

REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: FGF11

Product name(s): Telaron Alpha / Patton Alpha
Azoxystrobin 160 + Prothioconazole g/L 200 SC

Chemical active substance(s):

Azoxystrobin, 160 g/L
Prothioconazole, 200 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(new authorization)

Applicant: UPL Holdings Coöperatief U.A.

Submission date: November 2024, update April 2025

MS Finalisation date: January 2025, update April 2025

(initial Core Assessment)

August 2025 (final Core Assessment)

Version history

When	What
November 2024	Original Applicant version
January 2025	Initial zRMS assessment The report in the dRR format has been prepared by the Applicant, therefore all comments, additional evaluations and conclusions of the zRMS are presented in grey commenting boxes. Minor changes are introduced directly in the text and highlighted in grey. Not agreed or not relevant information are ~~struck through~~ and ~~shaded~~ for transparency. Following the evaluation and before sending the document for commenting, all colored highlighting was removed, from the parts updated by the Applicant, for better legibility.
April 2025	Updated Applicant's version including additional studies for bees.
April 2025	The report in the dRR format has been prepared by the Applicant, therefore all comments, additional evaluations and conclusions of the zRMS are presented in grey commenting boxes. Minor changes are introduced directly in the text and highlighted in grey. Not agreed or not relevant information are ~~struck through~~ and ~~shaded~~ for transparency. Following the evaluation and before sending the document for commenting, all colored highlighting was removed, from the parts updated by the Applicant, for better legibility.
August 2025	Final report (Core Assessment updated following the commenting period) Additional information/assessments included by the zRMS in the report in response to comments received from the CMS and the Applicant are highlighted in yellow. Not agreed or not relevant information are ~~struck through~~ and ~~shaded~~ for transparency.

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9 Ecotoxicology (KCP 10)

This document supports the registration of the product FGF11 in the Central Zone, according to Art.33 of the EU Reg.1107/2009.

The dossier has been drafted taking under consideration conditions for aa.ss. assessment as evaluated for azoxystrobin in EFSA Journal 2010; 8(4):1542, Addendum for confirmatory data (2014) and SANCO/1107/2001 (Rev.3, 2015) . For prothioconazole, the reference documents are EFSA Scientific Report (2007) 106, 1-98, Addendum for confirmatory data (2014) and SANCO/3923/07 updated in 2021.

The present submission reviews the ecotoxicological data in line with data requirements for PPP (refer to Commission Regulation (EU) No 284/2013).

This section summarises the ecotoxicological effects of the formulation FGF11 and evaluates potential risk to various representative terrestrial, aquatic and soil organisms.

The product is FGF11, an SC formulation containing 160 g/L azoxystrobin + 200 g/L prothioconazole. The proposed use patterns for FGF11 as fungicide in cereals and oilseed rape, are presented in the following GAP table.

Azoxystrobin

This document reviews the ecotoxicological studies for the product FGF11 containing azoxystrobin which was approved following Regulation 1107/2009 with the Commission implementing Regulation (EU) No 703/2011 of 20 July 2011, as regards the conditions of approval of the active substance azoxystrobin.

Where appropriate this document refers to the conclusions of the EU review of azoxystrobin. This will be where:

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

Relevant documents as:

-EFSA Journal 2010; 8(4):1542,
-Addendum for confirmatory data (2014)
- SANCO/1107/2001 (Rev.3, 2015)

are considered to provide the relevant review information or a reference to where such information can be found.

The Commission implementing Regulation (EU) No 703/2011, as regards the conditions of approval of the active substance azoxystrobin, provides specific provisions under Part B which need to be considered by the applicant in the preparation of their submission and by the MS prior to granting an authorisation:

For the implementation of the uniform principles as referred to in Article 29(6) of Regulation (EC) No1107/2009, the conclusions of the review report on azoxystrobin, and in particular Appendices I and II thereof, as finalised in the Standing Committee on the Food Chain and Animal Health on 17 June 2011 shall be taken into account. In this overall assessment Member States should pay particular attention to:

- *the protection of aquatic organisms. The Member States must ensure that the conditions of authorization include risk mitigation measures, where appropriate*

These concerns have been addressed within the current submission.

Prothioconazole

This document reviews the ecotoxicological studies for the product FGF11 containing prothioconazole which was included into Annex I of Directive 91/414 (Commission Directive 2008/44/EC of 4 April 2008) and in the Regulation 1107/2009 with the Commission implementing Regulation (EU) No 540/2011 of 25 May 2011, as regards the conditions of approval of the active substance prothioconazole

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

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

Relevant documents as:

- EFSA Scientific Report (2007) 106, 1-98
- Addendum for confirmatory data (2014)
- SANCO/3923/07 updated in 2021

are considered to provide the relevant review information or a reference to where such information can be found.

The Commission implementing Regulation (EU) No 540/2011, as regards the conditions of approval of the active substance prothioconazole, provides specific provisions under Part B which need to be considered by the applicant in the preparation of their submission and by the MS prior to granting an authorisation:

For the implementation of the uniform principles as referred to in Article 29(6) of Regulation (EC) No1107/2009, the conclusions of the review report on azoxystrobin, and in particular Appendices I and II thereof, as finalised in the Standing Committee on the Food Chain and Animal Health on 22 January 2008 shall be taken into account. In this overall assessment Member States should pay particular attention to:

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

These concerns have been addressed within the current submission.

Information on the detailed composition of FGF11 can be found in the confidential dossier of this submission (Registration Report - Part C).

9.1 Critical GAP and overall conclusions

Table 9.1-1: Table of critical GAPs

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Use- No. *	Member state(s)	Crop and/or sit- uation (crop destina- tion / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: develop- mental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ syn- ergist per ha	Conclusion						
					Method / Kind	Timing / Growth stage of crop & sea- son	Max. num- ber a) per use b) per crop/ season	Min. interval between ap- plications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organ- isms	Bees	Non-target ar- thropods	Soil organisms	Non-target plants
Zonal uses (field or outdoor uses, certain types of protected crops)																				
1	CEU (AT, BE, CZ, DE, HU, IE, NL, PL, RO, SI, SK) FR Maritime	Winter wheat, spring wheat, durum wheat, spelt TRZAW, TRZAS, TRSDU, TRZSP	F	Wheat: septoria leaf blotch (Zymoseptoria tritici), yellow rust (Puccinia striiformis) brown rust (Puccinia recondita)	Foliar spray application	30-69	a) 1 b) 1	-	a) 1.0 b) 1.0	160 g/ha azoxystrobin + 200 g/ha prothioconazole	100-400	35		A	A	R	R	A	A	A
2	CEU (AT, BE, CZ, DE, HU, IE, NL, PL, RO, SI, SK) FR Maritime	Winter triticale, spring triticale TTLWI, TTLSO	F	Wheat: septoria leaf blotch (Zymoseptoria tritici), yellow rust (Puccinia striiformis) brown rust (Puccinia recondita)	Foliar spray application	30-69	a) 1 b) 1	-	a) 1.0 b) 1.0	160 g/ha azoxystrobin + 200 g/ha prothioconazole	100-400	35		A	A	R	R	A	A	A
3	CEU (AT, BE, CZ, DE, HU, IE, NL, PL, RO, SI, SK) FR Maritime	Winter barley, spring barley HORVW, HORVS	F	Net blotch (Pyrenophora teres) Ramularia (Ramularia collo-cygni) Leaf blotch (Rhynchosporium secali) Rust (Puccinia hordei)	Foliar spray application	30-59	a) 1 b) 1	-	a) 1.0 b) 1.0	160 g/ha azoxystrobin + 200 g/ha prothioconazole	100-400	35		A	A	R	R	A	A	A
4	CEU (AT, BE, CZ, DE, HU, IE, NL, PL, RO, SI, SK) FR Maritime	Winter oilseed rape, spring oilseed rape BRSNW, BRSNS	F	Cottony rot (Sclerotinia sclerotiorum) Black spot (Alternaria brassicae)	Foliar spray application	61-73	a) 1 b) 1	-	a) 1.0 b) 1.0	160 g/ha azoxystrobin + 200 g/ha prothioconazole	100-400	35		A	A	R	R	A	A	A

