 monocot +4 dicot species Seedling emergence	>15000	2.77% (1m)	7.064	2123.4

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

Table 9.10-4: Assessment of the risk for non-target plants due to the use of FF-075 in winter oilseed rape (BBCH 55-69).

Intended use		Winter Oilseed Rape (BBCH 55-69)		
Active substance/product		FF-075		
Application rate (g/ha)		2 x 0.8 L/ha (14-day interval)		

MAF		1.7		
Test species	ER₅₀ (L/ha)	Drift rate	PER_{off-field} (L/ha)	TER criterion: TER ≥ 5
2 monocot +4 dicot species Seedling emergence	>8.52	2.77% (1m)	0.0377	>226
2 monocot +4 dicot species Vegetative vigour	>8.5	2.77% (1m)	0.0377	>225

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

Table 9.10-5: Assessment of the risk for non-target plants due to the use of FF-075 in winter and spring cereals (BBCH 30-69).

Intended use	Winter and spring cereals (BBCH 30-69)			
Active sub-stance/product	FF-075			
Application rate (g/ha)	2 x 1.0 L/ha (14-day interval)			
MAF	1.7			
Test species	ER₅₀ (L/ha)	Drift rate	PER_{off-field} (L/ha)	TER criterion: TER ≥ 5
2 monocot +4 dicot species Seedling emergence	>8.52	2.77% (1m)	0.04709	>180.93
<i>Brassica rapa</i> Vegetative vigour	>8.5	2.77% (1m)	0.04709	>180.51

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

9.10.2.3 Higher-tier risk assessment

Not relevant.

9.10.2.4 Risk mitigation measures

No risk mitigation needed.

9.10.3 Overall conclusions

The TER values considering the data gathered from the seedling emergence and vegetative vigour studies. for FF-075 are greater than the predicted exposure rates derived from the treatment of winter oilseed rape (BBCH 55-69) and winter and spring cereals (BBCH 30-69) at a distance of 1m from the treated field. Acceptable risk to non-target higher plants is concluded from uses of FF-075 in accordance with the GAP.

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

A study on the effects of the active substances, prothioconazole and azoxystrobin, on the respiration activity of activated sludge was available from the Draft Assessment Reports, (2005), and the EU evaluation of prothioconazole, (2007), and the EFSA Journal (EFSA Journal (2010); 8(4):1542) for azoxystrobin. The study was considered acceptable for the risk assessment of effects on biological methods of sewage treatment.

Table 9.11-1: Endpoints and effect values relevant for the risk assessment for activated sludge.

Media	Substance	Process	EC ₅₀ (mg/L)	Reference
Activated sludge	Prothioconazole	Respiration rate	EC ₅₀ = >10,000 mg a.s./L	EFSA Scientific Report (2007) 106, 1-98. DAR, V.3, Annex B, B.9, (2005). Mueller, 1999. 839 N/99
Activated sludge (<i>Pseudomonas sp</i>)	Azoxystrobin	Growth inhibition (6 hours)	NOEC = >3.2 mg a.s./L	EFSA Journal (2010); 8(4):1542 Morris, D.D., Sankey, S.A., and Latham, M., 1994. WAT95-50537

Values highlighted in bold are used in the risk assessment.

9.11.1 Overall conclusions

An assessment of the effects of prothioconazole and azoxystrobin on the inhibition of the respiration rate of aerobic wastewater micro-organisms resulted in EC₅₀ values of >10,000 mg a.s./L and >3.2 mg a.s./L, respectively. Therefore, it can be assumed that adverse effects on methods of sewage treatment are unlikely when FF-075 is applied according to GAP.

9.12 Monitoring data (KCP 10.8)

Monitoring studies are not available for prothioconazole, azoxystrobin or the formulated product, FF-075 and are not considered necessary in light of the acceptable risk concluded for all non-target organisms from uses of FF-075 in cereals (BBCH 30-69) and winter oilseed rape (BBCH 55-69) at the proposed label rates.

9.13 Classification and Labelling

Classification and labelling proposal of FF-075 in accordance to Regulation (EC) No: 1272/2008. The tiered approach outlined in the Guidance on the application of the CLP criteria (v5.0, July 2017)⁸, is outlined, below.

Acute Classification

1. *“Is aquatic toxicity test data available on the mixture as a whole?”*

YES. Acute aquatic invertebrate data and algal data is available for FF-075.

The guidance states that if the preparation contains two or more active substances and the most sensitive taxonomic groups for the active substances is not the same, testing on all tier 1 aquatic groups with the preparation is required.

For prothioconazole and azoxystrobin, the most sensitive taxonomic group to acute exposure is aquatic invertebrates, in both instances (1.3 and 0.055 mg a.s./L for prothioconazole and azoxystrobin, respectively). Therefore, data with the formulation for aquatic invertebrates is required for classification of FF-075 and further testing with fish is not required.

2. *Classify for short-term (acute) aquatic hazard*

“When the mixture as a whole has been tested to determine its aquatic toxicity, this information can be used for classifying the mixture according to the criteria that have been agreed for substances. The classification is normally based on the data for fish, crustacea and algae/plants.

3. *Classification for category Acute 1*

- (a) *When there are adequate acute toxicity test data (LC50 or EC50) available for the mixture as a whole showing $L(E)C50 \leq 1$ mg/l: Classify mixture as Acute*
- (b) *When there are acute toxicity test data (LC50(s) or EC50(s)) available for the mixture as a whole showing $L(E)C50(s) > 1$ mg/l for normally all trophic levels: No need to classify for short-term (acute) hazard..*

Acute aquatic data is available for the formulation, FF-075. The acute aquatic invertebrate endpoint is driving the acute risk assessment for the formulation and is greater than 1 mg/L, with an LC₅₀ of 2.97 mg FF-075/L. Therefore, in accordance with the above guidance, there is no need to classify for short-term (acute) hazard.

Chronic Classification

1. *“Is aquatic toxicity test data available on the mixture as a whole?”*

NO. Chronic data is not available for the formulation, FF-075.

2. *“Is sufficient data available in similar mixtures to estimate hazards?”*

NO. The Applicant has been unable to locate classification of a similar mixture.

3. *“Are either aquatic toxicity or classification data available for relevant components?”*


YES. Chronic toxicity data is available for both active substances. Additionally, agreed-upon classification data is available for both active substances. Prothioconazole was classified as Chronic 1 (H410, Chronic M Factor 1)⁹ and azoxystrobin is classified as Chronic 1 (H410, Chronic M Factor 10)¹⁰.

⁸ ECHA-17-G-21-EN. Guidance on the Application of CLP Criteria. July 2017.

4. “Apply Summation Method”

“First, all components classified as Chronic 1 are considered. If the sum of the concentrations (in %) of these components multiplied by their corresponding M-factors is equal to or greater than 25 %, the mixture is classified as Chronic 1. If the result of the calculation is a classification of the mixture as Chronic 1, the classification procedure is completed.”

As both active substances are classified as Chronic 1, both active substances, in the first instance are considered. The concentration (%) of prothioconazole and azoxystrobin are 18.8% and 13.64%, respectively. Therefore, the sum of the concentration of the active substances, multiplied by their M-factors is 155.2%. As this is greater than 25%, the formulation is classified as Chronic 1 and the classification procedure is complete.

Hazard symbol:			Justification Triggered by H410
Indication of danger:	Warning		Triggered by H410
Hazard Statement(s)	H410	Very toxic to aquatic life with long lasting effects.	Triggered by study data and CLP classification criteria (Please see rationale, above).
Precautionary Statement(s)	P273	Avoid release to the environment.	Recommended phrase for H410.
	P391	Collect spillage.	Recommended phrase for H410.
	P501	Dispose of contents/container in accordance with applicable regulations.	Recommended phrase for H410

zRMS comment:

We agree with the classification. of the product Eustakel Pro.

⁹ Committee for Risk Assessment (RAC) Opinion proposing harmonised classification and labelling at EU level of Prothioconazole (ISO); 2-(2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione. 15th March 2019.

¹⁰ Committee for Risk Assessment (RAC) Opinion proposing harmonised classification and labelling at EU level of Azoxystrobin (ISO); methyl (E)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate. 8th June 2018.

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
CP 10.2.1/01	Li, N	2021	<i>Daphnia</i> (<i>Daphnia magna</i>), acute immobilization test with prothioconazole 200 g/L + Azoxystrobin 150 g/L SC EC (FF-075) Rotam Research Laboratory (RRL), China Study report no.: 2859 GLP Unpublished	N	Rotam
CP 10.2.1/02	Li, N	2021	Fresh water algae (<i>Pseudokirchneriella subcapitata</i>) growth inhibition test with prothioconazole 200 g/L + azoxystrobin 150 g/L EC (FF-075) Rotam Research Laboratory (RRL), China Study report no.: 2858 GLP Unpublished	N	Rotam
CP 10.2.1/03	Li, N	2021	<i>Lemna minor</i> growth inhibition test with prothioconazole 200 g/L + azoxystrobin 150 g/L SC (FF-075). Rotam Research Laboratory (RRL), China Study report no.: 2867 GLP Unpublished	N	Rotam
CP 10.3.1.1.1/01	Parker, T	2020	Honeybees (<i>Apis mellifera</i>), acute oral toxicity test with Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF 075) Rotam Research Laboratory (RRL), China Study report No.: 2862 GLP Unpublished	N	Rotam

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
CP 10.3.1.1.1/02	Parker, T	2021	Honeybees (<i>Apis mellifera</i>), acute contact toxicity test with Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075) Rotam Research Laboratory (RRL), China Study report No.: 2862 GLP Unpublished	N	Rotam
CP 10.3.1.1.1.3/01	Wendling, K.	2020	Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC: Acute Oral and Contact Toxicity to the Bumble Bee, <i>Bombus terrestris</i> L. under Laboratory Conditions. Eurofins Agrosience Services Ecotox GmbH Study report No.: S19-03594 GLP Unpublished	N	Rotam
CP 10.3.1.2/01	Lozano, J.	2020	Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075): Honey Bee (<i>Apis mellifera</i> L.) Chronic Oral Toxicity Test (10-Day Feeding) under laboratory conditions. Trialcamp S.L.U., Spain Study report No.: S20-00395 GLP Unpublished	N	Rotam
CP 10.3.1.3/01	Lozano, J.	2020	Prothioconazole 200 g/L+ Azoxystrobin 150 g/L SC (FF-075): Honey Bee (<i>Apis mellifera</i> L.) Larval Toxicity Test following Repeated Exposure under laboratory conditions. Trialcamp S.L.U., Spain Study report No.: S20-00396 GLP Unpublished	N	Rotam
CP 10.3.2.1/01	Varela, S.	2021	Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF075): Toxicity to the Predatory Mite, <i>Typhlodromus pyri</i> Scheuten (Acari, Phytoseiidae) under Standard Laboratory Conditions. Eurofins Agrosience Services Ecotox GmbH, Study report No.: S20-09657. GLP	N	Rotam

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			Unpublished		
CP 10.3.2.1/02	Walter, C., and Stäbler, P.	2020	Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075): Toxicity to the Aphid Parasitoid <i>Aphidius rhopalosiphi</i> De Stefani Perez (Hymenoptera, Braconidae) under Laboratory Conditions. Eurofins Agrosience Services Ecotox GmbH Study report No.: S19-04385 GLP Unpublished	N	Rotam
CP 10.3.2.1/03	Walter, C. and Stäbler, P.	2020	Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075): Toxicity to the Predatory Mite, <i>Typhlodromus pyri</i> Scheuten (Acari, Phytoseiidae) under Extended Laboratory Conditions. Eurofins MITOX FOPSE Sarl, France Study report no. S19-04389 GLP Unpublished	N	Rotam
CP 10.3.2.1/04	Walter, C. and Stäbler, P.	2020	Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075): Toxicity to the Aphid Parasitoid <i>Aphidius rhopalosiphi</i> De Stefani Perez (Hymenoptera, Braconidae) under Extended Laboratory Conditions. Eurofins Agrosience Services Ecotox GmbH Study report No.: S19-04386 GLP Unpublished	N	Rotam
CP 10.3.2.1/05	Luna, F	2020	Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075): Toxicity to the Ladybird, <i>Coccinella septempunctata</i> L. (Coleoptera: Coccinellidae) Using an Extended Laboratory Test with Freshly Applied Spray Deposits. TrialCamp S.L.U., Spain Study report No.: S19- 04397 GLP Unpublished	N	Rotam

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
CP 10.3.2.1/06	Walter, C., and Stäbler, P.	2019	Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075): Toxicity to the Green Lacewing <i>Chrysoperla carnea</i> Steph. (Neuroptera, Chrysopidae) under Extended Laboratory Conditions. Eurofins Agrosience Services Ecotox GmbH Study report No.: S19-00968. GLP Unpublished	N	Rotam
CP 10.4.1.1/01	Parker, T	2021	Earthworm (<i>Eisenia fetida</i>), reproduction test with Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075) in artificial soil. Rotam Research Laboratory (RRL), China Study report no.: 2866 GLP Unpublished	N	Rotam
CP 10.4.2.1/01	Senn, L	2021	Prothioconazole 200g/L + Azoxystrobin 150g/L SC (FF-075): Reproduction toxicity to the collembolan species <i>Folsomia candida</i> in artificial soil. ECT Oekotoxikologie GmbH, Germany Study report No.: 20AV6CR. GLP Unpublished	N	Rotam
CP 10.4.2.1/02	Senn, L	2021	Prothioconazole 200g/L + Azoxystrobin 150g/L SC (FF-075): Reproduction toxicity to the predaceous mite <i>Hypoaspis</i> (Geolaelaps) aculeifer in artificial soil. ECT Oekotoxikologie GmbH, Germany; Study report No.: 20AV3HR. GLP Unpublished	N	Rotam
CP 10.5/01	Li, N	2021	Effects of Prothioconazole 200 g/L + Azoxystrobin 150 g/L EC (FF-075) on soil microorganisms: Nitrogen transformation test Rotam Research Laboratory (RRL), China Study report No.: 2864	N	Rotam

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			GLP Unpublished		
CP 10.6/01	Förster, B	2021	Prothioconazole 200 g/L + azoxystrobin 150 g/L SC (FF-075): Terrestrial plant seedling emergence and seedling growth test. Study report No.: 20AV6PA GLP Unpublished	N	Rotam
CP 10.6/02	Förster, B	2021	Prothioconazole 200 g/L + azoxystrobin 150 g/L SC (FF-075): Terrestrial Vegetative Vigour Test. Study report No.: 20AV6PB GLP Unpublished	N	Rotam

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

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
II A, 8.1.1 /01	xxxxxx	1999	JAU 6476 techn.ai.: Acute oral toxicity for bobwhite quail (<i>Colinus virginianus</i>) Bayer CropScience, Report No.: BAR/LD 028, Edition Number: M- 013030-01-1 Source: Redacted Date: 1999-06-17 GLP	Y	Bayer Crop- Science AG

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			Unpublished		
II A, 8.1.1 /02	xxxxxx	1990b	SXX 0665 (Technical Grade) acute oral LD50 to bobwhite quail Bayer CropScience, Report No.: VB-009, Edition Number: M- 013315-01-1 Date: 1990-11-30 GLP Unpublished	Y	Bayer Crop- Science AG
KIIA 8.1.1/03	xxxxxx	1992	ICIA5504: Acute oral toxicity (LD ₅₀) to bobwhite quail. ISN 288/921094 AVS95-00132 GLP Unpublished	Y	Syngenta
II A, 8.1.2 /01	xxxxxx	2001b	JAU 6476 techn.: 5-day dietary LC50 for bobwhite quail (<i>Colinus virginianus</i>) Bayer CropScience, Report No.: BAR/LC 005 Edition No.: M-054770-01-1 Source: Redacted GLP Unpublished	Y	Bayer Crop- Science AG
II A, 8.1.2 /02	xxxxxx	2001c	JAU 6476-desthio.: 5-day dietary LC50 for bobwhite quail (<i>Colinus virginianus</i>) Bayer CropScience, Report No.: BAR/LC 011 Edition No.: M-056229-02-1 Source: Redacted GLP Unpublished	Y	Bayer Crop- Science AG
KIIA 8.1.2/03	xxxxxx	1992	ICIA5504: Subacute dietary toxicity (LC ₅₀) to bobwhite quail. ISN 294/920972	Y	Syngenta

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			AVS95-00135 GLP Unpublished		
II A, 8.1.3 /02	xxxxxxx	2000b	Reproduction study in mallard duck with JAU 6476 (by dietary admixture) Bayer CropScience, Report No.: 259919, Edition Number: M- 035123-01-1 Date: 2000-11-07 Source: Redacted GLP Unpublished	Y	Bayer Crop- Science AG
II A, 8.1.3 /03	xxxxxxx	2002	JAU 6476-desthio techn. ai.: Effects of a subchronic dietary exposure to the northern bobwhite quail including effects on reproduction and behaviour Bayer CropScience, Report No.:BAR/REP 006, Edition Number: M090509-01-1 Date: 2002-01-07 Source: Redacted GLP Unpublished	Y	Bayer Crop- Science AG
KIIA 8.1.3/01	xxxxxxx	1994	ICIA5504: Effects on reproduction in bobwhite quail after dietary administration. ISN 314/942363 AVS95-00137 GLP Unpublished	Y	Syngenta

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
II A, 8.2.1 /01	xxxxxx	1999a	JAU 6476- Acute toxicity (96 hours) to Rainbow trout (<i>Oncorhynchus mykiss</i>) in a static test Bayer CropScience, Report No.: DOM 99076 Date: 1999-09-01 GLP Unpublished	Y	Bayer Crop- Science AG
II A, 8.2.1 /02	xxxxxx	1990a	SXX 0665: Acute toxicity to rainbow trout in a static test Bayer CropScience, Report No.: FF-298 Date: 1990-10-26 GLP Unpublished	Y	Bayer Crop- Science AG
II A, 8.2.1 /03	xxxxxx	2001d	JAU 6476-S-methyl- Acute toxicity (96 hours) to rainbow trout (<i>Oncorhynchus mykiss</i>) in a semi-static test. Bayer CropScience, Report No.: DOM 21047 Date: 2001-09-25 GLP Unpublished	Y	Bayer Crop- Science AG
II A, 8.2.1 /05	xxxxxx	1983a	Report for the test for cut of CGA 032 to rainbow trout Bayer CropScience, Report No.: 821418 Date: 1983-08-30 GLP Unpublished	Y	Bayer Crop- Science AG
IIA 8.2.1/06	xxxxxx	1994	ICIA5504: Acute toxicity to bluegill sunfish (<i>Lepomis macrochirus</i>) of a 500 g/kg WG formulation. BL5045/B WAT95-50736 GLP Unpublished	Y	Syngenta

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
IIA 8.2.1/07	xxxxxx	1993	ICIA5504: Acute toxicity to rainbow trout (<i>Oncorhynchus mykiss</i>). BL4602/B WAT95-50541 GLP Unpublished	Y	Syngenta
IIA 8.2.1/08	xxxxxx	1993	ICIA5504: Acute toxicity to bluegill sunfish (<i>Lepomis macrochirus</i>). BL4602/B WAT95-50583 GLP Unpublished	Y	Syngenta
KIIA 8.2.1/09	xxxxxx	1993	R234886: Acute toxicity to rainbow trout (<i>Oncorhynchus mykiss</i>). BL5010/B WAT95-50546 GLP Unpublished	Y	Syngenta
KIIA 8.2.1/10	xxxxxx	2002	R401553 (azoxystrobin metabolite): Acute toxicity to rainbow trout (<i>Oncorhynchus mykiss</i>) Syngenta Crop Protection AG, Basel, Switzerland 7252/B, 2013675 No SYN501657/0002 GLP Unpublished	Y	Syngenta

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
IIA 8.2.1.3/11	xxxxxx	2002	R402173 (Azoxystrobin metabolite): Acute toxicity to rainbow trout (<i>Oncorhynchus mykiss</i>) Syngenta Crop Protection AG, Basel, Switzerland No SYN511114/0001 7338/B, 2013671 GLP Unpublished	Y	Syngenta
II A, 8.2.2.2 /01	xxxxxx	2001e	JAU 6476- Early life stage toxicity to rainbow trout (<i>Oncorhynchus mykiss</i>) under flow-through conditions Bayer CropScience, Report No.: DOM 20028 Date: 2001-12-11 GLP Unpublished	Y	Bayer Crop- Science AG
II A, 8.2.2.2 /02	xxxxxx	2002	JAU 6476-desthio: Early life-stage toxicity test with rainbow trout (<i>Oncorhynchus mykiss</i>) under flow-through conditions xxxxxxx Bayer CropScience, Report No.: 1022.013.321 Date: 2002-02-15 GLP Unpublished	Y	Bayer Crop- Science AG
IIA 8.2.2/03	xxxxxx	1994	Early life stage toxicity of ICIA5504 to fathead minnow (<i>Pimephales promelas</i>) under flow through conditions. BL5284/B WAT95-50584 GLP Unpublished	Y	Syngenta

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
II A, 8.2.2.1 /01	xxxxxx	2002	1,2,4-Triazole- Juvenile growth test, fish (<i>Oncorhynchus mykiss</i>) Bayer CropScience, Report No.: DOM 21060 Date: 2002-01-14 GLP Unpublished	Y	Bayer Crop- Science AG
II A, 8.2.3 /01	xxxxxx	2001	(14C)-JAU 6476- Bioconcentration and biotransformation in bluegill (<i>Lepomis macrochirus</i>) under flow-through conditions Bayer CropScience, Report No.: DOM 21003 Date: 2001-11-13 GLP Unpublished	Y	Bayer Crop- Science AG
II A, 8.2.3 /02	xxxxxxx	2001	(14C)-JAU 6476-desthio- Bioconcentration and biotransformation in bluegill (<i>Lepomis macrochirus</i>) under flow-through conditions Bayer CropScience, Report No.: DOM 20006 Date: 2001-06-21 GLP Unpublished	Y	Bayer Crop- Science AG
II A, 8.2.3 /03	Schneider, J.	2002	Estimation of Partition Coefficient in Octanol-Water of JAU 6476-S-methyl Bayer CropScience, Report No.: MO-02- 002532, Date: 2002-02-06 GLP Unpublished	N	Bayer Crop- Science AG
II A, 8.2.3 /04	Koenig, N.	2013	Statement regarding the derivation of a bioconcentration factor (BCF) in fish for the metabolite prothio-conazole-S-methyl Bayer CropScience, Report No.: M-459145-01- 1, Edition Number: M- 459145-01-1 Date: 2013-07-10 Unpublished	N	Bayer Crop- Science AG

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
II A, 8.2.4 /01	Heimbach, F.	1999c	Acute toxicity of JAU 6476 (tech.) to water fleas (<i>Daphnia magna</i>) Bayer CropScience, Report No.: HBF/DM 212, Date: 1999-08-13 Unpublished	N	Bayer Crop- Science AG
II A, 8.2.4 /02	Heimbach, F.	1990a	Acute toxicity of SXX 0665 (tech.) to waterfleas (<i>Daphnia magna</i>) Bayer CropScience, Report No.: HBF/DM 95, Date: 1999-05-16 Unpublished	N	Bayer Crop- Science AG
II A, 8.2.4 /03	Dorgerloh, M.; Sommer, H.	2001b	Acute toxicity of JAU 6476-S-methyl (tech.) to waterfleas (<i>Daphnia magna</i>) Bayer CropScience, Report No.: DOM 21055 Date: 2001-09-03 Unpublished	N	Bayer Crop- Science AG
II A, 8.2.4 /05	Rufli, H.	1983b	Report on the test for acute toxicity of CGA 98032 to <i>Daphnia magna</i> Bayer CropScience, Report No.: 821416 Date: 1983-08-05 Unpublished	N	Bayer Crop- Science AG
IIA 8.2.4/06	Rapley, J.H., Kearson, L.L., and Hamer, M.J.	1995	ICIA5504: Comparative toxicity of the technical material in solution and a 500 g/kg WG formulation to the water flea, <i>Daphnia magna</i> . RJ1797B WAT95-50585 GLP Unpublished	N	Syngenta

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
IIA 8.2.4/07	Farrelly, E., and Hamer, M.J.	1995	ICIA5504: Comparative toxicity to first instar <i>Daphnia magna</i> in water-only and soil/water systems. RJ1655B WAT95-50597 GLP Unpublished	N	Syngenta
IIA 8.2.4/08	Farrelly, E., Kearson, L.L., Rapley, J.H., and Hamer, M.J.	1995	ICIA5504: Comparative toxicity of the technical material in solution and a 500 g/kg WG formulation to the freshwater copepod <i>Macrocyclus fuscus</i> . RJ1793B WAT95-50586 GLP Unpublished	N	Syngenta
KIIA 8.2.4/09	Kent SJ, Sankey SA, Grinell AJ	1993	ICIA5504: Acute Toxicity to Mysid Shrimp (<i>Mysidopsis bahia</i>) Zeneca AgroChemicals, Jealotts Hill, United Kingdom, BL4785/B NO ICI5504/0925 GLP Unpublished	N	Syngenta
KIIA 8.2.4/10	Kent SJ, Sankey SA, Grinell AJ	1994	ICIA5504: Acute Toxicity to Larvae of the Pacific Oyster (<i>Crassostrea gigas</i>) Zeneca AgroChemicals, Jealotts Hill, United Kingdom, BL4842/B No ICI5504/0927 GLP Unpublished	N	Syngenta
IIA 8.2.4/11	Rapley, J.H., Kearson, L.L., and Hamer, M.J.	1995a	ICIA5504: Comparative toxicity of the technical material in solution and a 500 g/kg formulation to the water flea, <i>Daphnia pulex</i> . RJ1798B WAT95-50596 GLP Unpublished	N	Syngenta

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
IIA 8.2.4/12	Farrelly, E., Kearson, L.L., Rapley, J.H., and Hamer, M.J.	1995d	ICIA5504: Comparative toxicity of the technical material in solution and a 500 g/kg WG formulation to the larvae of the midge <i>Chironomus riparius</i> . RJ1790B WAT95-50589 GLP Unpublished	N	Syngenta
IIA 8.2.4/13	Farrelly, E., Kearson, L.L., Rapley, J.H., and Hamer, M.J.	1995	ICIA5504: Comparative toxicity of the technical material in solution and a 500 g/kg WG formulation to the freshwater shrimp <i>Gammarus pulex</i> . RJ1782B WAT95-50587 GLP Unpublished	N	Syngenta
IIA 8.2.4/14	Farrelly, E., Kearson, L.L., Rapley, J.H., and Hamer, M.J.	1995	ICIA5504: Comparative toxicity of the technical material in solution and a 500 g/kg WG formulation to the phantom midge larva <i>Chaoborus crystallinus</i> . RJ1792B WAT95-50590 GLP Unpublished	N	Syngenta
IIA 8.2.4/15	Farrelly, E., Kearson, L.L., Rapley, J.H., and Hamer, M.J.	1995	ICIA5504: Comparative toxicity of the technical material in solution and a 500 g/kg WG formulation to the mayfly nymph <i>Cloen dipterum</i> . RJ1795B WAT95-50592 GLP Unpublished	N	Syngenta
IIA 8.2.4/16	Farrelly, E., Kearson, L.L., Rapley, J.H., and Hamer, M.J.	1995	ICIA5504: Comparative toxicity of the technical material in solution and a 500 g/kg WG formulation to the water louse <i>Asellus aquaticus</i> . RJ1789B WAT95-50588 GLP	N	Syngenta

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			Unpublished		
IIA 8.2.4/17	Farrelly, E., Kearson, L.L., Rapley, J.H., and Hamer, M.J.	1995	ICIA5504: Comparative toxicity of the technical material in solution and a 500 g/kg WG formulation to the damselfly nymph <i>Ischnura elegans</i> . RJ1794B WAT95-50591 GLP Unpublished	N	Syngenta
IIA 8.2.4/18	Farrelly, E., Kearson, L.L., Rapley, J.H., and Hamer, M.J.	1995	ICIA5504: Comparative toxicity of the technical material in solution and a 500 g/kg WG formulation to the water boatman <i>Notonecta glauca</i> . RJ1799B WAT95-50593 GLP Unpublished	N	Syngenta
IIA 8.2.4/19	Farrelly, E., Kearson, L.L., Rapley, J.H., and Hamer, M.J.	1995	ICIA5504: Comparative toxicity of the technical material in solution and a 500 g/kg WG formulation to the rotifer <i>Brachionus calyciflorus</i> . RJ1791B WAT95-50594 GLP Unpublished	N	Syngenta
IIA 8.2.4/20	Farrelly, E., Kearson, L.L., Rapley, J.H., and Hamer, M.J.	1995	ICIA5504: Comparative toxicity of the technical material in solution and a 500 g/kg WG formulation to the pond snail <i>Lymnaea stagnalis</i> . RJ1796B WAT95-50595 GLP Unpublished	N	Syngenta
IIA 8.2.4/21	Kent, S.J., Sankey, S.A., Banner, A.J., and Johnson, P.A.	1993	R234886: Acute toxicity to <i>Daphnia magna</i> . BL5008/B WAT95-50545	N	Syngenta

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			GLP Unpublished		
IIA 8.2.4/22	Wallace SJ	2002a	R402173 (Azoxystrobin metabolite): Acute toxicity to <i>Daphnia magna</i> . Syngenta Crop Protection AG, Basel, Switzerland, Brixham Environmental Laboratory, Brixham United Kingdom, BL7339/B 2013670 No SYN11114/0002 GLP Unpublished	N	Syngenta
KIIA 8.2.4/23	Bowles AJ, Wallace SJ	2002a	R401553 (Azoxystrobin metabolite): Acute toxicity to <i>Daphnia magna</i> , Syngenta Crop Protection AG, Basel, Switzerland, Brixham Environmental Laboratory, Brixham United Kingdom, BL7253/B 2013672 No SYN5016579003 GLP Unpublished	N	Syngenta
II A, 8.2.5 /01	Hendel, B.; Sommer, H.	2001	Influence of JAU 6476 (tech) on the reproduction rate of water fleas Bayer CropScience, Report No.: HBD/RDM Date: 2001-04-11 Unpublished	N	Bayer Crop- Science AG
II A, 8.2.5 /02	Dorgerloh, M.; Sommer, H.	2001c	Influence of JAU 6476-desthio on the reproduction rate of water fleas in a static renewal laboratory system Bayer CropScience, Report No.: DOM 21036 Date: 2001-09-10 Unpublished	N	Bayer Crop- Science AG
II A, 8.2.5 /06	Rapley, J.H., Farrelly, E., and Hamer, M.J.	1994	ICIA5504: Chronic toxicity to <i>Daphnia magna</i> . RJ1493B WAT95-50540	N	Syngenta

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			GLP Unpublished		
II A, 8.2.5 /07	Boeri RL, Magazu JP, Ward TJ	1997	Chronic Toxicity of Azoxystrobin to the Mysid <i>Mysidopsis bahia</i> Zeneca AgroChemicals, Jealotts Hill, United Kingdom, BL4842/B No ICI5504/0952 GLP Unpublished	N	Syngenta
II A, 8.2.6 /01	Dorgerloh, M.	2000b	JAU-6476- Influence on the growth of green alga, <i>Selenastrum capricornutum</i> Bayer CropScience, Report No.: DOM 99107 Date: 2000-10-25 Unpublished	N	Bayer Crop- Science AG
II A, 8.2.6 /02	Heimbach, F.	1990b	Growth inhibition of green algae (<i>Scenedesmus subspicatus</i>) by SXX 0665 (tech.) Bayer CropScience, Report No.: HBF/AL 78 Date: 1990-06-20 Unpublished	N	Bayer Crop- Science AG
II A, 8.2.6/03	Dorgerloh, M.,; Sommer, H.	2001a	JAU 6476-S-methyl- Influence on the growth of the green alga, <i>Selenastrum capricornutum</i> Bayer CropScience, Report No.: DOM 21028 Date: 2001-07-20 Unpublished	N	Bayer Crop- Science AG
II A, 8.2.6 /05	Palmer, S. J.; Kendall, T. Z.; Krueger, H. O.	2001	1,2,4-Triazole: A 96-hour toxicity test with the freshwater alga (<i>Selenastrum capricornutum</i>) Wildlife International Ltd., Easton, MD, USA Bayer CropScience, Report No.: 528A-101 Date: 2001-08-31 Unpublished	N	Bayer Crop- Science AG

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
II A, 8.2.6 /06	Smyth, D.D., Kent, S.J., Craig, N.C.D., Sankey, S.S., and Grinell, A.J.	1993	ICIA5504: Toxicity to the green alga <i>Selenastrum capricornutum</i> BL4800/B WAT95-50538 GLP Unpublished	N	Syngenta
II A, 8.2.6 /06	Smyth, D.D., Kent, S.J., Craig, N.C.D., Sankey, S.S., and Grinell, A.J.	1993	ICIA5504: Toxicity to the green alga <i>Selenastrum capricornutum</i> WAT95-50547 GLP Unpublished	N	Syngenta
II A, 8.2.6 /07	Smyth DV, Kent SJ, Sankey SA, Johnson PA	1994	ICIA5504: Toxicity to the Marine Alga <i>Skeletonema costatum</i> Zeneca AgroChemicals, Jealotts Hill, United Kingdom, BL5053/B No ICI5504/0966 GLP Unpublished	N	Syngenta
II A, 8.2.6 /08	Smyth DV, Sankey SA, Kent SJ, Sytanley RD	1994	ICIA5504: Toxicity to the freshwater Diatom <i>Navicula pelliculosa</i> Zeneca Agrochemicals, Jealotts Hill, United Kingdom, BL5054/B No ICI5504/0965 GLP Unpublished	N	Syngenta
II A, 8.2.6 /09	Smyth DV, Kent SI, Sankey, S.A, Shearing JM	1994	ICIA5504: Toxicity to the Blue Green Alga <i>Anabaena flos-aquae</i> Zeneca Agrochemicals, Jealotts Hill, United Kingdom, BL5054/B No ICI5504/0967 GLP Unpublished	N	Syngenta

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
II A, 8.2.6 /10	Smyth, D.V., Kent, S.J., Craig, N.C.D., Sankey, S.S., and Johnson, P.A.	1993	R234886: Toxicity to the green alga <i>Selenastrum capricornutum</i> . BL4800/B WAT95-50544 GLP Unpublished	N	Syngenta
II A, 8.2.6 /11	Bowler AJ, Wallace SJ	2002b	R401553 (Azoxystrobin metabolite): Toxicity to the green alga <i>Selenastrum capricornutum</i> Syngenta Crop Protection AG, Basel, Switzerland Brixham environmental Laboratory, Brixham, United Kingdom, BL7254/B. 2013669 No SYN501657/0004 GLP Unpublished	N	Syngenta
II A, 8.2.6 /12	Wallace SJ, Woodyer JM	2002	R402173 (Azoxystrobin metabolite): Toxicity to the green alga <i>Selenastrum capricornutum</i> Syngenta Crop Protection AG, Basel, Switzerland Brixham Environmental Laboratory, Brixham, United Kingdom, BL7340/B, 2013668 No SYN511114/0003 GLP Unpublished	N	Syngenta
II A, 8.2.7 /01	Hendel, B.	2000a	Influence of JAU 6476 (tech.) on development and emergence of larvae of <i>Chironomus riparius</i> in a water-sediment system Bayer CropScience, Report No.: HDB/CH 42 Date: 2000-09-14 Unpublished	N	Bayer Crop-Science AG
II A, 8.2.7 /02	Hendel, B.	2000b	Influence of SXX 0665 (tech.) on development and emergence of larvae of <i>Chironomus riparius</i> in a water-sediment system Bayer CropScience, Report No.: HDB/CH 43 Date: 2000-10-19 Unpublished	N	Bayer Crop-Science AG

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
II A, 8.2.7 /06	Farrelly, E., Kearson, L.L., Rapley, J.H., and Hamer, M.J.	1995d	ICIA5504: Comparative toxicity of the technical material in solution and a 500 g/kg WG formulation to the larvae of the midge <i>Chironomus riparius</i> . RJ1790B WAT95-50589 GLP Unpublished	N	Syngenta
II A, 8.2.7 /07	Smyth, D.V., Kent, S.J., and Sankey, R.D.	1994a	ICIA5504: Toxicity to the Duckweed <i>Lemna gibba</i> Zeneca Agrochemicals, Jealotts Hill, United Kingdom, BL5000/B No ICI5504/0963 GLP Unpublished	N	Syngenta
IIIA 10.2.3/01	Cole JFH, Everett CJ, Gentle W	2000	Azoxystrobin: An outdoor Pond Microcosm Study. Zeneca AgroChemicals, Jealotts Hill, United Kingdom, RJ2857B SYNICI5504/0976 GLP Unpublished	N	Syngenta
II A, 8.3.1.1 /01	Wilhelmy, H.	1999	JAU 6476 a.9.- Acute effects on the honeybee <i>Apis mellifera</i> NOACK Laboratorium, Sarstedt, Germany Bayer CropScience, Report No.: IBA64051 Date: 1999-11-10 Unpublished	N	Bayer Crop- Science AG
II A, 8.3.1.1 /02	Gough, H.J., Jackson, D., Lewis, G.B.,	1993	Acute contact and oral toxicity to Honey Bees (<i>Apis mellifera</i>) of technical material. RJ1517B BIE 96-00077 GLP Unpublished	N	Syngenta

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
IIIA 10.5.1/02	Stacey D.	2004	Azoxystrobin: A laboratory bioassay of the effects of fresh residues of a 250 g/L-1 SC formulation (A12705B) on the parasitic wasp <i>Aphidius rhopalosiphi</i> (Hymenoptera: Braconidae) Syngenta Crop Protection AG, Basel, Switzerland Syngenta – Jealotts Hill International, Bracknell, Berkshire, United Kingdom RJ3518B GLP Unpublished	N	Syngenta
KIIIA1 10.5.1/01	Taruza S.	2001	Azoxystrobin: A rate-response laboratory Test to evaluate the effects of a 250 g/l SC formulation on the Predatory Mite. <i>Typhlodromus pyri</i> Syngenta Crop Protection AG, Basel, Switzerland Mabo-Tox Ltd Southampton, UK SYN-01-45 GLP Unpublished	N	Syngenta
II A, 8.5.2 /02	Meisner, P.	2000d	Influence of JAU 6476-desthio on the reproduction of earthworms (<i>Eisenia fetida</i>) Bayer CropScience, Report Number: MPE/RG 332/00 Edition: M-026193-01-2 GLP Unpublished	N	Bayer Crop- Science AG
II A, 8.5.2 /02	Heimbach, F.	2000b	Influence of JAU 6476-S-Methyl on the reproduction of earthworms (<i>Eisenia fetida</i>) Bayer CropScience, Report Number: HBF/RG 317 Edition: M-021370-01-1 GLP Unpublished	N	Bayer Crop- Science AG

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
III A, 10.4.2.1/01	Moser T, Rombke J	2000	Azoxystrobin: Reproduction Toxicity of a Azoxystrobin 250g/l SC to the earthworm <i>Eisenia Andrei</i> in an artificial soil test. Zeneca AgroChemicals, Jealotts Hill, United Kingdom ECT Oekotoxikologie GmbH, Bad Sodenam Ts., Germany, F10RR No ICI5504/0903 GLP Unpublished	N	Syngenta
III A, 10.4.2.1/02	Nienstedt, K. M.	2002	Reproduction toxicity test exposing <i>Folsomia candida</i> (collembola) to JAU 6476 technical Bayer CropScience, Report Number: 1022.028.641 Edition: M-034235-01-1 GLP Unpublished	N	Bayer Crop- Science AG
III A, 10.4.2.1/04	Moser, T.; Roembke, J.	2001	Acute and reproduction toxicity of JAU 6476-Desthio to the collembolan species <i>Folsomia candida</i> according to the ISO Guideline 11267 Bayer CropScience, Report Number: P1CR Edition: M-035070-03-1 GLP Unpublished	N	Bayer Crop- Science AG
III A, 10.4.2.1/05	Moser, T.; Scheffczyk, A.	2001	Acute and reproduction toxicity of JAU 6476-S-methyl to the collembolan species <i>Folsomia candida</i> Bayer CropScience, Report Number: P35CR Edition: M-087207-01-1 GLP Unpublished	N	Bayer Crop- Science AG

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
III A, 10.4.2.1/06	Hoogendoorn, G. M.	2000	An extended laboratory study to evaluate the effects of JAU 6476 on the predaceous mite <i>Hypoaspis aculeifer</i> Canestrini (Acari: Laelapidae) MITOX tichting Bevoordoring Duurzame Plaagbestrijding, Amsterdam, Netherlands Bayer CropScience, Report No.: B060HAE, Edition Number: M- 037786-02-1 Date: 2000-05-29 GLP Unpublished	N	Bayer Crop- Science AG
IIIA 10.4.2.1/07	Barth M	2001	Azoxystrobin: Toxicity of a 250 g/l SC formulation (YF10537) on the reproduction of the Collembola <i>Folsomia candida</i> Syngenta Crop Protection AG, Basel, Switzerland. BioChem agrar, Gerichshain, Germany, 01 10 48 049, 2013722 No ICI5504/1319 GLP Unpublished	N	Syngenta
IIIA 10.4.2.1/08	Kollmann S.	2004	A12705A: Litterbag test on decomposition of organic material in the field by soil macro and microorganisms. Syngenta Crop Protection AG, Basel, Switzerland. Springborn Smithers Laboratories (Europe) AG, Hom, Switzerland, 1047.124.797 2033547 No ICI15504/2319 GLP Unpublished	N	Syngenta
II A, 8.6/01	Anderson, J. P. E.	1999b	Influence of JAU 6476 technical substance on the microbial mineralization of nitrogen in soils Bayer CropScience, Report No.: AJO/203199 Edition Number: M-024673-01-1	N	Bayer Crop- Science AG

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			Date: 1999-12-08 GLP Unpublished		
II A, 8.6 /02	Anderson, J. P. E.	2000	Influence of the metabolite JAU 6476-desthio on the microbial mineralization of nitrogen in soils Bayer AG, Leverkusen, Germany Report No.: AJO/219101, Edition Number: M- 057459-01-1 Date: 2000-05-09 GLP Unpublished	N	Bayer Crop- Science AG
II A, 8.6 /03	Anderson, J. P. E.	1999d	Influence of the metabolite JAU 6476-S-methyl on the microbial mineralization of nitrogen in soils Bayer AG, Leverkusen, Germany Report No.: AJO/203399, Edition Number: M- 024931-01-1 GLP Unpublished	N	Bayer Crop- Science AG
II A, 8.6 /04	Mason, G., Prevett, A., and Tarry, A.R.	1994	ICIA5504: Study of microbiological activities in soil. RJ 1654B BMF 95-00030 GLP Unpublished.	N	Syngenta
II A, 8.6 /05	Lemnitzer B	2002	Effects of R234886 (metabolite of Azoxystrobin) on the activity of soil microflora. Syngenta Crop Protection AG, Basel, Switzerland BioChem agrar, Gerichshain, Germany, 071048052S T003940-07 No R234886/0002 GLP Unpublished	N	Syngenta

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
II A, 8.6 /06	Schulz L.	2008	SYN501657 - Effects on the activity of soil microflora Syngenta - Jealott's Hill International, Bracknell, Berkshire, United Kingdom BioChem agrar, Gerichshain, Germany, 071048046C/N T003946-07 No SYN501657/0007 GLP Unpublished	N	Syngenta
II A, 8.6 /07	Schulz L.	2008a	SYN501114 - Effects on the activity of soil microflora Syngenta - Jealott's Hill International, Bracknell, Berkshire, United Kingdom BioChem agrar, Gerichshain, Germany, 071048045C/N T003947-07 No SYN501114/0002 GLP Unpublished	N	Syngenta
III A, 10.6.1/01	Meisner, P.; Kolb, U.	2000	Herbicidal screening data for JAU 6476 (tech.) Bayer AG, Leverkusen, Germany Report No.: MPE NTP 13/00 Date: 2000-07-07 GLP Unpublished	N	Bayer Crop-Science AG
IIIA 10.6.2/01	Porch JR, Krueger H O	2002	A Toxicity Test to Determine the Effects of Azoxystrobin on Seedling Emergence and Growth of Terrestrial Plants Syngenta Crop Protection AG, Basel, Switzerland Wildlife International Ltd, Easton MD USA No ICI5504/1376 GLP Unpublished	N	Syngenta
IIA 8.7/01	Morris, D.D., Sankey, S.A., and Lat-ham, M.	1994	ICIA5504: Toxicity to Pseudomonas putida. BL5209/B WAT95-50537 GLP Unpublished	N	Syngenta
IiI A,	Lechelt-Kuntze	2002	JAU 6476 EC 250: Effect on the earthworm fauna of a grassland area in one year	N	Bayer Crop-

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
10.7/01			Bayer CropScience, Report No.: LKC/RGF 58, Edition Number: M- 040814-03-1 Date: 2002-02-28 GLP Unpublished		Science AG
II A, 8.7 /01	Mueller, G	1999	Investigation of the ecological properties of JAU 6476 Bayer AG, Leverkusen, Germany Report No.: 839 N/99, Edition Number: M- 012578-01-1 Date: 1999-05-17 GLP Unpublished	N	Bayer Crop- Science AG

The following tables are to be completed by MS

List of data submitted by the applicant and not relied on

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

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

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

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

Appendix 2 Detailed evaluation of the new studies

A 2.1 KCP 10.1 Effects on birds and other terrestrial vertebrates

A 2.1.1 KCP 10.1.1 Effects on birds

A 2.1.1.1 KCP 10.1.1.1 Acute oral toxicity

A 2.1.1.2 KCP 10.1.1.2 Higher tier data on birds

A 2.1.2 KCP 10.1.2 Effects on terrestrial vertebrates other than birds

A 2.1.2.1 KCP 10.1.2.1 Acute oral toxicity to mammals

A 2.1.2.2 KCP 10.1.2.2 Higher tier data on mammals

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

A 2.2 KCP 10.2 Effects on aquatic organisms

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

Comments of zRMS:	The study is considered acceptable. All validity criteria were met.
	In the control, no immobilization or other signs of disease or stress was observed (e.g., discolouration or unusual behaviour such as trapping at the surface water). The dissolved oxygen concentration at the end of the test was ≥ 3 mg/L in the control and test solutions.
	Agreed endpoints: EC ₅₀ =2.97 mg/L with 95% confidence limit between 2.34 g/L and 3.60 mg/L. NOEC=1.20 mg/L LOEC =1.92 mg/L.