5	CEU (AT, BE, CZ, DE, HU, IE, NL, PL, RO, SI, SK) FR Mari-time	Winter rye, spring rye SECCW, SECCS	F	Brown rust (<i>Puccinia recondita</i>)	Foliar spray application	30-69	a) 1 b) 1	-	a) 1.0 b) 1.0	160 g/ha azoxystrobin + 200 g/ha prothioconazole	100-400	35		A	A	R	R	A	A	A
6	AT, BE, CZ, DE, HU, IE, NL, PL, RO, SI, SK FR Mari-time	Winter wheat, spring wheat, durum wheat, spelt TRZAW, TRZAS, TRSDU, TRZSP,	F	Fusarium (<i>Microdochium nivale</i> , <i>Fusarium roseum</i>)	Foliar spray application	59-69	a) 1 b) 1	-	a) 1.0 b) 1.0	160 g/ha azoxystrobin + 200 g/ha prothioconazole	100-400	35		A	A	R Aquatic Winter OSR A Spring OSR	R	A	A	A

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

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

Explanation for column 15 – 21 “Conclusion”

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

Remarks table:

- Numeration necessary to allow references
- Use official codes/nomenclatures of EU
- For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (e.g. fumigation of a structure)
- 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
- Scientific names and EPPO-Codes of target pests/diseases/ weeds or when relevant the common names of the pest groups (e.g. biting and sucking insects, soil born insects, foliar fungi, weeds) and the developmental stages of the pests and pest groups at the moment of application must be named
- Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench
Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plants - type of equipment used must be indicated

- Growth stage at first and last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
- The maximum number of application possible under practical conditions of use must be provided
- Minimum interval (in days) between applications of the same product.
- For specific uses other specifications might be possible, e.g.: g/m³ in case of fumigation of empty rooms. See also EPPO-Guideline PP 1/239 Dose expression for plant protection products
- The dimension (g, kg) must be clearly specified. (Maximum) dose of a.s. per treatment (usually g, kg or L product / ha).
- If water volume range depends on application equipments (e.g. ULVA or LVA) it should be mentioned under “application: method/kind”.
- PHI - minimum pre-harvest interval
- Remarks may include: Extent of use/economic importance/restrictions

9.1.1 Overall conclusions

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

Acute and chronic risk to birds and mammals following the proposed use of FGF11 were performed in accordance with EFSA guidance on birds and mammals (2009).

Birds

The acute and chronic risk from the two active substances (azoxystrobin and prothioconazole) was acceptable at the screening level for the intended uses in cereals and oilseed rape. The acute risk from the metabolite prothioconazole-desthio was acceptable at the screening level whereas the reproductive risk was acceptable at Tier 1. The risk to the mixture was calculated using the Finney's equation for acute toxicity and was acceptable at the screening level.

The risk from exposure to contaminated drinking water and secondary poisoning was also indicated to be acceptable following the proposed use of FGF11 according to GAP submitted.

Mammals

The acute and long-term risk for prothioconazole and azoxystrobin as well as the acute risk for metabolite prothioconazole-desthio were indicated to be acceptable for mammals at the screening level for the intended uses in cereals and oilseed rape. The long-term risks for the metabolite prothioconazole-desthio were solved in the Tier 1 assessment, except for the small herbivorous mammal "vole" scenario following application to cereals of prothioconazole-desthio. As such, additional refinement options were required for the long-term risk from the metabolite prothioconazole-desthio in cereals: refinement of the deposition factor together with refinement of DT₅₀ in food items resulted in a TER > 5 and an acceptable risk to mammals is presented for this scenario.

The TERa for mixture toxicities were above the trigger value of 10 indicating low risk for mammals for applications on cereals and oilseed rape at the screening level.

The risk from exposure to contaminated drinking water and secondary poisoning was considered acceptable following the proposed use of FGF11.

In conclusion, an acceptable overall risk for mammals is indicated for the intended GAP uses of FGF11.

9.1.1.2 Effects on aquatic organisms (KCP 10.2)

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).

Azoxystrobin and its metabolites

For azoxystrobin, based on the results of the risk assessments at FOCUS Step 3, the risk was acceptable based on the EU agreed weight-of-evidence approach for aquatic invertebrates, with no need to apply any further mitigation measures for application on spring cereals and winter and spring oilseed rape.

For application on winter cereals at Step 3 D1 Ditch and D2 Ditch scenarios were failing. It is to be noted that D1 and D2 Ditch scenarios are not relevant for Central Zone. In case, where D2 Ditch scenario needs to be assessed, at national MS level, the safety precaution phrase SPe2 shall be applied: „*To protect aquatic organisms do not apply to drained soils.*“

For the metabolites R401553, R402173 and R234886 of azoxystrobin an acceptable risk was indicated at FOCUS Step 1, 2 without applying any mitigation measures.

Prothioconazole and its metabolites

For prothioconazole and its metabolites prothioconazole-S-methyl, and 1,2,4-triazole, an acceptable risk was indicated at FOCUS Step 1, 2 for application to cereals and oilseed rape without applying any mitigation measures.

For the metabolite prothioconazole-desthio (M04), an acceptable risk was indicated based on FOCUS Step 4 PEC_{SW} for all scenarios when including the following risk mitigation measures:

- *Winter cereals* (BBCH > 30 < 69): 10 m vegetative buffer strip + 10 m no-spray buffer zone (R1, R3 and R4 scenarios)
- *Spring cereals* (BBCH > 30 < 69): 10 m vegetative buffer strip + 10 m no-spray buffer zone + 50% drift reduction (R4 scenario) and 10 m vegetative buffer strip + 10 m no-spray buffer zone for R1, R3 scenarios)
- *Winter oilseed rape* (BBCH 61-73): 10 m vegetative buffer strip + 10 m no-spray buffer zone + 50% drift reduction (R3 scenario)
- *Spring oilseed rape* (BBCH 61-73): None

Mixture assessment

Mixture toxicity assessment has been performed considering EFSA 2013 and the calculation sheet AGD_AquaMix (v. 1.22).

In line with the argumentation already provided earlier, mixture toxicity has been presented for azoxystrobin + prothioconazole and for azoxystrobin + prothioconazole-desthio

Azoxystrobin results to be the toxicity driver for the majority of the exposure scenario on fish, aquatic invertebrates and algae.

For Algae, MDR calculations indicate values between 0.2 and 5, i.e. the observed and calculated mixture toxicities are considered in agreement with the assumption of additive toxicity. Since synergism could be excluded, assessment for Algae has been finalised at the Screening Step, according to the simplified approach according to EFSA 2013.

For aquatic invertebrates, more-than additive (i.e. synergistic) mixture toxicity is theoretically indicated since MDR is greater than 5. But input data are very heterogenous, considering the exposure designs and the calculation of the endpoints thus not suitable enough to address properly the MDR on aquatic invertebrates, which is the most sensitive organism when exposed to azoxystrobin or formulation.

Finally, through ETRmix-CA approach a safe risk can be demonstrated for aquatic invertebrates and for fish.

Formulation assessment

Risk assessment performed with formulation toxicity data on aquatic invertebrates and fish demonstrated a safe risk when application of formulation FGF11 is implemented with mitigation measure as 10m buffer strip.

9.1.1.3 Effects on bees (KCP 10.3.1)

Risk assessment based on SANCO/10329/2002 rev.2 and EPPO scheme:

The acute hazard quotients (HQs) are lower than the trigger value of 50 for both honeybees and bumble bees, indicating that both active substances (azoxystrobin and prothioconazole) and the formulation FGF11 pose a low risk to bees following the application of the formulation according to the use pattern proposed.

The chronic risk assessment provided TER values above the triggers. Thus, a chronic risk for adult bees and honeybee larvae following the use of FGF11 must be excluded at National level of each Member state that requires that evaluation.

Risk assessment based on EFSA 2013:

The acute risk assessment for honeybees and bumble bees based on the available data set indicated an acceptable risk for acute oral and contact exposure of honey bees and bumble bees based on the screening Tier assessment.

For the remaining scenarios chronic exposure of honeybee adults and larvae, a risk was indicated for the exposure on treated crop and weeds in the field for cereals and on treated crop for application on oilseed rape. Two higher tier studies (a semi-field study and a field study)-have been performed for formulation.

The first one the semi-field study by Portail, B (2025) with FGF11 performed to determine the potential effects of FGF11 on the honeybee (*Apis mellifera* L.) after one foliar application at a rate of 160.02 g a.i./ha Azoxystrobin and 204.07 g a.i./ha Prothioconazole (13.10 g product/ha or 1 L product/ha) during bee flight under semi-field conditions in *Phacelia tanacetifolia* in Germany.

The second one by Roig, J. (2024) was performed for Azoxystrobin 160 + Prothioconazole 200 SC on the honeybee (*Apis mellifera* L.) in two test item application scenarios, T1 (outside of active bee foraging) and T2 (during active bee foraging), under semi-field conditions in full flowering *Phacelia tanacetifolia* at BBCH 65-66 according to CEB 230 in France. The target application rate of the test item Azoxystrobin 160 + Prothioconazole 200 SC was 1 L product/ha. The mortality in T1 and T2 were comparable to the C, although mortality in T2 was slightly higher as shown by the toxicity index (1.04 for T1 and 1.25 for T2). No effects on pupae and larvae were observed.

The forager bee mortality index (proportion of mortality after treatment, corrected by mortality before treatment / number of bees present on the crop before treatment) was slightly reduced in T1 and T2 in comparison with C (0.84 for T1 and 0.86 for T2).

The T1 treatment did not show any effect on the flight activity. The T2 treatment triggered a slight reduction of the flight activity as observed on the application day and during the following 3 days.

No abnormal behaviour related to the test item treatments was observed during the study period. Colonies remained healthy during the study period and contained normal levels of bees, brood and food.

-are currently ongoing and higher tier refinements will be addressed as soon final reports will be available. Based on current outcome of the risk assessment, a safe risk can be granted ~~with the implementation of mitigation measures.~~

In the same time, it should be noted that refinement of chronic risk assessment based on semi-field studies for bees is left for final decision of MSs according to recommendation given in CZHM, in Ecotoxicology, 2023.

Relevance of the risk assessment performed according to EFSA 2013 should be considered at National level.

9.1.1.4 Effects on arthropods other than bees (KCP 10.3.2)

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. Toxicity data from studies with FGF11 were used to perform risk assessment for the formulated product.

Toxicity data from studies with FGF11 were used to perform risk assessment for the formulated product. An acceptable in-field and off-field risk was shown for the intended use in cereals for *Aphidius rhopalosiphi*, *Typhlodromus pyri*, *Coccinella septempunctata* and *Chrysoperla carnea* thereby also covering the use in oilseed rape. The risk to non-target arthropods in the in-field as well as off-field area from exposure to FGF11 is considered safe.

9.1.1.5 Effects on soil microbial activity (KCP 10.5)

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).

Soil meso and macro fauna

Exposure to azoxystrobin, prothioconazole and their metabolites indicated an acceptable risk for earthworms and other non-target soil organisms for the intended uses in cereals and oilseed rape using Tier 1 assessments.

The risk to non-target soil meso- and macrofauna from the intended uses of the formulated product FGF11 was indicated to be acceptable at Tier 1 assessment.

Soil microbial activity

An acceptable risk for soil microorganisms (N-transformation) is indicated for azoxystrobin, prothioconazole and their metabolites as well as the formulation FGF11 for the intended uses in cereals and oilseed rape.

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

The risk assessment is based on the “Guidance Document on Terrestrial Ecotoxicology”, (SANCO/10329/2002 rev.2 final, 2002).

Risk assessments for FGF11 demonstrated an acceptable risk to non-target terrestrial plants for the intended uses in cereals and oilseed rape.

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

No other relevant data were identified in the EU review of the active substances and are considered necessary.

9.1.2 Grouping of intended uses for risk assessment

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

Where possible, the risk envelope approach has been used. Crops are pooled on the basis of similar application rates, plant morphology and of timing application. Use N.1 was used as surrogate for all uses on cereals, considering application rate of 1 kg product/ha and Use N.4 is considered for application on oilseed rape.