Report:	CP 10.2.1/01; Li, N. (2021)
Title:	<i>Daphnia</i> (<i>Daphnia magna</i>), acute immobilization test with prothioconazole 200 g/L + Azoxystrobin 150 g/L SC EC (FF-075)

Document No:	Rotam Research Laboratory (RRL), China; Study report No.: 2859.
Guideline:	OECD 202 (2004) and EEC C.2 (2008)
GLP	Yes. Laboratory certified by the Bundesinstitut für Risikobewertung, Berlin, Germany.

Test material

Test item:	Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC
Purity:	Prothioconazole 198 g/L (16.9%, w/w) + Azoxystrobin 148 g/L (12.7%, w/w)
Description	Off-white homogenous liquid
Batch No.:	20191211001

Test system

Organism (<i>Species</i>):	<i>Daphnia magna</i> Straus (<24 hours old at the beginning of the test)
Study type:	Acute toxicity (immobilisation)
Guideline deviations reported:	None
Duration of study:	48 hours
Parameters measured:	Immobilisation and behavioral changes/sublethal effects of daphnids during 48 hours of exposure
Observation intervals:	<i>Daphnia</i> were assessed for immobility and behavioural changes at 24 and 48 hours after the beginning of exposure.
Test concentrations:	Test item: 0.75, 1.20, 1.92, 3.07, 4.92 and 7.86 mg/L of Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (equivalent to 0.22, 0.36, 0.57, 0.91, 1.46 and 2.33 mg a.i./L) Reference item (potassium dichromate): 0.60, 0.78, 1.01, 1.31, 1.70 and 2.21 mg/L
Control:	ISO dilution water
Test Arenas:	50 mL capacity glass beakers containing 50 mL of test solution
Application of treatments:	Test concentrations were prepared by diluting appropriate volumes of the stock solution in ISO dilution water to achieve 500 mL of test solution.
No. of replicates:	Range finding test: 0 replicates Definitive test: 4 replicates, 5 daphnids per replicate
Temperature:	Water temperature 18 - 22 within 1°C (maintained in an incubator: Shanghai Yiheng Technical, Model: MGC-250P; thermometer: Hangzhou Logger Technology Co., Ltd, China, Model: L91-1)
pH:	6.0-9.0 within a variation of 1.5 unit, measured using a digital pH meter: Mettler-Toledo, Mode: SG2
Photoperiod:	16:8 h (light:dark)
Light intensity:	between 540 and 1080 lux
Dissolved oxygen:	≥ 3.0 mg/L
Water hardness:	160 mg/L CaCO ₃

Conductivity

361 μ S/cm

Methodology

Two days prior to definitive testing, gravid adults were separated from the culture and maintained in the same incubator where the culture was maintained with the conditions of temperature maintained at 20 – 20.1 °C and 16 hours of photoperiod daily with about 870 Lux. After 24 hours, first brood young ones produced were discarded and the adults retained in the beakers so that the daphnids produced from the second brood were <24 hours old at the initiation of exposure.

Based on the results of a range finding test, the definitive test was conducted with test concentrations of 0.75, 1.20, 1.92, 3.07, 4.92 and 7.86 mg/L of Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (equivalent to 0.22, 0.36, 0.57, 0.91, 1.46 and 2.33 mg a.i./L) along with a control. Potassium dichromate was used as the positive control (tested once every six months).

The content of Prothioconazole and Azoxystrobin in each test concentration was analyzed by a validated HPLC method. One sample from each test concentration were sampled for analysis prior to the introduction of test species at the beginning (0 hour about 200 mL) and at the termination (48 hours about 200 mL).

APPENDIX C: ANALYTICAL MEASUREMENTS

Table 4: Concentration of Prothioconazole in Test Medium

Nominal concentration		Measured concentration at 0 hour (mg a.i./L)	Recovered nominal concentration at 0 hour (%)	Measured concentration at 48 hours (mg a.i./L)	Recovered nominal concentration at 48 hours (%)
(mg/L)	(mg a.i./L)				
Control	BDL	BDL	BDL	BDL	BDL
0.75	0.127	0.123	97.0	0.119	93.9
1.20	0.203	0.197	97.1	0.193	95.2
1.92	0.324	0.295	90.9	0.293	90.3
3.07	0.519	0.472	91.0	0.500	96.4
4.92	0.831	0.766	92.1	0.818	98.4
7.86	1.328	1.240	93.3	1.311	98.7

BDL – Below detectable limit;

Nominal concentration (mg a.i./L) = Nominal concentration (mg/L) × A.I. content.

A.I. content: 16.9%

Table 5: Concentration of Azoxystrobin in Test Medium

Nominal concentration		Measured concentration at 0 hour (mg a.i./L)	Recovered nominal concentration at 0 hour (%)	Measured concentration at 48 hours (mg a.i./L)	Recovered nominal concentration at 48 hours (%)
(mg/L)	(mg a.i./L)				
Control	BDL	BDL	BDL	BDL	BDL
0.75	0.095	0.093	97.6	0.091	95.5
1.20	0.152	0.151	99.1	0.162	106.3
1.92	0.244	0.235	96.4	0.237	97.2
3.07	0.390	0.379	97.2	0.381	97.7
4.92	0.625	0.606	97.0	0.593	94.9
7.86	0.998	0.971	97.3	0.989	99.1

BDL – Below detectable limit;

Nominal concentration (mg a.i./L) = Nominal concentration (mg/L) × A.I. content.

A.I. content: 12.7%

The verifications of test concentration at 0 and 48 hours using a validated HPLC method showed that the measured concentration of Prothioconazole was 90.9% to 97.1% of nominal concentration at the start and 90.3% to 98.7% of nominal concentration at end of the test and the concentration of Azoxystrobin was 96.4% to 99.1% of nominal concentration at the start and 94.9% to 106.3% of nominal concentration at end of the test

TOC, hardness, free chlorine, conductivity and alkalinity of the test medium were measured and Ca/Mg ratio and Na/K ratio were calculated at the start of test. Light intensity inside the incubator was measured prior to the start of test. Environment parameters of the media such as temperature, pH and dissolved oxygen were measured at 0, 24 and 48 hours of exposure of test.

Daphnia were observed for immobility and behavioural changes at 24 and 48 hours after the beginning of exposure. Organisms that were unable to swim within 15 seconds after gentle agitation of the test beaker were considered immobile.

Statistical analyses

EC₅₀ values were determined statistically from the percent immobilization data obtained at 24 and 48 hours for the test species by probit analysis using NCSS software@2007. The 95% confidence limits were calculated using the formula $EC_{50} \pm 1.96 \times \text{standard error}$ (error from NCSS output). LOEC and NOEC were calculated using Dunnett's two-sided multiple comparison test using NCSS software@2007.

Results

In the control, no immobilization or other signs of disease or stress was observed (e.g., discolouration or unusual behaviour such as trapping at the surface water). The dissolved oxygen concentration at the end of the test was ≥ 3 mg/L in the control and test solutions.

Table CP 10.2.1/01-1: Cumulative Immobility in the Definitive Test

Test concentration (mg/L)	Number of <i>Daphnia</i> per test concentration	Cumulative Immobility of <i>Daphnia</i>											
		24 hours						48 hours					
		R1	R2	R3	R4	Total	%	R1	R2	R3	R4	Total	%
Control	20	0	0	0	0	0	0	0	0	0	0	0	0
0.75	20	0	0	0	0	0	0	1	0	0	0	1	5
1.20	20	1	0	0	1	2	10	1	0	0	1	2	10
1.92	20	2	1	1	2	6	30	2	2	1	2	7	35
3.07	20	2	1	2	2	7	35	2	2	2	3	9	45
4.92	20	3	4	3	4	14	70	4	4	3	4	15	75
7.86	20	4	4	5	4	17	85	5	4	5	4	18	90

R: Replicate

No immobility was observed in control during the exposure duration of 48 hours. Immobility observed in the test concentrations of 0.75, 1.20, 1.92, 3.07, 4.92 and 7.86 mg/L was 5%, 10%, 35%, 45%, 75% and 90% during 48 hours test period, respectively. Detailed data is presented in Table-1 and dose response is presented in Figure-1 using Curve fitting- One Independent Variable-Growth and Other Models 3 parameter Logistic (statistical tool NCSS@2007).

Daphnids in control and in the test concentrations of 0.75 and 1.20 mg/L did not exhibit any behavioural abnormalities during the 48 hours test period. At the bottom, sinking was observed in the test concentrations of 1.92, 3.07, 4.92 and 7.86 mg/L during the 48 hours test period.

The verifications of test concentration at 0 and 48 hours was conducted using a validated HPLC method. Test concentration verification showed that the measured concentration of Prothioconazole was 90.9% to 97.1% of nominal concentration at the start and 90.3% to 98.7% of nominal concentration at end of the test and the concentration of Azoxystrobin was 96.4% to 99.1% of nominal concentration at the start and 94.9% to 106.3% of nominal concentration at end of the test.

EC₅₀ values obtained by probit analysis using NCSS software@2007 and the associated 95% confidence limits calculated using the formula $EC_{50} \pm 1.96 \times \text{standard error}$ are presented in Table-2. EC₅₀ at 24 hours is found to be 3.44 mg/L with 95% confidence limit between 2.68 mg/L and 4.20 mg/L. EC₅₀ at 48 hours is found to be 2.97 mg/L with 95% confidence limit between 2.34 mg/L and 3.60 mg/L. The immobility data of 48 hours were subjected to analysis of variance by Dunnett's two sided multiple comparison test with control. NOEC is found to be 1.20 mg/L and the LOEC is found to be 1.92 mg/L.

Table CP 10.2.1/01-2: Cumulative Immobility in the Definitive Test

Test concentration (mg/L)	Number of <i>Daphnia</i> per test concentration	Cumulative Immobility of <i>Daphnia</i>											
		24 hours						48 hours					
		R1	R2	R3	R4	Total	%	R1	R2	R3	R4	Total	%
Control	20	0	0	0	0	0	0	0	0	0	0	0	0
0.75	20	0	0	0	0	0	0	1	0	0	0	1	5
1.20	20	1	0	0	1	2	10	1	0	0	1	2	10

1.92	20	2	1	1	2	6	30	2	2	1	2	7	35
3.07	20	2	1	2	2	7	35	2	2	2	3	9	45
4.92	20	3	4	3	4	14	70	4	4	3	4	15	75
7.86	20	4	4	5	4	17	85	5	4	5	4	18	90

R: Replicate

The results of the analytical verifications of test concentration during test period showed that the measured test concentrations at 24-hour intervals was between 83.35% to 100.57% of nominal concentration.

The measured TOC, hardness, free chlorine, conductivity and alkalinity of the test medium used for preparing the test concentrations were: TOC of 1.135 mg/L, hardness of 1.80×10^2 mg/L as CaCO₃, free chlorine of 0.000 ppm, conductivity of 360 µS/cm, alkalinity of 52.6 mg/L, Ca/Mg ratio of 4:1 and Na/K ratio of 10:1.

In the definitive test, no immobility was observed in the control and in the test concentration of 0.5 mg/L during the 48-hour exposure period. Immobility observed in the test concentrations of 1.1, 2.4, 5.3, 11.7 and 25.8 mg/L was 10%, 35%, 90%, 95% and 100%, respectively during the 48-hour exposure period. Daphnids in the control and test concentrations of 0.5 and 1.1 mg/L did not exhibit any behavioural abnormalities during the 48-hours exposure period. Sinking of test organisms was observed in the test concentrations of 2.4, 5.3, 11.7 and 25.8 mg/L during the 48-hour test period.

The resulting EC₅₀ based on immobilisation of *Daphnia* at 24 hours was found to be 15.5 mg/L with 95% confidence limit between 13.9 mg/L and 17.1 mg/L. The EC₅₀ at 48 hours was found to be 2.8 mg/L with 95% confidence limit between 2.4 mg/L and 3.2 mg/L. The NOEC was found to be 1.1 mg/L and the LOEC was found to be 2.4 mg/L.

Table CP 10.2.1/01-3: Cumulative Immobility in the definitive Test

Test concentration (mg/L)	Number of <i>Daphnia</i> per test concentration	Cumulative Immobility of <i>Daphnia</i>											
		24 hour						48 hour					
		R1	R2	R3	R4	Total	%	R1	R2	R3	R4	Total	%
Control	20	0	0	0	0	0	0	0	0	0	0	0	0
0.5	20	0	0	0	0	0	0	0	0	0	0	0	0
1.1	20	0	0	0	0	0	0	0	2	0	0	2	10
2.4	20	0	0	0	0	0	0	0	3	2	2	7	35
5.3	20	0	0	1	0	1	5	4	4	5	5	18	90
11.7	20	1	0	1	0	2	10	4	5	5	5	19	95
25.8	20	5	5	4	5	19	95	5	5	5	5	20	100

Table CP 10.2.1/01-4: EC₅₀ Value and the Associated 95% Confidence Limits (Formula $EC_{50} \pm 1.96 \times \text{standard error}$)

Period (hours)	EC ₅₀ value		Confidence limits			
			Lower		Upper	
	(mg/L)	(mg a.i./L)	(mg/L)	(mg a.i./L)	(mg/L)	(mg a.i./L)
24	3.44	1.02	2.68	1.24	4.20	0.79
48	2.97	0.88	2.34	1.07	3.60	0.69

Table CP 10.2.1/01-5 Estimated parameters of Probit Analysis (for mg/L)

Parameter	24 hours		48 hours	
	Estimate	Standard Error	Estimate	Standard Error
Alpha	3.486004	.255751	3.626488	.2417492
Beta	2.824624	.4606644	2.905859	.4576855
Dose 50	3.435573	.3914146	2.969409	.3224144

			E _r C ₂₀	1.82	1.55	2.09	0.54	0.46	0.62
			E _r C ₅₀	4.65	3.85	5.45	1.38	1.14	1.61
			E _r C ₉₀	19.42	11.70	27.14	5.75	3.46	8.03
		Yield	E _y C ₁₀	0.54	0.44	0.64	0.16	0.13	0.19
			E _y C ₂₀	0.75	0.65	0.85	0.22	0.19	0.25
			E _y C ₅₀	1.39	1.25	1.53	0.41	0.37	0.45
			E _y C ₉₀	3.56	3.05	4.07	1.05	0.90	1.20

Report:	CP 10.2.1/02; Li, N. (2021)
Title:	Fresh water algae (<i>Pseudokirchneriella subcapitata</i>) growth inhibition test with prothioconazole 200 g/L + azoxystrobin 150 g/L EC (FF-075)
Document No:	Rotam Research Laboratory (RRL), China; Study report No.: 2858.
Guideline:	OECD 201 (2006) and EEC C.3 (2008)
GLP	Yes. Laboratory certified by the Bundesinstitut für Risikobewertung, Berlin, Germany.

Test material

Test item:	Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC
Purity:	Prothioconazole 198 g/L (16.9%, w/w) + Azoxystrobin 148 g/L (12.7%, w/w)
Description	Off-white homogeneous liquid
Batch No.:	20191211001

Test system

Organism (<i>Species</i>):	<i>Pseudokirchneriella subcapitata</i> Strain No.: FACHB-271 Primary culture: Supplied by freshwater algae culture centre, Institute of Hydrobiology, No. 7, Donghu South road, Wu-chang District, Hubei province, China.
Study type:	Algal growth inhibition
Guideline deviations reported:	None
Duration of study:	96 hours
Parameters measured:	Percent inhibitions for growth rate and yield during 72 hours of exposure based on cell density
Observation intervals:	Algal cell count and cell appearance was assessed at 24-hour intervals.
Test concentrations:	Test item: 0.60, 0.96, 1.54, 2.46, 3.93 and 6.29 mg/L (corresponding to 0.18, 0.28, 0.46, 0.73, 1.16 and 1.86 mg a.i./L) with a factor 1.6 along with a control.

	Reference item (potassium dichromate): 0.1, 0.2, 0.4, 0.8, 1.6 and 3.2 mg/L
Control:	Untreated OECD TG 201 medium
Test Arenas:	250 mL capacity Erlenmeyer flasks closed with cotton plugs containing 100 mL OECD TG 201 test medium and kept in a shaker incubator for 96 hours under continuous illumination and agitation, at about 110 rpm.
Application of treatments:	Test concentrations were prepared (inside a clean chamber to maintain sterility) by diluting the stock solution. Algae were inoculated into test vessels and test solution subsequently dispensed to each replicate flask.
No. of replicates:	Range finding test: 3 for control and test concentrations Definitive test: 6 for control, 3 for test concentrations
Temperature:	21 – 24°C
pH:	8.1±0.1 for control (measured at start and end of the test using pH meter, Mettler Toledo, model: SG2. On initial day pH of bulk solutions were checked and on 96 hour, each replicates were checked)
Light intensity:	4440 – 8880 lux

Methodology

The OECD TG 201 test medium was prepared by adding the appropriate nutrients to a known volume of Milli-Q water. The solution was thoroughly mixed and the pH adjusted to 8.1±0.1 with 0.1 N NaOH. The medium was sterilised by filtering through a 0.22 µm pore size sterile membrane filter (Millipore) under aseptic conditions. Before test initiation, the pH of the culture medium was checked again and re-adjusted as necessary. All solutions were prepared in sterile containers to avoid contamination.

Based on the results of the range finding test, the definitive test was conducted with test concentrations of 0.60, 0.96, 1.54, 2.46, 3.93 and 6.29 mg/L (corresponding to 0.18, 0.28, 0.46, 0.73, 1.16 and 1.86 mg a.i./L) with a factor 1.6 along with a control. Potassium dichromate was used as a positive control (tested twice a year).

The test concentrations were prepared in OECD TG 201 medium by dilution of stock and transferred 100 mL of each test solution into 250 mL Erlenmeyer flasks.

The control and treatment flasks were inoculated with *Pseudokirchneriella subcapitata* pre-cultured for 3 days and tested at an initial cell density of 1×10^4 cells per mL and incubated under test condition for 72 hours.

The cells were counted using an Improved Neubaur's Haemocytometer under illumination of the microscope at 24, 48 and 72 hours after inoculation. During the test period, all the flasks were incubated in the shaker incubator under controlled temperature, light and agitation. The temperature during the test period ranged between 21.3°C to 23.5°C, the light intensity ranged between 7210 to 7650 Lux and agitation was at 110 RPM. The pH of the control at the beginning was 8.10 and the mean pH of the control at the termination of the experiment was 8.09. The pH of the test concentrations ranged between 7.95 to 8.08 at the beginning of the test and the mean pH of the test concentrations ranged between 7.92 to 8.07 at test termination.

The content of Prothioconazole in each test concentration was analyzed by a validated HPLC-UV meth-

od. For initial day dose verification, test concentrations were sampled after inoculation of alga. At the end of 96 hours exposure, the samples from the replicates of each treatment after observation were pooled, and then sampled for dose verification. At each occasion, test solution was sampled and alga was removed by centrifugation at low g. The active ingredient content available in test concentrations was analyzed after suitable treatments.

APPENDIX D: ANALYTICAL MEASUREMENTS

Table 17: Verification of Prothioconazole Concentration in OECD Test Medium

Nominal concentration		Measured concentration (mg a.i./L) (C ₁)		Measured concentrations (%) (C ₁ /C ₀ ×100)	
(mg/L)	(mg a.i./L) (C ₀)	0 hour	96 hours	0 hour	96 hours
Control		BDL	BDL	BDL	BDL
0.60	0.101	0.092	0.100	90.7	98.6
0.96	0.162	0.153	0.164	94.3	101.1
1.54	0.260	0.235	0.253	90.3	97.2
2.46	0.416	0.396	0.379	95.3	91.2
3.93	0.664	0.659	0.615	99.2	92.6
6.29	1.063	1.011	0.968	95.1	91.1

BDL – Below detectable limit; Nominal concentration (mg a.i./L) = Nominal concentration (mg/L) × purity (%);
Purity: 16.9%, w/w.

Table 18: Verification of Azoxystrobin Concentration in OECD Test Medium

Nominal concentration		Measured concentration (mg a.i./L) (C ₁)		Measured concentrations (%) (C ₁ /C ₀ ×100)	
(mg/L)	(mg a.i./L) (C ₀)	0 hour	96 hours	0 hour	96 hours
Control		BDL	BDL	BDL	BDL
0.60	0.076	0.079	0.073	103.7	95.8
0.96	0.122	0.118	0.122	96.8	100.1
1.54	0.196	0.185	0.189	94.6	96.6
2.46	0.312	0.295	0.305	94.4	97.6
3.93	0.499	0.469	0.469	94.0	94.0
6.29	0.799	0.758	0.786	94.9	98.4

BDL – Below detectable limit; Nominal concentration (mg a.i./L) = Nominal concentration (mg/L) × purity (%);
Purity: 12.7%, w/w.

The active ingredient content in the test medium was determined by a validated HPLC-UV system. The test concentration in the test medium at the initiation (0 hour) ranged between 90.3% to 99.2% of nominal concentration at the start and 91.1% to 101.1% of nominal concentration at end of the test of Prothioconazole and 94.0% to 103.7% of nominal concentration at the start and 94.0% to 100.1% of nominal concentration at end of the test of Azoxystrobin. As the measured concentrations were within 80 - 120% of the nominal concentration during the exposure period, the threshold concentrations were calculated based on nominal concentration. The results are presented in APPENDIX D, Table-17 and Table-18.

Statistical analyses

Probit analysis (statistical tool NCSS@2007) was used for the calculation of $EC_{90/50/20/10}$ values based on growth rate and yield. The 95% confidence limits were calculated using standard error and $EC_{90/50/20/10}$ values. The NOEC and LOEC were calculated using Duncan's Multiple-Comparison test with one-way analysis of variance with significance at alpha value at 0.05. The data was verified for homogeneity of variance and normality was accepted by ANOVA using Skewness, Kurtosis or Omnibus Normality of Residuals for normality and using Modified-Levene Equal-Variance test for homogeneity.

Results

The alga cells in the control increased by about 86 times at 72 hours which is well above the validity criteria of more than 16 times of the initial cell count during the 96 hours exposure period. The mean coefficient of variation for section by section growth rate for the control cultures over the test period (0 - 96 hours) is 31.60% (less than 35%). The coefficient of variation of average specific growth rate in control is 0.34% (less than 7%). These three findings satisfy the validity criteria of the test as required by OECD Guideline No.: 201.

Solubility of test item when tested at a concentration of 10 mg product/L in Alga test medium by HPLC analysis, showed that the solubility is 1.73 mg a.i./L (Solubility: 102.3%, against the prepared nominal concentration of 10 mg product/L) for Prothioconazole and 1.35 mg a.i./L (Solubility: 106.5%, against the prepared nominal concentration of 10 mg product/L) for Azoxystrobin. Stability of the test item in Alga test medium determined by analyzing the test concentrations of 0.2 mg product/L and 10 mg product/L at 96 hours showed that the recovered 102.4% and 99.9% of the initial measured concentration from 0.2 mg product/L and 10 mg product/L for Prothioconazole and 100.4% and 101.9% of the initial measured concentration from 0.2 mg product/L and 10 mg product/L for Azoxystrobin and hence the dose verification for definitive test was performed at 96 hours after inoculation of alga.

The active ingredient content in the test medium was determined by a validated HPLC-UV system. The test concentration in the test medium at the initiation (0 hour) ranged between 90.3% to 99.2% of nominal concentration at the start and 91.1% to 101.1% of nominal concentration at end of the test of Prothioconazole and 94.0% to 103.7% of nominal concentration at the start and 94.0% to 100.1% of nominal concentration at end of the test of Azoxystrobin. As the measured concentrations were within 80 - 120% of the nominal concentration during the exposure period, the threshold concentrations were calculated based on nominal concentration.

Results from the positive control (potassium dichromate) showed 72 h E_rC_{50} and E_yC_{50} values of 0.99 mg/L and 0.39 mg/L, respectively. The accepted range of EC_{50} values for potassium dichromate was between 0.2 mg/L to 1.03 mg/L (base on E_rC_{50}) and 0.2 to 0.75 mg/L (E_yC_{50}). The sensitivity of the test system was therefore concluded to be acceptable.

No significant changes in the appearance of algae cells were observed in control and in the test concentrations of 0.60, 0.96, 1.54, 2.46, 3.93 and 6.29 mg/L during 96-hour test period. The inhibition calculated based on the cell count is in the range of 1.72% to 54.62% in terms of growth rate and 8.72% to 94.87% in terms of yield at 96 hours for the test concentrations with respect to control.

The 72 hours effective nominal concentration (EC_{50}) based on inhibition of growth rate (E_rC_{50}) is 4.89 mg/L (1.45 mg a.i./L). The 72 hours effective nominal concentration (EC_{50}) based on inhibition of yield (E_yC_{50}) is 1.62 mg/L (0.48 mg a.i./L). No observed effect nominal concentration (NOEC) is 0.60 mg/L (0.18 mg a.i./L) of test item for growth rate and yield; Lowest observed effect nominal concentration (LOEC) is 0.96 mg/L (0.28 mg a.i./L) of test item for growth rate and yield.

Table CP 10.2.1/02-1: Effective Concentrations based on Inhibition of Growth Rate and Inhibition of Yield during 72 hours Exposure of Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC to *Pseudokirchneriella subcapitata*

Response variable based on % inhibition (0-72 h)		Effective concentration (mg/L)	95% confidence limits		Effective concentration (mg a.i./L)	95% confidence limits	
			Lower limit (mg/L)	Upper limit (mg/L)		Lower limit (mg a.i./L)	Upper limit (mg a.i./L)
Growth rate	E _r C ₁₀	1.16	0.92	1.40	0.34	0.27	0.41
	E _r C ₂₀	1.90	1.63	2.17	0.56	0.48	0.64
	E _r C ₅₀	4.89	4.01	5.77	1.45	1.19	1.71
	E _r C ₉₀	20.71	12.13	29.29	6.13	3.59	8.67
Yield	E _y C ₁₀	0.60	0.50	0.70	0.18	0.15	0.21
	E _y C ₂₀	0.84	0.72	0.96	0.25	0.21	0.28
	E _y C ₅₀	1.62	1.46	1.78	0.48	0.43	0.53
	E _y C ₉₀	4.37	3.68	5.06	1.29	1.09	1.50

The 96 hours effective nominal concentration (EC₅₀) based on inhibition of growth rate (E_rC₅₀) is 4.65 mg/L (1.38 mg a.i./L). The 96 hours effective nominal concentration (EC₅₀) based on inhibition of yield (E_yC₅₀) is 1.39 mg/L (0.41 mg a.i./L). No observed effect nominal concentration (NOEC) is 0.60 mg/L (0.18 mg a.i./L) of test item for growth rate and < 0.60 mg/L (0.18 mg a.i./L) for yield; Lowest observed effect nominal concentration (LOEC) is 0.96 mg/L (0.28 mg a.i./L) of test item for growth rate and 0.60 mg/L (0.18 mg a.i./L) for yield.

Table CP 10.2.1/02-2: Effective Concentrations based on Inhibition of Growth Rate and Inhibition of Yield during 96 hours Exposure of Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC to *Pseudokirchneriella subcapitata*

Response variable based on % inhibition (0-96 h)		Effective concentration (mg/L)	95% confidence limits		Effective concentration (mg a.i./L)	95% confidence limits	
			Lower limit (mg/L)	Upper limit (mg/L)		Lower limit (mg a.i./L)	Upper limit (mg a.i./L)
Growth rate	E _r C ₁₀	1.11	0.87	1.35	0.33	0.26	0.40
	E _r C ₂₀	1.82	1.55	2.09	0.54	0.46	0.62
	E _r C ₅₀	4.65	3.85	5.45	1.38	1.14	1.61
	E _r C ₉₀	19.42	11.70	27.14	5.75	3.46	8.03
Yield	E _y C ₁₀	0.54	0.44	0.64	0.16	0.13	0.19
	E _y C ₂₀	0.75	0.65	0.85	0.22	0.19	0.25
	E _y C ₅₀	1.39	1.25	1.53	0.41	0.37	0.45
	E _y C ₉₀	3.56	3.05	4.07	1.05	0.90	1.20

Conclusions

The 72 hours effective nominal concentration (EC₅₀) based on inhibition of growth rate (E_rC₅₀) is 4.89 mg/L (1.45 mg a.i./L). The 72 hours effective nominal concentration (EC₅₀) based on inhibition of yield (E_yC₅₀) is 1.62 mg/L (0.48 mg a.i./L). No observed effect nominal concentration (NOEC) is 0.60 mg/L (0.18 mg a.i./L) of test item for growth rate and yield; Lowest observed effect nominal concentration (LOEC) is 0.96 mg/L (0.28 mg a.i./L) of test item for growth rate and yield.

The 96 hours effective nominal concentration (EC₅₀) based on inhibition of growth rate (E_rC₅₀) is 4.65 mg/L (1.38 mg a.i./L). The 96 hours effective nominal concentration (EC₅₀) based on inhibition of yield (E_yC₅₀) is 1.39 mg/L (0.41 mg a.i./L). No observed effect nominal concentration (NOEC) is 0.60 mg/L (0.18 mg a.i./L) of test item for growth rate and < 0.60 mg/L (0.18 mg a.i./L) for yield; Lowest observed effect nominal concentration (LOEC) is 0.96 mg/L (0.28 mg a.i./L) of test item for growth rate and 0.60 mg/L (0.18 mg a.i./L) for yield.

Comments of zRMS:	<p>The study is considered acceptable. All validity criteria were met.</p> <ul style="list-style-type: none"> The doubling time was calculated based on the average specific growth rate during 7 days exposure of <i>Lemna</i> and the doubling time was 0.34 days which corresponds to greater than 7 fold increase in frond number during 7 days and an average specific growth rate of 2.04 per day <p>Agreed endpoints:</p> <p>NOEC = 0.05 mg/L (0.01 mg a.i./L) based on frond number for growth rate and yield. LOEC=0.15 mg/L (0.04 mg a.i./L) based on frond number for growth rate and yield. NOEC=0.05 mg/L (0.01 mg a.i./L) based on dry weight for growth rate and <0.05 mg/L (0.01 mg a.i./L) for yield. LOEC=0.15 mg/L (0.04 mg a.i./L) based on dry weight for growth rate and 0.05 mg/L (0.01 mg a.i./L) for yield. NOEC=0.05 mg/L (0.01 mg a.i./L) based on frond area for growth rate and yield. LOEC= 0.15 mg/L (0.04 mg a.i./L) based on frond area for growth rate and yield.</p>
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Report:	CP 10.2.1/02; Li, N. (2021)
Title:	<i>Lemna minor</i> growth inhibition test with prothioconazole 200 g/L + azoxystrobin 150 g/L SC (FF-075).
Document No:	Rotam Research Laboratory (RRL), China; Study report No.: 2867.
Guideline:	OECD 221 (2006)
GLP	Yes. Laboratory certified by the Bundesinstitut für Risikobewertung, Berlin, Germany.

Test material

Test item:	Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC
Purity:	Prothioconazole 198 g/L (16.9%, w/w) + Azoxystrobin 148 g/L (12.7%, w/w)
Description	Off-white homogeneous liquid
Batch No.:	20191211001

Test system

Organism (<i>Species</i>):	<i>Lemna minor</i> , Primary culture collected from field and cultured. The test species was identified by Taicang Aquaculture Inspection Institute, China.
Study type:	Growth inhibition
Guideline deviations reported:	None
Duration of study:	7 days
Parameters measured:	The frond counts and total frond area were record on day 0, 3, 5 and 7 after exposure. Dry weight was measured at test initiation and test termination as a measurement variable for assessing the effects of test item to <i>Lemna minor</i> .
Test concentrations:	Test item: 0.05, 0.15, 0.42, 1.22, 3.54, 10.26 and 29.74 mg/L test item concentrations in a geometric factor of 2.9. Reference item: 3,5-dichlorophenol (CAS No. [591-35-5]) was

	used as the positive control in order to evaluate the sensitivity of the test system. The test concentration of 3,5-dichlorophenol used for the study was 0.50, 1.10, 2.40, 5.30 and 11.70 mg/L
Control:	<i>Lemna minor</i> fronds from the culture beakers inoculated in freshly prepared sterilized SIS medium under aseptic conditions
Test Arenas:	300 mL glass beaker covered with petriplate placed in a light incubator during the study period. All the test vessels were randomly repositioned daily in the incubator.
Application of treatments:	The test item was dispersed in the test medium to which the <i>Lemna minor</i> plants are exposed.
No. of replicates:	Definitive test: 3 for control, 3 for test concentrations
Temperature:	24 -25.9 °C
pH:	6.32 – 6.53
Light intensity:	6500 – 10000 Lux, light intensity with a range of illumination $\pm 15\%$ (% RSD) over the incubation area

Methodology

This study was conducted to assess the effects of Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (sponsored by Rotam Agrochem International Co., Ltd.) on the growth of *Lemna minor* (freshwater aquatic plant), according to OECD Test Guideline 221, '*Lemna* sp. Growth Inhibition Test' (adopted 23 March, 2006).

Solubility and stability of the test item were referred from study No.: 2856. Solubility of Prothioconazole when tested at a concentration of 40 mg product/L by HPLC analysis, showed that the solubility is 6.40 mg a.i./L (Solubility: Solubility: 94.7%, against the prepared nominal concentration of 40 mg product/L). Solubility of Azoxystrobin when tested at a concentration of 40 mg product/L by HPLC analysis, showed that the solubility is 4.82 mg a.i./L (Solubility: Solubility: 94.9%, against the prepared nominal concentration of 40 mg product/L). Stability of the test item using the test concentrations of 0.04 and 40 mg product/L for a period of 7 days. Stability of Prothioconazole using the test concentrations of 0.04 and 40 mg product/L for a period of 7 days (Recovered 97.1% and 95.1% of the initial measured concentration from 0.04 mg product/L and 40 mg product/L at 7 days) and stability of Azoxystrobin using the test concentrations of 0.04 and 40 mg product/L for a period of 7 days (Recovered 110.4% and 102.3% of the initial measured concentration from 0.04 mg product/L and 40 mg product/L at 7 days). Hence the dose verification for definitive test was performed at the beginning and at the termination of the test and the test was conducted by static method without media renewal.

The definitive test was conducted with 0.05, 0.15, 0.42, 1.22, 3.54, 10.26 and 29.74 mg/L test item concentrations in a geometric factor of 2.9. The test was conducted with three replicates for test concentrations and control. The test was conducted with Swedish standard (SIS) growth medium by static method. The control and treatment dishes were inoculated with 7 days old pre-cultured *Lemna minor* with an initial frond number of 9 and the dishes were incubated under test condition for 7 days.

On the day of experiment, medium was prepared by adding a known volume of each nutrient stock solution, the Swedish standard growth medium was then brought to volume with Milli Q water and was adjusted to test pH (6.5 ± 0.2 with 0.1 N NaOH). After measurement of pH, the medium was sterilized through 0.22 μ m pore size sterile membrane filters under aseptic conditions. After filtration, medium with glass vessel were kept under aseptic conditions. All the solutions were prepared in sterile containers to

avoid contamination.

Pre-culture of *Lemna minor* was performed for seven days before initiation of the definitive test. For pre-culture, *Lemna minor* fronds from the culture beakers were inoculated in freshly prepared sterilized SIS medium under aseptic conditions. The inoculated beakers were kept in the growth cabinet and maintained with continuous illumination of 6500 – 10000 lux and at $24 \pm 2^\circ\text{C}$ for seven days.

The frond counts and total frond area were recorded on day 0, 3, 5 and 7 after exposure. Dry weight was measured at test initiation and test termination as a measurement variable for assessing the effects of test item to *Lemna minor*.

During the test period, all the flasks were incubated in a light incubator under controlled temperature and light. The temperature during the test period ranged between 24.0°C to 25.9°C and the light intensity ranged between 8050 to 8970 lux. The pH values of control and treated groups were between 6.32 to 6.50 and 6.33 to 6.53 respectively at the beginning and at the end of the test.

Statistical analyses

Based on the inhibitions (derived from frond number, total frond area and dry weight) of yield and growth rate E_{yC50} (7th day), E_{rC50} (7th day) and their 95% confidence limits (when possible) were calculated by Probit model. The EC_{50} values were reported based on the statistical model with the best fit. The 95% confidence limits were calculated using the following formula $EC_{50} \pm 1.96 \times \text{standard error}$ (error from NCSS output). The No Observed Effect Concentration (NOEC) and Lowest Observed Effect Concentration (LOEC) were calculated using a Dunnett's Two-sided multiple comparison test (with control) of variance (ANOVA) techniques. The calculations were done using NCSS software @ 2007.

Results

No morphological change in the appearance of *Lemna minor* fronds was observed in control and at the test concentrations of 0.05, 0.15, 0.42 and 1.22 mg/L, whereas morphological changes like chlorosis, short roots, no growth in the roots, single leafs and loss of buoyancy were found in test concentrations of 3.54, 10.26 and 29.74 mg/L during 7 days observation period.

At the tested concentrations of 0.05, 0.15, 0.42, 1.22, 3.54, 10.26 and 29.74 mg/L in definitive test based on frond number the result showed percent inhibitions of 1.17%, 6.48%, 15.24%, 19.47%, 22.51%, 30.20% and 67.06% in terms of growth rate and 3.07%, 15.71%, 33.33%, 40.61%, 45.59%, 56.32% and 87.74% in terms of yield, respectively.

At the tested concentrations of 0.05, 0.15, 0.42, 1.22, 3.54, 10.26 and 29.74 mg/L in definitive test based on total frond area the result showed percent inhibitions of 0.89%, 7.24%, 13.30%, 20.88%, 23.46%, 31.82% and 68.94% in terms of growth rate and 2.32%, 17.41%, 29.86%, 42.93%, 47.07%, 58.41% and 88.77% in terms of yield, respectively.

At the tested concentrations of 0.05, 0.15, 0.42, 1.22, 3.54, 10.26 and 29.74 mg/L in definitive test based on dry weight the result showed percent inhibitions of 1.68%, 3.19%, 9.67%, 12.30%, 18.31%, 29.81% and 67.99% in terms of growth rate and 4.56%, 8.49%, 23.74%, 29.23%, 40.51%, 57.78% and 89.32% in terms of yield, respectively.

APPENDIX D: ANALYTICAL MEASUREMENTS

Table 13: Verification of Prothioconazole Concentration in OECD Test Medium

Nominal concentration		Measured concentration (mg a.i./L) (C _i)		Measured concentrations (%) (C _i /C ₀ ×100)	
(mg/L)	(mg a.i./L) (C ₀)	0 day	7 days	0 day	7 days
Control		BDL	BDL	BDL	BDL
0.05	0.008	0.008	0.008	94.7	94.7
0.15	0.025	0.025	0.026	98.6	102.6
0.42	0.071	0.066	0.065	93.0	91.6
1.22	0.206	0.202	0.195	98.0	94.6
3.54	0.598	0.573	0.542	95.8	90.6
10.26	1.734	1.655	1.609	95.4	92.8
29.74	5.026	4.600	4.441	91.5	88.4

BDL – Below detectable limit; Nominal concentration (mg a.i./L) = Nominal concentration (mg/L) × purity (%);
Purity: 16.9%, w/w.

Table 14: Verification of Azoxystrobin Concentration in OECD Test Medium

Nominal concentration		Measured concentration (mg a.i./L) (C _i)		Measured concentrations (%) (C _i /C ₀ ×100)	
(mg/L)	(mg a.i./L) (C ₀)	0 day	7 days	0 day	7 days
Control		BDL	BDL	BDL	BDL
0.05	0.006	0.006	0.007	94.5	110.2
0.15	0.019	0.019	0.020	99.7	105.0
0.42	0.053	0.050	0.051	93.7	95.6
1.22	0.155	0.154	0.151	99.4	97.5
3.54	0.450	0.456	0.454	101.4	101.0
10.26	1.303	1.266	1.286	97.2	98.7
29.74	3.777	3.693	3.743	97.8	99.1

BDL – Below detectable limit; Nominal concentration (mg a.i./L) = Nominal concentration (mg/L) × purity (%);
Purity: 12.7%, w/w.

The doubling time was calculated based on the average specific growth rate during 7 days exposure of *Lemna minor* and the doubling time was 0.34 days which corresponds to greater than 7 fold increase in frond number during 7 days and an average specific growth rate of 2.04 per day which meets the validity criteria for the present study as per OECD 221.

The effective concentrations based on growth rate and yield were calculated from frond number, dry weight and frond area. The EC₅₀ values based on the inhibition of growth rate and yield were calculated using Probit analysis. The 95% confidence limits were calculated from the Probit analysis results. The NOEC and LOEC were calculated using Dunnett's Two-sided multiple comparison test (with control) of variance with significance at alpha value 0.05. The data was accepted for homogeneity of variance and normality for ANOVA. All the statistical analysis was done using NCSS@2007.