Table 9.1-2: Critical use pattern of FGF11

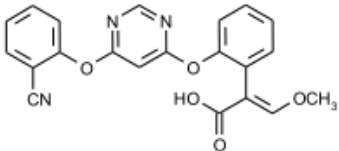
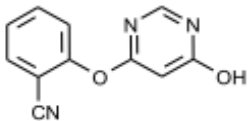
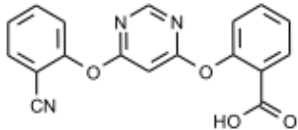
Grouping according to criterion			
Group	Intended uses	relevant use parameters for grouping	relevant parameter or value for sorting
Effects on birds and mammals			
Field crops	Post-emergence application, (winter/spring) cereals, considering 1 × 1 kg prod./ha, BBCH 30-69 Post-emergence application, (winter/spring) oilseed rape, considering 1 × 1.0 kg prod./ha, BBCH 61-73	Crop group according to EFSA Journal 2009; 7(12): 1438: cereals, oilseed rape	Maximum application rates to cereals and oilseed rape, considering indicator species (screening step) and generic focal species (Tier-1/Tier-2) relevant in treated fields according to EFSA exposure scenarios at time of application.
Effects on aquatic organisms			
Field crops	Post- emergence application, (winter/spring) cereals, considering 1 × 1 kg prod./ha, BBCH 30-69 Post-emergence application, (winter/spring) oilseed rape, considering 1 × 1.0 kg prod./ha, BBCH 61-73	Crop group according to FOCUS (2001 & 2015): (spring/winter) cereals and oilseed rape	Maximum application rates to cereals and oilseed rape, considering all relevant aquatic groups and calculated PEC _{sw} values at FOCUS Step-1 to 4
Effect on bees			
Field crops	Post- emergence application, (winter/spring) cereals, considering 1 × 1 kg prod./ha, BBCH 30-69 Post-emergence application, (winter/spring) oilseed rape, considering 1 × 1.0 kg prod./ha, BBCH 61-73	Crop group according to SANCO/10329/2002 rev.2 (final), October 17, 2002: Field crops	Maximum single application rate, i.e. 1 × 1.0 kg prod./ha [equivalent to 1 × 160 g azoxystrobin/ha + 1 × 200 g prothioconazole/ha] covering applications on cereals and oilseed rape
Effects on non-target arthropods			
Field crops	Post- emergence application, (winter/spring) cereals, considering 1 × 1 kg prod./ha, BBCH 30-69 Post-emergence application, (winter/spring) oilseed rape, considering 1 × 1.0 kg prod./ha, BBCH 61-73	Crop group according to ESCORT 2 (2000): Field crops	Maximum single application rate, i.e. 1 × 1.0 kg prod./ha [equivalent to 1 × 160 g azoxystrobin/ha + 1 × 200 g prothioconazole/ha] considered for applications on cereals and oilseed rape
Effects on terrestrial soil meso/macro fauna, soil microbial activity			
Field crops	Post- emergence application, (winter/spring) cereals, considering 1 × 1 kg prod./ha, BBCH 30-69 Post-emergence application,	Crop group according to SANCO/10329/2002 rev 2 (final), October 17, 2002: Oilseed rape	Maximum single application rate, i.e. 1 × 1.0 kg prod./ha [equivalent to 1 × 160 g azoxystrobin/ha + 1 × 200 g prothioconazole/ha] considered for applications on cereals and oilseed rape

Grouping according to criterion			
Group	Intended uses	relevant use parameters for grouping	relevant parameter or value for sorting
	(winter/spring) oilseed rape, considering 1 × 1.0 kg prod./ha, BBCH 61-73		
Effects on terrestrial non-target plants			
Field crops	Post- emergence application, (winter/spring) cereals, considering 1 × 1 kg prod./ha, BBCH 30-69 Post-emergence application, (winter/spring) oilseed rape, considering 1 × 1.0 kg prod./ha, BBCH 61-73	Crop group according to SANCO/10329/2002 rev 2 (final), October 17, 2002: Field crops	Maximum single application rate, i.e. 1 × 1.0 kg prod./ha [equivalent to 1 × 160 g azoxystrobin/ha + 1 × 200 g prothioconazole/ha] covering applications on cereals and oilseed rape

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 FGF11 is indicated in the table.

Table 9.1-3: Metabolites of azoxystrobin potentially relevant for risk assessment

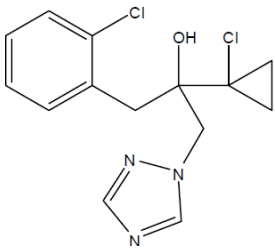
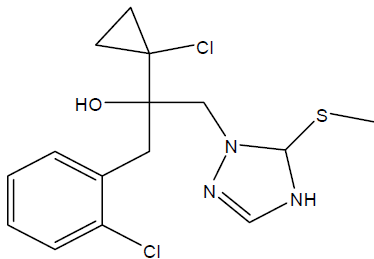
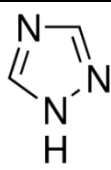
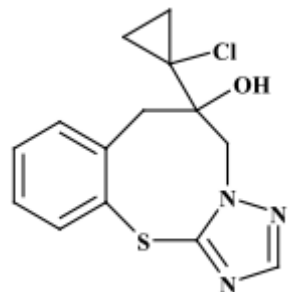
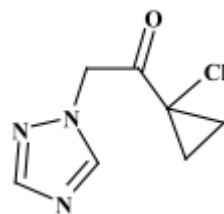
Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required for
R234886		389.4 g/mol	Soil: 28.8 % Water/sediment systems: 17.7% log Pow: 1.29 (estimated)	aquatic and soil organisms; drinking water for birds and mammals
R401553		213.2 g/mol	Soil: 17 % Water/sediment systems: 8.9% log Pow: 0.97 (estimated)	aquatic and soil organisms; drinking water for birds and mammals
R402173		333.3 g/mol	Soil: 17 % Water/sediment systems: 2.4 % log Pow: 1.46 (estimated)	aquatic and soil organisms; drinking water for birds and mammals

zRMS comments:

Metabolites relevant for soil and water compartment listed in Table 9.1-3 are the same as indicated in EFSA Journal 2010; 8(4):1542. For information agreed in this area please refer to the Core Assessment, Part B, Section 8, where all respective data are provided and used in calculation of PEC_{soil} and $PEC_{sw/seq}$ values, considered further in the risk assessment.

As the information on the maximum occurrence was not checked in detail, it was struck through in Table 9.1- **3** 4.

Table 9.1-4: Metabolites of prothioconazole potentially relevant for risk assessment

Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required for
Prothioconazole-desthio (M04)		312.2 g/mol	Soil: 100 % Water/sediment systems: 54.5 % log Pow: 3.04	aquatic organisms and soil organisms; drinking water and secondary poisoning for birds and mammals
Prothioconazole-S-methyl (M01)		358.3 g/mol	Soil: 14.6 % Water/sediment systems: 12.7 % log Pow: 4.3	soil and aquatic organisms; drinking water and secondary poisoning for birds and mammals
1,2,4- triazole (M13)		69.1 g/mol	Soil: 0.0001 % Water/sediment systems: 41.8 % log Pow: -0.71	aquatic organisms; drinking water for birds and mammals
Prothioconazole-thiazocine (M12)		307.8 g/mol	Soil: 0.0001 % Water/sediment systems: 14.1 % log Pow: 1.9	aquatic organisms; drinking water for birds and mammals
Prothioconazole-triazolylketone (M42)		185.7 g/mol	Soil: 0.0001 % Water/sediment systems: 9.1 % log Pow: 0.33	aquatic organisms; drinking water for birds and mammals

zRMS comments:

Metabolites relevant for soil and water compartment listed in Table 9.1-4 are the same as indicated in EFSA Scientific Report (2007) 106. It is noted that in the course of the EU review of prothioconazole metabolite JAU 6476-thiazocine was formed at >10% in photodegradation study in water, however according to EFSA Scientific Report (2007) 106, it was considered to be not relevant for evaluation in area of ecotoxicology.

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

As the information on the maximum occurrence was not checked in detail, it was struck through in Table 9.1-4 

9.2 Effects on birds (KCP 10.1.1)

Avian toxicity studies have been carried out with the active substances azoxystrobin and prothioconazole. Full details of these studies are provided in the respective EU DAR and related documents (EFSA Conclusion 2007, EFSA conclusion 2010 and RAR 2018).