Table CP 10.2.1/02-1: Effective Concentrations Based on Growth Rate and Yield During 7 Days Expose Period

Response Variable Based on % Inhibition			(mg/L)	(mg a.i./L)	95% confidence limits			
					Upper limit		Lower limit	
					(mg/L)	(mg a.i./L)	(mg/L)	(mg a.i./L)
Growth Rate	Based on Frond Number	E _r C ₅₀	18.98	5.62	28.11	8.32	9.85	2.92
	Based on Dry Weight	E _r C ₅₀	20.17	5.97	28.70	8.50	11.64	3.45
	Based on Frond Area	E _r C ₅₀	16.52	4.89	23.89	7.07	9.15	2.71
Yield	Based on Frond Number	E _y C ₅₀	2.96	0.88	3.84	1.14	2.08	0.62
	Based on Dry Weight	E _y C ₅₀	3.95	1.17	5.05	3.45	2.85	0.84
	Based on Frond Area	E _y C ₅₀	2.75	0.81	3.55	1.05	1.95	0.58

Conclusions

The results of the study shows that Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC at various concentrations has inhibitory effects on the growth rate and yield of *Lemna minor* during the 7 days exposure period. The validity criteria according to the OECD guideline 221 have been met in this study.

NOEC: No observed effect concentration was 0.05 mg/L (0.01 mg a.i./L) based on frond number for growth rate and yield.

LOEC: Lowest observed effect concentration was 0.15 mg/L (0.04 mg a.i./L) based on frond number for growth rate and yield.

NOEC: No observed effect concentration was 0.05 mg/L (0.01 mg a.i./L) based on dry weight for growth rate and <0.05 mg/L (0.01 mg a.i./L) for yield.

LOEC: Lowest observed effect concentration was 0.15 mg/L (0.04 mg a.i./L) based on dry weight for growth rate and 0.05 mg/L (0.01 mg a.i./L) for yield.

NOEC: No observed effect concentration was 0.05 mg/L (0.01 mg a.i./L) based on frond area for growth rate and yield.

LOEC: Lowest observed effect concentration was 0.15 mg/L (0.04 mg a.i./L) based on frond area for growth rate and yield.

Based on the test result of inhibition of growth rate, the test item, Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC as product basis is classified under category Acute 3 of GHS classification (2019).

Based on the test result of inhibition of growth rate, the test item, Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC as a.i basis is classified under category Acute 2 of GHS classification (2019).

A 2.2.2 KCP 10.2.2 Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms

A 2.2.3 KCP 10.2.3 Further testing on aquatic organisms

A 2.3 KCP 10.3 Effects on arthropods

A 2.3.1 KCP 10.3.1 Effects on bees

A 2.3.1.1 KCP 10.3.1.1 Acute toxicity to bees

A 2.3.1.1.1 KCP 10.3.1.1.1 Acute oral toxicity to bees

Comments of zRMS:	<p>The study is considered acceptable. All validity criteria were met.</p> <ul style="list-style-type: none"> The definitive test showed no mortality in control and all treatment dosed during 48 hours test period. No bees exhibited abnormal behavior in control and all treatments based on the actual consumed dose during the test period. From the mortality results of validation test, based on the actual consumed dose, the 24 hours oral LD₅₀ of Dimethoate Technical is 0.163 µg a.i./bee with 95% confidence limits between 0.155 and 0.171 µg a.i./bee. This result is within the validity criteria (0.10 – 0.35 µg a.i./bee) <p>Agreed endpoints:</p> <p>Oral LD₅₀ values for bees exposed to Prothioconazole 200 g/L and azoxystrobin 150 g/L SC.</p> <table> <tr> <th colspan="2" rowspan="2">Parameter</th><th colspan="4">LD₅₀ of test item</th></tr> <tr> <th>µg formulation/bee</th><th>µg total a.i./bee</th><th>µg Prothioconazole/bee</th><th>µg Azoxystrobin/bee</th></tr> <tr> <td>Test period</td><td>48 hours</td><td>>526.41</td><td>>156.61</td><td>>89.60</td><td>>67.01</td></tr> </table> <p>Remark: µg a.i./bee = µg product /bee × total A.I. content; Total A.I. content: 29.75%; Prothioconazole content: 17.02%; Azoxystrobin content: 12.73%.</p>					Parameter		LD ₅₀ of test item				µg formulation/bee	µg total a.i./bee	µg Prothioconazole/bee	µg Azoxystrobin/bee	Test period	48 hours	>526.41	>156.61	>89.60	>67.01
Parameter		LD ₅₀ of test item																			
		µg formulation/bee	µg total a.i./bee	µg Prothioconazole/bee	µg Azoxystrobin/bee																
Test period	48 hours	>526.41	>156.61	>89.60	>67.01																

Report:	CP 10.3.1.1/01; Parker, T. (2020)
Title:	Honeybees (<i>Apis mellifera</i>), acute oral toxicity test with Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075)
Document No:	Rotam Research Laboratory (RRL), China; Study report No.: 2862
Guidelines:	OECD Guideline No. 213 (1998) and EC C.16 (2008).
GLP	Yes. Laboratory certified by the Bundesinstitut für Risikobewertung, Berlin, Germany.

Test material

Test item:	Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC.
Purity:	Prothioconazole 198 g/L + Azoxystrobin 148 g/L
Product code:	FF-075
Description	Off-white homogeneous liquid.
Lot No./Batch No.:	20191211001.
Density	1.163 g/mL

Test system

Organism (<i>Species</i>):	Honeybee (<i>Apis mellifera</i>) Italian, 3 to 5-week old foraging worker bees obtained from a queen-right colony free of disease.
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Study type:	Acute oral toxicity.
Guideline deviations reported:	None.
Duration of study:	48 hours.
Parameters measured:	Mortality and behavioral symptoms.
Observation intervals:	4, 24 and 48 hours.
Test concentrations:	Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC corresponding to 0 (sucrose control), 168.06, 285.71, 485.70, 825.70 and 1403.7 µg formulation/bee (corresponding to 50, 85, 144.5, 245.65 and 417.61 µg a.i/bee)
Control:	5 mL sucrose solution 50% w/v
Reference item	Dimethoate Technical (98.06%) corresponding to 0.07, 0.12, 0.20, 0.34 µg a.i/bee.
Test units:	Stainless steel cages (10(l) × 9(h) × 6(w) cm) with a removable glass pane at the front and a mesh floor.
No. of replicates:	<u>Test, reference toxicant and both controls:</u> 3 cages per treatment, 10 bees per replicate.
Acclimation period/conditions:	2-hours. Bees were allocated to test cages and starved under test conditions.
Temperature:	23.4 – 26.8 °C
Relative humidity:	52 – 66 %.
Light intensity:	Continual darkness, other than at observation times and during dosing.

Methodology:

Adult worker honeybees were exposed to Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC at nominal doses corresponding to 168.06, 285.71, 485.70, 825.70 and 1403.7 µg formulation/bee (corresponding to 50, 85, 144.5, 245.65 and 417.61 µg a.i/bee) with a geometric factor of 1.7 along with control group using sucrose solution 50% w/v (without test item). This corresponded to 157.52, 274.60, 458.41, 550.25 and 526.41 µg formulation/bee based on the actual consumed dose (corresponding to 46.86, 81.69, 136.38, 163.70 and 156.61 µg a.i/bee). The bees of the reference toxicant groups were treated with dimethoate, corresponding to doses of 0.07, 0.12, 0.18 and 0.32 µg a.i./bee based on the actual consumed dose.

The bees were mildly anaesthetized by passing a minimum volume of carbon dioxide for a brief period, and 10 bees were allocated to each labeled test cages. Test cages were replicated 3-times per treatment. The bees were starved for about 2 hours under test conditions. The test solutions were offered to the test organisms of each test unit using Eppendorf tubes containing 200 µL of corresponding test solution. The tubes were weighed before and after adding the test solutions.

The tubes were carefully introduced to cages through their feeding hole to facilitate the consumption of test solution. The feeding was done in the following order: control, test item (from lowest dose to highest dose) and positive control (from lowest dose to highest dose). The control group was administered with sucrose solution 50% w/v without test item. After the consumption period of about 4 h, the Eppendorf tubes were removed carefully from the test cages and the diet consumption was calculated.

The amount of diet consumed in control and treatment were assessed from weight difference of the Eppendorf tubes with feed before and after the administration period. The dose administered to the individual bee was estimated as an average from the total diet consumed per replicate.

Food (50% w/v) aqueous sucrose solution) was provided *ad libitum* and the bees were maintained under controlled temperature and humidity conditions and in darkness for 48 hours.

Observations of mortality and behavioural abnormalities were made after 4, 24 and 48 hours.

The oral LD₅₀ values based on the actual consumed dose were determined statistically by probit analysis using NCSS software (Number Cruncher Statistical System, 2007 version). The 95% confidence limits were calculated using the formula: LD₅₀ ± 1.96 × standard error.

Test item was classified based on the honeybee LD₅₀ value for hazard estimation based on the classification scheme suggested by guidance for assessing pesticide risks to bees, United States Environmental Protection Agency June, 2014.

Non-toxic to bees: ≥ 11 µg/bee

Moderately toxic to bees: > 2 µg/bee to < 10.9 µg/bee

Highly toxic to bees: < 2 µg/bee

Results:

Mortality responses are presented in Table CP 8.3.1.1/01-1.

Following 48 h exposure, no mortalities were observed in the control or any of the treatments. The bees treated with Dimethoate Technical at the doses of 0.07, 0.12, 0.18 and 0.32 µg a.i./bee based on the actual consumed dose, showed 0%, 10.0%, 66.7% and 100.0% mortality at 24 h after end of dosing, respectively.

The 48 h acute oral LD₅₀ value of Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC for honeybees based on the actual dose consumed is greater than 156.61 µg total a.i./bee (89.60 µg Prothioconazole/bee and 67.01 µg Azoxystrobin/bee). On product basis, the 48 hours acute oral LD₅₀ value of Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC for honeybees based on the actual dose consumed is greater than 526.41 µg/bee. The 24 hours oral LD₅₀ value of Dimethoate Technical based on the actual consumed dose is 0.163 µg a.i./bee with 95% confidence limits between 0.155 and 0.171 µg a.i./bee.

Table CP 10.3.1.1/01-1: Acute oral toxicity (mortality) of honeybees exposed to control and Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC under laboratory conditions

Treatment code	Actual consumed dose		Consumption* (% of control)	Cumulative mortality ^a (%)		
	µg total a.i./bee	µg formulation/bee		4 h	24 h	48 h
T ₁	Control (sucrose solution 50% w/v)		-	0	0	0
T ₂	46.86	157.52	94.66	0	0	0
T ₃	81.69	274.60	93.29	0	0	0
T ₄	136.38	458.41	90.08	0	0	0
T ₅	163.70	550.25	64.73	0	0	0
T ₆	156.61	526.41	35.72	0	0	0

* Diet consumption comparison with treated and untreated group; a: Mean of three replications.

Table CP 10.3.1.1/01-2: Acute oral toxicity (mortality) of honeybees exposed to positive control under laboratory conditions.

Treatment code	Actual consumed dose	Consumption* (%)	Cumulative mortality ^a (%)		
	µg a.i./bee		4 hours	24 hours	48 hours
P ₁	0.07	98.94	0	0	3.3

P ₂	0.12	99.75	0	10.0	13.3
P ₃	0.18	95.24	6.7	66.7	70.0
P ₄	0.32	94.62	3.3	100.0	100.0

* Diet consumption comparison with treated and untreated group; a: Mean of three replications; P: Positive control.

Observations made at 4 hours after start of dosing and at 24 and 48 hours after end of dosing, showed no abnormal behavior in control and all treatment doses of test item during the test period. All the bees exposed to positive control doses also did not exhibit any abnormal behavior.

Table CP 10.3.1.1.1/01-3: Oral LD₅₀ values for bees exposed to Prothioconazole 200 g/L and azoxystrobin 150 g/L SC.

Parameter		LD ₅₀ of test item			
		µg formula- tion/bee	µg total a.i./bee	µg Prothiocona- zole/bee	µg Azoxystrobin /bee
Test peri- od	48 hours	>526.41	>156.61	>89.60	>67.01

Remark: µg a.i./bee = µg product /bee × total A.I. content; Total A.I. content: 29.75%; Prothioconazole content: 17.02%; Azoxystrobin content: 12.73%.

The study met the validity criteria as control mortality ≤10% and LD₅₀ value of the reference (24-h) was 0.163 µg a.i./bee (acceptable range 0.10 – 0.35 µg a.i./bee).

Conclusions:

The effects of Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC to honeybee assessed in an acute oral toxicity test showed that the 48 hours LD₅₀ value of Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC based on the actual dose consumed is greater than 526.41 µg formulation/bee equivalent to 156.61 µg total a.i./bee (89.60 µg Prothioconazole/bee and 67.01 µg Azoxystrobin /bee).

The hazard estimation of the test item based on the classification scheme by guidance for assessing pesticide risks to bees, United States Environmental Protection Agency June, 2014 and LD₅₀ value, the test item (Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC) is classified as Non-toxic to bees.

A 2.3.1.1.2 KCP 10.3.1.1.2 Acute contact toxicity to bees

Comments of zRMS:	The study is considered acceptable. All validity criteria were met.															
	<table><tr><th colspan="2">Validity criterion</th><th>Occurred/calculated</th><th>Acceptable range</th></tr><tr><td>Control mortality (48 h)</td><td>Milli-Q water 0.1% of wetting agent - Tween 80</td><td>0% (48 h)</td><td>≤10%</td></tr><tr><td>LD₅₀-value of reference (24 h)</td><td>Dimethoate Technical</td><td>0.20 µg a.i./bee</td><td>0.10 to 0.35 µg a.i./bee</td></tr></table>				Validity criterion		Occurred/calculated	Acceptable range	Control mortality (48 h)	Milli-Q water 0.1% of wetting agent - Tween 80	0% (48 h)	≤10%	LD ₅₀ -value of reference (24 h)	Dimethoate Technical	0.20 µg a.i./bee	0.10 to 0.35 µg a.i./bee
Validity criterion		Occurred/calculated	Acceptable range													
Control mortality (48 h)	Milli-Q water 0.1% of wetting agent - Tween 80	0% (48 h)	≤10%													
LD ₅₀ -value of reference (24 h)	Dimethoate Technical	0.20 µg a.i./bee	0.10 to 0.35 µg a.i./bee													
	Agreed endpoints:															
	48 h LD₅₀ > 1161.0 µg product/bee (345.4µg a.i./bee)															

Report:	CP 10.3.1.1.2/01; Parker, T. (2020)
Title:	Honeybees (<i>Apis mellifera</i>), acute contact toxicity test with Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075)
Document No:	Rotam Research Laboratory (RRL), China; Study report No.: 2863
Guidelines:	OECD Guideline No. 214 (1998) and EEC C.17 (2008).
GLP	Yes. Laboratory certified by the Bundesinstitut für Risikobewertung, Berlin, Germany.

Test material

Test item: Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075)
Purity: Prothioconazole 198 g/L + Azoxystrobin 148 g/L
Description: Off-white homogeneous liquid
Lot No./Batch No.: 20191211001

Test system

Organism (*Species*): Honey bee (*Apis mellifera*) Italian race, 3 to 5-week old foraging worker bees obtained from a queen-right colony free of disease.

Study type: Acute contact toxicity.

Guideline deviations reported: None.

Duration of study: 48 hours.

Parameters measured: Mortality and behavioural symptoms.

Observation intervals: 4, 24 and 48 hours.

Test concentrations: dose of 177.1, 283.4, 453.4, 725.7 and 1161.0 µg product/bee (equivalent to 52.7, 84.3, 134.9, 215.9 and 345.4 µg a.i./bee) with a factor 1.6 along with a control and solvent control.

Reference: Dimethoate Technical (98.06%)

Control: 1 µl Milli-Q water/bee.

Solvent control: 0.1% of wetting agent - Tween 80.

Test units: Stainless steel cages (10(l) × 9(h) × 6(w) cm) with a removable glass pane at the front and a mesh floor.

No. of replicates: Test, reference toxicant and both controls:
3 cages per treatment, 10 bees per replicate.

Acclimation period/conditions: The bees in the containers were acclimatized to test conditions, of a temperature between 24.1 °C to 25.3 °C and relative humidity of 55.7 % to 70.0 % for a period of about 1 hour before study initiation.

Temperature: 24.3 – 25.6°C.

Relative humidity: 60.3 – 69.2%.

Light intensity: Continual darkness, other than at observation times.

Methodology:

Adult worker honeybees were exposed to Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075) at nominal doses corresponding to 177.1, 283.4, 453.4, 725.7 and 1161.0 µg product/bee (equivalent to 52.7, 84.3, 134.9, 215.9 and 345.4 µg a.i./bee).

Applications were made to CO₂-anaesthetised bees from aqueous solutions of the test item, delivered to the dorsal thoracic surface with a single 1 µL droplet/bee by means of a microapplicator. Control and solvent control groups were similarly treated with Milli-Q water and 0.1% of wetting agent - Tween 80, respectively. The positive control test was conducted at 0.07, 0.12, 0.20 and 0.34 µg a.i./bee with a factor 1.7 along with a control (Milli-Q water, coded as *T1*) and a solvent control (0.1% of wetting agent - Tween 80, coded as *T2*).

After dosing, bees were transferred to test cages, with three replicates per treatment and 10 bees/cage. Food (50% w/v) aqueous sucrose solution) was provided *ad libitum* and the bees were maintained under controlled temperature and humidity conditions and in darkness for 48 hours.

Observations of mortality and behavioural abnormalities were made after 4, 24 and 48 hours.

Statistical analysis:

The contact LD50 values were determined statistically by probit analysis using NCSS software (Number Cruncher Statistical System, 2007 version). The 95% confidence limits were calculated using the formula: $LD50 \pm 1.96 \times \text{standard error}$.

Results:

The definitive test showed no mortality in the control, solvent control and treatment groups dosed with 177.1, 283.4, 453.4 and 725.7 µg product/bee (equivalent to 52.7, 84.3, 134.9 and 215.9 µg a.i./bee) during the test period. The mortality at 48 hours in dose of 1161.0 µg product/bee (equivalent to 345.4 µg a.i./bee) was 6.7%. The bees treated with Dimethoate Technical at the doses of 0.07, 0.12, 0.20 and 0.34 µg a.i./bee showed 3.3, 20.0 %, 46.7 % and 83.3 % mortality at 24 hours after dosing, respectively.

Observations for abnormal behaviour were made at 4, 24 and 48 hours after dosing. No abnormal behaviour was observed in the control, solvent control or in any of the treatment group during the test period. In the positive control, no abnormal behaviour was observed in bees tested with doses of 0.07, 0.12, 0.20 and 0.34 µg dimethoate/bee during the 48 hours test period.

During the test period, the bees were kept in constant darkness, except during application and observation period. The temperature during the definitive test was between 24.3°C and 25.6°C and relative humidity was between 60.3% and 69.2%.

Table CP 8.3.1.2/01-1: Acute contact toxicity (mortality) of honey bees exposed to Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC and Control under laboratory conditions

Treatment code	Nominal dose		Cumulative mortality* (%)		
	µg product /bee	µg a.i./bee	4 hours	24 hours	48 hours
T ₁	0	0	0	0	0
T ₂	0	0	0	0	0
T ₃	177.1	52.7	0	0	0
T ₄	283.4	84.3	0	0	0
T ₅	453.4	134.9	0	0	0
T ₆	725.7	215.9	0	0	0
T ₇	1161.0	345.4	3.3	6.7	6.7

*: Mean of three replications.

Table CA 8.3.1.2/01-2: Mortality of Honeybees treated with dimethoate technical.

Treatment code	Nominal dose	Cumulative mortality* (%)		
	µg a.i./bee	4 hours	24 hours	48 hours
P ₁	0.07	0	3.3	6.7
P ₂	0.12	0	20.0	23.3
P ₃	0.20	0	46.7	53.3
P ₄	0.34	13.3	83.3	86.7

*: Mean of three replications.

Table CA 8.3.1.2/01-3: Contact LD₅₀ values for test item (Prothioconazole 200 g/L azoxystrobin 150 g/L SC).

Parameter		LD ₅₀ of test item			
		µg product/bee	µg a.i./bee	µg Prothioconazole/bee	µg Azoxystrobin /bee
Test period	24 hours	>1161.0	>345.4	>197.7	>147.7
	48 hours	>1161.0	>345.4	>197.7	>147.7

Remark: µg a.i./bee = µg product /bee × A.I. content, A.I. content: 29.75%.

Table CA 8.3.1.2/01-3: Validity criteria of the acute contact toxicity test.

Validity criterion		Occurred/calculated	Acceptable range
Control mortality (48 h)	Milli-Q water 0.1% of wetting agent - Tween 80	0% (48 h)	≤10%
LD ₅₀ -value of reference (24 h)	Dimethoate Technical	0.20 µg a.i./bee	0.10 to 0.35 µg a.i./bee

Conclusions:

The effects of Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC to honeybee assessed in an acute contact toxicity test showed that the 48hours contact LD₅₀ value of Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC is more than 1161.0 µg/bee (345.4µg a.i./bee).

The hazard estimation based on the classification scheme by guidance for assessing pesticide risks to bees, United States Environmental Protection Agency June, 2014, based on the acute contact LD₅₀ value, the test item (Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC) is classified as Non-toxic to bees.

Comments of zRMS:

The study is considered acceptable. All validity criteria were met.

Parameter	Required	Observed	
		Oral	Contact
Average control mortality	≤ 10 %	Control (C): 0.00 %	Control (C): 3.33 %
Average reference item mortality	≥ 50 %	100.00	100.00 %

Agreed endpoints:

Contact toxicity test	µg product/ bumble bee	µg prothioconazole/ bumble bee	µg azoxystrobin/ bumble bee
24 h LD ₅₀ (95 % cl)	> 800 (n.d.)	> 136 (n.d.)	> 102 (n.d.)
48 h LD ₅₀ (95 % cl)	> 800 (n.d.)	> 136 (n.d.)	> 102 (n.d.)
24 h NOED	≥ 800	≥ 136	≥ 102
48 h NOED	≥ 800	≥ 136	≥ 102

cl: confidence limits
n.d.: not determined

Report:	CP 10.3.1.1.3/01; Wendling, K. (2020)
Title:	Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC: Acute Oral and Contact Toxicity to the Bumble Bee, <i>Bombus terrestris</i> L. under Laboratory Conditions.
Document No:	Eurofins Agrosience Services Ecotox GmbH, Study report No.: S19-03594
Guidelines:	OECD Guideline Document 246 (2017), OECD Guideline Document 247 (2017)
GLP	Yes. Laboratory certified by the Baden-Württemberg, Berlin, Germany.

Test material

Test item:	Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC
Purity:	Active ingredients (a.i.): 1. prothioconazole, content of a.i. (analysed): 198 g/L (nominal: 200 g/L), 2. azoxystrobin, content of a.i. (analysed): 148 g/L (nominal: 150 g/L)
Description	Liquid/white to off-white
Lot No./Batch No.:	20191211001

Test system

Organism (<i>Species</i>):	<i>Bombus terrestris</i> L. (Hymenoptera, Apidae), young adult worker bumblebees. Medium-size workers.
Study type:	Acute oral and contact toxicity.
Guideline deviations reported:	Behavioural abnormalities in the reference item treatment were not recorded since the reference item is known to be toxic to bumble bees and therefore effects are expected.
Duration of study:	Oral: 96-hours, Contact: 48 hours.

Parameters measured:	Mortality and behavioural symptoms.
Observation intervals:	4, 24 and 48 hours, and additionally at 72 and 96 hours for the oral test.
Test concentrations:	Oral test 50.0, 100, 200, 400 and 800 µg product/bumble bee (corresponding to 8.52, 17.0, 34.1, 68.2 and 136 µg prothioconazole/bumble bee and 6.37, 12.7, 25.5, 50.9 and 102 µg azoxystrobin/bumble bee). Contact test 100, 400 and 800 µg product/bumble bee (corresponding to 17.0, 68.2 and 136 µg prothioconazole/bumble bee and 12.7, 50.9 and 102 µg azoxystrobin/bumble bee).
Control:	Oral test: 50% (w/v) aqueous sucrose solution Control: 0.1% Triton X-100 solution
Reference item	Dimethoate 400 g/L Acute oral toxicity test: 1.5 µg a.i./bumble bee (target dose) 1.31 µg a.i./bumble bee (actual uptake) Acute contact toxicity test: 13 µg a.i./bumble bee
Test units:	In both test procedures, the bumble bees were kept individually (single housing) in Nicot cages (queen bee schooling cages: slightly conical perforated plastic cylinder; base: ~ 1 cm radius, height: 7 cm).
No. of replicates:	Oral test: 35 Contact test: 30.
Acclimation period/conditions:	The collected bumble bees were kept similar to test conditions until test start. During the acclimatisation period they were fed ad libitum with untreated 50 % (w/v) aqueous sucrose solution.
Temperature:	25.0 – 25.5°C
Relative humidity:	55.1 – 60.1%.
Light intensity:	Continual darkness, other than at observation times and during dosing.

Methodology:

Young adult worker Bumblebees (*Bombus terrestris* L.) obtained from commercial queen-right colonies were used as test organisms. Workers were selected one day before application. Bumblebees were obtained directly from the hives and they were randomly allocated to test cages. Overly small and overly big bumblebees as well as recently emerged individuals were excluded from the test group. The weights of the single individuals actually used for the test were recorded and did not differ by more than 0.15 g. The bumble bees were weighed in the application units directly after collection.

The acute oral and contact toxicity tests were carried out as dose-response tests with a test duration of 96 hours for the oral toxicity test and 48 hours for the contact toxicity test. Both tests comprised one control treatment group, five test item treatment groups and one reference item treatment group. In the acute oral

toxicity test each treatment group consisted of 35 test organisms (divided in 35 replicates, containing 1 test organism each). In the acute contact toxicity test each treatment group consisted of 30 test organisms (divided in 30 replicates, containing 1 test organism each). A higher number of replicates was chosen in the acute oral toxicity test than in the acute contact toxicity test to compensate for individuals with a low food uptake.

Plastic syringes (feeders) containing the corresponding application solution were used for application. The application volume was 40 µL/replicate (corresponding to 40 µL/bumble bee). The bumble bees were starved for approx. 2 hours prior to application start. Each unit was provided with the application solution for up to 4 hours, to ensure a sufficient uptake. The feeders were then removed and the bumble bees were provided ad libitum with an untreated 50 % (w/v) aqueous sucrose solution. Treatments started with the control followed by the test item (with increasing concentrations) and finally the reference item. For dose verification the amount of application solution(s) consumed was determined by weighing the feeders before and after feeding using calibrated equipment.

A hand operated micro-applicator was used for application. The application amount was 2 µL/bumble bee. After having been anaesthetised with CO₂ (the amount of anaesthetic used was minimised), the drop-let of the application solution was applied individually to the dorsal side of the thorax of each bumble bee. Treatments started with the control followed by the test item (with increasing concentrations) and finally the reference item.

The water-wetting agent Triton X-100 was directly mixed into the application solutions. This reduced the surface tension of the applied solution and ensured that the drop of the application solution was spread out immediately after the application. After the application the bees were returned to the test units.

Mortality was recorded 4 hours after application (after start of feeding in the acute oral toxicity test and after application in the acute contact toxicity test) and thereafter at 24, 48, 72 and 96 hours (\pm 30 min) for the oral toxicity test and at 24 and 48 hours (\pm 30 min) for the contact toxicity test.

Behavioural abnormalities such as symptoms of poisoning in comparison to the control were recorded at each observation interval.

In the reference item group, behavioural abnormalities assessments were not conducted as it was assumed that moribund and affected bees of the reference item group would die by the end of the test.

Analytical samples and retain samples of the application solutions of the controls and all the lowest and highest concentrated test item groups were taken directly after preparation. The sample size was 2 mL for each sample (analytical and retain sample, respectively). No samples of the reference item feeding solutions were taken.

The samples were deep frozen within 1 hour after sampling and stored in the freezer (\leq - 18 °C, minor fluctuations up to -1 °C and no period longer than ~ 8 hours 25 minutes) until transfer to the analytical laboratory.

The percentage of mortality after 24, 48, 72 and 96 hours for the oral toxicity test and after 24 and 48 hours for the contact toxicity test was calculated for each treatment group from the number of dead individuals in relation to the number of introduced bumble bees (acute contact toxicity test) or to the number of bumble bees which consumed \geq 80 % of the mean consumption of feeding solution (acute oral toxicity test), respectively.

The mortality of the contact toxicity test was corrected for corresponding control mortality according to the formula of SCHNEIDER-ORELLI (1947).

The consumption of application solution per bumble bee was determined by weighing the feeders at the start and at the end of the feeding application period. All bumble bees which entered the test were considered.

Only bumble bees which consumed ≥ 80 % of the mean consumption of feeding solution were considered for the further data evaluation.

Statistical Evaluation:

For the statistical evaluation the statistics program ToxRat professional, Version 3.3.0 was used.

The LD₅₀ values with 95 % confidence limits could not be calculated since there was no mortality above 50 % in any test item treatment group.

Fisher's Exact test with Bonferroni-Holm adjustment (one-sided greater, $\alpha = 0.05$) was used to evaluate whether there are significant differences between the mortality data of the control and the test item treatment group and to determine the oral NOED based on mortality.

Multiple Chi²-test with Bonferroni-Holm adjustment (one-sided greater, $\alpha = 0.05$) was used to evaluate whether there are significant differences between the mortality data of the control and the test item treatment group and to determine the contact NOED based on mortality.

Results:

The study is considered valid since the control and reference item validity criteria were met.

Table CP 10.3.1.1.3/01-1: Study validity check for parameters in the control and reference groups.

Parameter	Required	Observed	
		Oral	Contact
Average control mortality	≤ 10 %	Control (C): 0.00 %	Control (C): 3.33 %
Average reference item mortality	≥ 50 %	100.00	100.00 %

The actual concentrations of prothioconazole and azoxystrobin in the oral application solutions were equivalent to recoveries of 108 and 99 % and 114 and 104 % of nominal, respectively. The actual concentrations of prothioconazole and azoxystrobin in the contact application solutions were equivalent to recoveries of 110 and 99 % and 118 and 105 % of nominal, respectively. No residues of prothioconazole and azoxystrobin above the LOD (6.39 mg prothioconazole/L sucrose solution, 128 prothioconazole/L water, 4.77 mg azoxystrobin/L sucrose solution and 95.4 mg azoxystrobin/L water) were found in any of the control samples.

In the oral test in the test item treatment groups of 50.0, 100, 200, 400 and 800 µg product/bumble bee (corresponding to 8.52, 17.0, 34.1, 68.2 and 136 µg prothioconazole/bumble bee and 6.37, 12.7, 25.5, 50.9 and 102 µg azoxystrobin/bumble bee), 0.0, 15.6, 10.7, 30.8 and 24.0 % mortality was observed 96 hours after start of feeding, respectively.

Numerous affected or moribund bumble bees were observed during the 96 hour testing period at the target doses of 400 and 800 µg product/bumble bee (corresponding to 68.2 and 136 µg prothioconazole/bumble bee and 50.9 and 102 µg azoxystrobin/bumble bee).

Table CP 10.3.1.1.3/01-2: Mortality and actual uptake in the acute oral toxicity test in the control, test item and reference item groups.

Treatment group (Target dose)	Actual uptake	Mortality [%]			
		24 h	48 h	72 h	96 h
Control(s):					
Control	--	0.0	0.0	0.0	0.0
Test item: Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC [µg product/bumble bee]					
50.0	47.3	0.0	0.0	0.0	0.0
100	92.4	0.0	0.0	15.6	15.6
200	185	0.0	0.0	0.0	10.7
400	348	0.0	0.0	7.7	30.8
800	643	0.0	0.0	12.0	24.0
Reference item: dimethoate [µg dimethoate/bumble bee]					
1.5	1.31	100	100	100	100

Table CP 10.3.1.1.3/01-3: NOED and LD₅₀ values in the oral toxicity test with Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC.

Oral toxicity test ^a	µg product/ bumble bee	µg prothioconazole/ bumble bee	µg azoxystrobin/ bumble bee
24 h LD ₅₀ (95 % cl)	> 643 (n.d.)	110 (n.d.)	81.9 (n.d.)
48 h LD ₅₀ (95 % cl)	> 643 (n.d.)	110 (n.d.)	81.9 (n.d.)
72 h LD ₅₀ (95 % cl)	> 643 (n.d.)	110 (n.d.)	81.9 (n.d.)
96 h LD ₅₀ (95 % cl)	> 643 (n.d.)	110 (n.d.)	81.9 (n.d.)
96 h NOED	≥ 643	≥ 110	≥ 81.9
48 h NOED	≥ 643	≥ 110	≥ 81.9
72 h NOED	≥ 643	≥ 110	≥ 81.9
96 h NOED	185	31.5	23.6

^a Based on actual uptake due to a lack of mortality above 50 %
cl: confidence limits
n.d.: not determined

Table CP 10.3.1.1.3/01-3: Mortality in the acute contact toxicity test in the control, test item and reference item groups.

Treatment group	Mortality [%]	
	24 h	48 h
Control(s):		
Control	3.3	3.3
Test item: Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC [µg product/bumble bee]		
50.0	0.0	0.0
100	3.3	3.3
200	0.0	0.0
400	3.3	3.3
800	0.0	3.3
Reference item: dimethoate [µg dimethoate/bumble bee]		
13	100	100

Table CP 10.3.1.1.3/01-4: NOED and LD₅₀ values in the contact toxicity test with Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC.

Contact toxicity test	µg product/ bumble bee	µg prothioconazole/ bumble bee	µg azoxystrobin/ bumble bee
24 h LD ₅₀ (95 % cl)	> 800 (n.d.)	> 136 (n.d.)	> 102 (n.d.)
48 h LD ₅₀ (95 % cl)	> 800 (n.d.)	> 136 (n.d.)	> 102 (n.d.)
24 h NOED	≥ 800	≥ 136	≥ 102
48 h NOED	≥ 800	≥ 136	≥ 102

cl: confidence limits
n.d.: not determined

Conclusions:

In the oral toxicity test at the target doses of 50.0, 100, 200, 400 and 800 µg product/bumble bee (corresponding to 8.52, 17.0, 34.1, 68.2 and 136 µg prothioconazole/bumble bee and 6.37, 12.7, 25.5, 50.9 and

102 µg azoxystrobin/bumble bee), 0.0, 15.6, 10.7, 30.8 and 24.0 % mortality was observed 96 hours after start of feeding, respectively.

In the contact toxicity test at treatment groups of 100, 400 and 800 µg product/bumble bee (corresponding to 17.0, 68.2 and 136 µg prothioconazole/bumble bee and 12.7, 50.9 and 102 µg azoxystrobin/bumble bee), 3.3 % mortality was recorded at the end of the 48 hour test period, respectively.

According to the study results, the 96 hour NOED for Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC was determined to be 185 µg product/bumble bee (corresponding to 31.5 µg prothioconazole/bumble bee and 23.6 µg azoxystrobin/bumble bee).

The 48 hour contact NOED for Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC was determined to be ≥ 800 µg product/bumble bee (corresponding to ≥ 136 µg prothioconazole/bumble bee and ≥ 102 µg azoxystrobin/bumble bee).

A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

Comments of zRMS:	The study is considered acceptable. All validity criteria were met.			
	<ul style="list-style-type: none"> The mean control mortality was $\leq 15\%$ at the end of the test (actual 8.00%) and mean reference item mortality was $\geq 50\%$ at the end of the test (actual 100.00 %). 			
	Agreed endpoints:			
	Endpoints (D10)	Test item	a.i.1: prothioconazole	a.i.2: azoxystrobin
	NOEC ^a	425.52	72.51	54.20
	LC ₁₀ ^b	578.87	98.64	73.73
	[95 % confidence limits]	[409.27 – 818.76]	[69.74 – 139.51]	[52.13 – 104.28]
	LC ₂₀ ^b	962.86	164.07	122.64
	[95 % confidence limits]	[743.19 – 1247.45]	[126.64 – 212.56]	[94.66 – 158.88]
	LC ₅₀ ^b	2076.45	353.82	264.47
	[95 % confidence limits]	[1750.69 – 2462.83]	[298.31 – 419.66]	[222.98 – 313.68]
	Dose [µg/bee/day]			
	Endpoints (D10)	Test item	a.i.1: prothioconazole	a.i.2: azoxystrobin
	NOEDD ^a	6.10	1.04	0.78
	LDD ₁₀ ^b	9.13	1.56	1.16
	[95 % confidence limits]	[4.34 – 19.19]	[0.74 – 3.27]	[0.55 – 2.44]
	LDD ₂₀ ^b	12.73	2.17	1.62
	[95 % confidence limits]	[7.37 – 22.01]	[1.26 – 3.75]	[0.94 – 2.80]
	LDD ₅₀ ^b	21.05	3.59	2.68
	[95 % confidence limits]	[15.28 – 28.99]	[2.60 – 4.94]	[1.95 – 3.69]
a.i.: active ingredient.				
^a Step-down Rao-Scott-Cochran-Armitage test procedure (one sided greater, $\alpha = 0.05$).				
^b Weibull analysis using linear max. likelihood regression (95 %-confidence limits)				

Report:	CP 10.3.1.2/01; Lozano, J. (2020)
Title:	Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075): Honey Bee (<i>Apis mellifera</i> L.) Chronic Oral Toxicity Test (10-Day Feeding) under laboratory conditions.
Document No:	Trialcamp S.L.U., Spain; Study report No.: S20-00395
Guidelines:	OECD Guideline No. 245 (2017) and SANCO/3029/99, rev. 4 (2000).
GLP	Yes. Laboratory certified by the Entidad Nacional de Acreditación, Madrid, Spain.

Test material

Test item:	Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075)
Purity:	Active ingredient 1: prothioconazole; content of a.i.1: 198 g/L; active ingredient 2: azoxystrobin; content of a.i. 2: 148 g/L
Description	White to gray, liquid.
Lot No./Batch No.:	20191211001

Test system

Organism (<i>Species</i>):	Honeybee (<i>Apis mellifera</i> L.), young adult worker bees (not older than 48 hours)
Study type:	Chronic oral toxicity
Guideline deviations reported:	Behavioural abnormalities in the reference item treatment group were not recorded since the reference item is known to be toxic to honeybees and therefore effects are expected. Validity criteria for the reference item group were met.
Duration of study:	10 days
Parameters measured:	Mortality and behavioural abnormalities
Observation intervals:	Mortality and behaviour: daily, starting 24 h (± 2 h) after first application for whole the 10-day exposure period.
Test concentrations:	177.30, 425.52, 1021.24, 2450.98 and 5882.35 mg test item/kg feeding solution
Control:	C1: Negative control (untreated feeding solution). C2: Thickener control (feeding solution + 0.1 % Xanthan).
Reference item	0.90 mg dimethoate/kg feeding solution.
Test units:	Stainless steel cages (base: 8.5 cm x 4.5 cm; height: 6.0 cm, approximately). The front side of the cage was equipped with a transparent pane to enable observation. The bottom of the cage consisted of perforated steel, which guaranteed sufficient air supply. The cages were lined with filter paper. Two holes at the top of the cage allowed for the use of feeders.
No. of replicates:	<u>Test, reference toxicant and control:</u> 5 replicates per dose, 10 bees per replicate.
Acclimation period/conditions:	1-day. During acclimation, bees were fed <i>ad libitum</i> with 50% w/v sucrose solution.

Temperature:	30.0 * – 34.0 °C. *Short term deviation (<2 hours) during acclimatisation period.
Photoperiod	Constant darkness except during feeding and assessments.
Relative humidity:	48.8 * – 65.2 % *Short term deviation (<2 hours) during acclimatisation period.

Methodology:

A fresh test item stock solution was obtained daily by mixing a defined amount of test item with a defined amount of 50 % (w/v) aqueous sucrose solution + 0.1 % Xanthan. This stock solution was used also as the highest test item concentration feeding solution. The remaining test item feeding solutions were freshly prepared every day by mixing aliquots of the stock solution with 50 % (w/v) aqueous sucrose solution + 0.1 % Xanthan. The addition of Xanthan gum was intended to increase stability of the test item solutions. Five test item concentrations were tested with a constant spacing factor of 2.4, along with control group using 50% (w/v) aqueous sucrose solution (without test item).

Feeding solution samples for the thickener control and for all the concentrations of the test item treatments were taken every application day, from day 0 (D0) to day 9 (D9), directly after preparation. The samples were taken by duplicate, one main sample (A) and one for retention (R), with a volume of 2 mL each. Samples were taken directly after preparation of the whole set of test item solutions and stored in a freezer at $\leq -18^{\circ}\text{C}$ until chemical analysis. The analytical method used was validated according to SAN-CO/3029/99 rev 4 and quantification was performed by LC-MS/MS detection. The limit of quantification (LOQ) of the analytical method was 0.1 mg test item/kg (0.0170 mg prothioconazole/kg and 0.0127 mg azoxystrobin/kg) with a limit of detection (LOD) set at 0.03 mg test item/kg (30 % of the LOQ, 0.00510 mg prothioconazole/kg and 0.00381 mg azoxystrobin/kg).

Adult worker honeybees were introduced into test units, where feeding solutions were offered to test organisms of each test unit using 5 mL syringes containing 1 mL (0.1 mL/bee) of corresponding feeding solution. Syringes with the feeding solutions were weighed before application and then inserted into one of the holes in the upper surface of the testing cages. The bees in one replicate shared the food and thus receive similar doses. Feeding solutions remained in cages for 24 h (± 2 hours) and were then replaced with syringes containing fresh feeding solution. Replaced syringes were weighed to calculate food consumption. This was repeated daily. Additional test units without bees but with syringes containing 1 mL of feeding solution were maintained in the climatic chamber and these syringes changed daily concurrently with the test syringes and weighed before and after each replacement to calculate sucrose solution evaporation.

Mortality and behavioural abnormalities were recorded daily 24 hours after the first application and for the 10-day exposure period and dead bees removed. Behavioural abnormalities were not assessed in the reference group, as it was assumed that moribund and affected bees in the reference group would die by the end of the test. Behavioural abnormalities were recorded as: affected, apathetic, cramps, moribund and vomiting.

Statistical analyses

For the statistical evaluation, the Microsoft Office Excel 2013® Version 15.0 and the statistical software ToxRatPro® Version 3.3.0 was used. Fisher's exact binomial test ($\alpha = 0.05$, two sided) was performed in order to compare mortality response obtained between the two control groups (negative control and thickener control) for significant differences. The qualitative trend analysis by contrasts (monotonicity of concentration/response, $\alpha = 0.05$) revealed a linear trend between concentrations and mortality. As signs of extra binomial variance in the data were found (Tarone's procedure, $\alpha = 0.01$), the step-down Rao-Scott-Cochran-Armitage test procedure (one-sided greater, $\alpha = 0.05$) was used to evaluate whether there was a significant difference between the mortality data of the test item groups and the thickener control group in order to determine the No Observed Effect Concentration (NOEC) and the corresponding No

Observed Effect Dietary Dose (NOEDD). The median Lethal Concentration (LC_{50}) and the median Lethal Dietary Dose (LDD_{50}) values, and additionally the LC_{10}/LDD_{10} and LC_{20}/LDD_{20} values (Lethal Concentration/Dose that kills 10 and 20 % of exposed individuals, respectively), were calculated by Weibull analysis using linear max. Likelihood regression. Their 95 %-confidence limits were calculated by normal approximation.