Effects on birds of FGF11 were not evaluated as part of the EU assessment of azoxystrobin or prothioconazole. However, the provision of further data on the formulation is not considered essential, because the toxicity of the formulation can be read across from the data on the active substances, also in order to minimise unnecessary vertebrate testing. Neither prothioconazole nor azoxystrobin show a high acute toxicity and additionally, acute mixture toxicity based on active substance data was addressed in the risk assessment below.

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

9.2.1 Toxicity data

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

Species	Substance	Exposure System	Results	Reference	
Azoxystrobin					
Bobwhite quail (<i>Colinus virginianus</i>)	Azoxystrobin	Acute	LD ₅₀ > 2000 mg/kg bw	EFSA 2010	
Bobwhite quail (<i>Colinus virginianus</i>)	Azoxystrobin	Short term	LD ₅₀ >1179 mg/kg bw		
Bobwhite quail (<i>Colinus virginianus</i>)	Azoxystrobin	Reproductive	NOEL = 1200 mg/kg feed NOEL = 117 mg a.s./kg bw/d*G		
Prothioconazole					
Bobwhite quail (<i>Colinus virginianus</i>)	Prothioconazole	Acute	LD ₅₀ > 2000 mg/kg bw	EFSA 2007	
Bobwhite quail (<i>Colinus virginianus</i>)	Prothioconazole	Short term	LC ₅₀ > 5000 mg a.s./kg diet calc. LD ₅₀ > 1413 mg/kg bw/d		
Mallard (<i>Anas platyrhyn- chus</i>)	Prothioconazole	Short-term	LC ₅₀ > 5000 mg a.s./kg diet calc. LD ₅₀ > 2457 mg/kg bw/d		
Bobwhite quail (<i>Colinus virginianus</i>)	Prothioconazole	Reproductive	NOEL ≥ 86 mg/kg bw/d		
Mallard (<i>Anas platyrhyn- chus</i>)	Prothioconazole	Reproductive	NOEL = 78 mg/kg bw/d		
Prothioconazole metabolites					
Bobwhite quail (<i>Colinus virginianus</i>)	Prothioconazole- desthio	Acute	LD ₅₀ > 2000 mg/kg bw		
Bobwhite quail (<i>Colinus virginianus</i>)	Prothioconazole- desthio	Short-term	LC ₅₀ = 4090 mg p.m./kg diet LD ₅₀ > 297 mg p.m./kg bw/d		
Bobwhite quail (<i>Colinus virginianus</i>)	Prothioconazole- desthio	Reproductive	NOEL = 14.8 mg/kg bw/d		
Mallard (<i>Anas platyrhyn- chus</i>)	Prothioconazole- desthio	Reproductive	NOEL ≥ 63 mg/kg bw/d		

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

* In the list of endpoints for azoxystrobin, the NOEL is stated to be 1200 mg/kg bw/d and 117 mg/kg feed. This is a mistake, as it has to be 117 mg/kg bw/d (please refer to the DAR). The value of 1200 refers to ppm or mg/kg feed.

^{a)} EFSA conclusion for azoxystrobin; EFSA Journal 2010; 8(4):1542

^{b)} EFSA conclusion for prothioconazole; EFSA Scientific Report (2007) 106, 1-98

zRMS comments:

Avian toxicity data for azoxystrobin, prothioconazole and prothioconazole metabolite JAU 6476-desthio provided in Tables 9.2-1 above were confirmed by zRMS that they are in line with EU agreed endpoints reported in EFSA Journal 2010; 8(4):1542 and EFSA Scientific Report (2007) 106, respectively.

9.2.1.1 Justification for new endpoints

The endpoints used in the risk assessment are those reported in the EFSA conclusion (2007 and 2010) for prothioconazole and azoxystrobin, respectively.

9.2.2 Risk assessment for spray applications

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

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the surrogate use on cereals, AR 1 x 1 kg formulation (160 g azoxystrobin/ha + 200 g prothioconazole/ha) considering a BBCH interval 30-69 covers uses N.1,2,3,5,6 according to GAP.

For application on oilseed rape, patterns reported in the GAP: AR 1 x 1 kg formulation /ha, BBCH 61-73 have been addressed.

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

Screening Step

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

Table 9.2-2: Screening step assessment of the acute and long-term/reproductive risk for birds from azoxystrobin due to the use of FGF11 in cereals /oilseed rape

Intended use	Cereal / Oilseed rape				
Active substance/product	azoxystrobin				
Application rate (g/ha)	1 x 160 a.s./ha				
Acute toxicity (mg/kg bw)	>2000				
TER criterion	10				
Crop scenario	Indicator/generic focal species	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a
Growth stage					
Cereals/Oilseed rape	Small omnivorous bird	158.8	1	25.40	>78.7
Reprod. toxicity (mg/kg bw/d)	117				
TER criterion	5				
Crop scenario	Indicator/generic focal species	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{lt}
Growth stage					
Cereals	Small omnivorous bird	64.8	0.53	5.46	21.4

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.

zRMS comments:

The screening step risk assessment from exposure to azoxystrobin to birds for use in cereals and oilseed rape has been validated by zRMS.

TER_A and TER_{LT} values are above the trigger of 10 and 5 for acute and long-term exposure, indicating acceptable risk for birds for all intended uses.

Table 9.2-3: Screening step assessment of the acute and long-term/reproductive risk for birds from prothioconazole due to the use of FGF11 in cereals /oilseed rape

Intended use	Cereal /Oilseed rape				
Active substance/product	prothioconazole				
Application rate (g/ha)	1 x 200 a.s./ha				
Acute toxicity (mg/kg bw)	>2000				
TER criterion	10				
Crop scenario Growth stage	Indicator/generic focal species	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a
Cereals/Oilseed rape	Small omnivorous bird	158.8	1	31.75	> 63.0
Reprod. toxicity (mg/kg bw/d)	78				
TER criterion	5				
Crop scenario Growth stage	Indicator/generic focal species	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{lt}
Cereals / Oilseed rape	Small omnivorous bird	64.8	0.53	6.83	11.4

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

Table 9.2-4: Screening step assessment of the acute and long-term/reproductive risk for birds from prothioconazole-desthio due to the use of FGF11 in cereals / oilseed rape

Intended use	Cereal / Oilseed rape				
Active substance/product	Prothioconazole-desthio				
Application rate (g/ha)	1 x 181.4 a.s./ha*				
Acute toxicity (mg/kg bw)	>2000				
TER criterion	10				
Crop scenario Growth stage	Indicator/generic focal species	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a
Cereals/Oilseed rape	Small omnivorous bird	158.8	1	28.80	> 69.5
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 / Oilseed rape	Small omnivorous bird	64.8	0.53	6.19	2.4

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 rates are calculated based on the molecular weight ratio (312.194 g/mol : 344.26 g/mol = 0.907) with a 100% conversion from prothioconazole to prothioconazole-desthio

zRMS comments:

The screening step risk assessment for prothioconazole and metabolite JAU 6476-desthio has been validated by zRMS.

TER_A and TER_{LT} values for the exposure to prothioconazole are above the trigger of 10 and 5 for acute and long-term exposure, indicating acceptable risk for birds for all intended uses.

In case of metabolite JAU 6476-desthio TER_A value is above trigger of 10 but TER_{LT} value needs the Tier 1 calculations for all intended uses.

For this reason, further calculations have been performed in the Table 9.2-5 below:

First tier assessment

Table 9.2-5: First-tier assessment of long-term/reproductive risk for birds from prothioconazole-desthio due to the use of FGF11 in cereals and oilseed rape

Intended use		Cereal (BBCH 30-69) and Oilseed rape (BBCH 61-73)				
Active substance/product		Prothioconazole-desthio				
Application rate (g/ha)		1 x 181.4 a.s./ha*				
Reprod. toxicity (mg/kg bw/d)		14.8				
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 30-39	Small omnivorous bird	5.4	0.53	0.52	28.5 ~~136.5~~	
BBCH ≥ 40	Small omnivorous bird	3.3	0.53	0.32	46.2 ~~227.5~~	
Oilseed rape						
Late - late with seeds (BBCH 30-99)	Small insectivorous bird	2.7	0.53	0.26	57. 	
BBCH ≥ 40	Small omnivorous bird	2.7	0.53	0.26	57. 	
BBCH ≥ 40	Medium herbivorous/granivorous bird	0.9	0.53	0.09	164.4 ~~172.1~~	

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

* The rates are calculated based on the molecular weight ratio (312.194 g/mol : 344.26 g/mol = 0.907) with a 100% conversion from prothioconazole to prothioconazole-desthio

All acute and chronic ratios are above the relevant threshold for all substances, demonstrating that the risk to birds from dietary exposure is acceptable following the proposed use of FGF11.

zRMS comments:

Based on calculations presented in the Table 9.2-5, the TER_A and TER_{LT} values at Tier 1 for the exposure to prothioconazole-desthio are above the trigger of 10 and 5 for acute and long-term exposure, respectively, indicating acceptable risk for birds for all intended uses.

Mixture toxicity

As FGF11 contains two active substances, a mixture risk assessment is required. For acute risk assessment the Finney's equation is used to calculate a mixture toxicity ($LD_{50, \text{mix}}$) based on the toxicity of each substance and their proportions within the product. Due to the differences in toxicity between prothioconazole and its metabolite prothioconazole-desthio and the simple assumption that the metabolite forms rapidly at a rate of 1, the calculations are performed for both azoxystrobin + prothioconazole and azoxystrobin + prothioconazole-desthio.

For the acute exposure, according to the EFSA 2009, 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:

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

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

Table 9.2-6: Avian LD_{50} (mix) for azoxystrobin and prothioconazole when combined as FGF11

Active substance	Concentration a.s. in mixture [g/L]	Fraction a.s. in mixture	LD_{50} a.s. [mg/kg b.w.]	Fraction a.s./ LD_{50} a.s.	Surrogate LD_{50} [mg/kg b.w.]	Tox per fraction (a.s.)	Contribution to predicted toxicity (mix) [%]
Azoxystrobin	160	0.44	>2000	0.00022	2000	4500	44.4
Prothioconazole	200	0.56	>2000	0.00028		3600	55.6

Following Appendix B (step 1) in B&M guidance a surrogate $LD_{50} = 2000$ mg/kg b.w. is calculated based on the assumption of dose additivity. A combined acute risk assessment is not required if for one active substance the deviation between 'tox per fraction (a.s.)' and 'tox per fraction (mix)' is $\leq 10\%$ as in that case the risk is covered by the assessment for that active substance. For FGF11 this does not apply because the deviation for both active substances is more than 10%. Azoxystrobin contributes to 44.4 % to mixture toxicity, while the prothioconazole have an impact on the predicted risk of 55.6 %, therefore, surrogate LD_{50} was used in the acute risk assessment.

Table 9.2-7: Screening assessment and Tier 1 of the acute risk for birds due to the use of FGF11 in cereals and oilseed rape

Intended use		Cereals / Oilseed rape , 1.0 L _{PPP} /ha [equivalent to 160 g azoxystrobin + 200 g prothioconazole]			
Active substance/product		FGF11			
Application rate (g/ha)		1 x 360 (as sum of aa.ss.)			
LD ₅₀ (mix) (mg/kg bw)		2000			
TER criterion		10			
Screening step					
Crop scenario Growth stage	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a
Cereals / Oilseed rape	Small omnivorous birds	158.8	1	57.15	35

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

zRMS comments:

Combined acute risk assessment:

It is noted that for the combined acute risk assessment from exposure of active substances the Applicant selected acute toxicity endpoints LD₅₀> 2000 mg a.s./kg for both active compounds (prothioconazole and azoxystrobin), which is considered generally acceptable by zRMS although that for the a.s.- prothioconazole lower endpoint from short-term study with LD₅₀ of 1413 mg p.m./kg is available.

In zRMS's opinion as no treatment related mortalities were observed in the short-term toxicity study for the a.s. prothioconazole indicating that the dietary exposure has not resulted with increased mortality of tested birds and the acute LD₅₀>2000 kg a.s./kg bw is sufficiently protective to use in the risk assessment.

However, for some MSs that prefers endpoint from dietary studies with LD₅₀>1413 mg prothioconazole /kg bw in the acute combined risk assessment, the relevant calculations have been added by zRMS in the Tables 9.2-6-1 and 9.2.7-1 below:

Table 9.2-8-1: Avian LD₅₀ (mix) for azoxystrobin and prothioconazole when combined as FGF11

Active substance	Concentration a.s. in mixture [g/L]	Fraction a.s. in mixture	LD ₅₀ a.s. [mg/kg b.w.]	Fraction a.s./ LD ₅₀ a.s.	Surrogate LD ₅₀ [mg/kg b.w.]	Tox per fraction (a.s.)	Contribution to predicted toxicity (mix) [%]
Azoxystrobin	160	0.44	>2000	0.00022	1639.3	4500	36
Prothioconazole	200	0.56	>1413	0.000396		2500	64

Azoxystrobin contributes to 36% to mixture toxicity, while the prothioconazole have an impact on the predicted risk of 64%, therefore, surrogate LD₅₀mix value calculated by zRMS was used in the acute combined risk assessment.

Table 9.2-9-1: Screening assessment and Tier 1 of the acute risk for birds due to the use of FGF11 in cereals and oilseed rape

Intended use	Cereals / Oilseed rape , 1.0 L _{PPP} /ha [equivalent to 160 g azoxystrobin + 200 g prothioconazole]				
Active substance/product	FGF11				
Application rate (g/ha)	1 x 360 (as sum of a.s.)				
LD ₅₀ (mix) (mg/kg bw)	1639.3				

TER criterion		10			
Screening step					
Crop scenario Growth stage	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a
Cereals / Oilseed rape	Small omnivorous birds	158.8	1	57.15	28.70

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

Azoxystrobin + prothioconazole-desthio

Table 9.2-10: Avian LD₅₀ (mix) for azoxystrobin and prothioconazole-desthio when combined as FGF11

Active substance	Concentration a.s. in mixture [g/L]	Fraction a.s. in mixture	LD ₅₀ a.s. [mg/kg b.w.]	Fraction a.s./ LD ₅₀ a.s.	Surrogate LD ₅₀ [mg/kg b.w.]	Tox per fraction (a.s.)	Contribution to predicted toxicity (mix) [%]
Azoxystrobin	160	0.47	>2000	0.000235	2000	4267.5	46.9
Prothioconazole-desthio	181.4	0.53	>2000	0.000265		3764.1	53.1

Following Appendix B (step 1) in B&M guidance a surrogate LD₅₀ = 2000 mg/kg b.w. is calculated based on the assumption of dose additivity. A combined acute risk assessment is not required if for one active substance the deviation between ‘tox per fraction (a.s.)’ and ‘tox per fraction (mix)’ is ≤ 10% as in that case the risk is covered by the assessment for that active substance. For FGF11 this does not apply because the deviation for both active substances is more than 10%. Azoxystrobin contributes to 46.9 % to mixture toxicity, while the prothioconazole have an impact on the predicted risk of 53.1 %, therefore, surrogate LD₅₀ was used in the acute risk assessment.