Results

All validity criteria were met the mean control mortality was $\leq 15\%$ at the end of the test (actual 8.00%) and mean reference item mortality was $\geq 50\%$ at the end of the test (actual 100.00 %).

The results of dose verification showed recoveries between 82 % and 92 % in 50 % w/v aqueous sucrose solution containing 0.1 % xanthan for both analytes; this is, within $\pm 20\%$ of nominal test concentration used. Therefore, the results of dose verification show the correct dosage of test item to 50 % (w/v) aqueous sucrose solution + 0.1 % xanthan and the nominal concentrations were used in the calculation of the test results.

Symptoms of intoxication (mainly affected bees but also some moribund individuals) were observed, starting on day 1, with 7 affected bees in T5. Symptoms of intoxication had been observed, at some point during the exposure, in every test item treated group but with differing levels of occurrence. At the end of the test, in the last assessment on day 10; 3, 1, 1 and 3 affected bees were observed in treatments T1, T2, T3 and T4, respectively.

In comparison to the thickener control, treatment T3 (1021.24 mg test item/kg feeding solution), and all treatments above this concentration showed statistically significantly increased mortality after 10 days of exposure. Therefore, the No Observed Effect Concentration (NOEC) for mortality after 10 days of continuous exposure was determined to be 425.52 mg test item/kg feeding solution. The corresponding No Observed Effect Dietary Dose (NOEDD), based on the actual consumption of the feeding solutions, was determined to be 6.10 $\mu\text{g t.i./bee/day}$. The 10-day LC_{10} -value (Lethal Concentration that kills 10 % of exposed individuals) with 95 % confidence interval was determined to be 578.87 [409.27 – 818.76] mg FF-075/kg feeding solution. The corresponding 10-day LDD_{10} -value (Lethal Dietary Dose that kills 10 % of exposed individuals) with 95 % confidence interval, based on the actual consumption of the feeding solutions, was determined to be 9.13 [4.34 – 19.19] $\mu\text{g FF-075/bee/day}$. The 10-day LC_{20} -value (Lethal Concentration that kills 20 % of exposed individuals) with 95 % confidence interval was determined to be 962.86 [743.19 – 1247.45] mg FF-075/kg feeding solution. The corresponding 10-day LDD_{20} -value (Lethal Dietary Dose that kills 20 % of exposed individuals) with 95 % confidence interval, based on the actual consumption of the feeding solutions, was determined to be 12.73 [7.37 – 22.01] $\mu\text{g FF-075/bee/day}$.

The 10-day LC_{50} -value (Lethal Concentration that kills 50 % of exposed individuals) with 95 % confidence interval was determined to be 2076.45 [1750.69 – 2462.83] mg FF-075/kg feeding solution. The corresponding 10-day LDD_{50} -value (Lethal Dietary Dose that kills 50 % of exposed individuals) with 95 % confidence interval, based on the actual consumption of the feeding solutions, was determined to be 21.05 [15.28 – 28.99] $\mu\text{g FF-075/bee/day}$.

Table CP 10.3.1.2/01-1: Cumulative and corrected cumulative mortality in the control, the test item and the reference item treatment groups

Treatment group (code)		Concentration [mg t.i./kg f.s.]	Total number of bees dosed	Total number of dead bees	Cumulative mortality [%]	SE	Corrected mortality ^a [%]
Negative control	(C1)	–	50	4	8.00	3.74	0.00
Thickener control	(C2)	–	50	4	8.00	2.00	–
Pooled control	(CP)	–	100	8	8.00	1.41	–
Test item FF-075	(T1)	177.30	50	2	4.00	4.00	-4.35
	(T2)	425.52	50	9	18.00	11.14	10.87
	(T3)	1021.24	50	12	24.00 ^b	5.10	17.39
	(T4)	2450.98	50	32	64.00 ^b	5.10	60.87
	(T5)	5882.35	50	48	96.00 ^b	2.45	95.65
Reference item (dimethoate) ^c	(R)	0.90	50	50	100.00	0.00	100.00

t.i.: test item; f.s.: feeding solution; SE: standard error.

^a Corrected for thickener control group according to Abbott's formula (1925) modified by Schneider-Orelli (1947). Negative values indicate lower mortality compared to thickener control group.

^b Significantly increased compared to the thickener control group (step-down Rao-Scott-Cochran-Armitage test procedure, one sided greater, $\alpha = 0.05$).

^c For the reference item the concentration value is expressed in active ingredient (dimethoate).

Table CP 10.3.1.2/01-2: Behavioural Abnormalities

Treatment group (code)		Concentration [mg t.i./kg f.s.]	Affected bees [%] ^a									
			D1	D2	D3	D4	D5	D6	D7	D8	D9	D10
Negative control	C 1	–	0.00	0.00	0.0 0	0.0 0	0.0 0	0.0 0	0.00	0.00	0.00	0.00
Thickener control	C 2	–	0.00	0.00	0.0 0	0.0 0	0.0 0	0.0 0	0.00	0.00	0.00	0.00
Test item FF-075	T1	177.30	0.00	0.00	0.0 0	0.0 0	0.0 0	0.0 0	0.00	0.00	0.00	6.25
	T2	425.52	0.00	2.04	0.0 0	2.0 8	0.0 0	0.0 0	0.00	0.00	0.00	2.44
	T3	1021.24	0.00	0.00	0.0 0	0.0 0	0.0 0	0.0 0	0.00	4.35	4.76	2.63
	T4	2450.98	0.00	0.00	2.0 4	0.0 0	0.0 0	2.1 3	7.89	2.78	0.00	16.6 7
	T5	5882.35	15.2 2	14.6 3	5.8 8	3.5 7	3.8 5	0.0 0	25.0 0	66.6 7	50.0 0	0.00

t.i.: test item; fs: feeding solution.

^a Percentage of affected individuals respect to the living ones.

Conclusions:

The chronic toxicity of the test item 'Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075)' to honey bees was tested under laboratory conditions over a period of 10 days.

All validity criteria were met and sensitivity of the test organisms was confirmed. Accordingly, the study was deemed valid. The test item concentrations were analytically confirmed and therefore the doses/concentrations were based on nominal values.

At the end of the test (day 10); 3, 1, 1 and 3 affected bees were observed in treatments T1, T2, T3 and T4, respectively.

Table CP 10.3.1.2/01-3: List of endpoints.

Endpoints (D10)	Concentration [mg/kg feeding solution]		
	Test item	a.i.1: prothioconazole	a.i.2: azoxystrobin
NOEC ^a	425.52	72.51	54.20
LC ₁₀ ^b [95 % confidence limits]	578.87 [409.27 – 818.76]	98.64 [69.74 – 139.51]	73.73 [52.13 – 104.28]
LC ₂₀ ^b [95 % confidence limits]	962.86 [743.19 – 1247.45]	164.07 [126.64 – 212.56]	122.64 [94.66 – 158.88]
LC ₅₀ ^b [95 % confidence limits]	2076.45 [1750.69 – 2462.83]	353.82 [298.31 – 419.66]	264.47 [222.98 – 313.68]
Endpoints (D10)	Dose [µg/bee/day]		
	Test item	a.i.1: prothioconazole	a.i.2: azoxystrobin
NOEDD ^a	6.10	1.04	0.78
LDD ₁₀ ^b [95 % confidence limits]	9.13 [4.34 – 19.19]	1.56 [0.74 – 3.27]	1.16 [0.55 – 2.44]
LDD ₂₀ ^b [95 % confidence limits]	12.73 [7.37 – 22.01]	2.17 [1.26 – 3.75]	1.62 [0.94 – 2.80]
LDD ₅₀ ^b [95 % confidence limits]	21.05 [15.28 – 28.99]	3.59 [2.60 – 4.94]	2.68 [1.95 – 3.69]

a.i.: active ingredient.

^a Step-down Rao-Scott-Cochran-Armitage test procedure (one sided greater, $\alpha = 0.05$).

^b Weibull analysis using linear max. likelihood regression (95 %-confidence limits)

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

Comments of zRMS:	<p>The study is considered acceptable. All validity criteria were met.</p> <ul style="list-style-type: none"> On day 8, there was no mortality in the control group (C). On day 22, the adult emergence rate of the initial grafted larvae was 89.58 % in the control group (C). Therefore, the validity criteria for the control group were met for both test periods: Cumulative mortality in the Reference Item group also met the validity
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criteria (>50 % at day 8, actual value 68.75 %)			
Agreed endpoints:			
	Concentration		
Endpoints (D22)	mg t.i./kg diet	mg a.i.1/kg diet	mg a.i.2/kg diet
NOEC ^a	509.96	86.90	64.95
LOEC ^a	1274.91	217.24	162.38
EC ₁₀ ^b	509.96 < EC ₁₀ < 1274.91	86.90 < EC ₁₀ < 217.24	64.95 < EC ₁₀ < 162.38
[95 % confidence limits]	n.d.	n.d.	n.d.
EC ₂₀ ^b	1274.91 < EC ₂₀ < 3187.27	217.24 < EC ₂₀ < 543.10	162.38 < EC ₂₀ < 405.95
[95 % confidence limits]	n.d.	n.d.	n.d.
EC ₅₀ ^c	1464.31	249.51	186.50
[95 % confidence limits]	[1266.60 – 1692.88]	[215.82 – 288.46]	[161.32 – 215.62]
	Dose		
Endpoints (D22)	µg t.i./larva	µg a.i.1/larva	µg a.i.2/larva
NOED ^a	78.53	13.38	10.00
LOED ^a	196.34	33.45	25.01
ED ₁₀ ^b	78.53 < EC ₁₀ < 196.34	13.38 < EC ₁₀ < 33.45	10.00 < EC ₁₀ < 25.01
[95 % confidence limits]	n.d.	n.d.	n.d.
ED ₂₀ ^b	196.34 < EC ₂₀ < 490.84	33.45 < EC ₂₀ < 83.64	25.01 < EC ₂₀ < 62.52
[95 % confidence limits]	n.d.	n.d.	n.d.
ED ₅₀ ^c	225.50	38.42	28.72
[95 % confidence limits]	[195.06 – 260.70]	[33.24 – 44.42]	[24.84 – 33.20]
t.i.: test item; a.i.: active ingredient (a.i.1: prothioconazole; a.i.2: azoxystrobin); n.d.: not determined.			
^a Step-down Cochran-Armitage test procedure ($\alpha = 0.050$; one-sided greater).			
^b Estimated empirically from the results.			
^b Untrimmed Spearman-Karber procedure (95 %-confidence limits approximated by $\pm 2 \times \text{SE}(\text{Ln}(\text{EC}_{50}))$).			

Report:	CP 10.3.1.2/02; Lozano, J. (2020)
Title:	Prothioconazole 200 g/L+ Azoxystrobin 150 g/L SC (FF-075): Honey Bee (<i>Apis mellifera</i> L.) Larval Toxicity Test following Repeated Exposure under laboratory conditions.
Document No:	Trialcamp S.L.U., Spain; Study report No.: S20-00396
Guidelines:	OECD Guideline No. 239 (2016) and SANCO/3029/99, rev. 4 (2000).
GLP	Yes. Laboratory certified by the Entidad Nacional de Acreditación, Madrid, Spain.

Test material

Test item:	Prothioconazole 200 g/L+ Azoxystrobin 150 g/L SC
Purity:	Prothioconazole, content of a.i.: 200 g/L (nominal), 198 g/L (analysed). Azoxystrobin, content of a.i.: 150 g/L (nominal), 148 g/L

	(analysed).
Description	White to gray, liquid
Lot No./Batch No.:	20191211001
Test system	
Organism (<i>Species</i>):	Honeybee (<i>Apis mellifera</i> L.), synchronized first instar (L1) larvae not older than 30 hours at grafting
Study type:	Honeybee larval toxicity
Guideline deviations reported:	The reduction of the relative humidity conditions from $95 \pm 5\%$ to $80 \pm 5\%$ was done on day 7 (D7) of the test instead of on day 8 (D8). The reported deviation to the guideline has no impact on the outcome of the study since validity criteria for the control were met.
Duration of study:	22 days
Parameters measured:	Mortality and behavioural abnormalities Emergence
Observation intervals:	Larval mortality and behavioural abnormalities: days 4, 5, 6, 7 and 8 Presence of uneaten food: day 8 Mortality during pupation phase: day 15 Emergence: day 22
Test concentrations:	Test Item: Nominal concentrations of 93.09, 232.73, 581.82, 1454.55 and 3636.36 mg FF-075/kg diet. Based on the cumulative application volume of 140 μ L/larva, the corresponding nominal cumulative doses were 14.34, 35.84, 89.60, 224.00 and 560.00 μ g FF-075/larva.
Control:	Untreated diet
Reference item	BAS 152 I (a.i. content: Dimethoate, 99.7% w/w): 48.0 mg dimethoate/kg diet (equivalent to 7.39 μ g dimethoate/larva).
Test units:	Crystal polystyrene grafting cells (NICOTPLAST) (diameter: 9 mm). Cells were sterilised by submerging in ethanol 70% (v/v) for 30 min then dried. Each cell was placed into a well of a sterile 48-well cellular culture plate (Greiner Bio One), then sterilised by exposure to Ultra-violet light for 15 minutes. Open plates were placed into hermetically sealed Plexiglas desiccators, containing a dish filled with a saturated potassium sulphate (K_2SO_4) solution in order to keep a water saturated atmosphere from day 1 until day 7. On day 7, the well plates were transferred to another Plexiglas desiccator, containing a dish with a saturated sodium chloride (NaCl) solution in order to maintain a slightly lower relative humidity until day 15. On day 15, each plate was transferred into a polypropylene emergence box (18 x 13 x 7 cm, approximately) in an incubator. Bees that emerged in the emergence box had access to aqueous sucrose solution <i>ad libitum</i> .
No. of replicates:	<u>Test, reference toxicant and control:</u>

	3 replicates (hives) per dose, 16 larvae per replicate.
Acclimation period/conditions:	None
Temperature:	33.5 °C – 35.1 °C
Photoperiod	Constant darkness except during feeding and assessments
Relative humidity:	52.9 % – 100.0 %

Methodology:

Test item and reference item stock solutions and test item dilutions were freshly prepared daily using deionised water as a solvent. Seven doses of the test item with a spacing factor of 2.5 were assayed. In addition, a unique stock solution of the reference item was prepared on D3 and stored in a refrigerator for use up to D6. The same volume of deionised water was added to the diet of the control group from D3 until D6. Test solutions were added to the diet using a micropipette just before feeding, from D3 until D6. The volume of application solution in the diet did not exceed 10% of the final diet volume. The diet was homogenized using a vortex mixer.

Larval mortality was assessed before feeding on D4, D5, D6, and also on D7 and D8. and recorded as dead if no respiration (movement of spiracles) was observed. On D8, during mortality assessment, the presence of uneaten food was qualitatively recorded.

Other observations (larval appearance and size) were recorded to aid in the interpretation of mortality in comparison to the control group. Dead larvae and pupae were removed at each assessment time.

Duplicate samples (2 mL each) of the control group and of each concentration of the test item treated larval diet from D3 to D6 were taken directly after their preparation for analytical dose verification. Analytical samples were stored in a freezer at $\leq -18^{\circ}\text{C}$ within 18 minutes after sampling until chemical analysis. The analytical method used was validated according to SANCO/3029/99 rev 4 and quantification was performed by LC-MS/MS detection. The limit of quantification (LOQ) of the analytical method was 0.1 mg test item/kg (0.0170 mg prothioconazole/kg and 0.0127 mg azoxystrobin/kg) with a limit of detection (LOD) set at 0.03 mg test item/kg (30 % of the LOQ, 0.00510 mg prothioconazole/kg and 0.00381 mg azoxystrobin/kg).

The measured concentration for the active ingredient azoxystrobin in the diet was within the range of 80 – 120 % of nominal test concentration used for all the analysed samples (azoxystrobin actual min. 90 %, max. 120 %). However, the measured concentration for the active ingredient prothioconazole in the diet was slightly below the range of 80 – 120 % of nominal test concentration used, in 13 out of the 20 analysed samples (prothioconazole actual min. 66 %, max. 86 %). Recovery discrepancies were observed between the two active ingredients for all the samples analysed. Such differences could be explained by the high complexity of the analysed matrix (diet) that interacts with prothioconazole, altering its correct quantification. However, since the correct dosage of nominal test item to diet could not be fully assured, the concentrations, doses and resulting endpoints are shown corrected for the mean analysed recovery (87.65 % of nominal) along the report.

The cumulative larval mortality [%] for each treatment group was calculated from the number of dead larvae on day 8 (D8) in relation to the total number of larvae per treatment group across all replicates after selection on day 3 (D3).

Mortality during the pupation phase was evaluated on day 15 (D15) and on day 22 (D22). The cumulative pupae mortality [%] for each treatment group was calculated from the number of larvae that have not

transformed into pupae on day 15 (D15) and those bees without emergence on day 22 (D22) in relation to the total number of entered pupae after pre-pupa stage on day 8 (D8). The adult emergence [%] for each treatment group was calculated from the number of emerged bees on day 22 (D22) in relation to the total number of larvae per treatment group after selection on day 3 (D3).

In case control mortality occurred, the cumulative mortality for each test item group is expressed as percentage of the control populations after an adjustment according to the formula of Abbott (1925), modified by Schneider-Orelli (1947).

Statistical analyses:

Statistical evaluation was made using Microsoft Office Excel® v.15.0 and the statistical software Tox-RatPro® v. 3.3.0.

A step-down Cochran-Armitage test procedure ($\alpha = 0.050$; one-sided greater) was used for the estimation of the NOED / NOEC values. To justify the use of the step-down Cochran-Armitage test, at first, a trend analysis by contrasts using proportions ($\alpha = 0.05$) was performed, revealing a linear trend. Subsequently, Tarone's test procedure ($\alpha = 0.01$) was performed, not founding sings of extra-binomial variance.

The ED_{10} / EC_{10} , ED_{20} / EC_{20} and ED_{50} / EC_{50} could not be calculated by linear regression. Alternatively, the ED_{50} / EC_{50} were calculated by interpolation using the untrimmed Spearman-Kärber procedure and their 95 % confidence intervals were approximated by $\pm 2 \times SE(\ln(EC_{50}))$. The ED_{10} / EC_{10} and ED_{20} / EC_{20} were estimated empirically from the results.

Results:

On day 8, there was no mortality in the control group (C). On day 22, the adult emergence rate of the initial grafted larvae was 89.58 % in the control group (C). Therefore, the validity criteria for the control group were met for both test periods: the D8 mortality was lower than 15.00 % and the D22 days emergence rate was greater than 70.00 %, across all replicates. Cumulative mortality in the Reference Item group also met the validity criteria (>50 % at day 8, actual value 68.75 %).

Mean corrected cumulative larval mortality on day 8 (D8) of the test item treated groups was 0.00, 10.42, 8.33, 4.17 and 95.83 % in 81.59, 203.99, 509.96, 1274.91 and 3187.27 mg FF-075/kg diet (corrected for mean analysed recovery), respectively.

Mean corrected pupal mortality on day 15 (D15) of the test item treated groups was 0.00, 6.82, 9.09, 9.09 and 100.00 % in 81.59, 203.99, 509.96, 1274.91 and 3187.27 mg FF-075/kg diet (corrected for mean analysed recovery), respectively.

Mean corrected mortality at the end of the test (D22) of the test item treated groups was -2.33, 6.98, 9.30, 18.60 and 100.00 % in 81.59, 203.99, 509.96, 1274.91 and 3187.27 mg FF-075/kg diet (corrected for mean analysed recovery), respectively.

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

In comparison to the control group, the treatment groups at the two highest concentrations tested, T4 and T5, with 1274.91 and 3187.27 mg FF-075/L diet, respectively (corrected for mean analysed recovery); showed statistically significantly increased mortality at the end of the test on day 22 (D22). Therefore, the Lowest Observed Effect Concentration (LOEC) value was determined as 1274.91 mg FF-075/L diet (corrected for mean analysed recovery). The corresponding Lowest Observed Effect Dose (LOED) value,

based on the cumulative feeding volume provided per larva, was determined as 196.34 µg FF-075/larva (corrected for mean analysed recovery). Accordingly, the No Observed Effect Concentration (NOEC) value was determined as 509.96 mg FF-075/L diet (corrected for mean analysed recovery). The corresponding No Observed Effect Dose (NOED), based on the cumulative feeding volume provided per larva, was determined as 78.53 µg FF-075/larva (corrected for mean analysed recovery).

The EC₁₀-value, estimated empirically from the results, was assumed to be between 509.96 and 1274.91 mg FF-075/L diet (corrected for mean analysed recovery). The ED₁₀-value, estimated empirically from the results, was assumed to be between 78.53 and 196.34 µg FF-075/larva (corrected for mean analysed recovery).

The EC₂₀-value, estimated empirically from the results, was assumed to be between 1274.91 and 3187.27 mg FF-075/L diet (corrected for mean analysed recovery). The ED₂₀-value, estimated empirically from the results, was assumed to be between 196.34 and 490.84 µg FF-075/larva (corrected for mean analysed recovery).

The EC₅₀-value with 95 % confidence interval was determined to be 1464.31 [1266.60 – 1692.88] mg FF-075/L diet (corrected for mean analysed recovery). The ED₅₀-value with 95 % confidence interval was determined to be 225.50 [195.06 – 260.70] mg FF-075/L diet (corrected for mean analysed recovery).

Table CP 10.3.1.2/02-1: Effects of Prothioconazole 200 g/L+ Azoxystrobin 150 g/L SC (FF-075) on Honey Bee (*Apis mellifera* L.) Larvae from Repeated Exposure.

Treatment Group	Concentration ^a [mg test item/kg diet]	Cumulative Mortality [%]						
		D4	D5	D6	D7	D8	D15	D22
Control	--	0.00	0.00	0.00	0.00	0.00	8.33	10.42
Test item FF-075	81.59	0.00	0.00	0.00	0.00	0.00	8.33	8.33
	203.99	4.17	6.25	6.25	8.33	10.42	14.58	16.67
	509.96	0.00	2.08	2.08	4.17	8.33	16.67	18.75
	1274.91	0.00	0.00	0.00	2.08	4.17	16.67	27.08 ^c
	3187.27	4.17	18.75	60.42	91.67	95.83	100.00	100.00 ^c
Reference item (dimethoate)	48.00 ^b	25.00	50.00	60.42	68.75	68.75	93.75	95.83

^a Test item concentrations corrected for mean analysed recovery (87.65 % of nominal).

^b mg dimethoate/kg diet.

^c Significantly increased compared to the control group (step-down Cochran-Armitage test procedure, one sided greater, $\alpha = 0.05$).

Treatment Group	Concentration ^a [mg test item/kg diet]	Corrected Mortality [%] ^b						
		D4	D5	D6	D7	D8	D15	D22
Test item FF-075	81.59	0.00	0.00	0.00	0.00	0.00	0.00	-2.33
	203.99	4.17	6.25	6.25	8.33	10.42	6.82	6.98
	509.96	0.00	2.08	2.08	4.17	8.33	9.09	9.30
	1274.91	0.00	0.00	0.00	2.08	4.17	9.09	18.60 ^c
	3187.27	4.17	18.75	60.42	91.67	95.83	100.00	100.00 ^c

^a Test item concentrations corrected for mean analysed recovery (87.65 % of nominal).

^b Corrected for control mortality according to Abbott's formula (1925) modified by Schneider-Orelli (1947). Negative values indicate lower mortality compared to control group.

^c Significantly increased compared to the control group (step-down Cochran-Armitage test procedure, one sided greater, $\alpha = 0.05$).

Table CP 10.3.1.2/02-2: Mortality during pupation phase (D8-D22) and emergence rate (D22).

Treatment Group	Concentration ^a [mg test item/kg diet]	Pupae mortality D8-D22 [%]	Emergence D22 [%]
Control	--	10.42	89.58
Test item FF-075	81.59	8.33	91.67
	203.99	6.98	83.33
	509.96	11.36	81.25
	1274.91	23.91	72.92
	3187.27	100.00	0.00

^a Test item concentrations corrected for mean analysed recovery (87.65 % of nominal).

Conclusions:

All validity criteria were met and sensitivity of the test organisms was confirmed. Accordingly, the study was deemed valid.

The correct dosage of nominal test item to diet could not be fully assured, therefore, the concentrations, doses and resulting endpoints are shown corrected for the mean analysed recovery (87.65 % of nominal).

On day 8, no individuals were observed with uneaten food or other affections. At the end of the test, in the final assessment of the emergence on day 22, no emerged bees were recorded as being affected.

The Endpoints of the study are summarised in the following table.

Table CP 10.3.1.2/02-3: Endpoints at Emergence on day 22 (D22)

Endpoints (D22)	Concentration		
	mg t.i./kg diet	mg a.i.1/kg diet	mg a.i.2/kg diet
NOEC ^a	509.96	86.90	64.95
LOEC ^a	1274.91	217.24	162.38
EC ₁₀ ^b	509.96 < EC ₁₀ < 1274.91	86.90 < EC ₁₀ < 217.24	64.95 < EC ₁₀ < 162.38
[95 % confidence limits]	n.d.	n.d.	n.d.
EC ₂₀ ^b	1274.91 < EC ₂₀ < 3187.27	217.24 < EC ₂₀ < 543.10	162.38 < EC ₂₀ < 405.95
[95 % confidence limits]	n.d.	n.d.	n.d.
EC ₅₀ ^c	1464.31	249.51	186.50
[95 % confidence limits]	[1266.60 – 1692.88]	[215.82 – 288.46]	[161.32 – 215.62]
Endpoints (D22)	Dose		
	µg t.i./larva	µg a.i.1/larva	µg a.i.2/larva
NOED ^a	78.53	13.38	10.00

LOED ^a	196.34	33.45	25.01
ED ₁₀ ^b [95 % confidence limits]	78.53 < EC ₁₀ < 196.34 n.d.	13.38 < EC ₁₀ < 33.45 n.d.	10.00 < EC ₁₀ < 25.01 n.d.
ED ₂₀ ^b [95 % confidence limits]	196.34 < EC ₂₀ < 490.84 n.d.	33.45 < EC ₂₀ < 83.64 n.d.	25.01 < EC ₂₀ < 62.52 n.d.
ED ₅₀ ^c [95 % confidence limits]	225.50 [195.06 – 260.70]	38.42 [33.24 – 44.42]	28.72 [24.84 – 33.20]

t.i.: test item; a.i.: active ingredient (a.i.1: prothioconazole; a.i.2: azoxystrobin); n.d.: not determined.

^a Step-down Cochran-Armitage test procedure ($\alpha = 0.050$; one-sided greater).

^b Estimated empirically from the results.

^b Untrimmed Spearman-Kärber procedure (95 %-confidence limits approximated by $\pm 2 \times \text{SE}(\text{Ln}(\text{EC}_{50}))$).

A 2.3.1.4 KCP 10.3.1.4 Sub-lethal effects

A 2.3.1.5 KCP 10.3.1.5 Cage and tunnel tests

A 2.3.1.6 KCP 10.3.1.6 Field tests with honeybees

A 2.3.2.1 KCP 10.3.2.1 Effects on non-target arthropods other than bees

Comments of zRMS:	<p>The study is considered acceptable. All validity criteria were met.</p> <ul style="list-style-type: none"> Mortality below 20% (15.0 %) was achieved 7 days after the application An acceptable reproductive capacity (6.88 eggs /female) was assessed over a further 7days in the control group The toxic reference product caused 100% mortality (corrected relative to control) and confirmed the sensitivity of the test species and the test conditions. <p>Agreed endpoints:</p> <table border="1"> <thead> <tr> <th rowspan="2">Endpoint</th><th colspan="3">Rate</th></tr> <tr> <th>[L test item/ha]^a</th><th>[g prothioconazole/ha]^b</th><th>[g azoxystrobin/ha]^b</th></tr> </thead> <tbody> <tr> <td>LR₅₀ (lower –upper 95 % cl)</td><td>2.189 (1.572 – 3.134)</td><td>433.42 (311.26 – 620.53)</td><td>323.97 (232.66 – 463.83)</td></tr> <tr> <td>ER₅₀</td><td>n.d.; [> 1.3000]</td><td>n.d.; [> 257.40]</td><td>n.d.; [> 192.40]</td></tr> <tr> <td>NOER (mortality)</td><td>< 0.3250</td><td>< 64.35</td><td>< 48.10</td></tr> <tr> <td>NOER (reproduction)</td><td>≥ 1.3000</td><td>≥ 257.40</td><td>≥ 192.40</td></tr> </tbody> </table> <p>^a Rate in L of formulated product /ha ^b Active ingredient content according to the certificate of analysis (Prothioconazole 198.0 g/L, Azoxystrobin 148.0 g/L) n.d.: not determined as corrected reduction on reproduction were below 50% up to and including 1.300 L of formulated product /ha (relative to the control)</p>			Endpoint	Rate			[L test item/ha] ^a	[g prothioconazole/ha] ^b	[g azoxystrobin/ha] ^b	LR ₅₀ (lower –upper 95 % cl)	2.189 (1.572 – 3.134)	433.42 (311.26 – 620.53)	323.97 (232.66 – 463.83)	ER ₅₀	n.d.; [> 1.3000]	n.d.; [> 257.40]	n.d.; [> 192.40]	NOER (mortality)	< 0.3250	< 64.35	< 48.10	NOER (reproduction)	≥ 1.3000	≥ 257.40	≥ 192.40
Endpoint	Rate																									
	[L test item/ha] ^a	[g prothioconazole/ha] ^b	[g azoxystrobin/ha] ^b																							
LR ₅₀ (lower –upper 95 % cl)	2.189 (1.572 – 3.134)	433.42 (311.26 – 620.53)	323.97 (232.66 – 463.83)																							
ER ₅₀	n.d.; [> 1.3000]	n.d.; [> 257.40]	n.d.; [> 192.40]																							
NOER (mortality)	< 0.3250	< 64.35	< 48.10																							
NOER (reproduction)	≥ 1.3000	≥ 257.40	≥ 192.40																							

Report:	CP 10.3.2.1/01; Varela, S. (2021)
Title:	Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF075): Toxicity to the Predatory Mite, <i>Typhlodromus pyri</i> Scheuten (Acari, Phytoseiidae) under Standard Laboratory Conditions

Document No:	Eurofins Agroscience Services Ecotox GmbH, Germany; Study report No.: S20-09657.
Guideline:	IOBC (BLÜMEL et al., 2000)
GLP	Yes. Laboratory certified by the Baden-Württemberg, Berlin, Germany.

Test material

Test item:	Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075).
Purity:	Prothioconazole 198.0 g/L, Azoxystrobin 148.0 g/L.
Description	White to grey, liquid
Batch No.:	20191211001

Test system

Organism (<i>Species</i>):	<i>Typhlodromus pyri</i> Scheuten, Protonymphs (≤ 24 hours old)
Study type:	Lethal and sub-lethal toxicity.
Guideline deviations reported:	Once the residues have dried, the glass slides will be moved into a piece of green fluffy material soaked with water, instead of a Petri dishes fitted with cotton wool soaked with water slightly coloured with ink. The actual ink available in the laboratory needs to be cleared with water continuously, which make it very clear and difficult the correct visualization of mites. No impact on the study. The treated glass and the mites are not in contact with the fluffy material.
Duration of study:	14 days.
Parameters measured:	Mortality and any change in behaviour with respect to the control were assessed after 1, 3 and 7 days. Reproduction was assessed on day 9, 11 and 14.
Observation intervals:	Mortality and changes to behaviour: 1, 3 and 7 days. Reproduction: between day 9 and 14 with a maximum 3-day interval.
Test concentrations:	0.325, 0.650, 1.300, 2.600, 5.200 and 10.400 L test item /ha equivalent to 64.35, 128.70, 257.40, 514.80, 1029.60 and 2059.20 g prothioconazole/ha and 48.10, 96.20, 192.40, 384.80, 769.60 and 1539.20 g azoxystrobin/ha (according to the analytical concentration).
Control:	Deionised water
Positive control:	Dimethoate 40 % w/v EC: 0.009 L of reference item/ha equivalent to 3.726 g dimethoate /ha (according to the analytical concentration).
Test Arenas:	Glass slides measuring 7.6 x .62 cm, treated with dried residues were placed in petri dishes. A non-drying glue gel (prevent escaping) was applied on the glass slides and filter paper was extended from the slide to the petri dish to provide access to water.

Application of treatments:	The application was conducted with a laboratory track-sprayer (Company Schachtner, Ludwigsburg, Germany) to glass plates.
No. of replicates:	5 replicates for each treatment group. 20 protonymphs per replicate.
Temperature:	25.2 – 25.5 °C.
Light Intensity:	Light intensity 1765 – 2399 lux.
Relative humidity:	65.7 -81.0 %

Methodology

The study was conducted as a rate-response test with eight treatment groups, including the test item at six application rates, the reference item (Dimethoate 40 % w/v EC) at a single application rate and the control (deionised water). Each treatment group included 5 replicates containing 20 impartially selected protonymphs. The mites were exposed on the treated glass plates for 14 days. The cumulative juvenile mortality was assessed for the first 7 days of exposure. The cumulative mean number of eggs per female was assessed on days 9, 11 and 14 of exposure.

For exposure: The glass slides (7.6 × 2.6 cm approx.) were placed onto a piece of green fluffy material. Once the residues have dried, the glass slides were moved into Petri dishes fitted with pieces of green fluffy material that were soaked with water, allowing the correct visualization of mites.

A small strip of moist filter paper was partly laid on the glass slide (5 mm distance) and extended onto the cotton wool (approx. 20 mm distance) in order to give the mites access to water. A glue ring, made of a sticky gel, was drawn around the edge of the glass slides (passing over the strips of filter paper) as a barrier to dispersal of mites.

After the test units had been set up, the protonymphs were transferred onto the glass plate with a fine-bristled brush under a stereo-microscope. Healthy protonymphs of the correct age were chosen without bias from the synchronised culture. The exposure in each treatment group started after the application when residues were dried and was completed within approx. 50 minutes. Following mite introduction pollen was supplied as food and the units were incubated under the test conditions for the duration of the test.

The spray solutions were prepared with deionised water and the application was conducted with a laboratory track-sprayer (Company Schachtner, Ludwigsburg, Germany) with an application rate of 200 L/ha. The control plates were treated with deionised water.

The mortality was assessed on day one, three and seven of exposure. Dead and surviving mites were counted. The number of escaped mites was determined. Dead mites were removed after counting. Any change in behaviour with respect to the control was recorded if observable. All assessments were conducted using a stereomicroscope.

Reproduction was assessed only for the treatment groups with a corrected mortality ≤ 50 %. In this study, the reproduction was studied only with the treatments T1 to T3 of the test item and compared to control. On day seven of exposure, the sex of the test organisms was determined by the shape and size of the body (females: big, bulging, pear-shaped body; males: small, oval-flat body). The sex-ratio was always above 5 females to 1 male and therefore, it was not necessary to be adapted for the treatments by transferring males within the treatment group. The number of offspring per female was determined by counting the number of females and eggs/larvae on days 9, 11 and 14. Eggs laid until day seven inclusive were removed from the test arena and were not counted. Males and females were counted and the number of eggs and larvae was determined. Dead animals, eggs and larvae were removed after counting. All assessments

were conducted using a stereomicroscope. The cumulative reproduction per female (from day 7 to day 14) was evaluated at the end of the reproduction period.

The cumulative number of eggs laid per female during the reproduction period was calculated for each replicate and for each treatment group the cumulative mean reproduction value and the standard deviation were calculated.

The cumulative juvenile mortality and escaping rate was calculated for each mortality assessment day. The study endpoint was the cumulative juvenile mortality at day 7.

The percentage of mortality was calculated for each replicate from the combined number of dead and escaped individuals in correlation to the number of introduced test organisms. The escape rate was also calculated. A mean value and the standard deviation were calculated for each treatment group on day seven after the exposure.

The corrected mortality and escape rate were obtained by comparing the value observed in each treatment group with that in the control group, according to the formula of Abbott (1925), modified by Schneider-Orelli (1947).

Statistical analyses

Data were analysed using unrounded values; therefore, manual recalculation of rounded values presented in the report may result in slightly different values.

Step-down Cochran-Armitage Test with survival at 7 d (one-sided greater, $\alpha=0.05$) was used to detect significant differences between mortality data of the test item group and the control.

The LR_{50} and their 95%-confidence limits were calculated by Probit analysis with survival at 7 d.

Reproduction data met normality (Shapiro-Wilk's Test) and homogeneity (Levene's Test). The analysis of contrasts did not reveal a linear trend, thus the Dunnett's Multiple t-test (one-sided smaller, $\alpha=0.05$) was performed with cumulative offspring/female at 14 d.

It was not possible to determine the 14-day- ER_{50} since reductions of reproduction with the tested rates of the test item were less than 50% (the reduction of more than 50% obtained in T2 is considered not related with the product). Therefore, the 14-day- ER_{50} was estimated in accordance with the reduction in reproduction relative to the control.

For evaluation, Microsoft® Excel version 16.0 and the statistical program ToxRat® Professional 3.3.0 were used.

Results

All validity criteria were met since a) mean adult mortality in controls was 15% (test guideline requires $\leq 20\%$); b) The corrected cumulative mean mortality in the reference item group should range between 50 % and 100 % on day 7 after application (actual: 100 % corrected mortality), c) The cumulative mean number of eggs per female in the control (from day 7 to day 14) should be ≥ 4.0 eggs/female (actual: 6.88 eggs/female).

The mortality in the reference item group was 100 % which confirms the sensitivity of the test system. The mites in the test item groups showed no abnormal behaviour compared to the control group.

Observed mortality was below 50% up to and including the tested rate of 1.3000 L test item/ha (32.94 % corrected to the control). As a consequence, statistical analyses regarding mortality data and Standard Probit analysis (Finney, 1971) were performed in order to obtain the 7-d LR_{50} value. Therefore, the LR_{50}

was determined as 2.189 L test item /ha, equivalent to 433.42 g of prothioconazole/ha and 323.97 g azoxystrobin/ha, according to the analysed content.

Statistically significant lethal effects compared to control were observed up to and including the tested rate of 10.4000 L test item/ha (Step-down Cochran-Armitage Test, one-sided greater, $\alpha = 0.05$). Therefore, the NOER for lethal effects was estimated to be lower than 0.3250 L test item/ha, equivalent to 64.35 g of prothioconazole/ha and 48.10 g azoxystrobin/ha, according to the analysed content.

Table CP 10.2.1/01-1: Cumulative juvenile mortality of *Typhlodromus pyri* after 7 days of the exposure.

Treatment group	Application rate [L formulat- ed/ha]	Cumulative juvenile mortality ^a [%]	± SD (Standard deviation)	Corrected mortality [%]
Control (deionised water)	0	15.00	3.54	---
Test item (Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF- 075))	0.3250	26.00 ^{sd}	6.52	12.94
	0.6500	33.00 ^{sd}	18.57	21.18
	1.3000	43.00 ^{sd}	13.51	32.94
	2.6000	61.00 ^{sd}	6.52	54.12
	5.2000	61.00 ^{sd}	6.52	54.12
	10.4000	64.00 ^{sd}	9.62	57.65
Reference item (Dimethoate 40 % w/w EC)	0.009	100.00	0.00	100.00

^a Based on the sum of dead and escaped mites.

^{sd}: Statistically significantly increased compared to control (Step-down Cochran-Armitage Test, one-sided greater, $\alpha = 0.05$)

The rate of escaping in the test item groups was quite homogeneous for all the treatments. A minimum rate of escaping was detected at 0.3250 and 1.3000 L test item /ha with 20%, while the maximum rate of escaping was observed at 2.6000 L test item/ha with 27.00 %.

All the individuals were assessed as mature after 7 days of the exposure for the control group. An increased percentage of immature individuals were assessed after 7 days of the exposure from the rate 0.6500 L test item/ha and above.

Table CP 10.3.2.1/01-2: Rate of escaping of *Typhlodromus pyri* after 7-days of the exposure.

Treatment group	Application rate [L formulat- ed /ha]	Escaping rate [%]	± SD (Standard deviation)	Corrected escaping rate [%]	Immature [%]
Control (deionised water)	0	11.00	4.18	---	0.00
Test item (Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075))	0.3250	20.00	8.16	10.11	0.00
	0.6500	25.00	12.75	15.73	3.10
	1.3000	20.00	7.91	10.11	10.37
	2.6000	27.00	7.58	17.98	26.27
	5.2000	23.00	5.70	13.48	28.37

	10.4000	26.00	6.52	16.85	44.90
Reference item (Dimethoate 40 % w/w EC)	0.009	26.00	17.46	16.85	0.00

The reproduction was studied with the rates of 0.3250 to 1.3000 L of test item/ha and compared to control since the mortality of *T. pyri* was higher than 50% relative to the control from 2.6000 L test item/ha and above.

Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075) did not decrease the reproduction of *Typhlodromus pyri* at the rates of 0.3250 and 1.3000 L test item/ha when compared to the control. The reproduction was significantly decreased with the tested rate of 0.6500 L test item /ha (Dunnett's Multiple t-test, one-sided smaller, $\alpha = 0.05$), however, such effect is considered not related with the test item, since no effect was found when a higher rate of the test item was tested. Therefore, the NOER for sub-lethal effects (cumulative offspring/female) was estimated to be higher than or equal to 1.3000 L test item/ha, equivalent to 257.40 g of prothioconazole/ha and 192.40 g of azoxystrobin/ha, according to the analysed content.

According to these results of reproduction the 14-day-ER50 of Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075) was estimated to be higher than 1.3000 L test item/ha, equivalent to 257.40 g of prothioconazole/ha and 192.40 g of azoxystrobin/ha, according to the analysed content.

Table CP 10.3.2.1/01-3: Reproduction of *Typhlodromus pyri* adults after a 7-day exposure

Treatment group	Application rate [L formulated /ha]	Mean no. of eggs/female	± SD (Standard deviation)	Reduction in Reproduction ^a [%]
Control (deionised water)	0	6.88	0.82	---
Test item (Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075))	0.3250	6.77	0.66	1.60
	0.6500	3.24	0.46	52.95 ^{sd}
	1.3000	6.93	1.99	- 0.73
	2.6000	Not studied	--	--
	5.2000	Not studied	--	--
	10.4000	Not studied	--	--

^a Negative value means increase in reproduction rate relative to the control group

^{sd} Significantly decreased compared to control (Dunnett's Multiple t-test, one-sided smaller, $\alpha = 0.05$). The effect is considered not related with the test item.

Conclusions

The study was conducted as a rate response test under standard laboratory test conditions with eight treatment groups on *Typhlodromus pyri*, including the test item Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075) at six application rates, the reference item (Dimethoate 40 % w/v EC) at a single application rate and the control, applied with deionised water.

Mortality below 20% (15.0 %) was achieved 7 days after the application and an acceptable reproductive capacity (6.88 eggs /female) was assessed over a further 7 days in the control group, meeting the validity criteria. The toxic reference product caused 100% mortality (corrected relative to control) and confirmed the sensitivity of the test species and the test conditions.

Under these standard laboratory test conditions, LR_{50} (rate producing 50 % mortality) was determined to be 2.189 L test item/ha with the lower – upper 95 % confidence limits 1.572 – 3.134 L test item/ha respectively, and equivalent to 433.42 g prothioconazole/ha and 323.97 g azoxystrobin/ha, according to the analysed content.

The NOER for lethal effects was estimated as lower than 0.3250 L test item/ha (equivalent to 64.35 g prothioconazole/ha and 48.10 g azoxystrobin/ha according to the analysed content) since significant effects on mortality of *Typhlodromus pyri* when compared to the control at the tested rates of the test item from 0.3250 to 10.4000 L test item/ha were obtained (Step-down Cochran-Armitage Test, one-sided greater, $\alpha=0.05$).