Table 9.2-11: Screening assessment and Tier 1 of the acute risk for birds due to the use of FGF11 in cereals and oilseed rape

Intended use		Cereals/ Oilseed rape , 1.0 L _{PPP} /ha [equivalent to 160 g azoxystrobin + 181.4 g prothioconazole-desthio]			
Active substance/product		FGF11			
Application rate (g/ha)		1 x 341.4			
LD ₅₀ (mix) (mg/kg bw)		2000			
TER criterion		10			
Screening step					
Crop scenario Growth stage	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a
Cereals / Oilseed rape	Small omnivorous birds	158.8	1	54.20	36.9

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

As regards the risk to reproduction from exposure to more than one active substance, it is currently not recommended to consider the use of predicted toxicity values as surrogates in the risk assessment. The chronic combined risk is considered to be addressed by the risk assessment of the individual substances.

zRMS comments:

LD_{50mix} for the mixture of azoxystrobin and JAU 6476-desthio proposed by the Applicant should be considered lower toxicity endpoint LD₅₀ of 297 pm./kg bw for metabolite which was obtained from dietary study for birds. For this reason, LD_{50mix} was recalculated by zRMS with consideration of this endpoint and a total conversion of prothioconazole to the JAU 6476-desthio metabolite.

zRMS calculations are presented below.

Table 9.2-12-1: Avian LD₅₀ (mix) for azoxystrobin and prothioconazole-desthio when combined as FGF11

Active substance	Concentration a.s. in mixture [g/L]	Fraction a.s. in mixture	LD ₅₀ a.s. [mg/kg b.w.]	Fraction a.s./ LD ₅₀ a.s.	Surrogate LD ₅₀ [mg/kg b.w.]	Tox per fraction (a.s.)	Contribution to predicted toxicity (mix) [%]
Azoxystrobin	160	0.47	>2000	0.000235	516.8	4267.5	12
Prothioconazole-desthio	181.4	0.53	>297	0.00178		560.37	88

Azoxystrobin contributes to 36% to mixture toxicity, while the prothioconazole has an impact on the predicted risk of 64%, therefore, surrogate LD_{50mix} value calculated by zRMS was used in the acute combined risk assessment.

Table 9.2-13-1: Screening assessment and Tier 1 of the acute risk for birds due to the use of FGF11 in cereals and oilseed rape

Intended use		Cereals/ Oilseed rape, 1.0 L _{PPP} /ha [equivalent to 160 g azoxystrobin + 181.4 g prothioconazole-desthio]			
Active substance/product		FGF11			
Application rate (g/ha)		1 x 341.4			
LD ₅₀ (mix) (mg/kg bw)		516.8			
TER criterion		10			
Screening step					
Crop scenario Growth stage	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a
Cereals / Oilseed rape	Small omnivorous birds	158.8	1	54.20	9.53
Tier 1 - cereals					
BBCH 30-39	Small omnivorous bird "lark"	12.0	1	4.1	126.05
BBCH ≥40	Small omnivorous bird "lark"	7.2	1	2.45	211
Cereals late season seed heads	Small granivorous/insectivorous bird “bunting	13	1	4.44	116.4
Tier 1 - oilseed rape					
BBCH ≥40	medium herbivorous/grani- vorous bird “pigeon	55.5	1	18.95	27.27
Late-late(with seeds) (BBCH 30-99)	Small insectivorous bird “dunnock)	7.4	1	2.52	205.08

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

TER_{mix} approach:

TER_{mix} approach for combined long-term risk assessment agreed by among of MSs in Central Zone based on the lowest TER_{LT} values for each compound are presented below

Cereals				Σ1/TER	Σ1/TER ⁻¹	Trigger
Azoxystrobin		Prothioconazole				
21.4	0.046	11.4	0.0877	0.1337	7.5	5

¹⁾ Lowest Tier 1 TER_{LT}

Cereals				Σ1/TER	Σ1/TER ⁻¹	Trigger
Azoxystrobin		JAU 6476-desthio				
21.4	0.046	136.5	0.0073	0.0533	18.8	5

¹⁾ Lowest TER_{LT}

Oilseed rape				Σ1/TER	Σ1/TER ⁻¹	Trigger
Azoxystrobin		Prothioconazole				
21.4	0.046	11.4	0.0877	0.1337	7.5	5

¹⁾ Lowest Tier 1 TER_{LT}

Oilseed rape				Σ1/TER	Σ1/TER ⁻¹	Trigger
Azoxystrobin		JAU 6476-desthio				
21.4	0.046	57.4	0.01754 ⁴²	0.06354 ⁴²	15.8	5

¹⁾ Lowest TER_{LT}

According to results, no unacceptable combined long-term risk is obtained in all crops according to the proposed GAP.

9.2.2.2 Higher-tier risk assessment

Not relevant

9.2.2.3 Effects of secondary poisoning

Active substances

Based on a $\log P_{ow} > 3$ for the active substance prothioconazole (i.e. $\log P_{ow}$ of 3.82 at pH 7; for details, see EFSA Scientific report (2007) 106, 1- 98), a potential for bioaccumulation has to be considered for this compound on a hypothetical basis. By contrast, azoxystrobin indicate no potential for bioaccumulation with respect to a $\log P_{ow} < 3$ (i.e. 2.5; for details, see EFSA Journal 2010; 8(4):1542). Thus, only for prothioconazole a risk assessment was performed for exposure from accumulation in food chains in agreement with the current guidance document. No mixture toxicity risk assessment is considered to be required.

Prothioconazole metabolites

The $\log P_{ow}$ values of prothioconazole-desthio and prothioconazole-S-methyl amount to 3.04 and 4.19, respectively, and thus do exceed the trigger value of 3. The remaining metabolites of prothioconazole do not show $\log P_{ow}$ values above 3 with -0.71 for 1,2,4-triazole, 1.9 for prothioconazole-thiazocine and 0.33 for prothioconazole-triazolylketone. In conclusion, a potential for bioaccumulation may be expected for the active substance prothioconazole and its metabolites prothioconazole-desthio (M04) and prothioconazole-S-methyl (M01). Consequently, a deterministic risk assessment by calculating TER values was performed only for these compounds of concern.