The 14-day- ER_{50} of Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075) was estimated to be higher than 1.3000 L test item/ha, based on the results in reproduction relative to the control, since fecundity reduction was less than 50 % with the test item when it was possible to be studied; tested rates of 0.3250 and 1.3000 L test item/ha with corrected mortality below 50 % compared to the control. The fecundity reduction was higher than 50% with the tested rate of 0.6500 L test item /ha, however, such effect was considered not related with the test item, since the effect was lower when a higher rate of the test item was tested.

The NOER for sub-lethal effects (cumulative offspring/female) was estimated as higher than 1.3000 L test item/ha (equivalent to 257.40 g prothioconazole/ha and 192.40 g azoxystrobin/ha according to the analysed content) since no significant effect when compared to the control at the tested rates of 0.3250 and 1.3000 L test item/ha were obtained (Dunnett's Multiple t-test, one-sided smaller, $\alpha = 0.05$). The significant effect observed with the tested rate of 0.6500 L test item/ha is considered not related with the test item, since no effect was found when a higher rate of the test item was tested.

Comments of zRMS:	The study is considered acceptable. All validity criteria were met.
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Control mortality:	The mean mortality in the control group should be $\leq 13\%$ after 48 hours of exposure (actual: 2.5 % mortality).
Reference item mortality:	The reference item should cause a mean corrected 48-hour mortality $> 50\%$ (actual: 100.0 %).
Control reproduction:	The mean number of mummies per female in the control should be ≥ 5.0 (actual: 10.1 mummies per female). No more than two females should fail to produce mummies (actual: one female failed to produce mummies).

Agreed endpoints:

Treatment group	Application rates [mL product/ha]	Mean mortality [%]	Corrected mortality [%]	Reproduction [mummies/female]	Reduction in reproduction rate [%]
Control	0	2.5	-	10.1	-
Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075)	750	0.0	-2.6	5.1 ^b	49.5
	1500	5.0	2.6	6.1 ^b	39.6
	3000	12.5 ^a	10.3	4.9 ^b	51.5
	6000	40.0 ^a	38.5	2.4 ^b	76.2
	12000	25.0 ^a	23.1	4.1 ^b	59.4
Endpoints		[mL Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075)/ha]			
LR ₅₀ (95 % confidence interval)		n.d., but assumed > 12000			
ER ₅₀ (95 % confidence interval)		1495 (982 – 2091)			

n.d.: not determined, since mortality was below 50 %

^a statistically significantly increased compared to the control (Cochran-Armitage test, one-sided greater, $\alpha = 0.05$)

^b Statistically significantly reduced compared to the control (Jonckheere-Terpstra test, one-sided smaller, $\alpha=0.05$)

Report:	CP 10.3.2.1/02; Walter, C., and Stabler, P. (2020)
Title:	Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075): Toxicity to the Aphid Parasitoid <i>Aphidius rhopalosiphi</i> De Stefani Perez (Hymenoptera, Braconidae) under Laboratory Conditions.
Document No:	Eurofins Agrosience Services Ecotox GmbH, Germany; Study report No.: S19-04385
Guideline:	IOBC (Mead-Briggs et al., 2000)
GLP	Yes. Laboratory certified by the Baden-Wurttemberg, Berlin, Germany.

Test material

Test item:	Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075).
Purity:	Active ingredients (a.i.): 1. prothioconazole, content of a.i. (analysed): 198 g/L (nominal: 200 g/L), 2. azoxystrobin, content of a.i. (analysed): 148 g/L (nominal: 150 g/L)
Description	Liquid/white to off-white
Batch No.:	20191211001

Test system

Organism (<i>Species</i>):	<i>Aphidius rhopalosiphi</i> De Stefani-Perez, Life stage at test start: adult wasps (< 48 hours after hatching)
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Study type:	Lethal and sub-lethal toxicity.
Guideline deviations reported:	None reported.
Duration of study:	15 days.
Parameters measured:	Mortality was assessed 2, 24 and 48 hours after the end of release. The reproduction was evaluated 14–15 days after the start of exposure (11–12 days after the end of the parasitisation period).
Test concentrations:	750, 1500, 3000, 6000 and 12000 mL. product/ha.
Control:	Deionised water
Positive control:	BAS 152 11 and deionised water applied at 0.300 mL/ha.
Test Arenas:	Mortality: two treated square glass plates (length: 13 cm, serving as upper and lower covers with treated surface inwards) were assembled with an aluminium frame (length: 13 cm, height: 1.5 cm, thickness: 1 cm) to an exposure unit as soon as the spray residues were dry. Reproduction: a Plexiglas tube (diameter: ~10 cm; length: ~25 cm) was placed upon a pot containing aphid infested barley seedlings. The soil was covered with sand. The top of the tube was covered with gauze. There was an access hole in the Plexiglas tube. Once the female had been introduced, the hole was closed with foam material.
Application of treatments:	The application was conducted with a laboratory track-sprayer (Company Schachtner, Ludwigsburg, Germany) to glass plates.
No. of replicates:	4 replicates for each treatment group. 10 adult wasps.
Temperature:	20.1 – 21.6 °C.
Photoperiod:	16h:8h, L:D
Light intensity:	During exposure: 1200 – 1500 lux During parasitisation: 1089 – 2090 lux During development of aphid mummies: 6000 – 7000 lux
Relative humidity:	64.3 -80.2 %

Methodology

Adult wasps were exposed to dried spray residues of test item on glass plates for 48 hours. A control and a reference item treatment were included in the test. Mortality was assessed after 2, 24 and 48 hours. A sample of surviving females was individually confined over untreated aphid-infested plants for approx. 24 hours, and the number of parasitised aphids (mummies) that subsequently developed was recorded after 11–12 days. The study was performed according to the IOBC guideline (MEAD-BRIGGS et al. 2000). *Aphidius rhopalosiphi* is a recommended sensitive indicator species for testing the side effects of plant protection products on non-target arthropods. Data on toxicity of *Aphidius rhopalosiphi* were generated to comply with EU Regulations 283/2013 and 284/2013 implementing EU Reg. (EC) 1107/2009.

The study was conducted as a rate-response test with seven treatment groups, including the test item at five application rates, the reference item (BAS 152 65 I) at a single application rate and the control (treated with deionised water). Each test item group, control group and reference group included 4 replicates, containing 10 adult wasps (2 males and 8 females) each. Reproduction was assessed for the all test item groups and the control group. For the reproduction test 17 individually confined female wasps (inspected as either alive or affected) were taken from each test item group and control group and placed in the reproduction units consisting of barley seedlings infested with aphids.

Two treated square glass plates (length: 13 cm, serving as upper and lower covers with treated surface inwards) were assembled with an aluminium frame (length: 13 cm, height: 1.5 cm, thickness: 1 cm) to an exposure unit as soon as the spray residues were dry. Three sides of the aluminium frame contained three screened aeration holes (diameter: 1 cm) each. The fourth side of the frame contained two aeration holes and one opening (diameter: 1 cm) for the introduction of the test organisms and subsequent feeding.

The upper glass plate of the test unit was covered with a squared black cardboard frame. This reduced the light at the untreated areas of the aluminium frame in order to attract the wasps to the treated center of the glass plates. For ventilation of the exposure cages, an aquarium aeration pump was connected by means of a flexible tube to one aeration hole in the frame.

For the assessment of the reproduction of *Aphidius rhopalosiphii* a Plexiglas tube (diameter: ~10 cm; length: ~25 cm) was placed upon a pot containing aphid infested barley seedlings. The soil was covered with sand. The top of the tube was covered with gauze. There was an access hole in the Plexiglas tube. Once the female had been introduced, the hole was closed with foam material.

The test organisms were collected in glass tubes for sex determination and introduction in the exposure and reproduction units. Introduction of the wasps was completed within one hour after the application of the corresponding treatment group. During the exposure period the wasps were fed with a 20 % aqueous sucrose solution *ad libitum*.

The mortality was evaluated at 2, 24 and 48 hours after end of release. The percentage of mortality after 48 hours of exposure was calculated for each replicate from the combined number of dead and moribund individuals in relation to the number of introduced test organisms. A mean value and the standard deviation were calculated for each treatment group.

The reproduction was evaluated 14–15 days after the start of exposure (11–12 days after the end of the parasitisation period). The number of aphid mummies obtained from each treatment group within the parasitisation period of approx. 24 hours was used to calculate the mean value (\pm standard deviation) for each test item group and the control group. Only results for the females found alive at the end of the 24-hour parasitisation period were used for calculation of reproduction.

Statistical analyses

Cochran-Armitage test (one-sided greater, $\alpha=0.05$) was used to detect significant differences between mortality data of the test item groups and the control.

Reproduction data didn't meet normality (Shapiro-Wilk's Test, $\alpha=0.01$), but variance homogeneity (Levene Test, $\alpha=0.01$). Thus, Jonckheere-Terpstra test (one-sided smaller, $\alpha=0.05$) was used to compare reproduction data between the treatment groups and the control group.

The LR₅₀ value could not be calculated since mortality was below 50 % in all test item treatment groups and no rate-response relationship was observed.

The ER₅₀ value was calculated by 3-parameter logistic cumulative distribution function (weighted non-linear regression with weighting by variability (1/Var(Y), optimization method: Downhill Simplex). Confidence limits were estimated by bootstrapping and were bias-corrected (1000 resamples).

For evaluation the statistical program ToxRat Professional 3.3.0 was used.

Results

All validity criteria were met since a) the mean mortality in the control group should be ≤ 13 % after 48 hours of exposure (actual: 2.5 % mortality), b) the reference item should cause a mean corrected 48-hour mortality > 50 % (actual: 100.0 %).c) the mean number of mummies per female in the control should be ≥ 5.0 (actual: 10.1 mummies per female), d) no more than two females should fail to produce mummies (actual: one female failed to produce mummies).

Reproduction was statistically significantly reduced compared to the control at all test item rates tested (Jonckheere-Terpstra test, one-sided smaller, $\alpha = 0.05$).

Since mortality of *Aphidius rhopalosiphi* was below 50 % in all test item treatment groups and no rate-response relationship was observed, the 48-hour LR₅₀ could not be determined, but is assumed to be > 12000 mL Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075)/ha.

The ER₅₀ value (with 95 % confidence interval) for reproduction of *Aphidius rhopalosiphi* was calculated to be 1495 (982 – 2091) mL Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075)/ha (3-parameter logistic cumulative distribution function).

Reproduction of *A. rhopalosiphi* was not statistically significantly reduced compared to the control at any test item rate up to and including 700 g Prothioconazole 200 g/L +azoxystrobin 150 g/L SC (FF-075)/ha, the highest rate included in the reproduction test.

Table CP 10.3.2.1/02-1: Mortality of *Aphidius rhopalosiphi* adults after a 48-hour exposure to Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075)

Treatment group	Application rate [mL product/ha]	Mean mortality [%]	\pm SD	Corrected mortality [%]
Control	0	2.5	5.0	-
Test item Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075)	750	0.0	0.0	-2.6
	1500	5.0	5.8	2.6
	3000	12.5 *	5.0	10.3
	6000	40.0 *	14.1	38.5

	12000	25.0 *	19.1	23.1
Reference item	0.300	100.0	0.0	100.0

* Statistically significantly increased compared to the control (Cochran-Armitage test, one-sided greater, $\alpha = 0.05$)

Table CP 10.3.2.1/02-2: Reproduction of *Aphidius rhopalosiphi* following exposure to Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075).

Treatment group	Application rate [mL product/ha]	Total no. of females considered	Total no. of aphid mummies observed	Mean no. of aphid mummies per female	±SD	Reduction in reproduction [%]
Control	0	16	161	10.1	5.1	-
Test item Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075)	750	16	82	5.1 *	7.6	49.5
	1500	17	104	6.1 *	5.7	39.6
	3000	17	83	4.9 *	5.6	51.5
	6000	17	41	2.4 *	2.0	76.2
	12000	14	57	4.1 *	4.8	59.4

* Statistically significantly reduced compared to the control (Jonckheere-Terpstra test, one-sided smaller, $\alpha = 0.05$)

Conclusions:

Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075) caused a statistically significant effect on the mortality of *Aphidius rhopalosiphi* compared to the control at application rates of 3000, 6000 and 12000 mL/ha. Reproduction was statistically significantly reduced compared to the control at each rate tested, i.e. from 750 up to 12000 mL Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075)/ha. Since mortality was below 50 % in all test item treatment groups, the 48-hour LR₅₀ could not be determined, but is assumed to be > 12000 mL Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075)/ha. The ER₅₀ value (with 95 % confidence interval) for reproduction of *Aphidius rhopalosiphi* was calculated to be 1495 (982 – 2091) mL Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075)/ha.

Comments of zRMS:	The study is considered acceptable. All validity criteria were met.	
	Control mortality	The mean mortality (dead and escaped individuals) in the control should be ≤ 20 % on day 7 after treatment (actual: 10.0 % mortality).
	Reference item mortality	The corrected cumulative mean mortality in the reference item group should range between 50 % and 100 % on day 7 after application (actual: 98.1 % corrected mortality).
	Control reproduction	The cumulative mean number of eggs per female in the control (from day 7 to day 14) should be ≥ 4.0 eggs/female (actual: 8.2 eggs/female).

Agreed endpoints:					
Treatment group	Application rates [mL product/ha]	Cumulative juvenile mortality ^a [%]	Corrected mortality ^b [%]	Reproduction [eggs/female]	Reduction in reproduction rate [%]
Control	0	10.0	-	8.2	-
Test item Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075)	750	8.3	-1.9	7.1	13.4
	1500	8.3	-1.9	6.3	23.2
	3000	15.0	5.6	7.8	4.9
	6000	23.3 *	14.8	8.2	0.0
	12000	36.7 *	29.7	7.1	13.4
Endpoints		[mL product/ha]			
7-day LR ₅₀		n.d., but assumed > 12000			
14-day ER ₅₀		n.d., but assumed > 12000			
^a based on the sum of dead and escaped mites					
^b negative values indicate reduced mortality compared to the control					
[*] statistically significantly increased compared to the control (Cochran-Armitage test, one-sided greater, $\alpha = 0.05$)					
n.d.: not determined (since corrected mortality was < 50 %)					

Report:	CP 10.3.2.1/03; Walter, C. and Stäbler, P. (2020)
Title:	Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075): Toxicity to the Predatory Mite, <i>Typhlodromus pyri</i> Scheuten (Acari, Phytoseiidae) under Extended Laboratory Conditions.
Document No:	Eurofins MITOX FOPSE Sarl, France; Study report No.: S19-04389
Guideline:	No Guideline available but based on IOBC (BLÜMEL et al., 2000).
GLP	Yes. Laboratory certified by the Ministry of Health, Welfare and Sport, Utrecht, The Netherlands.

Test material

Test item:	Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075).
Purity:	Active ingredients (a.i.): 1. prothioconazole, content of a.i. (analysed): 198 g/L (nominal: 200 g/L), 2. azoxystrobin, content of a.i. (analysed): 148 g/L (nominal: 150 g/L)
Description	Liquid/ white to off-white
Batch No.:	20191211001

Test system

Organism (<i>Species</i>):	<i>Typhlodromus pyri</i> Scheuten (Acari: Phytoseiidae), Life stage at test start: <24 hours old Protonymphs
Study type:	Lethal and sub-lethal toxicity
Guideline deviations reported:	None with impact on the outcome of the study

Duration of study:	14 days
Parameters measured:	Mortality was assessed after 3 and 7 days of exposure; Reproduction (number of eggs per female produced over a 7-day period – day 7-14) of all surviving females from all test rates that caused ≤50% corrected mortality.
Test concentrations:	Test item: 750, 1500, 3000, 6000, and 12000 mL Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075)/ha Reference item: 30 mL BAS 152 65 I/ha
Control:	Deionised water

Test Arenas:	Mortality: The exposure was conducted on leaves of untreated common bean plants (<i>Phaseolus vulgaris</i> , variety: Maxi, growth stage: BBCH 12 to 13 according to MEIER, 2001) grown at the testing facility. The leaf disc was positioned on top of the wet cotton wool pad in the Petri dish after treatment and drying of the residues, with the treated leaf surface facing upwards
Application of treatments:	The application was conducted with a calibrated Schachtner laboratory track-sprayer at an application volume of 200 L/ha to the top side of detached bean leaves.
No. of replicates:	Mortality: 6 replicates for the control, each treatment group and the reference group. 10 <i>T. pyri</i> per replicate.
Temperature:	24.7 – 25.8 °C
Photoperiod:	16 h light / 8 h darkness
Light intensity:	3000 - 3500 lux
Relative humidity:	70.9 – 82.1 %

Methodology

Test and reference item solutions were prepared by diluting appropriate amounts of stock solution in deionised water and were applied with a calibrated laboratory track-sprayer to the upper side of bean leaves in a spray volume of 200 L/ha. A control group treated with deionised water was included in the study. Bean leaves were selected from an untreated culture maintained by the test facility.

Typhlodromus pyri Scheuten were obtained from a synchronized cohort of eggs was obtained from a laboratory culture at the test facility. Stock keeping was performed according to OVERMEER (1985). Young protonymphs <24 hours old were used for testing and were introduced to exposure units in groups of 10 to dry residues. The bioassay was initiated once residues had dried, and the leaf discs were cut out from the treated leaves. Six replicates of each treatments and control group were used. Food was added to each unit before the mites were introduced.

Test units were inspected 3 and 7 days after initiation of the bioassay, and food was added to the test units. Dead and surviving mites were counted. The number of escaped mites was determined. Dead mites were removed after counting. Any change in behaviour with respect to the control was recorded if observable. All assessments were conducted using a stereomicroscope.

Reproduction was assessed for the control group and all test item groups, since the corrected mortality was < 50 % up to the highest test item rate. On day seven of exposure the sex of the test organisms was determined by the shape and size of the body (females: big, bulging, pear-shaped body; males: small, oval-flat body). Nymphs which had not developed into adults due to a potential treatment effect were recorded as juveniles until a sex determination was possible. The sex ratio was adapted in test item group T2 by transferring one male within the treatment group. No adaptation was required for the control group and all other test item groups as the sex ratio was already in line with the requirement of ≥ 5 females to 1 male. The number of offspring per female was determined by counting the number of females and eggs/larvae on day 10, 12 and 14. The number of surviving adult males were determined as well. Eggs laid until day seven inclusive were removed from the test arena and were not counted. Dead individuals, eggs and larvae were removed after counting. All assessments were conducted using a stereomicroscope.

The study endpoint was the cumulative juvenile mortality at day 7. Additionally, the escape rate was determined for each treatment group. The percentage of mortality was calculated for each replicate from the

combined number of dead and escaped individuals in correlation to the number of introduced test organisms. The escape rate was calculated as well. The mean value and standard deviation were calculated for each treatment group. The corrected mortality and escape rate were obtained by comparing the value observed in each treatment group with that in the control group, according to the formula of ABBOTT (1925), modified by SCHNEIDER-ORELLI (1947).

The cumulative reproduction per female (from day 7 to day 14) was evaluated at the end of the reproduction period.

The cumulative number of eggs laid per female during the reproduction period was calculated per replicate with the formula below. For each treatment group the cumulative mean reproduction value and the standard deviation were calculated. Evaluation of reproduction data based only on replicates containing females and males over the whole reproduction phase. Nymphs that did not develop into adults until day 7 of exposure were not considered for evaluation of reproduction until a sex determination was possible.

Statistical analyses

Mortality data (number of dead and escaped individuals after 7 days compared to the number of introduced individuals) and escape rate data (number of escaped individuals after 7 days compared to the number of introduced individuals) of the test item groups were tested for monotonicity (Trend analysis by Contrasts, $\alpha = 0.05$). Since mortality data showed a linear trend, Cochran-Armitage test (one-sided greater, $\alpha = 0.05$) was used for comparison of test item groups and the control group. Escape rate data did not show a linear trend, thus multiple Chi²-test with Bonferroni-Holm adjustment (one-sided greater, $\alpha = 0.05$) was used for comparison of test item groups and the control group.

Cumulative reproduction data (cumulative number of eggs per female) met normality (Shapiro-Wilk's Test, $\alpha = 0.01$) and variance homogeneity (Levene's Test, $\alpha = 0.01$). Since data did not show a linear trend (Trend analysis by Contrasts, $\alpha = 0.05$), statistical analysis was conducted using Dunnett's t-test (one-sided smaller, $\alpha = 0.05$).

The 7-day LR₅₀ and the 14-day ER₅₀ could not be calculated, since mortality and reduction in reproduction rate were below 50 % in all test item treatment groups. For evaluation the statistical program ToxRat Professional 3.3.0 was used.

Results

All validity criteria were met since a) the mean mortality in the control should be $\leq 20\%$ (actual: 10.0 % mortality), b) the reference item should cause a cumulative corrected mortality $> 50\%$ (actual: 98.1% corrected mortality), c) the mean reproduction (as number of eggs/female/7 days) in the control should be ≥ 4 (actual: 8.2).

Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075) caused a statistically significant increase in the mortality of *T. pyri* at application rates of 6000 and 12000 mL product/ha (Cochran-Armitage test, one-sided greater, $\alpha = 0.05$) but no statistically significant difference in the escape rate at any test item rate compared to the control (Multiple Chi²-test with Bonferroni-Holm adjustment, one-sided greater, $\alpha = 0.05$).

Cumulative juvenile mortality in the reference item group was 98.3 % (98.1 % corrected with control). No abnormal behaviour of the test organisms was observed compared to the control.

Reproduction of *T. pyri* was not statistically significantly reduced compared to the control up to and including the highest test item rate of 12000 g Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075)/ha (Dunnett's t-test, one-sided smaller, $\alpha = 0.05$).

Since mortality and reduction in reproduction rate were below 50 % in all test item treatment groups, the 7-day LR₅₀ and the 14-day ER₅₀ could not be calculated, but are assumed to be > 12000 g Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075)/ha, the highest rate tested

Table CP 10.3.2.1/03-1: Escape Rate of *Typhlodromus pyri* after a 7 day exposure to Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075)

Treatment group	Application rate [mL product/ha]	Escape rate [%]	±SD	Corrected escape rate [%] ^a
Control	0	10.0	6.3	-
Test item Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF- 075)	750	8.3	4.1	-1.9
	1500	6.7	8.2	-3.7
	3000	13.3	12.1	3.7
	6000	13.3	5.2	3.7
	12000	10.0	11.0	0.0
Reference item	30.0	30.0	6.3	22.2

^a Negative values indicate that the escape rate was lower compared to the control

Table CP 10.3.2.1/03-2: Reproduction of *Typhlodromus pyri* adults after a 14-day exposure to Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075)

Treatment group	Application rate [mL product/ha]	Reproduction [eggs/female]	±SD	Reduction in reproduc- tion rate [%]
Control	0	8.2	1.8	-
Test item Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF- 075)	750	7.1	1.6	13.4
	1500	6.3	3.3	23.2
	3000	7.8	2.4	4.9
	6000	8.2	3.0	0.0
	12000	7.1	3.1	13.4

Conclusions:

Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075) caused a statistically significant increase in the mortality of *T. pyri* at application rates of 6000 and 12000 mL product/ha but no statistically significant difference in the escape rate at any test item rate compared to the control. Reproduction of *T. pyri* was not statistically significantly reduced compared to the control up to the highest test item rate of 12000 g Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075)/ha. Since mortality and reduction in reproduction rate were below 50 % in all test item treatment groups, the 7-day LR₅₀ and the 14-day ER₅₀ could not be calculated, but are assumed to be > 12000 g Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075)/ha, the highest rate tested.

Comments of zRMS:	The study is considered acceptable. All validity criteria were met.					
	Control mortality:	The mean mortality in the control should be ≤ 10 % after 48 hours of exposure (actual: 0.0 % mortality).				
	Reference item mortality:	The reference item should cause a cumulative 48-hour corrected mortality > 50 % (actual: 100.0 % corrected mortality).				
	Control reproduction:	The mean number of mummies per female in the control should be ≥ 5.0 mummies/female (actual: 17.4 mummies/female).				
		No more than 2 females should fail to produce mummies (actual: 2 females failed to produce mummies).				
	Agreed endpoints:					
	Treatment group	Application rates	Settling on plants during initial 3 h	Mean mortality	Reproduction	Reduction in reproduction rate
		[mL product/ha]	[%]	[%]	[mummies per female]	[%]
	Control	0	40.4	0.0	17.4	-
	Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075)	750	33.8	0.0	17.9	-2.9
	1500	21.9 ^a	0.0	17.4	0.0	
	3000	38.4	0.0	16.7	4.0	
	6000	51.4	16.0 ^b	15.6	10.3	
	12000	33.7	24.0 ^b	9.9 ^c	43.1	
Reference BAS 152 65 I	18.0	35.1	100.0	n.d.	n.d.	
Endpoints	[mL Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075)/ha]					
LR ₅₀	n.d. ^d , but assumed > 12000					
ER ₅₀	n.d. ^e , but assumed > 12000					
^a Since settling on the plants was less than 30 % during the initial 3 hours, two further assessments were carried out after 24 and 48 hours, each resulting in settling rates > 30 % for this treatment group						
^b Statistically significantly increased compared to the control (Cochran-Armitage test, one-sided smaller, α = 0.05)						
^c Statistically significantly reduced compared to the control (Williams' test, one-sided smaller, α = 0.05)						
^d LR ₅₀ not determined as corrected mortality was below 50 % in all test item treatment groups						
^e ER ₅₀ not determined as reduction in reproduction was below 50 % in all test item treatment groups						
n.d.: not determined						

Report:	CP 10.3.2.1/04; Walter, C. and Stäbler, P (2020)
Title:	Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075): Toxicity to the Aphid Parasitoid <i>Aphidius rhopalosiphii</i> De Stefani Perez (Hymenoptera, Braconidae) under Extended Laboratory Conditions.
Document No:	Eurofins Agrosience Services Ecotox GmbH, Germany; Study report No.: S19-04386
Guideline:	IOBC (Mead-Briggs et al., 2009)
GLP	Yes. Laboratory certified by the Baden-Württemberg, Berlin, Germany.

Test material

Test item: Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075); Batch No. 20191211001; active ingredients (a.i.):

1. prothioconazole, content of a.i. (analysed): 198 g/L (nominal: 200 g/L),
2. azoxystrobin, content of a.i. (analysed): 148 g/L (nominal: 150 g/L)

Purity: Prothioconazole 200 g/L + 150 g/L Azoxystrobin (nominal),
Prothioconazole 198 g/L + Azoxystrobin 148 g/L analysed.

Description: Liquid white to off white

Batch No.: 20191211001

Test system

Organism (*Species*): *Aphidius rhopalosiphi* De Stefani-Perez,
Life stage at test start: adult wasps (< 48 hours after hatching)

Study type: Lethal and sub-lethal toxicity.

Guideline deviations reported: The time buffer for the 24 h-parasitisation period was exceeded for more than 15 minutes in the control group (24 h + 16 min) and in test item treatment groups T1 (24 h + 21 min), T3 (24 h + 27 min), T4 (24 h + 26 min) and T5 (24 h + 26 min). No impact on the study is expected since exceedance of the time buffer has no impact on the study results.

Duration of study: 14 days.

Parameters measured: Mortality was assessed 2, 24 and 48 hours after exposure.
Any change in behaviour with respect to the control was documented if observable (e.g. intense grooming, inactivity).
Reproduction was assessed 14 days after the start of exposure.

Observation intervals: Mortality and changes to behaviour: 2, 24 and 48 hours after introduction.
Reproduction: 14 days after start of exposure (11 days after end of parasitisation).

Test concentrations: Test item: 750, 1500, 3000, 6000 and 12000 mL product/ha
Reference item: 18.0 mL BAS 152 11 l/ha

Control: Deionised water

Test Arenas: Mortality: Before application the soil of the pots was covered with sand. After application and drying of treatment residues the pots with the barley seedlings were enclosed within clear Plexiglas cylinders (diameter: approx. 10 cm; length: approx. 25 cm). The tops of the cylinders were cov-

ered with wasp-proof gauze. For introduction of the wasps and the connection to the ventilation system a hole in the wall of the cylinder was used.

Reproduction: For the assessment of the parasitic capacity of *Aphidius rhopalosiphii* a Plexiglas cylinder (diameter: ~10 cm; length: ~25 cm) was placed upon a pot containing aphid infested barley seedlings. The soil was covered with sand. The top of the cylinder was covered with gauze. There was an access hole in the Plexiglas cylinder. Once the female had been introduced, the hole was closed with foam material.

Application of treatments:	The application was conducted with a laboratory track-sprayer (Company Schachtner, Ludwigsburg, Germany) to barley seedlings.
No. of replicates:	5 replicates for each treatment group. 5 female adult wasps.
Temperature:	19.6 – 21.5 °C.
Photoperiod:	16 h light / 8 h darkness
Light intensity:	770 – 2200 lux during exposure, 680 – 2100 lux during parasitisation, 5100 – 10860 lux during development of mummies.
Relative humidity:	63.5 – 81.2 %

Dates of work 27 Apr 2020 – 11 May 2020

Methodology

The test and reference item were diluted in deionised water and applied with a laboratory track sprayer to barley seedlings in a spray volume of 400 L/ha. A control group treated with deionised water was included in the study. After assembling of test units five adult female wasps were introduced into each test unit (5 replicates per treatment). The settling behaviour of the wasps was assessed during the initial three hours after their introduction. Direct treatment effects and any change in behaviour with respect to the control were assessed 2, 24 and 48 hours after start of exposure. Reproduction (mummies/female) was assessed 11 days following a 24-hour parasitisation period. Reproduction was assessed for the control group and each test item group, where the corrected mortality was below 50 %.

Adult wasps less than 48 hours old (after hatching) were used for the test. The delivered mummies were transferred to hatching containers. The freshly hatched wasps were provided with honey water gelatine solution (100 g honey, 50 g Aqua dest., 1.5 g gelatine) on a small piece of plastic (ad libitum). On the day of application the test organisms were collected in glass tubes for sex determination and introduction in the exposure units.

The study was conducted as a rate-response test including a water control group C (deionised water), 5 test item groups (T1–T5) and one reference item group R. Each treatment group included 5 replicates, containing 5 female adults each. For the reproduction test 17 individually confined female survivors (alive or affected) were taken from each treatment group without bias. The reproduction was assessed for the test item groups, where the corrected mortality was calculated as ≤ 50 %.

Barley (*Hordeum vulgare*, variety “Avalon”) seedlings were used for the exposure and reproduction units. For the exposure units the seedlings were trimmed to a uniform height of 10 to 12 cm before use. Each

exposure unit contained 8 to 10 seedlings at the 2nd leaf growth stage (BBCH 11 to 12 according to MEIER, U., 2001).

For the reproduction units 10–40 barley seedlings were sown per pot in the test facility, 8 days before start of the reproduction phase. The seedlings were 10–15 cm tall at start of the parasitisation period..

Rhopalosiphum padi (Homoptera, Aphididae) in the life stage of 2nd instar to adult were used for parasitisation in the reproduction test. The aphids were obtained from a stock culture at the test facility. Each reproduction unit was infested with >100 aphids (both adults and nymphs) at start of the parasitisation period.

Before application the soil of the pots was covered with sand. After application and drying of treatment residues the pots with the barley seedlings were enclosed within clear Plexiglas cylinders (diameter: approx. 10 cm; length: approx. 25 cm). The tops of the cylinders were covered with wasp-proof gauze. For introduction of the wasps and the connection to the ventilation system a hole in the wall of the cylinder was used.

Treatments were applied to test units using a calibrated laboratory track sprayer at a rate of 400 L/ha.

For the assessment of the parasitic capacity of *Aphidius rhopalosiphi* a Plexiglas cylinder (diameter: ~10 cm; length: ~25 cm) was placed upon a pot containing aphid infested barley seedlings. The soil was covered with sand. The top of the cylinder was covered with gauze. There was an access hole in the Plexiglas cylinder. Once the female had been introduced, the hole was closed with foam material.

As food source for the test organisms the test plants were sprayed with a 10 % (w/w) fructose solution shortly before application. By this, plants became more attractive for parasitoids. During the 24-hour parasitisation period no food was provided. However, the test organisms could feed on the honeydew of the aphids.

Mortality was evaluated at 2, 24 and 48 hours after the start of exposure. Endpoint was the mortality after 48 hours of exposure. The percentage of mortality after 48 hours was calculated for each replicate from the combined number of dead and moribund individuals in correlation to the number of introduced test organisms. The mean value and standard deviation were calculated for each treatment group.

The condition of the test organisms was recorded 2, 24 and 48 hours after introduction. Any change in behaviour with respect to the control was documented if observable (e.g. intense grooming, inactivity).

Reproduction was assessed for treatment groups with a corrected mortality ≤ 50 %. After the 48-hour mortality assessment the surviving females were removed from the exposure units and transferred individually to the reproduction units. After a 24-hour parasitisation period females were removed from the reproduction units and their condition (alive or dead) was recorded. The number of parasitised aphids was counted in each replicate 14 days after application (11 days after end of parasitisation period).

Statistical analyses

The mean values of settling observations were square root arcsine transformed prior to comparison by a one-way analysis of variance (SOKAL & ROHLF, 1981). Dunnett's t-test (3-h and 24-h assessments) and Williams' test (48-h assessment) (both one-sided smaller, $\alpha = 0.05$) were used to detect significant differences in the settling rates between the test item groups and the control group.

Mortality data showed a linear trend (Trend analysis by Contrasts, $\alpha = 0.05$), thus Cochran-Armitage test (one-sided greater, $\alpha = 0.05$) was used to detect significant differences between the test item groups and the control.

Reproduction data met normality (Shapiro-Wilk's test, $\alpha = 0.01$) and homoscedasticity (Levene's test, $\alpha = 0.01$). Since data showed a linear trend (Trend analysis by Contrasts, $\alpha = 0.05$), statistical analysis was conducted using Williams' test (one-sided smaller, $\alpha = 0.05$).

The 48-hour LR_{50} could not be calculated as the corrected mortality was below 50 % in all test item treatment groups.

The ER_{50} could not be calculated as reduction in reproduction was below 50 % in all test item treatment groups.

Results

All validity criteria were met since a) the mean mortality in the control should be ≤ 10 % after 48 hours of exposure (actual: 0.0 % mortality), b) the reference item should cause a cumulative 48-hour corrected mortality > 50 % (actual: 100.0 % corrected mortality), c) the mean number of mummies per female in the control should be ≥ 5.0 mummies/female (actual: 17.4 mummies/female), d) no more than 2 females should fail to produce mummies (actual: 2 females failed to produce mummies).

The mortality in the reference item group was 100 %.

Since mean settling on the plants was less than 30 % during the initial 3 hours at the test item rate of 1500 mL product/ha (T2), two further assessments were carried out after 24 and 48 hours for all treatment groups. The settling on plants during the initial three hours as well as 24 and 48 hours after start of exposure (prolonged evaluation) was not statistically significantly reduced in the test item treatment groups, when compared to the control group (Dunnett's t-test for the 3-h and 24-h assessments, Williams' test for the 48-h assessment, one-sided smaller, $\alpha = 0.05$). Details of all assessments are given in Table 3 to Table 15 in Appendix C. In the prolonged assessment after 24 and 48 hours a higher settling rate on plants above 30 % was observed in test item group T2.

Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075) caused a statistically significant increase in the mortality of *Aphidius rhopalosiphi* compared to the control at rates of 6000 and 12000 mL/ha. (Cochran-Armitage test, one-sided greater, $\alpha = 0.05$).

No abnormal behaviour was observed in any test item group compared to the control group, but at the 48-h assessment of settling behaviour and mortality, one test organism in each of the test item groups T4 and T5 and in the reference item group was found dead sticking to a plant.

Reproduction of *Aphidius rhopalosiphi* was statistically significantly reduced compared to the control at the highest test item rate of 12000 mL Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075)/ha (Williams' test, one-sided smaller, $\alpha = 0.05$).

As no effects ≥ 50 % on mortality of *Aphidius rhopalosiphi* could be observed at any test item rate, the 48-hour LR_{50} of Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075) could not be calculated but is assumed to be > 12000 mL/ha.

Table CP 10.3.2.1/04-1: Settling behaviour of *Aphidius rhopalosiphi* after 24 hours.

Treatment group	Application rate [mL product/ha]	% of wasps settled on the plants after 24 h of exposure in each replicate					Mean value
		1	2	3	4	5	
Control	-	0.0	40.0	60.0	20.0	20.0	28.0
Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075)	750	40.0	40.0	40.0	40.0	40.0	40.0
	1500	20.0	60.0	40.0	40.0	40.0	40.0
	3000	60.0	75.0	20.0	20.0	0.0	35.0
	6000	60.0	40.0	60.0	40.0	60.0	52.0
	12000	40.0	20.0	40.0	20.0	0.0	24.0

Table CP 10.3.2.1/04-2: Settling behaviour of *Aphidius rhopalosiphi* after 48 hours.

Treatment group	Application rate [mL product/ha]	% of wasps settled on the plants after 48 h of exposure in each replicate					Mean value
		1	2	3	4	5	
Control	-	40.0	20.0	40.0	20.0	0.0	24.0
Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075)	750	20.0	60.0	40.0	20.0	40.0	36.0
	1500	60.0	40.0	80.0	60.0	40.0	56.0
	3000	40.0	60.0	40.0	50.0	40.0	46.0
	6000	20.0	60.0	40.0	60.0	50.0	46.0
	12000	20.0	50.0	40.0	33.3	66.7	42.0

Table CP 10.3.2.1/04-3: Mortality of *Aphidius rhopalosiphi* adults after a 48-hour exposure to Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075).

Treatment group	Application rate [mL/ha]	Mean mortality [%]	±SD	Corrected mortality [%]
Control	-	0.0	0.0	-
Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075)	750	0.0	0.0	0.0
	1500	0.0	0.0	0.0
	3000	0.0	0.0	0.0
	6000	16.0 *	16.7	16.0
	12000	24.0 *	16.7	24.0
Reference item	18.0	100.0	0.0	100.0

* Statistically significantly increased compared to the control (Cochran-Armitage test, one-sided smaller, $\alpha = 0.05$)

Table CP 10.3.2.1/04-4: Reproduction of *Aphidius rhopalosiphi* after exposure to Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075).

Treatment group	Application rate [mL/ha]	Total females found alive	Total mummies	Mean no. of mummies per female	±SD	Reduction in reproduction [%]
Control	-	17	296	17.4	8.9	-
Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075)	750	17	305	17.9	7.3	-2.9
	1500	16	278	17.4	9.9	0.0
	3000	17	284	16.7	8.3	4.0
	6000	16	249	15.6	11.3	10.3
	12000	15	149	9.9 *	6.4	43.1

* Statistically significantly reduced compared to the control (Williams' test, one-sided smaller, $\alpha = 0.05$)

Conclusions

Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075) caused a statistically significant increase in the mortality of *Aphidius rhopalosiphi* compared to the control at rates of 6000 and 12000 mL/ha. The test item caused no repellent effect on *Aphidius rhopalosiphi* during the initial 3 hours and after 24 and 48 hours of exposure. Reproduction was statistically significantly reduced compared to the control at the highest test item rate of 12000 mL Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075)/ha.

No effects on mortality of *Aphidius rhopalosiphi* equal or above 50 % could be observed at any test item rate. Therefore, the 48-hour LR₅₀ of Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075) could not be calculated but is assumed to be > 12000 mL/ha.

Reduction in reproduction was below 50 % in all test item treatment groups. Therefore, the ER₅₀ of Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075) could not be calculated but is assumed to be > 12000 mL/ha.

Comments of zRMS:

The study is considered acceptable. All validity criteria were met.

All validity criteria were met and the sensitivity of the test organisms was confirmed: 0.00 % mortality in the control group and 25.2 fertile eggs per female per day. Mortality in the reference item group was ≥ 50 % (actual value: 100 %). Hence, the validity criteria were fulfilled and accordingly, the study was deemed valid.

Agreed endpoints:

Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075) Extended conditions; fresh and dried residues on bean leaflets	
Endpoint	[L test item/ha] ^a
NOER Lethal effects	NOER ≥ 16 L FP/ha
LR₅₀	LR ₅₀ > 16 L FP/ha
Reproduction^b	No impact on reproduction up to and including 16.0 L test item/ha in accordance with the validity criteria for the control group: ≥ 2 fertile eggs/female/day

^a Rate in L of formulated product (FP)/ha

^b Reproduction was evaluated only qualitatively and no statistical analysis was performed

Report:	CP 10.3.2.1/05; Luna, F. (2020)
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Title:	Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075): Toxicity to the Ladybird, <i>Coccinella septempunctata</i> L. (Coleoptera: Coccinellidae) Using an Extended Laboratory Test with Freshly Applied Spray Deposits
Document No:	TrialCamp S.L.U., Spain; Study report No.: S19- 04397.
Guideline:	IOBC (Schmuck, R., et al., 2000) modified for the use of natural substrate
GLP	Yes. Laboratory certified by Entidad Nacional de Acreditación, Spain.

Test material

Test item:	Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075).
Purity:	Prothioconazole 198 g/L + Azoxystrobin 148 g/L, analysed.
Description	White to grey, liquid.
Batch No.:	20191211001

Test system

Organism (<i>Species</i>):	<i>Coccinella septempunctata</i> L. (Coleoptera, Coccinellidae) life stage at start of exposure: larvae (4 days old).
Study type:	Lethal and sub-lethal toxicity.
Guideline deviations reported:	All the eggs laid during the two weeks were not used to assess hatching rate. Eggs laid on walls of the containers were taken into account for fecundity calculations (eggs/female/day) but they were not taken into account to assess hatching rate. The eggs laid on walls could be damaged when removed. There were sufficient eggs from the egg laying substrate, bean sprouts or paper to study the hatching rate. Date of report has been delayed with respect to the indicated in the Study Plan. The Study Monitor and Sponsor's Representative was absent for several months and agreed with this delay.
Duration of study:	7-8 weeks
Parameters measured:	Mortality was assessed 2-hours after exposure and every working day to the completion of the adult stage or the last evaluation of larval or pupal mortality, 12 days after exposure. Pupation and hatching Juvenile mortality Abnormal appearance of larvae. Reproduction was assessed from day 7 to day 21. Fecundity Hatching rate
Test concentrations:	Test item: 1.0, 2.0, 4.0, 8.0 and 16.0 L of formulated product (FP)/ha of formulated product (FP)/ha, nominal.
Control:	Reference item: 0.030 L product/ha Deionised water

Positive control:	Dimethoate 40 % w/v EC (BAS 152 65 I) Batch No. 10248664A Content of a.i. (analysed): Dimethoate: 414.0 g /L
Test Arenas:	The exposure was conducted on detached leaves from bean plants (<i>Phaseolus vulgaris</i> , Fabaceae) of the variety CONTENDER. The leaves were placed with the treated surface (adaxial face) upwards on the top of a wet cotton pad in a Petri dish which was moistened regularly with deionised water throughout the test. Plastic vessels (size: 32.5 x 26.5 x 20 cm) closed with gauze covered lids were used as maintenance and breeding units. On the bottom of the container, a piece of paper or a tissue (to absorb the excreta and to facilitate the cleaning process) was placed. On the top of the tissue, black plastic sheets rolled up to form a cylinder were provided for egg laying.
Application of treatments:	The application was conducted with a laboratory track-sprayer (Company Schachtner, Ludwigsburg, Germany) to bean leaves at 200 L/ha
No. of replicates:	40 replicates for each treatment group. 1 larva per replicate.
Temperature:	24.5 – 25.2 °C.
Photoperiod:	16 h light / 8 h darkness
Light intensity:	1335 - 4083 lux
Relative humidity:	74.3 – 89.8 %

Methodology

The objective of the study was to determine the effects of freshly applied spray deposits of Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075) on mortality and reproduction of the ladybird *Coccinella septempunctata* L. under extended laboratory conditions on bean leaflets; to determine the No Observed Effect Rate (NOER) and the Median Lethal Rate (LR50), where possible. Reproductive results were compared to the threshold values described in the guideline method as validity criteria for the control treatment, where possible.

Larvae of *Coccinella septempunctata* L. (Coleoptera, *Coccinellidae*) were used as test organism. Forty larvae per treatment (4 days old) of *C. septempunctata* from synchronized eggs were selected for the study. Egg clusters with more than 40 eggs per treatment were confined until the exposure of larvae. They were fed, at least, every working day with fresh aphids (*Acyrtosiphon pisum*). On the day of application, the test organisms were carefully transferred to the test units using a fine brush.

The study was conducted as a rate response test with seven treatment groups, including the test item at five application rates, the reference item (BAS 152 65 I: Dimethoate 40 % w/v EC) at a single application rate and the control (treated with deionised water). Each treatment group included 40 replicates, containing one larva each. The exposure was conducted on detached leaves from bean plants (*Phaseolus vulgaris*, Fabaceae) of the variety CONTENDER grown at the testing facility. One leaf was used per replicate test unit. Immediately before application of the test solutions, the bean leaves were cut off and placed on wet paper on petri dishes for the application.

The application was conducted with a laboratory track-sprayer (Company Schachtner, Ludwigsburg, Germany). The track-sprayer was calibrated with water before application by adjusting the spraying pressure and distance to target to provide an output of $200 \text{ L} \pm 10 \%$ per ha ($2 \text{ mg/cm}^2 \pm 10 \%$).

After spray residues had dried (approx. 39 – 1 hr 11 mins after application), the leaves were placed with the treated surface (adaxial face) upwards on the top of a wet cotton pad in a Petri dish which was moistened regularly with deionised water throughout the test. To prevent the test organisms from escaping, a ring made of acrylic glass (approx. 4 cm diameter, 4 cm height) with the inner surface covered with Fluon was attached after application to the surface of the leaf and was tightly fixed with the help of rubber bands. Aphids of the species *Acyrtosiphon pisum ad libitum* on sprouts of broad bean (*Vicia faba*) were used as food during larval development.

The reproduction performance was assessed for treatment groups with a corrected mortality $\leq 50 \%$. The reproduction test was started seven days after the first egg batch had been observed in the control group. Sex was determined and the beetles were transferred to reproduction units. Beetles with deformities were excluded. Food and egg laying substrate were provided.

The adults of *C. septempunctata* were fed with freshly caught aphids (*Acyrtosiphon pisum*) on sprouts of broad bean (*Vicia faba*) and a supplement of pollen and water with honey (50% w/w) was provided. Aphids were supply at least, every working day, and pollen and water with honey were replaced when necessary, at least every two or three days.

Larval mortality was assessed from the same day of each exposure (2 hours after the exposures) and at least every working day to the completion of the adult stage or last evaluation of larval or pupal mortality, 12 days after the exposure. The reproduction test was started seven days after the first egg batch had been observed in the control group. Sex was determined and the beetles were transferred to reproduction units; one or two units per treatment. Beetles with deformities were excluded. Food and egg laying substrate was provided. Egg production (fecundity) was assessed every day (24-hour period) except weekends during a two weeks period and each assessment covered a twenty-four hour period (8 assessments). For determination of the hatching rate the egg batches were clipped out from the egg laying substrate and transferred individually into cell plates. The egg batches on the wall of the containers were not taken into account to assess the hatching rate because they could be damaged when removed. The eggs were kept at test conditions until hatch. After 4 to 6 days of starting each egg-laying period, as soon as the majority had hatched, the number of “not emerged” eggs was recorded. The percentage of fertile eggs was determined by assessing the number of eggs that hatched from the removed eggs into cell

Statistical analyses

Fisher’s Exact Binomial Test with Bonferroni Correction (one-sided greater, $\alpha=0.05$) was used to determine a significant increase in the mortality of the test item groups compared to the control group.

It was not possible to determine the LR50 by probit analysis since reductions of mortality with the tested rates of the test item were less than 50%.

For evaluation, Microsoft® Excel version 16.0 and the statistical program ToxRat® Professional 3.3.0 was used.

The reproduction test was evaluated only qualitatively due to the very high species-inherent variability in egg laying performance (Schmuck R., et al., 2000). Statistical evaluations were therefore not conducted.

Results

All validity criteria were met and the sensitivity of the test organisms was confirmed: 0.00 % mortality in the control group, cumulative mortality in the reference group was 100% and the mean number of fertile eggs per female per day in the control group was 25.2.

Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075) applied to detached bean leaflets did not cause statistically significant effect on mortality of *Coccinella septempunctata* L. at the tested item rates between 1.0 and 16.0 L test item/ha when compared to the control (Fisher's Exact Binomial Test with Bonferroni Correction, $\alpha = 0.050$, one-sided greater). Therefore, the NOER (No Observed Effect Rate) was estimated to be higher than or equal to 16.0 L test item/ha.

No mortality was observed at the rate of 1.0 L test item/ha, as in the control group, and maximum mortality was 12.5 % at the maximum tested rate of 16.0 L test item/ha. Therefore, mortality was less than 50 % and less than 30 % (validity criterion for mortality in the control group).

The rate producing 50 % mortality (LR₅₀) was estimated to be greater than the maximum tested rate of 16.0 L test item/ha.

The main response to the test item was always observed on larvae and only during the first week of exposure.

A slight delay (approx. one day compared to the control group) was observed in reaching adult state with the test item with exception of the test item at the rate of 1.0 L test item/ha where adults were emerged, with small differences, as in the control group.

No behavioural abnormalities, malformations or any pathological symptoms of the test organisms were observed in the control group and in any of the test item groups during the mortality phase.

The mean fecundity in the test item groups (1.0 to 16.0 L test item/ha) was between 19.1 and 37.1 eggs per female per day compared to 25.2 eggs per female per day in the control group. The mean hatching rate was 100 % or nearly 100 % (99.9 %) in all test item groups, and 100 % in the control group.

The mean fertility in the test item groups was between 19.1 and 37.1 fertile eggs per female per day compared to 25.2 fertile eggs per female per day in the control group. That means a reduction of 24.1 % (treatment T1) and an increase between 10.9 % (T4) and 47.5 % (T3) in relation to the control treatment. Therefore, the incidence of side effects (reproduction) was not dose-related.

Reproduction values with the test item rates were always greater than the control validity criterion of 2 fertile eggs per female per day. Even an increase in fertility eggs number was observed with the test item at rates between 2.0 and 16.0 L test item/ha compared to the control group, treated with deionised water. Therefore, it can be assumed that there are no adverse effects on reproduction.

Table CP 10.3.2.1/05-1: Mortality of *Coccinella septempunctata* after exposure to Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075).

Treatment group	Treatment Code	Application rate [L FP/ha] ^a	Response on larvae ^b [number]	Response on pupae/adult [number] ^c	Mortality ^d [%]	Corrected mortality ^e [%]
Control (deionised water)	C	(0)	0	0	0.00	--
Test item (Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075))	T1	1.0	0	0	0.00	0.00
	T2	2.0	2	0	5.00 ^{ns}	5.00
	T3	4.0	3	0	7.50 ^{ns}	7.50
	T4	8.0	3	0	7.50 ^{ns}	7.50
	T5	16.0	5	0	12.50 ^{ns}	12.50

Reference item (Dimethoate 40 %w/v EC)	R	0.030	40	0	100.00	100
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^a Rate of the test and reference items in L of formulated product (FP) per ha

^b Dead or moribund larvae, and larvae non-viable (do not develop to pupa nor adult)

^c Dead pupae, dead adult just when it was emerging or adult non-viable

^b Total *C. septempunctata* mortality up to the completion of adult emergence (pre-imaginal mortality)

“ns”: Not significantly different compared to control (Fisher’s Exact Binomial Test with Bonferroni Correction, 1-sided greater, $\alpha = 0.05$)

^c Corrected mortality according to Abbott (1925), modified by Schneider-Orelli (1947):

Corrected Mortality [%] = $[(Mt - Mc) / (100 - Mc)] \times 100$ [Mt = Mortality [%] in treated, Mc = Mortality [%] in control].

Table CP 10.3.2.1/05-2: Reproduction of *Coccinella septempunctata* after exposure to Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075).

Treatment group	Treatment Code	Application rate [L FP/ha] ^a	Fecundity [Mean eggs/female/day]	Mean hatching rate [%]	Mean fertile eggs/female/day
Control: deionised water	C	(0)	25.2	100.0	25.2
Test item: Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075)	T1	1.0	19.1	99.9	19.1
	T2	2.0	32.6	100.0	32.6
	T3	4.0	37.1	100.0	37.1
	T4	8.0	27.9	100.0	27.9
	T5	16.0	33.6	100.0	33.6

^a Rate of the test and reference items in L of formulated product (FP) per ha

Conclusions

Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075) applied to bean leaflets did not cause significant effects on mortality of *Coccinella septempunctata* L. when compared to the control at the tested rates of the test item from 1.0 to 16.0 L test item/ha. Therefore, the NOER (No Observed Effect Rate) was estimated to be higher than or equal to 16.0 L test item/ha.

Mortality (corrected to the control) was always below 50 % with up to and including the rate of 16.0 L test item/ha. Therefore, the rate producing 50 % mortality (LR₅₀) was estimated to be greater than the maximum tested rate of 16.0 L test item/ha.

It can be assumed that there are no adverse effects on the reproductive performance of the test organism at the rates of the test item up to and including 16.0 L test item/ha, since the mean fertility was above the control validity criterion of 2 fertile eggs per female per day.

Table CP 10.3.2.1/05-3: End points after exposure of *Coccinella septempunctata* to Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075).

Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075) Extended conditions; fresh and dried residues on bean leaflets	
Endpoint	[L test item/ha] ^a
NOER Lethal effects	NOER ≥ 16 L FP/ha
LR₅₀	LR ₅₀ > 16 L FP/ha
Reproduction ^b	No impact on reproduction up to and including 16.0 L test item/ha in accordance with the validity criteria for the control group: ≥ 2 fertile eggs/female/day

^a Rate in L of formulated product (FP)/ha

^b Reproduction was evaluated only qualitatively and no statistical analysis was performed

Comments of zRMS:

The study is considered acceptable. All validity criteria were met.

Control mortality:

The maximum cumulative mortality in the control group was $\leq 20\%$ (actual: 16.7 %).

Reference item mortality:

The cumulative mortality in the reference item group was $\geq 50\%$ (actual: 63.3 %).

Control reproduction:

The mean number of eggs per female per day in the control group was ≥ 15 (actual: 33.5) and the mean hatching rate in the control group was $\geq 70\%$ (actual: 93.9 %).

Agreed endpoints:

Treatment group	Application rates [mL/ha]	Mortality [%]	Corrected mortality [%]	Fecundity [Mean no. of eggs/female/day]	Fertility [Mean hatching rate in %]
Control	0	16.7	-	33.5	93.9
Prothioconazole 200 g/L + Azoxystrobin 150g/L SC (FF-075)	750	23.3	7.9	26.5	91.3
	1500	13.3	-4.1	32.2	95.1
	3000	37.9	25.5	24.2	81.8
	6000	23.3	7.9	28.9	92.2
	12000	40.0	28.0	38.3	93.3
Endpoint	[mL Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075)/ha]				
LR ₅₀	n.d., assumed > 12000				

n.d.: not determined, since effects on mortality were below 50 %

Report:	CP 10.3.2.1/06; Walter, C., and Stäbler, P., (2019)
Title:	Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075): Toxicity to the Green Lacewing Chrysoperla carnea Steph. (Neuroptera, Chrysopidae) under Extended Laboratory Conditions.
Document No:	Eurofins Agrosience Services Ecotox GmbH, Germany; Study report No.: S19-00968.

Guideline:	IOBC (VOGT <i>et al.</i> , 2000) modified for the exposure on natural substrate
GLP	Yes. Laboratory certified by the Baden-Württemberg, Berlin, Germany.

Test material

Test item:	Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075).
Purity:	Active ingredients (a.i.): 1. prothioconazole, content of a.i. (analysed): 198 g/L (nominal: 200 g/L), 2. azoxystrobin, content of a.i. (analysed): 148 g/L (nominal: 150 g/L)
Description	Liquid/ white to off-white
Batch No.:	20191211001

Test system

Organism (<i>Species</i>):	<i>Chrysoperla carnea</i> Steph. (Neuroptera, Chrysopidae) life stage at start of exposure: 1 st instar larvae (2–3 days old)
Study type:	Lethal and sub-lethal toxicity.
Guideline deviations reported:	None with impact on the outcome of the study.
Duration of study:	Mortality: 21 days Reproduction: 15 days
Parameters measured:	Percentage mortality, the mean number of eggs/female/day, the percentage of fertile eggs/female/day and the LR ₅₀ (median lethal rate), if possible.
Test concentrations:	Test item: 750, 1500, 3000, 6000 and 12000 mL product/ha Reference item: 70.0 mL BAS 152 65 I/ha
Control:	Deionised water
Test Arenas:	Mortality: Common bean (<i>Phaseolus vulgaris</i> , variety: 'Maxi', growth stage: BBCH 12–13) leaves grown at the testing facility were used for exposure, one leaf per replicate test unit. Leaves were cut off and placed on glass plates immediately before test solution application. After spray residues had dried, leaves were placed (treated surface upwards) on a wet cotton pad in a Petri dish which was moistened regularly with tap water throughout the test. A ring made of acrylic glass (approx. 3.5 cm diameter, 2.5 – 3.5 cm height) with the inner surface covered with Polytetrafluorethylene was attached to the surface of the leaf after application and tightly fixed with rubber bands to prevent the test organism escape. Emergence and reproduction: Plastic vessels (17 cm x 12.5 cm x 6 cm) with gauze covered lids were used for emergence and reproduction. The containers were closed with cotton gauze covered lids to encourage egg laying.
Application of treatments:	The application was conducted with a laboratory track-sprayer (Company Schachtner, Ludwigsburg, Germany) to detached bean leaves.
No. of replicates:	30 replicates per treatment group

Temperature:	24.6 – 26.0 °C
Photoperiod:	16 h light / 8 h darkness
Light intensity:	1800 – 3400 lux during exposure
Relative humidity:	67.3 – 83.6 %

Methodology

Test and reference item were diluted in water and applied with a laboratory track sprayer to detached bean leaves. A control group treated with deionised water was included in the study. All applications were performed with a spray volume of 200 L/ha. After drying of the treated leaves the test units were assembled. Each treatment group included 30 replicates containing one larva each. The larvae were exposed to the dried residues on the bean leaves and were fed with small quantities of *Sitotroga cerealella* eggs

Mortality and any behavioural abnormalities were determined from the larval stage until pupation at intervals of 1–3 days, with percentage mortality assessed after 21 days of exposure. Adult emergence was recorded beginning from 5 days after transfer of the last pupae. Abnormal appearance like deformations of larvae, pupae or adults, and incomplete pupae was recorded. Throughout testing, the condition of the test organisms was recorded as alive or dead. Larvae were considered dead if there was no reaction to a mechanical stimulus, whereas dead pupae were considered as the absence of adult emergence, including adults which died during emergence.

Reproduction was assessed for the control group and all test item groups since the corrected mortality was $\leq 50\%$ in each of the treatment groups. All adults from a respective treatment group were transferred to a reproduction unit. Adult lacewings with deformities were excluded. The reproduction test started eight days after the first egg batch had been observed. At the beginning of the egg laying period, the sex of the test organisms was determined by the shape of the abdomen and the number of males and females was recorded. The minimum requirement of three females and two males was reached for each treatment group. Number of eggs/female/day: Two egg samples (each covering a 24-hour egg laying period) were taken within one week. The reproduction units were covered with new gauze for a 24-hour period. All eggs deposited within that time on the gauze and on the wall of the container were counted. The eggs deposited on the gauze were kept at test conditions until emergence of the larvae. After 2–3 days of incubation food (*S. cerealella* eggs) was added to the eggs in order to avoid egg predation by hatched larvae. At the end of the 5–6-day incubation period the egg samples were evaluated by counting the number of eggs from which no larvae emerged (eggs still green or grey to black, mostly wizened and larvae not successfully hatched, dead larvae still sticking to the eggshell).

Prolongation of the reproduction period was not necessary since control egg production was above the threshold value of ≥ 15 eggs/female/day and the hatching rate was $\geq 70\%$ for each assessment date.

The percentage mortality was calculated for each treatment group from the cumulative number of dead larvae, not emerged pupae and adults which died during emergence or did not successfully moult in correlation to the number of test organisms initially exposed.

If larvae escaped during the test or had been inadvertently killed during feeding or cleaning procedure, their number was subtracted from the number of originally exposed larvae before calculating mortality.

The corrected mortality was obtained by correcting the values observed in the treated group with those in the control group, according to the formula of ABBOTT (1925), modified by SCHNEIDER-ORELLI (1947).

The number of eggs per female was calculated for each observation day from the total number of eggs (deposited on egg laying substrate and reproduction unit) and the number of females corrected for mortality during egg laying. In addition the percentage of fertile eggs was determined by calculating the hatching rate from the eggs laid on the substrate.

The mean number of fertile eggs/female/day was calculated by averaging the values of single assessments obtained during the reproduction phase.

Statistical analyses

Mortality data were analysed for a linear trend using Trend analysis by Contrasts ($\alpha = 0.01$). Since data did not show a significant linear trend, Multiple Fisher's exact test with Bonferroni-Holm adjustment (one-sided greater, $\alpha=0.05$) was used to detect significant differences between mortality data of the test item groups and the control.

The LR_{50} could not be calculated since mortality was below 50 % in all test item treatment groups.

A statistical analysis of the reproduction data was not conducted.

Results

All validity criteria were met since a) the maximum cumulative mortality in the control group was $\leq 20\%$ (actual: 16.7 %), b) the cumulative mortality in the reference item group was $\geq 50\%$ (actual: 63.3 %), c) the mean number of eggs per female per day in the control group was ≥ 15 (actual: 33.5) and the mean hatching rate in the control group was $\geq 70\%$ (actual: 93.9 %).

Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075) applied to detached bean leaves did not cause a statistically significant increase in the mortality of *Chrysoperla carnea* compared to the control group at test item rates up to 12000 mL/ha (Multiple Fisher's exact test with Bonferroni-Holm adjustment, one-sided greater, $\alpha = 0.05$).

The mortality in the reference item group was 63.3 % (55.9 % corrected with control).

The LR_{50} of Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075) could not be calculated as the mortality in all treatment groups was below 50 % and is therefore assumed to be > 12000 mL/ha.

The results of the reproduction test are shown in the following table.

The mean fecundity was between 24.2 and 38.3 eggs per female per day compared to 33.5 eggs per female per day in the control group. The mean fertility (hatching rate) was between 81.8 and 95.1 % in the test item treatment groups compared to 93.9 % in the control group.

Table CP 10.3.2.1/06-1: Mortality of *Chrysoperla carnea* after exposure to Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075).

Treatment group	Application rate [mL/ha]	Mortality [%]	Corrected mortality [%]
Control	0	16.7	-
Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF- 075)	750	23.3	7.9
	1500	13.3	-4.1
	3000	37.9	25.5
	6000	23.3	7.9

	12000	40.0	28.0
Reference item	70.0	63.3	55.9

Table CP 10.3.2.1/06-2: Reproduction of *Chrysoperla carnea* after exposure to Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075).

Treatment group	Application rate [mL/ha]	Fecundity [Mean no. of eggs/female/day]	Fertility [Mean hatching rate in %]
Control	0	33.5	93.9
Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF- 075)	750	26.5	91.3
	1500	32.2	95.1
	3000	24.2	81.8
	6000	28.9	92.2
	12000	38.3	93.3

Conclusions

Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075) applied to bean leaves did not cause a statistically significant increase in the mortality of *Chrysoperla carnea* compared to the control group at test item rates up to 12000 mL/ha. No effects on mortality of *Chrysoperla carnea* equal or above 50 % could be observed at any test item rate. Therefore, the LR₅₀ of Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075) could not be calculated but is assumed to be > 12000 mL/ha.

It can be assumed that there are no adverse effects on the reproductive performance of the test organism up to 12000 mL Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075)/ha, since the mean fecundity in all treatment groups was above the critical value of 15 eggs per female per day and the mean fertility was above 70 %.

A 2.4 KCP 10.4 Effects on non-target soil meso- and macrofauna

A 2.4.1 KCP 10.4.1 Earthworms

A 2.4.1.1 KCP 10.4.1.1 Earthworms - sub-lethal effects

Comments of zRMS:	<p>The study is considered acceptable. All validity criteria were met.</p> <ul style="list-style-type: none"> The number of juveniles produced per replicate is in the range of 55 to 64 in control The coefficient of variation of reproduction in control is 4.7% Adult mortality over the initial 4 weeks of the test in control is 0.0%
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Agreed endpoints:					
EC ₁₀ , EC ₂₀ , EC ₅₀ , LOEC and NOEC Values for Test Item.					
Parameters	mg/kg dry weight of artificial soil	mg a.i./kg dry weight of artificial soil	mg Prothioconazole /kg dry weight of artificial soil	mg Azoxystrobin /kg dry weight of artificial soil	
EC ₁₀	95.90	28.39	16.21	12.18	
95% Confident Limit (lower limit)	75.34	22.30	12.73	9.57	
95% Confident Limit (upper limit)	116.46	34.47	19.68	14.79	
EC ₂₀	173.58	51.38	29.34	22.04	
95% Confident Limit (lower limit)	146.18	43.27	24.70	18.56	
95% Confident Limit (upper limit)	200.98	59.49	33.97	25.52	
EC ₅₀	540.01	159.84	91.26	68.58	
95% Confident Limit (lower limit)	446.28	132.10	75.42	56.68	
95% Confident Limit (upper limit)	633.74	187.59	107.1	80.48	
LOEC	89.6	26.52	15.14	11.38	
NOEC	56	16.58	9.46	7.11	
LOEC	89.6	26.52	15.14	11.38	
NOEC	56	16.58	9.46	7.11	

Report:	CP 10.4.1.1/01; Parker, T. (2021)
Title:	Earthworm (<i>Eisenia fetida</i>), reproduction test with Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075) in artificial soil
Document No:	Rotam Research Laboratory (RRL), China; Study report No.: 2866
Guideline:	OECD Guideline No. 222: "Earthworm Reproduction Test" (2016)
GLP	Yes. Laboratory certified by the Bundesinstitut für Risikobewertung, Berlin, Germany.

Test material

Test item:	Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC
Purity:	Prothioconazole 198 g/L (16.9%, w/w) + Azoxystrobin 148 g/L (12.7%, w/w)
Description	Off-white homogeneous liquid
Batch No.:	20191211001

Test system

Organism (<i>Species</i>):	<i>Eisenia fetida</i> (Savigny 1826) Age: 2 to 12 months old with well-developed clitellum (Individuals in a test group did not differ in age by more than 4 weeks) body weight range on day 0: 300 – 600 mg/worm (including gut content)
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	Source: Earthworm culture maintained in the laboratory, primarily supplied by Kunshan Jieqiang Aquarium Technology Co., Ltd, No. 82, Ma'anshan west road, Kunshan city, China
Study type:	Earthworm chronic toxicity test (reproduction test)
Guideline deviations reported:	None
Duration of study:	56 days
Parameters measured:	Mortality, abnormal behavior, biomass change, reproduction effects (number of juveniles produced)
Observation intervals:	Mortality, abnormal behaviour and biomass change of adult worms were assessed on day 28. Reproduction effects were assessed on day 56.
Test concentrations:	Test item: 38, 56, 89.6, 143.36, 229.38, 367, 587.2 and 939.52 mg of Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075)/kg dry weight of artificial soil (11.25, 16.58, 26.52, 42.43, 67.90, 108.63, 173.81 and 278.10 mg total a.i/kg dry soil) Reference item: 0.82, 1.07, 1.39, 1.80, 2.34, 3.04, 3.96 and 5.15 mg Carbendazim Technical /kg soil dry weight
Control:	Artificial soil with Milli-Q water
Test Arenas:	Glass test container of approximately 1 litre capacity (diameter 11.5 cm), giving a substrate depth about 5-6 cm when moist test soil added. Test containers were covered with a perforated plastic lid to enable exchange of air and minimize evaporation of moisture. The wet weight of soil in each replicate was documented at the beginning of the test.
Application of treatments:	Pre-moistened artificial soil was mixed with test solution and blended thoroughly for approximately 5 minutes. After mixing, the treated artificial soil was divided 8 replicates for control and 4 replicates for treatments, then the weight of wet soil was recorded.
No. of replicates:	10 earthworms per replicate 8 replicates per control 4 replicates per test concentration
Temperature:	20 ± 2°C
Photoperiod:	16:8 h (light:dark)
pH:	6.0 ± 0.5
Moisture	40 to 60% maximum water holding capacity
Light intensity:	400 to 800 Lux
Relative humidity:	Not applicable

Methodology

Artificial soil was prepared by blending the following ingredients in a soil blender for 20 minutes.

- 10% Sphagnum-peat, air-dried and finely ground (pH: 5.5 – 6.0);
- 20% Kaolin clay, extra pure, (Kaolinite content >30%);
- 70% fine quartz-sand (50% of particle size between 50 to 200 microns)

The pH and maximum water holding capacity of the artificial soil were determined (pH (mean value): 6.42, maximum water holding capacity: 50.1%).

The artificial soil was pre-moistened with half the volume of water required to achieve 50% moisture of dry mass (maximum water holding capacity). An amount of 4 kg of soil was moistened with 500 mL of water for control and 2 kg of soil was moistened with 250 mL of water for each treatment. The test item was weighed or diluted and dissolved in the remaining portion of water and mixed into soil.

Twenty-four hours before start of the test, 11- month old healthy, clitellated earthworms were removed from the breeding box and placed in plastic bowl container filled with moist artificial soil (pH: 6.42, moisture content: 25.4%). The earthworms were acclimatized under test condition with temperature in the range of 18.6oC to 20.6oC with light intensity of 568 lux measured from above the container. During acclimation period, food (wet powdered cow manure) was spread on the top-soil layer and moistened with Milli-Q water. The age of selected earthworms did not differ by more than 4 weeks, approximately.

Acclimatized, healthy worms with a clitellum were selected for each replicate. Earthworms were cleaned to remove the adhering soil particles without causing any injury and were blotted carefully by placing on a soft blotting paper to remove the excess moisture. The randomly selected earthworms from a synchronised culture with a relatively homogeneous age were weighed individually in a glass petri plate by placing on soft blotting paper. It was ensured that the wet weight of each worm was within 300 to 600 mg and that the worms were distributed homogenously with respect to average weight. The earthworms were released to each test container after test item application. The earthworms were not individually identified during test period. After earthworm release, the test containers were covered with perforated plastic lid to prevent the test medium from drying out and to avoid escape of earthworms from the containers under test conditions.

Approximately 200 g of powdered cow manure, moistened with about 200 mL Milli-Q water and approximately 10.0 g feed was spread on the soil surface of each test container. Feed was provided one day after test item application and thereafter once in a week for 4 weeks. The observation for feed consumption was made visually once in a week. On 28th day, unconsumed feed was carefully removed from each test container followed by removal of the adult worms. After removal of adult worms on 28th day observation, 200 g of food was mixed, moistened with 200 mL Milli-Q water and approximately 10 g feed was spread on the soil surface of each test container and no further feed was added during the remaining 4 weeks of the test.

Once a week the water content of the treated artificial soil was checked by weighing each test container and evaporated water was replenished, ensuring that the difference in water content between experimental start and end was <10%.

The test medium (treated artificial soil) was emptied from test container into a stainless steel tray covered with blotting paper and the mortality and behavioral abnormalities were observed and counted visually on the 14th day for range finding test and 28th day after exposure for definitive test. Earthworms were considered dead if they failed to respond to gentle stimulation. Due to rapid decomposition under test conditions, missing earthworms were also considered dead. The number of live and dead earthworms in each replicate was recorded. The live adult worms were washed, weighed and disposed (safely). After the mortality assessment, the cocoons/juveniles along with the test medium were retained in the respective test container and were maintained up to 56 days. After an additional 28 days, the assessment for reproduction was made.

Live earthworms in each test container were weighed and mean weight as a group for each replicate was calculated between 0 and 28th day using the same procedure used in start of the test.

At the end of day 56 of experimental period, the number of hatched juveniles were counted. Counting of juveniles was conducted by transferring the medium to a stainless-steel tray placed in a hot water bath initially set at a temperature of 40°C and gradually raised to 60°C for a period of about 20 minutes. As the

soil heated, juveniles came to the soil surface and were counted and the percent reduction in reproduction subsequently calculated.

The maximum water holding capacity of artificial soil was determined before testing. pH of test soil was determined at day 0 (start of test) and day 56 (end of test).

Statistical analyses

EC_x values, EC₁₀, EC₂₀, EC₅₀ (Effect Concentration) were obtained using Probit analysis using statistical method from NCSS software @2007 and the associated 95% confidence limits were calculated using the formula $EC_{10} / EC_{20} / EC_{50} \pm 1.96 \times \text{standard error}$ (Handbook of biological statistics, 2014).

NOEC, LOEC based on the reduction of reproduction results were evaluated statistically using ANOVA (one way analysis of variance), Dunnett's test with a significance at alpha value at 0.05 after the ANOVA acceptance of normality and homogeneity tests;

NOEC, LOEC based on the biomass change results were evaluated statistically using AVOVA (one way analysis of variance), Dunn's test with a significance at Z-value at 1.96 after the ANOVA rejection of normality and homogeneity tests.

Results

- 1) The number of juveniles produced per replicate is in the range of 55 to 64 in control;
- 2) The coefficient of variation of reproduction in control is 4.7%;
- 3) Adult mortality over the initial 4 weeks of the test in control is 0.0%.

The above results satisfy the validity criteria and test is considered as valid.

During the test period the temperature and light intensity was maintained in the range of 18.0°C to 21.7°C and 540 Lux to 586 Lux. The pH at the start of the test (day 0) was 6.40 to 6.43 and end of the test (day 56) was 6.40 to 6.42. Moisture content at the start of the test (day 0) was 24.9% to 26.5% and at end of the test (day 56) was 24.5% to 25.9%.

After 28 days of exposure, the mortality in control and in the treated concentrations of 38, 56, 89.6, 143.36, 229.38, 367, 587.2 and 939.52 mg/kg dry weight of artificial soil was 0%. No behavioral abnormalities were observed in control and all treated concentrations during the exposure period.

Reproduction assessment on 56th day showed that in the control group, the mean number of juveniles produced was 60 and in the treated concentrations was between 14 and 58. The reduction of reproduction over control in the treated concentrations was between 3.1% and 76.7%.

The individual body weight of earthworm on day 0 and day 28 are presented in Table-8. Earthworm average body wet weight ranged between 396 and 493 mg/worm on day 0. Based on the body weight, the mean biomass gain in the treatments was in the range of -11.10% (939.52 mg/kg dry weight of artificial soil) to 1.95% (38.00 mg/kg dry weight of artificial soil) and in the control the biomass gain was 4.96% at the end of the test.

Table CP 10.4.1.1/01-1: Behavioural Abnormalities and Mortality – Definitive test

Nominal concentration (mg/kg dry weight of artificial soil)	Replication	Behavioural abnormalities	Mortality	Average mor- tality %	Standard deviation
		Day 28			
Control	R1	N (10)	0	0.0	0.0

	R2	N (10)	0		
	R3	N (10)	0		
	R4	N (10)	0		
	R5	N (10)	0		
	R6	N (10)	0		
	R7	N (10)	0		
	R8	N (10)	0		
38	R1	N (10)	0	0.0	0.0
	R2	N (10)	0		
	R3	N (10)	0		
	R4	N (10)	0		
56	R1	N (10)	0	0.0	0.0
	R2	N (10)	0		
	R3	N (10)	0		
	R4	N (10)	0		
89.6	R1	N (10)	0	0.0	0.0
	R2	N (10)	0		
	R3	N (10)	0		
	R4	N (10)	0		
143.36	R1	N (10)	0	0.0	0.0
	R2	N (10)	0		
	R3	N (10)	0		
	R4	N (10)	0		

Nominal concentration (mg/kg dry weight of artificial soil)	Replication	Behavioural abnor- malities	Mortality	Average mortality %	Standard deviation
		Day 28			
229.38	R1	N (10)	0	0.0	0.0
	R2	N (10)	0		
	R3	N (10)	0		
	R4	N (10)	0		
367	R1	N (10)	0	0.0	0.0
	R2	N (10)	0		
	R3	N (10)	0		
	R4	N (10)	0		

587.2	R1	N (10)	0	0.0	0.0
	R2	N (10)	0		
	R3	N (10)	0		
	R4	N (10)	0		
939.52	R1	N (10)	0	0.0	0.0
	R2	N (10)	0		
	R3	N (10)	0		
	R4	N (10)	0		

N – Normal; () – Number of earthworm.

Table CP 10.4.1.1/01-2: Record for Reproductive Assessment

Nominal concentration (mg/kg dry weight of artificial soil)	Juveniles produced								Mean	SD	RSD%	Reduction of reproduction over control (%)
	R1	R2	R3	R4	R5	R6	R7	R8				
Control	62	61	64	60	62	58	59	55	60	2.8	4.7	-
38	58	54	59	62	-	-	-	-	58	3.3	5.7	3.1
56	52	58	56	57	-	-	-	-	56	2.6	4.7	7.3
89.6	52	54	51	55	-	-	-	-	53	1.8	3.4	11.9 ^b
143.36	48	52	52	50	-	-	-	-	51	1.9	3.8	16.0 ^b
229.38	42	51	50	49	-	-	-	-	48	4.1	8.5	20.2 ^b
367	45	39	48	46	-	-	-	-	45	3.9	8.7	26.0 ^b
587.2	25	38	21	29	-	-	-	-	28	7.3	25.8	53.0 ^b
939.52	14	16	18	8	-	-	-	-	14	4.3	30.9	76.7 ^b

R – Replication; SD- Standard Deviation; RSD – Relative standard deviation; - Not applicable; b – Statistically significance was observed when compared to control using ANOVA (one way analysis of variance), Dunnett's test with a significance at alpha value at 0.05.

Table CP 10.4.1.1/01-3: Mean Body Weight and Biomass Change

Nominal concentration (mg/kg dry weight of artificial soil)	Weight change from initial weight (%)								Mean (%)	Standard deviation
	R1	R2	R3	R4	R5	R6	R7	R8		
Control	+4.54	+6.09	+3.84	+3.70	+5.81	+6.04	+4.95	+4.67	+4.96	0.95
38	+2.31	+3.33	+0.43	+1.71	-	-	-	-	+1.95	1.21
56	+1.27	+1.25	+2.09	+0.62	-	-	-	-	+1.31	0.60
89.6	+0.47	-2.02	-1.61	+0.23	-	-	-	-	-0.73 ^a	1.26
143.36	-2.71	-2.08	-3.95	-2.08	-	-	-	-	-2.72 ^a	0.88
229.38	-2.66	-1.88	-3.84	-0.51	-	-	-	-	-2.22 ^a	1.40
367	-6.01	-4.08	-3.13	-3.76	-	-	-	-	-4.24 ^a	1.24

587.2	-4.45	-4.84	-2.69	-8.33	-	-	-	-	-5.08 ^a	2.36
939.52	-10.26	-16.78	-9.31	-8.07	-	-	-	-	-11.10 ^a	3.89

R – Replication; SD- Standard Deviation; RSD – Relative standard deviation; - Not applicable; a – Statistically significance was observed when compared to control using ANOVA (one way analysis of variance), Dunn's test with a significance at Z-value at 1.96.

Conclusions

The reproduction test with test item, Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075) to earthworm, *Eisenia fetida* was investigated in an artificial soil test.

The 56-day EC₅₀ is 540.01 mg/kg dry weight of artificial soil with 95% confidence limit between 446.28 mg/kg dry weight of artificial soil and 633.74 mg/kg dry weight of artificial soil based on reproduction.

The 56-day EC₁₀ is 95.90 mg/kg dry weight of artificial soil with 95% confidence limit between 75.34 mg/kg dry weight of artificial soil and 116.46 mg/kg dry weight of artificial soil based on reproduction.

The 56-day EC₂₀ is 173.58 mg/kg dry weight of artificial soil with 95% confidence limit between 146.18 mg/kg dry weight of artificial soil and 200.98 mg/kg dry weight of artificial soil based on reproduction.

The LOEC (Lowest Observed Effect Concentration) of the test item based on the reproduction is 89.6 mg/kg dry weight of artificial soil. The NOEC (No Observed Effect Concentration) of the test item based on the reproduction is 56 mg/kg dry weight of artificial soil, after 56 days.

The LOEC (Lowest Observed Effect Concentration) of the test item based on the biomass change is 89.6 mg/kg dry weight of artificial soil. The NOEC (No Observed Effect Concentration) of the test item based on the biomass change is 56 mg/kg dry weight of artificial soil, after 28 days.

Table CP 10.4.1.1/01-4: EC₁₀, EC₂₀, EC₅₀, LOEC and NOEC Values for Test Item.

Parameters	mg/kg dry weight of artificial soil	mg a.i./kg dry weight of artificial soil	mg Prothioconazole /kg dry weight of artificial soil	mg Azoxystrobin /kg dry weight of artificial soil
EC ₁₀	95.90	28.39	16.21	12.18
95% Confident Limit (lower limit)	75.34	22.30	12.73	9.57
95% Confident Limit (upper limit)	116.46	34.47	19.68	14.79
EC ₂₀	173.58	51.38	29.34	22.04
95% Confident Limit (lower limit)	146.18	43.27	24.70	18.56
95% Confident Limit (upper limit)	200.98	59.49	33.97	25.52
EC ₅₀	540.01	159.84	91.26	68.58
95% Confident Limit (lower limit)	446.28	132.10	75.42	56.68
95% Confident Limit (upper limit)	633.74	187.59	107.1	80.48
LOEC	89.6	26.52	15.14	11.38
NOEC	56	16.58	9.46	7.11
LOEC	89.6	26.52	15.14	11.38

NOEC	56	16.58	9.46	7.11
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A 2.4.1.2 KCP 10.4.1.2 Earthworms - field studies

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

A 2.4.2.1 KCP 10.4.2.1 Species level testing

Comments of zRMS:	<p>The study is considered acceptable. All validity criteria were met.</p> <ul style="list-style-type: none"> Mean adult mortality in controls was 2.5% (test guideline requires $\leq 20\%$); The mean number of juveniles in the controls was 697.5 ± 79.4 (test guideline requires ≥ 100); The coefficient of variation for the juvenile numbers in the control was 11.4% (test guideline requires $<30\%$). In the reference test (ECT Study No. ICR2002) the EC_{50} value was calculated by 3-parameter normal cumulative distribution (CDF) as 66.1 mg boric acid/kg artificial soil (dw) (95 % confidence limits = 54.8 – 79.7 mg boric acid/kg artificial soil (dw)). <p>Agreed endpoints:</p> <table> <tr> <th></th><th>mg test item/ kg soil (dw)</th><th>mg Prothioconazole/ kg soil (dw)</th><th>mg Azoxystrobin/ kg soil (dw)</th></tr> <tr> <td>NOEC_{Mortality}</td><td>≥ 1000</td><td>≥ 170.4</td><td>≥ 127.4</td></tr> <tr> <td>LOEC_{Mortality}</td><td>> 1000</td><td>> 170.4</td><td>> 127.4</td></tr> <tr> <td>LC₅₀</td><td>> 1000</td><td>> 170.4</td><td>> 127.4</td></tr> <tr> <td>NOEC_{Reproduction}</td><td>100.0</td><td>17.0</td><td>12.7</td></tr> <tr> <td>LOEC_{Reproduction}</td><td>177.8</td><td>30.3</td><td>22.6</td></tr> <tr> <td>EC₁₀ (+ CI)</td><td>134.6 (91.2 – 197.5)</td><td>22.9 (15.5 – 33.7)</td><td>17.1 (11.6 – 25.0)</td></tr> <tr> <td>EC₂₀ (+ CI)</td><td>189.9 (143.1 – 253.9)</td><td>32.4 (24.4 – 43.3)</td><td>24.2 (18.2 – 32.0)</td></tr> <tr> <td>EC₅₀ (+ CI)</td><td>367.2 (303.7 – 443.9)</td><td>62.6 (51.7 – 75.6)</td><td>46.8 (38.7 – 56.0)</td></tr> </table>				mg test item/ kg soil (dw)	mg Prothioconazole/ kg soil (dw)	mg Azoxystrobin/ kg soil (dw)	NOEC _{Mortality}	≥ 1000	≥ 170.4	≥ 127.4	LOEC _{Mortality}	> 1000	> 170.4	> 127.4	LC ₅₀	> 1000	> 170.4	> 127.4	NOEC _{Reproduction}	100.0	17.0	12.7	LOEC _{Reproduction}	177.8	30.3	22.6	EC ₁₀ (+ CI)	134.6 (91.2 – 197.5)	22.9 (15.5 – 33.7)	17.1 (11.6 – 25.0)	EC ₂₀ (+ CI)	189.9 (143.1 – 253.9)	32.4 (24.4 – 43.3)	24.2 (18.2 – 32.0)	EC ₅₀ (+ CI)	367.2 (303.7 – 443.9)	62.6 (51.7 – 75.6)	46.8 (38.7 – 56.0)
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Report:	CP 10.4.2/01; Senn, L (2021)
Title:	Prothioconazole 200g/L + Azoxystrobin 150g/L SC (FF-075): Reproduction toxicity to the collembolan species <i>Folsomia candida</i> in artificial soil.
Document No:	ECT Oekotoxikologie GmbH, Germany; Study report No.: 20AV6CR
Guideline:	OECD Guideline No. 232 (2016).
GLP	Yes. Laboratory certified by the Hessisches Ministerium für Umwelt, Klimaschutz, Landwirtschaft und Verbraucherschutz, Wiesbaden, Germany.

Test material

Test item: Prothioconazole 200g/L + Azoxystrobin 150g/L SC (FF-075)

Purity: Prothioconazole (CAS-no.: 178928-70-6), azoxystrobin

	(CAS-no.: 131860-33-8) Prothioconazole: 198 g/L Azoxystrobin: 148 g/L.
Description	White to off-white homogeneous suspension liquid.
Batch No.:	20191211001
Test system	
Organism (<i>Species</i>):	<i>Folsomia candida</i> (Isotomidae, Collembola). Juvenile mites (age 12 d) from a synchronised breeding culture maintained at the test facility, originally established with organisms obtained from Eurofins GmbH.
Study type:	Sub-lethal toxicity.
Guideline deviations reported:	None reported.
Duration of study:	28 days.
Parameters measured:	Adult springtail mortality, behavioural effects and juvenile production after an exposure period of 28 days.
Observation intervals:	<u>At test start</u> : determination of physico-chemical parameters (water content, pH) of the artificial soil; <u>Weekly</u> : (up to 3 weeks after start of exposure): moisture loss and compensation; <u>At 4 weeks</u> : number of surviving adult springtails per replicate; numbers of juvenile springtails per replicate.
Test concentrations:	Control (untreated substrate); 17.8, 31.6, 56.2, 100.0, 177.8, 316.2, 562.3 and 1000 mg test item/kg artificial soil dry weight (dw) Reference item: boric acid: 17.8, 31.6, 56.2, 100 and 178 mg boric acid/kg artificial soil (dw); (separate GLP study)
Artificial substrate:	<ul style="list-style-type: none">- 5% air-dried and shredded sphagnum peat;- 20% kaolin clay (kaolinite content > 30 %);- 0.24% calcium carbonate;- <i>ca.</i> 74.76% quartz sand (fine sand content with particles between 50 and 200 µm higher than 50 %).
Soil moisture content:	guideline requirement: 40-60% of WHC _{max} test start: 48.5 – 51.5% of WHC _{max} test end: 45.7 – 49.7% of WHC _{max} .
No. of replicates:	8 replicates for control; 4 replicates for each test item treatment. 10 collembolans per replicate.
Temperature:	18.7 – 20.6 °C, mean: 19.6 °C (recommended 20 ± 2 °C)
Photoperiod:	Light : dark = 16 h : 8 h. Light intensity 612 - 754 lux
pH value:	guideline requirement: 6.0 ± 0.5. test start: 5.6 – 5.7; test end: 5.7 – 6.0.

Methodology

Based on the outcome of a prior range-finding test, collembolans were exposed to eight concentrations of soil-incorporated Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC (FF-075): 17.8, 31.6, 56.2, 100.0, 177.8, 316.2, 562.3 and 1000 mg test item/kg artificial soil dry weight (dw), with four replicates per treatment group. These were compared to an untreated control (8 replicates). The sensitivity of the test organisms was checked in a separate study, using collembolans from the same stock culture, exposed to boric acid mixed into artificial soil at concentrations of 17.8, 31.6, 56.2, 100 and 178 mg/kg soil dw.

Batches of test soil were prepared by incorporation (thorough mixing for 3 minutes) of volumes of a stock solution and serial dilutions derived from it that contained the test item dissolved in deionised water. The control soil was amended with water only. Aliquots of soil mixtures (30 g dry weight equivalent) were transferred to glass test vessels (5 cm diameter × 10 cm height and 200 mL capacity). There were 8 replicate vessels for the untreated control group and 4 replicates for each of the test item treatments. Ten juvenile springtails were transferred to the soil surface in each vessel and the vessels were then closed with tightly applied Parafilm, perforated to permit aeration. An additional single vessel per treatment received no test animals but was used to provide measurements of soil pH and moisture content at the end of the test. All vessels were incubated under controlled climate conditions.

The collembolans were fed with 8-10 mg granulated dry baker's yeast per vessel at the start of the test and feeding was repeated on D 14. Test vessels were weighed at weekly intervals and deionised water was added as necessary to restore moisture losses.

On D 28 the contents of each vessel were mixed with water and transferred to individual crystallising dishes. The contents were gently stirred to release the springtails from the soil substrate and black dye was added to the water to improve the contrast and aid counting. Surviving adults were counted directly whereas juvenile counts were made with the aid of a binocular microscope and a plastic enumeration grid.

Statistical analyses

Mortality: For determination of the LOEC/NOEC Mortality data were checked for monotone dose-response by qualitative trend analysis by contrasts. Afterwards, Fisher's Exact Binomial Test with Bonferroni Correction ($\alpha = 0.05$, one-sided) was applied.

Reproduction: For determination of the LOEC/NOEC_{Reproduction} data were checked for normality by Shapiro-Wilk's test procedure and for homogeneity by Levene's test. Treatment means were compared by ANOVA and Williams t-test ($\alpha = 0.05$, one-sided) and tested for statistically significant differences compared to the control (Sachs 1982). The EC_x values were calculated by 3-parametric normal CDF.

The statistical software package ToxRat Professional Version 3.3.0 was used for these calculations.

Results

All validity criteria were met since a) mean adult mortality in controls was 2.5% (test guideline requires $\leq 20\%$); b) the mean number of juveniles in the controls was 697.5 ± 79.4 (test guideline requires ≥ 100); c) the coefficient of variation for the juvenile numbers in the control was 11.4% (test guideline requires $<30\%$).

In the reference test (ECT Study No. ICR2002) the EC₅₀ value was calculated by 3-parameter normal cumulative distribution (CDF) as 66.1 mg boric acid/kg artificial soil (dw) (95 % confidence limits = 54.8 – 79.7 mg boric acid/kg artificial soil (dw)).

Mean adult mortality was 2.5 % in the untreated control group at the end of the test. In the test item treatment groups the mortality ranged from 2.5 to 15.0 %. Statistical analysis (Fisher's Exact Binomial test; $\alpha = 0.05$, one-sided) showed no significant differences concerning the number of juveniles between the control and all test item concentrations. The NOEC_{mortality} and LC₅₀ were determined to be >1000 mg test

item/kg soil (dw).

Table CP 10.4.2.1/01-1: Number of dead adult collembolans after 28 days. Data are given as absolute value of all replicates of each treatment and in percent of the total initial number ($n = 80$ for control and $n = 40$ for treatment).

Concentration [mg test item/kg soil (dw)]	Number of dead adult collembolans	Mortality [%]
Control	2	2.5
17.8	3	7.5
31.6	4	10.0
56.2	5	12.5
100.0	3	7.5
177.8	6	15.0
316.2	1	2.5
562.3	2	5.0
1000	2	5.0

A mean number of 697.5 juveniles was observed at the control. The reproduction in the test item treatment groups ranged from mean values of 81.3 to 659.5 juveniles per replicate.

Statistical analysis (Williams t-test; $\alpha = 0.05$, one-sided) showed significant differences concerning the number of juveniles between the control and the four highest treatments, i.e. 177.8, 316.2, 562.3 and 1000 mg test item/kg soil (dw).

Therefore, the $\text{NOEC}_{\text{Reproduction}}$ was determined to be 100.0 mg test item/kg soil (dw) and accordingly the $\text{LOEC}_{\text{Reproduction}}$ to be 177.8 mg test item/kg soil (dw). The EC_{10} was calculated to be 134.6 mg test item/kg soil (dw) (95% confidence limits = 91.2 – 197.5 mg test item/kg soil (dw)). The EC_{20} was calculated to be 189.9 mg test item/kg soil (dw) (95% confidence limits = 143.1 – 253.9 mg test item/kg soil (dw)). The EC_{50} was calculated to be 367.2 mg test item/kg soil (dw) (95% confidence limits = 303.7 – 443.9 mg test item/kg soil (dw)).

Table CP 10.4.2.1/01-2: Reproduction after 28 days. Data are given as mean number of juveniles \pm standard deviation per test vessel and as percentage of the control ($n = 8$ for control; $n = 4$ for treatment).

Concentration [mg test item/kg soil (dw)]	Number of Juveniles [mean \pm sd]	Number of Juveniles [% of Control]	Coefficient of Variation
Control	697.5 \pm 79.4	-	11.4
17.8	659.5 \pm 78.3	94.6	11.9
31.6	653.8 \pm 76.2	93.7	11.7
56.2	625.0 \pm 32.6	89.6	5.2
100.0	657.0 \pm 76.8	94.2	11.7
177.8	536.8 \pm 124.6	77.0	23.2
316.2	409.8 \pm 193.2	58.7	47.2
562.3	172.8 \pm 61.6	24.8	35.6
1000	81.3 \pm 14.3	11.6	17.6

No effects on behaviour (including feeding activity) or morphology of the collembolans were observed during the test.

Conclusions

The NOEC_{mortality} and LC₅₀ were determined to be >1000 mg test item/kg soil (dw).

The 28 day NOEC_{reproduction} for *Folsomia candida* exposed to Prothioconazole 200 g/L +Azoxystrobin 150 g/L SC (FF-075) was 100 mg/kg dw soil. The corresponding EC₁₀ (reproduction) calculated from the response curve was 134.6 mg/kg dw soil (95% confidence limits 91.2 – 197.5).

Table CP 10.4.2.1/01-3: Calculated endpoints in mg test item/kg soil (dw) converted to the active ingredients Prothioconazole and Azoxystrobin. For the concentrations of the active ingredients, the contents of 198 g/L Prothioconazole and 148 g/L Azoxystrobin were used as reported in the Certificate of Analysis, see also section 20, and the density of the product (1.162 g/mL) was taken into account.

	mg test item/ kg soil (dw)	mg Prothioconazole/ kg soil (dw)	mg Azoxystrobin/ kg soil (dw)
NOEC _{Mortality}	≥ 1000	≥ 170.4	≥ 127.4
LOEC _{Mortality}	> 1000	> 170.4	> 127.4
LC ₅₀	> 1000	> 170.4	> 127.4
NOEC _{Reproduction}	100.0	17.0	12.7
LOEC _{Reproduction}	177.8	30.3	22.6
EC ₁₀ (+ CI)	134.6 (91.2 – 197.5)	22.9 (15.5 – 33.7)	17.1 (11.6 – 25.2)
EC ₂₀ (+ CI)	189.9 (143.1 – 253.9)	32.4 (24.4 – 43.3)	24.2 (18.2 – 32.3)
EC ₅₀ (+ CI)	367.2 (303.7 – 443.9)	62.6 (51.7 – 75.6)	46.8 (38.7 – 56.5)

Comments of zRMS:	<p>The study is considered acceptable. All validity criteria were met.</p> <ul style="list-style-type: none"> • Mean adult mortality in controls was 2.5 % (test guideline requires \leq %); • The number of juveniles in the controls was 152.8 ± 19.1 (test guideline requires ≥ 50); • The coefficient of variation for the juvenile numbers in the control was 12.5% (test guideline requires $\leq 30\%$) • In the separate reference test the EC_{50 reproduction} was established at
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	<p>333.8 mg boric acid/kg soil dw (95% C.L.; 193.5 – 575.8 mg/kg soil dw), which is consistent with the expected response according to the guideline and verifies that the test system was suitably sensitive.</p> <p>Agreed endpoints:</p> <p>The 14 day NOEC_{reproduction} for <i>Hypoaspis aculeifer</i> exposed to Prothioconazole 200 g/L + azoxystrobin 150 g/L SC (FF-075) was >1000 mg/kg dw soil.</p>
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Report:	CP 10.4.2.1/02; Senn, L (2021)
Title:	Prothioconazole 200g/L + Azoxystrobin 150g/L SC (FF-075): Reproduction toxicity to the predaceous mite <i>Hypoaspis</i> (<i>Geolaelaps</i>) <i>aculeifer</i> in artificial soil.
Document No:	ECT Oekotoxikologie GmbH, Germany; Study report No.: 20AV3HR.
Guideline:	OECD Guideline No. 226 (2016).
GLP	Yes. Laboratory certified by the Hessisches Ministerium für Umwelt, Klimaschutz, Landwirtschaft und Verbraucherschutz, Wiesbaden, Germany.

Test material

Test item:	Prothioconazole 200g/L + Azoxystrobin 150g/L SC
Purity:	Prothioconazole: 198 g/L, Azoxystrobin: 148 g/L.
Description	White to off-white homogeneous suspension liquid
Batch No.:	20191211001

Test system

Organism (<i>Species</i>):	<p><i>Hypoaspis</i> (<i>Geolaelaps</i>) <i>aculeifer</i> CANESTRINI (Acari: Laelapidae).</p> <p>Adult, mated female mites (7 – 14 d beyond reaching adult stage) from a synchronised culture maintained at the test facility, originally established with organisms obtained from Eurofins GmbH.</p>
Study type:	Sub-lethal toxicity.
Guideline deviations reported:	The substrate of the highest test item concentration, i.e. 1000 mg test item/kg soil (dw), had at the beginning of the test with 38.2 % a slightly lower moisture content than recommended by the guideline (40 – 60 % of WHC _{max}). Since no conspicuous results occurred at this concentration and the measured value for the moisture content at the end of the test was in the recommended range, it can be assumed that this deviation at test start is due to a human error and has no impact on the reliability of the study results.
Duration of study:	14 days.
Parameters measured:	Adult mite mortality, behaviour effects and juvenile production after an exposure period of 14 days.
Observation intervals:	<u>At test start</u> : determination of physico-chemical parameters (water content, pH) of the artificial soil;

	<u>At 1 week:</u> moisture loss and compensation
	<u>At 2 weeks:</u> number of surviving adult mites per replicate; numbers of juvenile mites per replicate. Morphology and behaviour: pathological or other symptoms or distinct changes in behaviour of the test organisms during the course of the study.
Test concentrations:	Control (untreated substrate); 62.5, 125, 250, 500 and 1000 mg Prothioconazole 200 + 150 g/L SC/kg dw soil Reference item: boric acid: 56.2, 100, 178, 316 and 562 mg boric acid/kg (dry weight). (tested in a separate study).
Artificial substrate:	<ul style="list-style-type: none">- 5% sphagnum peat;- 20% kaolin clay (kaolinite content > 30 %);- 0.24% calcium carbonate;- 74.76% quartz sand (fine sand dominant with more than 50% of the particles between 50 and 200 µm).
Water content (g/10 g dry soil):	guideline requirement: 40-60% of WHC _{max} test start: 38.2 – 51.5% of WHC _{max} test end: 47.1 – 49.1% of WHC _{max} .
No. of replicates:	8 replicates for control; 4 replicates for each test item treatment. 10 adult female mites per replicate.
Temperature:	18.8 – 20.6°C
Photoperiod:	Light : dark = 16 h : 8 h. Light intensity 680 – 756 lux.
pH value:	guideline requirement: 6.0 ± 0.5. test start: 5.5 – 5.7; test end: 5.8 – 6.1.

Methodology

Based on the outcome of a prior range-finding test, soil mites were exposed to ten concentrations of soil-incorporated Prothioconazole 200 g/L + azoxystrobin 150 g/L SC (FF-075): 62.5, 125, 250, 500 and 1000 mg Prothioconazole 200 + 150 g/L SC/kg dw soil, with four replicates per treatment group. These were compared to an untreated control (8 replicates). The sensitivity of the test organisms was checked in a separate study, using mites from the same stock culture, exposed to boric acid mixed into artificial soil at concentrations of 56.2, 100, 178, 316 and 562 mg/kg soil dw.

Batches of test soil were prepared by incorporation (thorough mixing for 3 minutes) of volumes of a stock solution and serial dilutions derived from it that contained the test item dissolved in deionised water. The control soil was amended with water only. Aliquots of soil mixtures (20 g dry weight equivalent) were transferred to glass test vessels (5 cm diameter × 10 cm height and 200 mL capacity). There were 8 replicate vessels for the untreated control group and 4 replicates for each of the test item treatments. Ten adult female mites were transferred to the soil surface in each vessel and the vessels were then closed with tightly applied Parafilm, perforated to permit aeration. An additional single vessel per treatment received no test animals, but was used to provide measurements of soil pH and moisture content at the end of the test. All vessels were incubated under controlled climate conditions.

The mites were fed with prey mites (*Tyrophagus putrescentiae*) at the start of the test and feeding was repeated on D 2, D 7 and D 9. Test vessels were weighed on D 7 and deionised water was added as necessary to restore moisture losses.

On D14 the contents of each vessel were transferred to individual extraction funnels mounted in a Kempson extractor and heated from above by means of light bulbs for 22 h and 20 minutes to drive the test organisms down through the soil matrix. The adult and juvenile mites obtained from each funnel were counted.

Statistical analyses

Mortality: For determination of the LOEC/NOEC_{Mortality} data were checked for monotone dose-response by qualitative trend analysis by contrasts. Afterwards, Fisher's Exact Binomial Test with Bonferroni Correction ($\alpha = 0.05$, one-sided) was applied.

Reproduction: For determination of the LOEC/NOEC_{Reproduction} data were checked for normality by Shapiro-Wilk's test procedure and for homogeneity by Levene's test. Treatment means were compared by ANOVA and Dunnett's t-test ($\alpha = 0.05$, one-sided) and tested for statistically significant differences compared to the control (Sachs 1982).

The statistical software package ToxRat Professional Version 3.3.0 was used for these calculations.

Results

All validity criteria were met since a) mean adult mortality in controls was 2.5 % (test guideline requires $\leq 20\%$); b) the number of juveniles in the controls was 152.8 ± 19.1 (test guideline requires ≥ 50); c) the coefficient of variation for the juvenile numbers in the control was 12.5% (test guideline requires $\leq 30\%$).

In the separate reference test the EC_{50 reproduction} was established at 333.8 mg boric acid/kg soil dw (95% C.L.; 193.5 – 575.8 mg/kg soil dw), which is consistent with the expected response according to the guideline and verifies that the test system was suitably sensitive.

Mean adult mortality was 2.5 % in the untreated control group at the end of the test. Statistical analysis (Fisher's Exact Binomial test; $\alpha = 0.05$, one-sided) showed significant differences concerning mortality between the control and all test item concentrations. Since mortality occurred without a concentration-response relationship and did not rise above the 20 % allowed for the control (as a validity criterion), it can be assumed that the significant differences are due to the low variance of the control and were not induced by the test item. In addition, no effects on the usually more sensitive endpoint reproduction could be observed, therefore, the NOEC_{Mortality} is considered to be ≥ 1000 mg test item/kg soil (dw) and the LOEC_{Mortality} to be > 1000 mg test item/kg soil (dw).

Table CP 10.4.2.1/02-1: Number of dead adult female mites after 14 days of exposure (n = 80 for control; n = 40 for treatment). Data are given as absolute values and in percent of the total number (mortality).

Concentration [mg test item/kg soil (dw)]	Number of dead adults	Percent mortality
Control	2	2.5
62.5	6	15.0
125	7	17.5
250	5	12.5
500	6	15.0
1000	7	17.5

Statistical analysis (Fisher's Exact Binomial test; $\alpha = 0.05$, one-sided) showed significant differences concerning mortality between the control and all test item concentrations. Since mortality occurred with-

out a concentration-response relationship and did not rise above the 20 % allowed for the control (as a validity criterion), it can be assumed that the significant differences are due to the low variance of the control and were not induced by the test item. In addition, no effects on the usually more sensitive end-point reproduction could be observed, therefore, the NOEC_{Mortality} is considered to be ≥ 1000 mg test item/kg soil (dw) and the LOEC_{Mortality} to be > 1000 mg test item/kg soil (dw).

Table CP 10.4.2.1/02-2: Reproduction after 14 days. Data are given as mean number of juveniles \pm standard deviation per test vessel and as percentage of the solvent control (n = 8 for control; n = 4 for treatment).

Concentration [mg test item/kg soil (dw)]	Number of Juveniles [mean \pm sd]	Number of Juveniles [% of control]	Coefficient of Variation
Control	152.8 \pm 19.1	100.0	12.5
62.5	139.3 \pm 33.0	91.2	23.7
125	133.3 \pm 22.6	87.2	16.9
250	153.3 \pm 23.4	100.3	15.3
500	133.0 \pm 13.6	87.1	10.3
1000	159.3 \pm 22.3	104.3	14.0

Conclusions

The 14 day NOEC_{reproduction} for *Hypoaspis aculeifer* exposed to Prothioconazole 200 g/L + azoxystrobin 150 g/L SC (FF-075) was >1000 mg/kg dw soil.

A 2.4.2.2 KCP 10.4.2.2 Higher tier testing

A 2.5 KCP 10.5 Effects on soil nitrogen transformation

Comments of zRMS:	<p>The study is considered acceptable. All validity criteria were met.</p> <ul style="list-style-type: none"> The validity criteria for the test was met as the variation (% relative standard deviation) between the control replicates on day 28 was $<15\%$ (1.33%) <p>Agreed endpoints: There was no significant difference in nitrate formation in the test concentration of 1.369 mg test item/kg soil dry weight and significant difference in nitrate formation of 6.845 mg test item/kg soil dry weight when compared to control after 28 days of exposure.</p>
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Report:	CP 10.5/01; Li, N. (2021)
Title:	Effects of Prothioconazole 200 g/L + Azoxystrobin 150 g/L EC (FF-075) on soil microorganisms: Nitrogen transformation test
Document No:	Rotam Research Laboratory (RRL), China; Study report No.: 2864
Guideline:	OECD Guideline No. 216: 'Soil Microorganisms: Nitrogen Transformation Test' (2000)

GLP	Yes. Laboratory certified by the German Federal Institute for Risk Assessment, Berlin, Germany.
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Test material

Test item:	Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC
Purity:	Prothioconazole 198 g/L (16.9%, w/w) + Azoxystrobin 148 g/L (12.7%, w/w)
Description	Off white homogenous liquid.
Batch No.:	20191211001

Test system

Organism (<i>Species</i>):	Soil microorganisms in sandy clay loam soil (sand: 53.53%, clay: 27.42% and silt: 19.05%).
Study type:	Nitrogen transformation test.
Guideline deviations reported:	None reported.
Duration of study:	28 days.
Parameters measured:	Nitrate formation rate
Observation intervals:	0, 7, 14 and 28 th day.
Test concentrations:	The maximum predicted environmental concentration (PEC) in soil was derived from the maximum recommended application rate and maximum number of applications. The test concentrations selected were PEC (1.369 mg test item/kg soil dry weight) and 5 times of PEC (6.845 mg test item/kg soil dry weight). Maximum PEC of test item in soil was calculated assuming uniform incorporation to a depth of 5 cm and a soil bulk density of 1.5.
Control:	Untreated soil
Test Arenas:	500 mL glass beakers covered with perforated plastic lids. Bulk soil of about 2.5 to 3.0 kg was transferred into 5 L glass beaker for extended sampling occasions. All the beakers were weighed initially and were incubated under test conditions.
Application of treatments:	Weighted 13.92 mg of test item into 500 mL volumetric flask and made up to mark with Milli-Q water. Weighted 27.83 mg test item and mixed with 200 mL Milli-Q water. All the test solution (200 mL) were mixed with pre-incubated soil to get the test concentration of 1.369 and 6.845 mg test item/kg soil dry weigh. The total water content of the soil was maintained at 40 - 60% of maximum water holding capacity. The same quantity of water without the test item was added to the third container of soil and homogenized to make the control sample.
No. of replicates:	3 replicates per test concentration and control
Temperature:	20 ± 2°C
Photoperiod:	Tests were incubated in darkness
Moisture	Moisture content of soil samples maintained between 40 – 60% of maximum water holding capacity during the test

Light intensity: Not applicable

Relative humidity: Not applicable

Methodology

Soil samples were collected from the field (Xiangzidian village, Yinhua town, Shanyang city, Shanxi province, China) at a depth of 20-25 cm and transported to the test facility in sealed thermocol boxes. The sampling site was grass land which had not been treated with any crop protection products, organic or inorganic fertilizers 12 months prior to testing. Soil samples were cleared of large objects and sieved through a 2 mm sieve. Lucerne grass meal was mixed with the soil at a concentration of 4 g/kg soil dry weight in all test concentrations and the prepared soil was used for testing. Soil was stored at $4\pm 2^{\circ}\text{C}$ after receiving and the soil was pre incubated for 7 days under the test conditions. At the start of pre-incubation about 4.5 kg of soil (<2 mm) was weighed each for control and test concentrations and moistened with Milli-Q water, required to achieve 40% - 60% of maximum water holding capacity.

13.92 mg of test item into 500 mL volumetric flask and made up to mark with Milli-Q water. 27.83 mg test item and mixed with 200 mL Milli-Q water. All the test solution (200 mL) were mixed with pre-incubated soil to get the test concentration of 1.369 and 6.845 mg test item/kg soil dry weigh. The total water content of the soil was maintained at 40 - 60% of maximum water holding capacity. The same quantity of water without the test item was added to the third container of soil and homogenized to make the control sample.

The total nitrogen content and soil microbial biomass were determined. From each treated and control soil, sub samples of about 200 g were transferred to 500 mL glass beakers and covered with perforated plastic lids. The bulk soil of about 2.5 to 3.0 kg was transferred into 5 L glass beaker for extended sampling occasions. All the beakers were weighed initially and were incubated under test conditions. The soil from each treatment was tested for pH, moisture content and nitrate concentration before incubation. Once a week the moisture content was determined by re-weighing the test containers and adjusted with Milli-Q water to the initial weight. A sample of each replicate from each treatment was taken on the 7th, 14th and 28th day after application to determine the pH, moisture content and nitrate formation was tested.

About 40 g of the soil was weighed and extracted using 100 mL 0.04 M $(\text{NH}_4)_2\text{SO}_4$. The soil and solvent were shaken continuously for approximately 30 minutes using a shaker. The aqueous solution was filtered through whatman No.2 filter paper of size 8 μm and nitrate content was measured using the calibrated nitrate selective electrode, and the nitrate concentration and rate of nitrate formation subsequently calculated.

A study with a reference substance (Eptam Technical) of known microbial toxicity to demonstrate the test results under the laboratory test conditions is conducted once a year.

Statistical analyses

The nitrogen formation and nitrogen formation rate of test concentrations were statistically compared with control using Dunnett's Two-sided Multiple Comparison test using analysis of variance (ANOVA) techniques. The calculations were done using NCSS software @2007.

Results

The validity criteria for the test was met as the variation (% relative standard deviation) between the control replicates on day 28 was <15% (1.33%), thereby meeting the criteria as per OECD 216 guideline.

The moisture content, water holding capacity and pH were measured on 0, 7th, 14th and 28th day. The mean moisture content ranged from 22.0% to 23.0% during 28 days test period. Mean moisture content ranged from 50.4% to 52.6% of its maximum water holding capacity during the 28 days test period. The average pH ranged from 6.47 to 6.50 during 28 days test period. The test was conducted in dark at a controlled temperature maintained between 19.1°C to 20.7°C.

The percent deviation of nitrate formation rate of the concentration of 1.369 and 6.845 mg test item/kg soil dry weight compared to control on day 28 was -2.62% and -5.52%, respectively. The effects are considerably less than 25% (the threshold limit specified by OECD guideline No.: 216). So the test item Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC was evaluated as having no long-term influence on nitrogen transformation in soils.

CP 10.5/01-1: Initial soil nitrate concentration

Day	R	mg NO ₃ ⁻ /100 mL (C)	Weight of soil (g) (W)	D	D _w	mg nitrate/kg soil dry weight	Mean mg nitrate/kg soil dry weight	SD	%RSD
Initial	R1	7.56	40.0	1	90.3	47.30	47.66	0.32	0.67
	R2	7.66	40.0	1	90.3	47.93			
	R3	7.63	40.0	1	90.3	47.74			

R: Replicate; D_w = Dry weight of soil; D = Dilution factor of soil extract; SD: Standard deviation.

CP 10.5/01-2: Rate of Nitrate Formation at Time Intervals after Application

At each interval time	Control	1.369 mg test item/kg soil dry weight			6.845 mg test item/kg soil dry weight	
	mg nitrate/kg soil dry weight/day	mg nitrate/kg soil dry weight/day	Deviation from control (%)		mg nitrate/kg soil dry weight/day	Deviation from control (%)
0 to 7	0.30	0.06	-80.00		0.02	-93.07
0 to 14	1.15	1.02	-10.53		0.95	-17.43
0 to 28	0.84	0.80 ^{n.s}	-5.05		0.73 ^s	-12.67

-: Inhibitory effects, n.s: no statistically significant on day 28 at p>0.05; s: statistically significant on day 28 at p≤0.05.

CP 10.5/01-3 Rate of Nitrate Formation at Different Time Intervals after Application

At each interval time	Interval of days	Control	1.369 mg test item/kg soil dry weight			6.845 mg test item/kg soil dry weight	
		mg nitrate/kg soil dry weight/day	mg nitrate/kg soil dry weight/day	Deviation from control (%)		mg nitrate/kg soil dry weight/day	Deviation from control (%)
7 to 0	7	0.30	0.06	-80.00		0.02	-93.07
14 to 7	7	1.99	1.99	-0.15		1.87	-6.12
28 to 14	14	0.54	0.57	+6.67		0.52	-2.50

-: Inhibitory effects, +: Stimulatory effects.

CP 10.5/01-3: Calculation of Nitrate Formation on Each Sampling Occasion

Day	Nominal concentration (mg test item/kg soil dry weight)	R	mg NO ₃ ⁻ /100 mL (C)	Weight of soil (g) (W)	D	D _w	mg nitrate/kg soil dry weight	Mean mg nitrate/kg soil dry weight	SD	%RSD	Deviation from control (%)
0	Control	R1	7.69	40.0	1	90.3	48.12	48.20	0.10	0.20	-

		R2	7.70	40.0	1	90.3	48.18				
		R3	7.72	40.0	1	90.3	48.30				
		R1	7.56	40.0	1	90.3	47.30				
	1.369	R2	7.62	40.0	1	90.3	47.68	47.51	0.19	0.40	-1.43
		R3	7.60	40.0	1	90.3	47.55				
		R1	7.55	40.0	1	90.3	47.24				
	6.845	R2	7.59	40.0	1	90.3	47.49	47.22	0.28	0.60	-2.03
		R3	7.50	40.0	1	90.3	46.93				
		R1	8.02	40.0	1	90.3	50.18				
7	Control	R2	8.10	40.0	1	90.3	50.68	50.28	0.36	0.71	-
		R3	7.99	40.0	1	90.3	49.99				
		R1	7.70	40.0	1	90.3	48.18				
	1.369	R2	7.65	40.0	1	90.3	47.87	47.93	0.23	0.47	-4.69
		R3	7.63	40.0	1	90.3	47.74				
		R1	7.60	40.0	1	90.3	47.55				
	6.845	R2	7.56	40.0	1	90.3	47.30	47.36	0.17	0.35	-5.81
		R3	7.55	40.0	1	90.3	47.24				
		R4	10.2	40.0	1	90.3	63.82	64.24	1.30	2.03	-
14	Control	R5	10.5	40.0	1	90.3	65.70				
		R6	10.1	40.0	1	90.3	63.19				
	1.369	R4	9.92	40.0	1	90.3	62.07	61.86	0.19	0.31	-3.70
		R5	9.86	40.0	1	90.3	61.69				
		R6	9.88	40.0	1	90.3	61.82				
	6.845	R4	9.72	40.0	1	90.3	60.82	60.46	0.32	0.53	-5.88
		R5	9.62	40.0	1	90.3	60.19				
		R6	9.65	40.0	1	90.3	60.38				

Day	Nominal concentration (mg test item/kg soil dry weight)	R	mg nitrate/100 mL (C)	Weight of soil (g) (W)	D	D _w	mg nitrate/kg soil dry weight	Mean mg nitrate/kg soil dry weight	SD	%RSD	Deviation from control (%)
28	Control	R7	11.5	40.0	1	90.3	71.95	71.75	0.96	1.33	-
		R8	11.6	40.0	1	90.3	72.58				
		R9	11.3	40.0	1	90.3	70.70				
	1.369	R7	11.2	40.0	1	90.3	70.08	69.87 ^{n.s}	0.96	1.37	-2.62
		R8	11.3	40.0	1	90.3	70.70				
		R9	11.0	40.0	1	90.3	68.83				
	6.845	R7	10.8	40.0	1	90.3	67.57	67.78 ^s	0.96	1.41	-5.52
		R8	10.7	40.0	1	90.3	66.95				
		R9	11.0	40.0	1	90.3	68.83				

R: Replicate; SD: Standard deviation; %RSD (Relative standard deviation); n.s: no statistically significant on day 28 at p>0.05; s: statistically significant on day 28 at p≤0.05; D_w = Dry weight of soil, D = Dilution factor of soil extract, F (Conversion factor for nitrate) = 0.226.

mg nitrate/kg soil dry weight = (C×F×1000×D×100)/(D_w×W).

Table CP 10.5/1-4: Soil characteristics and substrate (Lucerne grass) details

Sand content	53.53%
Silt content	19.05%
Clay content	27.42%
pH	6.5 (pH meter: METTLER-TOLEDO, Shanghai; Model: SG2)
Maximum water holding capacity (WHC _{max})	43.7%
Dry matter	93.3%
Cation exchange capacity (CEC)	0.81 mmol/kg soil as mg N/L (SHIMADZU Corporation, Japan; Model: UV-2450)
Total organic carbon content (TOC)	8.13 g/kg dry soil weight Analytic Jena AG, Germany; Model: HT1300)
Total Nitrogen content (TN)	0.076% (Analytic Jena AG, Germany; Model: Multi N/C 3100)
Total carbon content of soil	13.16 g/kg dry soil weight (Analytic Jena AG, Germany; Model: HT1300)
Microbial Biomass and nitrate content prior to testing	
Microbial biomass	182.37 mg/g soil dry weight (2.24% of TOC)
Initial nitrate content of soil	47.66 mg nitrate/kg soil dry weight (HANNA Instrument Co., Ltd., Romania; Model: Ht1300)
Total Nitrogen content of test item	2.16%
Substrate (Lucerne grass) details	
Supplier	Bozhou limit plant Co., Ltd
Size	<2 mm
Content of Lucerne grass in test concentrations	4 g/kg test soil dry weight
Carbon content (C)	418.94 g/kg
Nitrogen content (N)	2.829%
Ratio of C/N	14.8:1

Conclusions

There was no significant difference in nitrate formation in the test concentration of 1.369 mg test item/kg soil dry weight and significant difference in nitrate formation of 6.845 mg test item/kg soil dry weight when compared to control after 28 days of exposure.

The percent deviation of nitrate formation rate between concentration of 1.369 and 6.845 mg test item/kg soil dry weight and control on day 28 was -2.62% and -5.52%, respectively.

The percent deviation of nitrate formation rate is less than 25%. Therefore, according to OECD guideline No. 216, the test item Prothioconazole 200 g/L + Azoxystrobin 150 g/L SC was evaluated as having no long-term influence on nitrogen transformation in soils.

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

A 2.6.1 KCP 10.6.1 Summary of screening data

A 2.6.2 KCP 10.6.2 Testing on non-target plants

zRMS comments:

The study is considered acceptable. The validity criteria were fulfilled :

- The seedling emergence in the control was $\geq 75\%$ ($\geq 70\%$ required)
- The seedling survival in the control was 100% ($\geq 90\%$ required)
- No phytotoxicity was observed in the controls.
- environmental conditions for a particular species were identical and growing media contained the same amount of soil matrix and support media.

Agreed endpoints:

Summary of the NOEC, LOEC and EC₅₀ of the endpoints emergence, survival and shoot fresh weight, referring to the nominal test item application rate [L FF-075/ha].

Species	NOER	LOER	ER ₁₀	ER ₅₀
<u>Emergence</u>				
All species	≥ 8.5	> 8.5	n.d.	n.d.
<u>Survival</u>				
All species	≥ 8.5	> 8.5	n.d.	n.d.
<u>Shoot fresh weight</u>				
<i>A. cepa</i>	≥ 8.5	> 8.5	n.d.	n.d.
<i>A. sativa</i>	≥ 8.5	> 8.5	n.d.	n.d.
<i>B. rapa</i>	≥ 8.5	> 8.5	n.d.	n.d.
<i>C. sativus</i>	1.8	3.8	n.d.	n.d.
<i>P. sativum</i>	≥ 8.5	> 8.5	n.d.	n.d.
<i>S. lycopersicum</i>	3.8	8.5	3.2 (1.0/9.8)	n.d.

n.d. = not detected (e.g. due to the lacking of adverse effects)

Visual Phytotoxicity:

There were no visual damages in any of the six test species at any of the test item application rates other than normal phenotypical variance which could be observed in the controls as well.

Report:	CP 10.6/01; Förster, B (2021)
Title:	Prothioconazole 200 g/L + azoxystrobin 150 g/L SC (FF-075): Terrestrial plant seedling emergence and seedling growth test.
Document No:	ECT Oekotoxikologie GmbH; Study report No.: 20AV6PA
Guideline:	OECD Guideline for the Testing of Chemicals No. 208 Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test
GLP	Yes. Hessisches Ministerium für Umwelt, Klima-schutz, Landwirtschaft und Verbraucherschutz, Wiesbaden, Germany, 28 May 2018.

Test material

Test item: Prothioconazole 200g/L + Azoxystrobin 150g/L SC (FF-075).
Purity: Active substance content: 194.3±2.0 g/L (Prothioconazole) and 149.1±1.0 g/L (Azoxystrobin).
Description: White to off-white homogeneous suspension liquid
Batch No.: 20191211001

Test system

Organism (*Species*):
Allium cepa (onion)
Avena sativa (oat)
Brassica rapa (turnip)
Cucumis sativus (cucumber)
Pisum sativum (pea)
Solanum lycopersicum (tomato)

Study type: Seedling emergence and seedling growth

Guideline deviations reported: Relative air humidity in the test room partly fell below the lower threshold value of 30% and air temperature partly exceeded the upper threshold value of 32°C given in the study plan during the first days of the test, i.e. before emergence of seedlings. The soil had been covered with lids during this period to prevent soil from desiccation. Therefore the deviation had no impact on the integrity of the study.
Accidentally, no seeds were planted in three out of seven pots of treatment T2 in *Cucumis sativus*. therefore the number of replicates (pots) for treatment T2 was four instead of seven and the total number of seeds was 12 instead of 21. Since there was no adverse effect of the test item observed at any of the higher application rates the deviation is considered to have no impact on the results or on the integrity of the study.

Duration of study: 16 days

Parameters measured: Emergence and survival, visual appearance, shoot fresh weight.

Observation intervals: 0, 7, 14 and at test end.

Test concentrations: 8.52, 3.78, 1.76, 0.8 and 0.36 L FF-075/ha.

Control: Control soil was sprayed with water without test item.

Test Arenas: Common plant pots made of polypropylene (diameter 11 cm,

	height 8.5 cm) were used as test pots. Each pot was filled with 480±9 g soil fresh weight (approximately 450 g dry weight) and placed in a separate polystyrene beaker (Kastelplast GmbH, Mainz, Germany) serving as water reservoir. Each pot was labelled with the study number, the treatments code, a consecutive number and the plants species
Test soil	Standard soil LUFA Type 2.3, a sandy loam, was purchased from the Landwirtschaftliche Untersuchungs- und Forschungsanstalt (LUFA), D-67346 Speyer, Germany.
Application of treatments:	The test item was sprayed onto the soil surface immediately after planting the seeds. Control soil was sprayed with water without the test item.
No. of replicates:	4 replicate (pots) per treatment with 6 seeds per pot for the monocotyledons and 7 replicates (pots) per treatment with 3 seeds per pot for the dicotyledonous species.
Temperature:	19.9 – 26.5°C
Photoperiod:	16/8 h light/dark.
Light intensity:	207 to 237 µE m ⁻² s ⁻¹
Relative humidity:	25.8 – 63.5% (air humidity below 30% occurred only during the first days of the test before seedling emergence)

Methodology

The study was conducted in order to determine possible effects of the test item Prothioconazole 200g/L + Azoxystrobin 150g/L SC (FF-075) on the seedling emergence and early growth of higher terrestrial plants. Therefore, the effect of the test item was tested with plant species representing six plant families: two monocotyledonous test species, *Allium cepa* (onion) and *Avena sativa* (oat), and four dicotyledonous species, *Brassica rapa* (turnip), *Cucumis sativus* (cucumber), *Pisum sativum* (pea) and *Solanum lycopersicum* (tomato).

The test item was sprayed onto the soil surface immediately after planting the seeds. Control soil was sprayed with water without the test item.

Measurement endpoints were the emergence rate, survival of seedlings, shoot fresh weight, and visual detrimental effects (such as e.g. chlorosis, necrosis, and development abnormalities).

Assessment endpoints comprised the No-Observed-Effect-Rate (NOER), and the 10% and 50% Effect-Rate (ER₁₀ and ER₅₀) for the end points emergence rate, survival of seedlings, and the shoot fresh weight.

Day 0 of the test was defined as the day by which at least 50% of the seedlings in the untreated control pots of a particular species had emerged.

Visual detrimental effects were assessed on day 7 and at the end of the test for the respective test species. Shoot fresh weight was evaluated at the end of the test.

Seeds of two monocotyledonous species, *Allium cepa* (onion) and *Avena sativa* (oat), as well as four dicotyledonous species, *Brassica rapa* (turnip), *Cucumis sativus* (cucumber), *Pisum sativum* (pea) and *Solanum lycopersicum* (tomato) were planted in a natural sandy loam soil (standard soil LUFA Sp 2.3). The test item was applied immediately thereafter at rates ranging from 0.36 to 8.52 L FF-075/ha for all spe-

cies. Seedlings were left to emerge and grow under controlled conditions for 14 days following 50 % emergence of the seedlings in the respective control soil.

Soils were supplied with water and a nutrient solution as needed by bottom watering. The test was performed in a growth chamber equipped with artificial lighting and air conditioning.

Light intensity: 207 to 237 $\mu\text{E m}^{-2} \text{ s}^{-1}$ (16/8 h light/dark).

Air temperature: 19.9°C to 26.5°C (18.7°C to 34.6°C for *P. sativum*)

Rel air humidity: 25.8% to 63.5% (17.5% to 63.5% for *P. sativum*)

The pots were placed randomly at the beginning of the test and were re-arranged weekly. On day 7 (7 days after 50% emergence of control seedlings) seedlings were evaluated visually. At day 14 (day 16 for *P. sativum*), seedlings were counted, evaluated visually, and harvested to determine shoot fresh weight.

Two samples of the two stock solution were taken immediately prior to spray application and stored at $\leq -18^\circ\text{C}$. One of the duplicate samples was shipped under frozen conditions to the PI on 26 January 2021 (arrival at the analytical test site on 28 January 2021). Chemical analysis was performed under the responsibility of the Principal Investigator (PI) for analysis.

Statistical analyses

Number of emerged and number of survived seedlings as well as shoot fresh weight at the end of the test was considered for statistical data analysis. Statistical methods were applied to evaluate whether differences between test item treatments and control were significant. Arithmetic means for each pot were calculated from the single plants within a pot. Since each pot constituted a replicate, the mean values were used for statistical analysis.

Emergence and survival data were checked by the Step-down Cochran-Armitage Test Procedure or in case of a non-monotone dose response with the Chi2-test with Bonferroni correction to determine the NOEC and LOEC. Significance level was $\alpha = 0.05$, one sided greater. The 3-Parameter normal cumulative distribution function was used to determine the effective concentrations EC_{10} and EC_{50} for the end point shoot fresh weight.

Normal distribution and homogeneity of variances were checked by the Shapiro-Wilk's Test and Levene's test, respectively. A trend analysis by contrast was performed to check for monotony of rate/response.

NOEC and LOEC of shoot fresh weight were determined either by applying either the Williams multiple sequential t-test (monotonous dose-response relationship) or the Dunnett's test. In case of inhomogeneous variances, the step-down Jonckheere-Terpstra test was applied, in case data differed significantly from normal distribution the Multiple Sequentially-rejective U-test after Bonferroni-Holm was applied. Significance level was $\alpha = 0.05$, one-sided smaller.

Statistical evaluations were performed using the ToxRat Pro software Version 3.30. (ToxRat Solutions GmbH, 2010).

Results

The validity criteria were fulfilled as seedling emergence in the control was $\geq 75\%$ ($\geq 70\%$ required) and seedling survival in the control was 100% ($\geq 90\%$ required) for all six species. No phytotoxicity was observed in the controls. Environmental conditions for a particular species were identical and growing media contained the same amount of soil matrix and support media.

Measured test item concentration of prothioconazole and azoxystrobin for the highest application rate was 89% and 71%, respectively, of the nominal test item concentration. Therefore, effective rates (ER_x) and limit rates (LOER, NOER) were based on nominal test item concentration.

CP 10.6/01-01. Nominal and measured concentration of Prothioconazole and Azoxystrobin in the stock solution for spray application.

Test run	Nominal concentration [g FF-075/L]	Nominal concentration [g a.s./L]		Measured concentration [g a.s./L]		Measured concentration [% of nominal]	
	Formulated product	Prothio	Azoxy	Prothio	Azoxy	Prothio	Azoxy
DT1	28.0	4.66	3.57	4.16	2.53	89	71
DT2	28.2	4.69	3.60	n.d.*	n.d.	n.a.	n.a.

Prothio = Prothioconazole; Azoxy = Azoxystrobin; DT1 = all test species except *P. sativum*; DT2 = *P. sativum*; n.d. = not determined (verification by weight of the test item used for stock solution); n.a. = not applicable.

CP 10.6/01-02. Number of seeds introduced, number of emerged seeds, and number of survived seedlings in the untreated control pots.

Plant species	Seeds [No.] ¹	Day 0 [d] ²	Emerged [No.] ³	Survived [No.] ⁴	Emerged [%]	Survived [%]
<i>A. cepa</i>	24	7	18	18	75.0	100
<i>A. sativa</i>	24	4	24	24	100	100
<i>B. rapa</i>	21	4	21	21	100	100
<i>C. sativus</i>	21	4	18	18	85.7	100
<i>P. sativum</i>	21	6	16	16	76.2	100
<i>S. lycopersicum</i>	21	5	21	21	100	100

- (1) Total number of seeds introduced; (2) day 0 of the test given as days after sowing; (3) emerged until test end; (4) survived until test end.

No statistically significant effect on either emergence or survival of seedlings in any of the test species was observed at any test item application rate up to and including 8.5 L formulated product per hectare.

CP 10.6/01-03. Seedling emergence and seedling survival.

Test item [L FF-075/ha]	<i>A. cepa</i>	<i>A. sativa</i>	<i>B. rapa</i>	<i>C. sativus</i>	<i>P. sativum</i>	<i>S. lycopersicum</i>
Number of seeds sown						
C 0.0	24	24	21	21	21	21
T1 0.36	24	24	21	21	21	21
T2 0.80	24	24	21	12 ^(a)	21	21
T3 1.76	24	24	21	21	21	21
T4 3.78	24	24	21	21	20 ^(b)	21
T5 8.52	24	24	21	21	21	21
Number of emerged seedlings						
C 0.0	18	24	21	18	16	21
T1 0.36	16	22	20	20	19	17
T2 0.80	19	23	18	11	19	19
T3 1.76	21	23	19	18	19	19
T4 3.78	20	21	18	20	17	20
T5 8.52	18	23	20	20	20	21
Number of live seedlings at test end						
C 0.0	18	24	21	18	16	21
T1 0.36	16	22	20	20	18	17

T2	0.80	19	23	18	11	19	19
T3	1.76	19	23	19	18	19	19
T4	3.78	16	21	18	20	17	20
T5	8.52	16	23	20	20	20	21
Relative number of emerged seeds [% of seeds sown]							
C	0.0	75.0	100	100	85.7	76.2	100
T1	0.36	66.7	91.7	95.2	95.2	90.5	81.0
T2	0.80	79.2	95.8	85.7	91.7	90.5	90.5
T3	1.76	87.5	95.8	90.5	85.7	90.5	90.5
T4	3.78	83.3	87.5	85.7	95.2	85.0	95.2
T5	8.52	75.0	95.8	95.2	95.2	95.2	100
Relative number of live seedlings at test end [% of emerged]							
C	0.0	100	100	100	100	100	100
T1	0.36	100	100	100	100	94.7	100
T2	0.80	100	100	100	100	100	100
T3	1.76	90.5	100	100	100	100	100
T4	3.78	80.0	100	100	100	100	100
T5	8.52	88.9	100	100	100	100	100

(a) = Accidentally, no seeds were planted in three out of seven pots; (b) = a discrepancy was discovered between the recorded initial and final number of seedlings in one out of the seven pots, therefore this pot was not considered; n.a = not applicable.

CP 10.6/01-04. Number of seedlings per rating score on day 7 and at the end of the test.

Test species	Rate code	Rate FF-075 [L/ha]	Rating score day 7					Rating score end of test				
			2	3	4	5	6	2	3	4	5	6
<i>A. cepa</i>	C	0.0	--	--	--	--	--	--	--	--	--	--
	T1	0.36	1	--	--	--	--	1	--	--	--	--
	T2	0.80	--	--	--	--	--	--	--	--	--	--
	T3	1.76	3	1	4	1	--	4	--	--	1	2
	T4	3.78	8	--	1	--	--	10	--	--	--	4
	T5	8.52	6	1	2	1	--	9	3	--	--	2
<i>A. sativa</i>	C	0.0	--	--	--	--	--	--	--	--	--	--
	T1	0.36	--	--	--	--	--	--	--	--	--	--
	T2	0.80	--	--	--	--	--	--	--	--	--	--
	T3	1.76	--	--	--	--	--	--	--	--	--	--
	T4	3.78	--	--	--	--	--	--	--	--	--	--
	T5	8.52	--	--	--	--	--	--	--	--	--	--
<i>B. rapa</i>	C	0.0	1	--	--	--	--	--	--	--	--	--
	T1	0.36	--	--	--	--	--	--	--	--	1	--
	T2	0.80	1	--	--	--	--	--	--	--	--	--
	T3	1.76	1	--	--	--	--	--	--	--	--	--
	T4	3.78	--	--	--	--	--	--	--	1	--	--
	T5	8.52	2	--	--	--	--	--	--	--	--	--
<i>C. sativus</i>	C	0.0	1	--	--	--	--	--	--	--	--	--
	T1	0.36	--	--	--	--	--	--	--	--	--	--
	T2	0.80	--	--	--	--	--	1	--	--	--	--
	T3	1.76	--	--	--	--	--	1	--	--	--	--
	T4	3.78	1	--	--	--	--	2	--	1	1	--
	T5	8.52	--	--	--	--	--	2	--	1	--	--
<i>P. sativum</i>	C	0.0	2	--	--	--	--	2	--	--	--	--
	T1	0.36	--	--	--	--	1	2	--	--	--	1

	T2	0.80	1	--	--	--	--	3	--	--	--	--
	T3	1.76	2	--	--	--	--	2	--	--	--	--
	T4	3.78	--	--	--	--	--	1	--	--	--	--
	T5	8.52	3	--	--	--	--	4	--	--	--	--
<i>S. lycopers.</i>	C	0.0	--	--	--	1	--	--	--	--	2	--
	T1	0.36	1	--	--	--	--	--	--	--	--	--
	T2	0.80	--	--	--	--	--	1	--	--	--	--
	T3	1.76	--	--	--	--	--	--	--	--	2	--
	T4	3.78	3	1	--	--	--	1	1	--	2	--
	T5	8.52	6	--	--	--	--	7	--	--	1	--

Rating scores: 1 = no damages (not shown); 2/3 = slight damages; 4/5 = severe damages; 6 = dead seedling; (--) = no seedling allocated to the rating score; (for details of the rating score system see section **Błąd! Nie można odnaleźć źródła odwołania.**); *S.lycopers.* = *S. lycopersicum*.

Shoot fresh weight was statistically significantly reduced compared to the control in *Cucumis sativus* by 15.7% 19.0% and at application rates of 3.78 L FF-075/ha and 8.52 L FF-075/ha. In *Solanum lycopersicum* shoot fresh weight was statistically significantly reduced by 26.8% at 8.52 L FF-075/ha. No statistically significant deviation from the control was found for the shoot fresh weight in any of the other four test species at application rates up to and including 8.52 L FF-075/ha which was the highest tested rate. The lowest NOER was 1.76 L formulated product/ha.

No statistically significant effect on shoot fresh weight was observed in any of the other four test species at any of the test item application rates up to and including 8.5 L FF-075/ha which was the highest tested application rate.

CP 10.6/01-04. Shoot fresh weight at the end of the test, mean and standard deviation [g], and relative reduction related to the control [%].

Treatment code	C	T1	T2	T3	T4	T5
Test item [L FF-075/ha]	0.0	0.36	0.80	1.76	3.78	8.52
<i>Allium cepa</i>						
Mean [g]	0.157	0.196	0.238	0.149	0.151	0.110
SD [g]	0.074	0.035	0.033	0.061	0.028	0.040
Reduction [%] ^a	n.a.	-24.4	-51.3	5.6	4.0	30.0
<i>Avena sativa</i>						
Mean [g]	0.832	0.834	0.876	0.774	0.782	0.756
SD [g]	0.109	0.068	0.085	0.087	0.149	0.075
Reduction [%] ^a	n.a.	-0.4	-5.4	6.9	6.0	9.1
<i>Brassica rapa</i>						
Mean [g]	1.83	1.69	2.01	1.84	1.74	1.62
SD [g]	0.13	0.30	0.62	0.65	0.41	0.36
Reduction [%] ^a	n.a.	7.5	-9.8	-0.6	5.1	11.3
<i>Cucumis sativus</i>						
Mean [g]	2.03	1.91	2.00	2.06	1.71 [#]	1.64 [#]
SD [g]	0.24	0.18	0.38	0.26	0.30	0.16
Reduction [%] ^a	n.a.	5.8	1.4	-1.6	15.7	19.0
<i>Pisum sativum</i>						
Mean [g]	2.68	2.76	2.17	2.17	2.57	2.12
SD [g]	0.90	0.50	0.46	0.51	0.44	0.48
Reduction [%] ^a	n.a.	-3.2	18.9	18.8	3.8	20.6

Solanum lycopersicum

Mean [g]	1.20	1.59	1.46	1.38	1.21	0.88*
SD [g]	0.16	0.45	0.24	0.36	0.33	0.31
Reduction [%] ^a	n.a.	-33.0	-21.5	-15.6	-1.3	26.8

C = control; T1 to T5 = test item treated; a = related to C (control), negative values represent increase; n.a. = not applicable; * = significantly different from the control (Williams Multiple Sequential t-test, $\alpha = 0.05$, one-sided smaller); n.a. not applicable; # = significantly different from the control (Step-down Jonckheere-Terpstra test, $\alpha = 0.05$, one-sided smaller).

There were no visual damages in any of the six test species at any of the test item application rates other than normal phenotypical variance which could be observed in the controls as well.

Conclusions

No adverse effects on either seedling emergence or survival of seedlings were observed at test item application rates up to and including 8.5 L FF-075/ha.

No ER₅₀ could be determined for any of the end points in any of the six test species due to the lacking of an effect of at least 50% at application rates of up to and including 8.5 L FF-075/ha which was the highest tested application rate.

The lowest NOER was 1.8 L FF-075/ha (equivalent to nominally 350 g Prothioconazole/ha and 268 g Azoxystrobin/ha) and was observed with shoot fresh weight of *Cucumis sativus*.

The lowest ER₁₀ was 3.2 L FF-075/ha (equivalent to nominally 622 g Prothioconazole/ha and 477 g Azoxystrobin/ha) and was observed with shoot fresh weight of *Solanum lycopersicum*.

CP 10.6/01-05. Summary of the NOEC, LOEC and EC₅₀ of the endpoints emergence, survival and shoot fresh weight, referring to the nominal test item application rate [L FF-075/ha].

Species	NOER	LOER	ER ₁₀	ER ₅₀
<u>Emergence</u>				
All species	≥8.5	>8.5	n.d.	n.d.
<u>Survival</u>				
All species	≥8.5	>8.5	n.d.	n.d.
<u>Shoot fresh weight</u>				
<i>A. cepa</i>	≥8.5	>8.5	n.d.	n.d.
<i>A. sativa</i>	≥8.5	>8.5	n.d.	n.d.
<i>B. rapa</i>	≥8.5	>8.5	n.d.	n.d.
<i>C. sativus</i>	1.8	3.8	n.d.	n.d.
<i>P. sativum</i>	≥8.5	>8.5	n.d.	n.d.
<i>S. lycopersicum</i>	3.8	8.5	3.2 (1.0/9.8)	n.d.

n.d. = not detected (e.g. due to the lacking of adverse effects)

zRMS comments:

The validity criteria were fulfilled. The study is considered acceptable.

- The survival of plants in the control was 100% (required $\geq 90\%$) for all six species.
- No phytotoxicity was observed in the controls.
- Environmental conditions for a particular species were identical and growing media contained the same amount of soil matrix and support media.

Test item [L FF-075/ha]	<i>A. cepa</i>	<i>A. sativa</i>	<i>B. rapa</i>	<i>C. sativus</i>	<i>P. sativum</i>	<i>S. lycopersicum</i>
Survival						
LR ₁₀	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
LR ₅₀	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
LOER	>8.52	>8.52	>8.52	>8.52	>8.52	>8.52
NOER	≥ 8.52	≥ 8.52	≥ 8.52	≥ 8.52	≥ 8.52	≥ 8.52
Shoot fresh weight						
ER ₁₀	n.d.	n.d.	n.d.	0.6 (0.2/2.7)	n.d.	n.d.
ER ₅₀	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
LOER	>8.52	>8.52	>8.52	0.80	>8.52	>8.52
NOER	≥ 8.52	≥ 8.52	≥ 8.52	0.36	≥ 8.52	≥ 8.52

n.d. not determined / calculation not feasible due to inappropriate data (e.g. no mortality or no meaningful rate/response was observed).

Visual phytotoxicity:

Typical damages were necrosis and/or wilting of leaves which appeared at test item application rates of ≥ 0.8 L FF-075/ha. Damages such as decolouration and wilting of leaves seemed to appear only in those aboveground portions of the plants which had been reached directly by the spray during test item application. Leaves developing post application did not show such damages, while growth seemed to be reduced. At test item application rates of ≥ 1.76 L FF-075/ha some (partly) wilting leaves were seen in *Cucumis sativus*.

Report:	CP 10.6/02; Förster, B and Chambers, J.G. (2021)
Title:	Prothioconazole 200 g/L + azoxystrobin 150 g/L SC (FF-075): Terrestrial Vegetative Vigour Test.
Document No:	ECT Oekotoxikologie GmbH; Study report No.: 20AV6PB
Guideline:	OECD Guideline for the Testing of Chemicals No. 208 Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test
GLP	Yes. Hessisches Ministerium für Umwelt, Klima–schutz, Landwirtschaft und Verbraucherschutz, Wiesbaden, Germany, 28 May 2018.

Test material

Test item: Prothioconazole 200g/L + Azoxystrobin 150g/L SC (FF-075).

Purity:	Prothioconazole: 16.62±0.17 %, w/w (194.3±2.0 g/L) Azoxystrobin: 12.76±0.09 %, w/w (149.1±1.0 g/L)
Description	White to off-white homogeneous suspension liquid
Batch No.:	20191211001
Test system	
Organism (<i>Species</i>):	<i>Allium cepa</i> (onion) <i>Avena sativa</i> (oat) <i>Brassica rapa</i> (turnip) <i>Cucumis sativus</i> (cucumber) <i>Pisum sativum</i> (pea) <i>Solanum lycopersicum</i> (tomato)
Study type:	OECD Guideline for the Testing of Chemicals No. 227; Terrestrial Plant Test: Vegetative Vigour Test.
Guideline deviations reported:	The analytical phase plan was authorized by the principal investigator using a scanned signature instead of a wet signature. The deviation has no impact on the integrity of the study.
Duration of study:	21 days
Parameters measured:	Survival of plants, visual appearance, shoot fresh weight.
Observation intervals:	0, 7, 14, 21 and 22 days.
Test concentrations:	8.52, 3.78, 1.76, 0.8 and 0.36 L FF-075/ha.
Control:	Control soil was sprayed with water without test item.
Test Arenas:	Common plant pots made of polypropylene (diameter 15 or 11 cm diameter) were used as test pots. Each pot was filled with soil and placed in a separate polystyrene beaker (Kastelplast GmbH, Mainz, Germany) serving as water reservoir. Each pot was labelled with the study number, the treatments code, a consecutive number and the plants species
Test soil	Standard soil LUFA Type 2.3, a sandy loam, was purchased from the Landwirtschaftliche Untersuchungs- und Forschungsanstalt (LUFA), D-67346 Speyer, Germany.
Application of treatments:	The treatments were sprayed over the test species on three occasions. Control plants were sprayed with water without the test item.
No. of replicates:	Between 1 and 6 plants per pot, and 4, 7 or 20 pots per treatment.
Temperature:	17.7 – 31.2°C
Photoperiod:	16/8 h light/dark.
Light intensity:	202 to 308 µE m ⁻² s ⁻¹
Relative humidity:	25.5 – 65.1%

Methodology

The study was conducted in order to determine possible effects of the test item on the vegetative vigour of higher terrestrial plants. Therefore, the test item was tested with six plant species, representing six plant families. Two monocotyledon species, *Allium cepa* (onion) and *Avena sativa* (oat), and four dicotyledon species, *Brassica rapa* (turnip), *Cucumis sativus* (cucumber), *Pisum sativum* (pea) and *Solanum lycopersicum* (tomato).

The test item was sprayed at five different rates (dose-response test) per species onto the leaves and above-ground portions of the plants at their 2- to 4- true leaf stage. Control plants were sprayed with water.

Nominal test item application rates ranged from 0.36 L FF-075/ha to 8.52 L FF-075/ha. The test item concentration of the spray solution for the highest application rate was verified by chemical analysis.

Survival of plants and visual detrimental effects such as e.g. chlorosis and development abnormalities were evaluated on day 7, 14, and at test end, i.e. day 21 or 22. At test end, shoot biomass (fresh weight) was evaluated.

During the test, deionised water and a nutrient solution were added to the reservoirs as necessary to maintain the soil sufficiently moist and to ensure normal plant growth. All treatments of a particular species received the same volume of nutrient solution.

Two samples of the stock solution were taken immediately prior to spray application to plants and stored at $\leq -18^{\circ}\text{C}$ until chemical analysis. Spray application for the different test species took place at three time points with the test solution prepared freshly on the day of application. Each time two analytical samples were taken. One of each of the duplicate samples was shipped under frozen conditions to the PI on 26 January 2021 (arrival at the analytical test site on 28 January 2021).

Statistical analyses

Survival and shoot fresh weight of plants at the end of the test was considered for statistical data analysis to evaluate whether differences between the test item rates and the control were significant. Arithmetic means for each pot were calculated from the single plants within a pot (except for *B. rapa* and *C. sativus* with one plant per pot). Since each pot constituted a replicate, the mean values (or single values in case of *B. rapa* and *C. sativus*) were used for statistical analysis.

Survival data were checked by the Step-down Cochran-Armitage Test Procedure or in case of a non-monotone dose response with the Chi2-test with Bonferroni correction to determine the NOER and LOER. Significance level was $\alpha = 0.05$, one sided greater. The 3-Parameter normal cumulative distribution function was used to determine the effective rates ER₁₀ and ER₅₀ for the end point shoot fresh weight.

Normal distribution and homogeneity of variances were checked by the Shapiro-Wilk's Test and Levene's test, respectively. NOER and LOER of shoot fresh weight were determined by applying either the Williams multiple sequential t-test (monotonous) followed by the Jonckheere-Terpstra test. The Dunnett's test was applied if the dose-response relationship was not monotonous. In case of inhomogeneous variances, the Welch t-Test was applied, in case data differed significantly from normal distribution the Multiple Sequentially-rejective U-test after Bonferroni-Holm was applied. Significance level was $\alpha = 0.05$, one-sided smaller.

Statistical evaluations were performed using the ToxRat Pro software Version 3.3.0 (ToxRat Solutions GmbH, 2013).

Results

The validity criteria were fulfilled as survival of plants in the control was 100% (required $\geq 90\%$) for all six species. No phytotoxicity was observed in the controls. Environmental conditions for a particular species were identical and growing media contained the same amount of soil matrix and support media.

Seedling emergence was not relevant for this test. A surplus of plants had been pre-cultured in order to obtain a sufficient number of plants of the respective species and in their 2- to 4 true leaf stage available at test start.

Stock solution and test solutions were prepared on the day of spray application. Nominal test item concentration in the stock solution was verified by chemical analysis. Measured test item concentrations of the two samples of the highest application rate were 100% and 102% of nominal respectively, for Prothioconazole and 84% and 92%, respectively, for Azoxystrobin. Therefore, effective rates (ER_x) and limit rates (LOER, NOER) were based on nominal test item concentrations.

CP 10.6/02-01. Nominal and measured concentration of the active substance (a.s.) in the stock solutions.

	Application date		
	30 Sep 2020	08 Oct 2020	25 Nov 2020
Nominal concentration [g FF-075/L]	28.2	28.2	28.2
<u>Prothioconazole</u>			
Nominal concentration [g a.s./L]	4.69	4.69	4.69
Measured concentration [g a.s./L]	4.680	n.d.*	4.800
Measured concentration [% of nominal]	100	n.a.	102
<u>Azoxystrobin</u>			
Nominal concentration [g a.s./L]	3.60	3.60	3.60
Measured concentration [g a.s./L]	3.02	n.d.	3.32
Measured concentration [% of nominal]	84	n.a.	92

n.d. = not determined (verification by weight of test item used for stock solution); n.a. = not applicable.

All plants were evaluated visually on day 7, 14 and 21 and the observations were expressed by allocating each single plant to a scoring number of the standardized 6-point evaluation scheme.

Typical damages were necrosis and/or wilting of leaves which appeared at test item application rates of ≥ 0.8 L FF-075/ha. Damages such as decolouration and wilting of leaves seemed to appear only in those aboveground portions of the plants which had been reached directly by the spray during test item application. Leaves developing post application did not show such damages, while growth seemed to be reduced (see section 11.4). At test item application rates of ≥ 1.76 L FF-075/ha some (partly) wilting leaves were seen in *Cucumis sativus*.

CP 10.6/02-02. Visual appearance of *Allium cepa* and *Avena sativa* according to a 6-point scoring system on day 7, 14 and 21.

Test item [L FF-075/ha]	Category	<i>Allium cepa</i>			<i>Avena sativa</i>		
		d7	d14	d22	d7	d14	d21
0.0 (C)	1	22	22	24	24	24	24
	2	2	2	--	--	--	--
	3	--	--	--	--	--	--
	4	--	--	--	--	--	--
	5	--	--	--	--	--	--
	6	--	--	--	--	--	--
0.36	1	18	15	19	20	22	22
	2	5	8	4	4	2	2
	3	--	--	--	--	--	--
	4	--	--	--	--	--	--
	5	--	--	--	--	--	--
	6	1	1	1			
0.80	1	20	17	19	21	22	17
	2	4	7	5	2	1	6
	3	--	--	--	--	--	--
	4	--	--	--	--	--	--
	5	--	--	--	--	--	--
	6	--	--	--	1	1	1
1.76	1	19	18	17	22	23	21
	2	4	5	6	2	1	3
	3	--	--	--	--	--	--
	4	--	--	--	--	--	--
	5	1	--	--	--	--	--
	6	--	1	1	--	--	--
3.78	1	19	17	15	20	21	18
	2	5	7	9	4	3	6
	3	--	--	--	--	--	--
	4	--	--	--	--	--	--
	5	--	--	--	--	--	--
	6	--	--	--	--	--	--
8.52	1	14	13	14	19	17	16
	2	10	11	10	5	7	8
	3	--	--	--	--	--	--
	4	--	--	--	--	--	--

5	--	--	--	--	--	--	--
6	--	--	--	--	--	--	--

(--) no plant allocated to the particular category.

CP 10.6/02-03. Visual appearance of *Brassica rapa* and *Cucumis sativus* according to a 6 point scoring system on day 7, 14 and 21.

Test item [L FF-075/ha]	Category	<i>Brassica rapa</i>				<i>Cucumis sativus</i>		
		d7	d14	d21		d7	d14	d21
0.0 (C)	1	18	17	12		20	13	14
	2	1	3	8		--	7	6
	3	1	--	--	--	--	--	--
	4	--	--	--	--	--	--	--
	5	--	--	--	--	--	--	--
	6	--	--	--	--	--	--	--
0.36	1	13	12	12		20	6	9
	2	7	8	7		--	14	11
	3	--	--	--		--	--	--
	4	--	--	1		--	--	--
	5	--	--	--		--	--	--
	6	--	--	--		--	--	--
0.80	1	11	11	11		17	6	7
	2	9	8	8		3	11	10
	3	--	1	1		--	2	2
	4	--	--	--		--	1	1
	5	--	--	--		--	--	--
	6	--	--	--		--	--	--
1.76	1	10	7	6		16	--	2
	2	10	13	13		4	16	12
	3	--	--	--		--	1	3
	4	--	--	1		--	3	3
	5	--	--	--		--	--	--
	6	--	--	--		--	--	--
3.78	1	10	6	7		19	1	2
	2	10	13	11		1	7	4
	3	--	1	2		--	3	6
	4	--	--	--		--	9	8
	5	--	--	--		--	--	--
	6	--	--	--		--	--	--
8.52	1	2	3	10		8	--	--
	2	16	13	8		12	5	3
	3	2	4	1		--	7	8
	4	--	--	1		--	8	9
	5	--	--	--		--	--	--
	6	--	--	--		--	--	--

(--) no plant allocated to the particular category.

CP 10.6/02-04. Visual appearance of *Pisum sativum* and *Solanum lycopersicum* according to a 6 point scoring system on day 7, 14 and 21.

Test item [L FF-075/ha]	Category	<i>Pisum sativum</i>			<i>Solanum lycopersicum</i>		
		d7	d14	d21	d7	d14	d21
0.0 (C)	1	20	20	14	19	21	19
	2	1	1	4	2	--	2
	3	--	--	--	--	--	--
	4	--	--	3	--	--	--
	5	--	--	--	--	--	--
	6	--	--	--	--	--	--
0.36	1	18	19	15	17	19	14
	2	3	2	3	4	2	7
	3	--	--	--	--	--	--
	4	--	--	3	--	--	--
	5	--	--	--	--	--	--
	6	--	--	--	--	--	--
0.80	1	19	18	14	16	16	15
	2	2	3	4	5	5	6
	3	--	--	--	--	--	--
	4	--	--	3	--	--	--
	5	--	--	--	--	--	--
	6	--	--	--	--	--	--
1.76	1	20	20	16	15	18	12
	2	1	1	5	6	3	9
	3	--	--	--	--	--	--
	4	--	--	--	--	--	--
	5	--	--	--	--	--	--
	6	--	--	--	--	--	--
3.78	1	14	14	16	14	17	5
	2	7	7	4	7	4	16
	3	--	--	1	--	--	--
	4	--	--	--	--	--	--
	5	--	--	--	--	--	--
	6	--	--	--	--	--	--
8.52	1	10	13	10	8	15	4
	2	10	8	10	13	6	17
	3	1	--	1	--	--	--
	4	--	--	--	--	--	--
	5	--	--	--	--	--	--
	6	--	--	--	--	--	--

(--) no plant allocated to the particular category.

Survival of plants was significantly reduced compared to the control in *B. rapa* at test item application rates of ≥ 3.78 L FF-075/ha. No adverse effects on survival were observed in any other of the six test species.

CP 10.6/02-05.Survival of plants in the test.

Test item rate [L FF-075/ha]	A. <i>cepa</i>	A. <i>sativa</i>	B. <i>rapa</i>	C. <i>sativus</i>	P. <i>sativum</i>	S. <i>lycopersicum</i>
Number of plants introduced per treatment (test item rate) on day 0 (day of test item application) (all rates)	24	24	20	20	21	21
Number of live plants at the end of the test (21 days after application)						
0.0	24	24	20	20	21	21
0.36	23	24	20	20	21	21
0.80	24	23	20	20	21	21
1.76	23	24	20	20	21	21
3.78	24	24	20	20	21	21
8.52	24	24	20	20	21	21
Survival until the end of the test [% of introduced]						
0.0	100	100	100	100	100	100
0.36	96	100	100	100	100	100
0.80	100	96	100	100	100	100
1.76	96	100	100	100	100	100
3.78	100	100	100	100	100	100
8.52	100	100	100	100	100	100

* = significantly different from control (Step-down Cochran-Armitage test, $\alpha = 0.05$, one-sided greater).

Shoot fresh weight was significantly reduced in *C. sativus* at test item rates of ≥ 0.8 L FF-075/ha. Deviation from control was -14% at 0.8 L FF-075/ha and -23% at 8.52 L FF-075/ha, which was the highest tested rate).

No statistically significant reduction in shoot fresh weight was observed in any of the other five test species at any application rate of the test item up to and including 8.52 L FF-075/ha

CP 10.6/02-06. Nominal test item application rates [L FF-075/ha] and mean shoot fresh weight [g] at the end of the test (21 days after application; 22 days for *A. cepa*).

Test item rate [L FF-075/ha]	A. <i>cepa</i>	A. <i>sativa</i>	B. <i>rapa</i>	C. <i>sativus</i>	P. <i>sativum</i>	S. <i>lycopersicum</i>
0.0	2.374	6.498	26.908	30.882	9.138	16.994
0.36	2.865	6.640	25.689	32.856	10.187	17.404
0.80	2.128	6.621	26.030	26.560*	9.642	16.668
1.76	2.736	6.236	25.463	24.921*	10.928	17.310
3.78	2.757	6.666	24.306	23.956*	11.378	17.210
8.52	2.642	6.458	23.984	23.732*	9.976	17.026

* = Significantly different from the control (Williams multiple sequential t-test, $\alpha=0.05$, one-sided smaller).

CP 10.6/02-07. Relative reduction [%] compared to the control of mean shoot fresh weight at the end of the test (21 days after application; 22 days for *A. cepa*).

Test item rate [L FF-075/ha]	A. <i>cepa</i>	A. <i>sativa</i>	B. <i>rapa</i>	C. <i>sativus</i>	P. <i>sativum</i>	S. <i>lycopersicum</i>
0.36	-20.67	-2.18	4.53	-6.39	-11.48	-2.41

0.80	10.37	-1.90	3.26	13.99*	-5.52	1.92
1.76	-15.25	4.03	5.37	19.30*	-19.59	-1.86
3.78	-16.14	-2.58	9.67	22.43*	-24.51	-1.27
8.52	-11.30	0.62	10.87	23.15*	-9.17	-0.18

* = Significantly different from the control (Williams multiple sequential t-test, $\alpha=0.05$, one-sided smaller); negative values represent increased shoot fresh weight compared to the control.

The following validity criteria defined in the study guideline were fulfilled and the study can therefore be considered to be valid:

- Less than 10% of the control plants died.
- No phytotoxicity was observed in the controls.
- Environmental conditions for a particular species were identical and growing media contained the same amount of soil.

Conclusions:

Number of survived plants and shoot fresh weight at the end of the test were considered for statistical data analysis. An overview of effective application rates ER_{10} and ER_{50} , as well as the limit rates LOER and NOER.

No adverse effect on survival was observed in any of the six test species at any test item application rate up to and including 8.52 L FF-075/ha which was the highest tested application rate.

The lowest LOER, NOER and ER_{10} were 0.80 L FF-075/ha, 0.36 L FF-075/ha, and 0.6 L FF-075/ha respectively, and were observed with shoot fresh weight in *Cucumis sativus*.

No ER_{50} could be determined due to the lack of an effect >23%. Consequently, the ER_{50} is considered to be >8.52 L FF-075/ha equivalent to 1.655 kg Prothioconazole/ha and 1.270 kg Azoxystrobin/ha.

CP 10.6/02-08. Summary of the ER₁₀, ER₅₀ (with 95% confidence intervals, CI), LOER, and NOER of the endpoints survival and shoot fresh weight, referring to nominal test item application rates [L FF-075/ha].

Test item [L FF- 075/ha]	<i>A. cepa</i>	<i>A. sativa</i>	<i>B. rapa</i>	<i>C. sativus</i>	<i>P. sativum</i>	<i>S. lycopersicum</i>
Survival						
LR ₁₀	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
LR ₅₀	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
LOER	>8.52	>8.52	>8.52	>8.52	>8.52	>8.52
NOER	≥8.52	≥8.52	≥8.52	≥8.52	≥8.52	≥8.52
Shoot fresh weight						
ER ₁₀	n.d.	n.d.	n.d.	0.6 (0.2/2.7)	n.d.	n.d.
ER ₅₀	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
LOER	>8.52	>8.52	>8.52	0.80	>8.52	>8.52
NOER	≥8.52	≥8.52	≥8.52	0.36	≥8.52	≥8.52

n.d. not determined / calculation not feasible due to inappropriate data (e.g. no mortality or no meaningful rate/response was observed).

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

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

A 2.8 KCP 10.8 Monitoring data

Appendix 3 Combined Aquatic Toxicity Assessments

(Please refer to folder enclosed)

Appendix 4

Applicant's response on the calculation of the effect rates based on phytotoxicity in the seedling emergence and vegetative vigour studies:

The cMS, Czech Republic, has requested for the calculation of the effect rates (ERx) based on phytotoxicity in the seedling emergence (OECD 208) and vegetative vigour studies (OECD 227). This request is based from the statement in the “Outcome of the Pesticides Peer Review Meeting on general recurring issues in ecotoxicology” EFSA Supporting publication 2019:EN-1673, page 26). The following should be considered:

1. There is still no guidance on how to calculate the ECx based on phytotoxicity.
2. According to OECD 208 and OECD 227, phytotoxicity is based on detrimental deviations from the normal pattern of appearance and growth of plants in a response to a give substance. There is no definitive scoring system proposed in the guidance and thus this is established by the laboratories performing the test. For the studies in discussion here, they used the 6-point scoring system with categories defined as follows:

Table 1: Scoring scheme for the visual evaluation of the test species.

Category	Evaluation	Description
1	Healthy	Plant with normal growth, without damages apart from what is within the normal range of variability (e.g. wilted cotyledons are no damage).
2	Slightly damaged	Chlorotic and/or necrotic leaves (less than ¼ of the plant).
3	Slightly damaged	Single wilted leaves (less than ¼ of the plant)
4	Severely damaged	Chlorotic and/or necrotic and/or wilted leaves (more than ¼ of the plant).
5	Severely damaged	Plant with developmental abnormalities.
6	Dead	All above-ground parts of the plant are wilted.

Although there is no guidance yet on how the ERx should be calculated, the applicant instead evaluated the percentage of plants affected for each of the category reported, Tables 2 - 3. For seedling emergence and vegetative vigour, less than 50% of the plants were affected or could be classified as severely damaged (Categories 3 – 5) in Days 7, 14, and 21 (relevant for vegetative vigour only). This means that no ER50 could be calculated due to the lack of significant effects. The applicant also tried to run a dose response analysis on the statistical programming software R but no relevant dose response model could be fitted to the data. In conclusion, the phytotoxic effect cannot be reported as an ER50 and is therefore not a relevant endpoint for the risk assessment of the formulation FF-075.

Table 2a: Vegetative vigour (Day 7): percentage of plants affected based on categories.

Concentration (L FF-075/ha)		0	0.36	0.8	1.76	3.78	8.52
Species/Category	<i>Allium cepa</i>						
	1	91.7%	75.0%	83.3%	79.2%	79.2%	58.3%
	2	8.3%	20.8%	16.7%	16.7%	20.8%	41.7%
	5	0.0%	0.0%	0.0%	4.2%	0.0%	0.0%
	6	0.0%	4.2%	0.0%	0.0%	0.0%	0.0%
	<i>Avena sativa</i>						
	1	100.0%	83.3%	87.5%	91.7%	83.3%	79.2%
	2	0.0%	16.7%	8.3%	8.3%	16.7%	20.8%
	6	0.0%	0.0%	4.2%	0.0%	0.0%	0.0%
	<i>Brassica rapa</i>						
	1	90.0%	65.0%	55.0%	50.0%	50.0%	10.0%
	2	5.0%	35.0%	45.0%	50.0%	50.0%	80.0%
	3	5.0%	0.0%	0.0%	0.0%	0.0%	10.0%
	<i>Cucumis sativus</i>						
	1	100.0%	100.0%	85.0%	80.0%	100.0%	40.0%
	2	0.0%	0.0%	15.0%	20.0%	0.0%	60.0%
	<i>Pisum sativum</i>						
	1	95.2%	85.7%	90.5%	95.2%	66.7%	47.6%
	2	4.8%	14.3%	9.5%	4.8%	33.3%	47.6%
	3	0.0%	0.0%	0.0%	0.0%	0.0%	4.8%
	<i>Solanum lycopersicum</i>						
	1	90.5%	81.0%	76.2%	71.4%	66.7%	38.1%
	2	9.5%	19.0%	23.8%	28.6%	33.3%	61.9%

Table 2b: Vegetative vigour (Day 14): percentage of plants affected based on categories.

Concentration (L FF-075/ha)		0	0.36	0.8	1.76	3.78	8.52
Species/Category	<i>Allium cepa</i>						
	1	91.7%	62.5%	70.8%	70.8%	70.8%	54.2%
	2	8.3%	33.3%	29.2%	29.2%	29.2%	45.8%
	6	0.0%	4.2%	0.0%	0.0%	0.0%	0.0%
	<i>Avena sativa</i>						
	1	100.0%	91.7%	91.7%	87.5%	87.5%	70.8%
	2	0.0%	8.3%	4.2%	12.5%	12.5%	29.2%
	6	0.0%	0.0%	4.2%	0.0%	0.0%	0.0%
	<i>Brassica rapa</i>						
	1	85.0%	60.0%	55.0%	35.0%	35.0%	15.0%
	2	15.0%	40.0%	40.0%	65.0%	65.0%	65.0%
	3	0.0%	0.0%	5.0%	0.0%	0.0%	20.0%
	<i>Cucumis sativus</i>						
	1	65.0%	30.0%	30.0%	0.0%	5.0%	0.0%
	2	35.0%	70.0%	55.0%	80.0%	35.0%	25.0%
	3	0.0%	0.0%	10.0%	5.0%	15.0%	35.0%
	4	0.0%	0.0%	5.0%	15.0%	45.0%	40.0%
	<i>Pisum sativum</i>						
	1	95.2%	90.5%	85.7%	95.2%	66.7%	57.1%
	2	4.8%	9.5%	14.3%	4.8%	33.3%	42.9%

<i>Solanum lycopersicum</i>						
1	100.0%	90.5%	76.2%	85.7%	81.0%	71.4%
2	0.0%	9.5%	23.8%	14.3%	19.0%	28.6%

Table 2c: Vegetative vigour (Day 21): percentage of plants affected based on categories.

Concentration (L FF-075/ha)		0	0.36	0.8	1.76	3.78	8.52
Species/Category	<i>Allium cepa</i>						
	1	100.0%	79.2%	79.2%	79.2%	62.5%	58.3%
	2	0.0%	16.7%	20.8%	20.8%	37.5%	41.7%
	6	0.0%	4.2%	0.0%	0.0%	0.0%	0.0%
	<i>Avena sativa</i>						
	1	100.0%	91.7%	87.5%	75.0%	75.0%	66.7%
	2	0.0%	8.3%	12.5%	25.0%	25.0%	33.3%
	<i>Brassica rapa</i>						
	1	60.0%	60.0%	55.0%	35.0%	35.0%	50.0%
	2	40.0%	35.0%	40.0%	65.0%	55.0%	40.0%
	3	0.0%	0.0%	5.0%	0.0%	10.0%	5.0%
	4	0.0%	5.0%	0.0%	0.0%	0.0%	5.0%
	<i>Cucumis sativus</i>						
	1	70.0%	45.0%	35.0%	10.0%	10.0%	0.0%
	2	30.0%	55.0%	50.0%	60.0%	20.0%	15.0%
	3	0.0%	0.0%	10.0%	15.0%	30.0%	40.0%
	4	0.0%	0.0%	5.0%	15.0%	40.0%	45.0%
	<i>Pisum sativum</i>						
	1	66.7%	71.4%	66.7%	76.2%	81.0%	47.6%
	2	19.0%	14.3%	19.0%	23.8%	19.0%	47.6%
	3	0.0%	0.0%	0.0%	0.0%	0.0%	4.8%
	4	14.3%	14.3%	14.3%	0.0%	0.0%	0.0%
	<i>Solanum lycopersicum</i>						
	1	90.5%	66.7%	71.4%	57.1%	23.8%	19.0%
	2	9.5%	33.3%	28.6%	42.9%	76.2%	81.0%

Table 3a: Seedling emergence (Day 7): percentage of plants affected based on categories.

Concentration (L FF-075/ha)		0	0.36	0.8	1.76	3.78	8.52
Species/Category	<i>Allium cepa</i>						
	1	100.00%	89.47%	100.00%	23.08%	31.03%	22.22%
	2	0.00%	10.53%	0.00%	15.38%	55.17%	33.33%
	3	0.00%	0.00%	0.00%	7.69%	0.00%	8.33%
	4	0.00%	0.00%	0.00%	41.03%	13.79%	22.22%
	5	0.00%	0.00%	0.00%	12.82%	0.00%	13.89%
	<i>Avena sativa</i>						
	1	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%
	<i>Brassica rapa</i>						
	1	90.91%	100.00%	90.91%	100.00%	100.00%	82.61%
	2	9.09%	0.00%	9.09%	0.00%	0.00%	17.39%
	<i>Cucumis sativus</i>						
	1	88.24%	100.00%	100.00%	100.00%	88.24%	100.00%

2	11.76%	0.00%	0.00%	0.00%	11.76%	0.00%
<i>Pisum sativum</i>						
1	82.61%	76.92%	90.91%	82.61%	100.00%	75.00%
2	17.39%	0.00%	9.09%	17.39%	0.00%	25.00%
6	0.00%	23.08%	0.00%	0.00%	0.00%	0.00%
<i>Solanum lycopersicum</i>						
1	80.00%	90.91%	100.00%	100.00%	62.96%	55.56%
2	0.00%	9.09%	0.00%	0.00%	22.22%	44.44%
4	0.00%	0.00%	0.00%	0.00%	14.81%	0.00%
5	20.00%	0.00%	0.00%	0.00%	0.00%	0.00%

Table 3b: Seedling emergence (Day 14): percentage of plants affected based on categories.

Concentration (L FF-075/ha)		0	0.36	0.8	1.76	3.78	8.52
Species/Category	<i>Allium cepa</i>						
	1	100.00%	89.47%	100.00%	46.15%	13.79%	11.11%
	2	0.00%	10.53%	0.00%	23.08%	72.41%	63.89%
	3	0.00%	0.00%	0.00%	0.00%	0.00%	19.44%
	5	0.00%	0.00%	0.00%	10.26%	0.00%	0.00%
	6	0.00%	0.00%	0.00%	20.51%	13.79%	5.56%
	<i>Avena sativa</i>						
	1	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%
	<i>Brassica rapa</i>						
	1	100.00%	95.24%	100.00%	95.24%	95.00%	100.00%
	4	0.00%	0.00%	0.00%	4.76%	5.00%	0.00%
	5	0.00%	4.76%	0.00%	0.00%	0.00%	0.00%
	<i>Cucumis sativus</i>						
	1	100.00%	100.00%	93.75%	93.75%	70.59%	81.25%
	2	0.00%	0.00%	6.25%	6.25%	17.65%	12.50%
	3	0.00%	0.00%	0.00%	0.00%	0.00%	6.25%
	4	0.00%	0.00%	0.00%	0.00%	5.88%	0.00%
	5	0.00%	0.00%	0.00%	0.00%	5.88%	0.00%
	<i>Pisum sativum</i>						
	1	82.61%	76.92%	81.82%	82.61%	95.24%	66.67%
	2	17.39%	0.00%	18.18%	17.39%	4.76%	33.33%
	6	0.00%	23.08%	0.00%	0.00%	0.00%	0.00%
	<i>Solanum lycopersicum</i>						
	1	76.00%	100.00%	95.24%	90.48%	62.96%	48.15%
	2	0.00%	0.00%	4.76%	0.00%	7.41%	48.15%
	3	0.00%	0.00%	0.00%	0.00%	7.41%	0.00%
	5	24.00%	0.00%	0.00%	9.52%	22.22%	3.70%