Azoxystrobin metabolites

As outlined in the underlying residue definitions in the EFSA Journal 2010; 8(4):1542, the metabolites of azoxystrobin, i.e. R234886, R401553 and R402173 have estimated $\log P_{ow}$ of 1.29, 0.97 and 1.46, respectively. Based on a $\log P_{ow} < 3$ no potential for bioaccumulation has to be considered for these compounds.

Risk assessment for earthworm-eating birds via secondary poisoning

According to EFSA/2009/1438, the risk for vermivorous birds is assessed for a bird of 100 g body weight

with a daily food consumption of 104.6 g. Bioaccumulation in earthworms is estimated based on measured/predicted concentrations in soil/porewater / is based on experimental data.

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

Table 9.2-14: Assessment of the risk for earthworm-eating birds due to exposure to prothioconazole and prothioconazole metabolites via bioaccumulation in earthworms (secondary poisoning) for the intended use cereals (worst case)

Parameter	Prothioconazole	Prothioconazole-desthio (M04)	Prothioconazole - S-methyl (M01)	comments
PEC _{soil initial} (mg/kg soil)	0.053	0.028	0.008	Part B, Section 8 (worst case)
log P _{ow} / P _{ow}	3.82/6607 3.4 / 2511.9	3.04 / 1096.5	4.19 / 15448.2	EFSA Scientific Report (2007) 106, 1-98
K _{oc}	1765	575.4	2556.3	Geomean (M04 and M01) EFSA Scientific Report (2007) 106, 1-98
f _{oc}	0.02	0.02	0.02	Default
BCF _{worm}	2.27 0.878	1.216	3.642	$BCF_{worm/soil} = (0.84 + 0.12 \times P_{ow}) / f_{oc} \times K_{oc}$
PEC _{worm}	2.38 0.047	0.034	0.029	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	2.5 0.049	0.036	0.031	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	78	14.8	7.8	EFSA Scientific Report (2007) 106, 1-98. NOEL of the parent compound prothioconazole was divided by a factor of 10 (worst-case assumption) for M01
TER _{it}	31.2 1596.9	413.9	254.9	

TER values shown in bold fall below the relevant trigger.

The TER for all substances was > 5, hence no further refinements are required.

zRMS comments:

The Applicants' approach in evaluation of the risk of secondary poisoning is in line with EFSA (2009).

Compounds selected for this assessment are agreed by the zRMS. Evaluation was not triggered for remaining metabolites of active substance due to their log P_{ow} <3.

Overall, acceptable risk for earthworms - eating birds via secondary poisoning for all relevant compounds could be concluded for birds.

Risk assessment for fish-eating birds via secondary poisoning

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

No risk assessment is considered required for prothioconazole-S-methyl, as predicted surface water concentrations are low and therefore the risk from secondary poisoning for fish-eating birds is considered low

as well. According to degradation pathway, as reported in Part B8, prothioconazole is readily degraded into the metabolite prothioconazole-S-methyl (M01) which then is promptly converted into metabolite prothioconazole-desthio (M04). Due to its low concentration and considering that a.s. and metabolite prothioconazole-desthio are already assessed for secondary poisoning, the risk assessment for metabolite M01 is considered to be covered.

Table 9.2-15: Assessment of the risk for fish-eating birds due to exposure to Prothioconazole and Prothioconazole-desthio (M04) via bioaccumulation in fish (secondary poisoning) cereals (worst case)

Parameter	Prothioconazole	Prothioconazole-desthio (M04)	comments
PEC _{sw} (mg/L)	0.0217	0.0391	Part B – Section 8 (Step 1 as a worst case)
BCF _{fish}	19.7	65	EFSA Scientific report (2007) 106, 1-98
BMF	-	-	biomagnification factor (relevant for BCF ≥ 2000)
PEC _{fish}	0.428 0.227	2.54 1.347	PEC _{fish} = PEC _{water} × BCF _{fish}
Daily dietary dose (mg/kg bw/d)	0.068 0.036	0.40 0.214	DDD = PEC _{fish} × 0.159
NOEL (mg/kg bw/d)	78	14.8	EFSA Scientific Report (2007) 106, 1-98
TER _{It}	1147.0 2165.2	37 69.1	

TER values shown in bold fall below the relevant trigger.

The TER for both substances was > 5, hence no further refinements are required.

zRMS comments:

The Applicants' approach in evaluation of the risk of secondary poisoning is in line with EFSA (2009). Compounds selected for this assessment are agreed by the zRMS. Evaluation was not triggered for remaining metabolites of active substance due to their log Pow <3. Overall, acceptable risk for fish-eating birds via secondary poisoning for all relevant compounds could be concluded for birds.

9.2.2.4 Biomagnification in terrestrial food chains

Not relevant.

9.2.2.5 Drinking water exposure

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

Leaf scenario

Since FGF11 is not intended to be applied on leafy vegetables forming heads or crop plants with comparable water collecting structures at principal growth stage 4 or later, the leaf scenario does not have to be considered.

Puddle scenario

Due to the characteristics of the exposure scenario in connection with the standard assumptions for water

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

With a geometric K_{oc} of 391.5 and 1765 mL/g for azoxystrobin and prothioconazole, respectively, azoxystrobin belongs to the groups of less sorptive substances whilst prothioconazole belongs to the group of more sorptive substances. The relevant metabolites for surface water belonging to the group of less sorptive substances are R234886 ($K_{oc} = 21$), R401553 ($K_{oc} = 188 \text{ mL/g}$), R402173 ($K_{oc} = 25 \text{ mL/g}$), prothioconazole-thiazocine ($K_{oc} = 165 \text{ mL/g}$), prothioconazole-triazolylketone ($K_{oc} = 1$) and 1,2,4-triazole ($K_{oc} = 83$). The relevant metabolites for surface water belonging to the group of more sorptive substances are prothioconazole-desthio ($K_{oc} = 574 \text{ mL/g}$) and prothioconazole-S-methyl ($K_{oc} = 2526 \text{ mL/g}$). To achieve a concise risk assessment, the risk envelope approach is applied using the highest application rate on cereals ($1 \times 1000 \text{ g}_{\text{PPP}}/\text{ha}$). Here, the assessment for the use on cereals covers the risk for birds from application on oilseed rape also.

For the metabolites the effective application rate is calculated based on the parental application rate taking into account the molecular weight ratio and the maximum occurrence in surface water. In addition, for those metabolites for which no measured effect endpoints are available, a ten times higher toxicity was assumed compared to the parent substance.

Table 9.2-16:: Assessment of the risk for birds due to exposure via contaminated drinking water in puddles: consideration of ratio between application rate and toxicity endpoint

Azoxystrobin			
Effective application rate (g/ha)	160		
Acute toxicity (mg/kg bw)	2000 ~~>1179~~	quotient	0.08 ~~<0.135~~
Reprod. toxicity (mg/kg bw/d)	117	quotient	1.37
Prothioconazole			
Effective application rate (g/ha)	200		
Acute toxicity (mg/kg bw)	>1413	quotient	<0.14
Reprod. toxicity (mg/kg bw/d)	78	quotient	2.56
Prothioconazole-desthio			
Effective application rate (g/ha)	181.4 ~~98.85~~		
Acute toxicity (mg/kg bw)	297	quotient	0.61 ~~0.33~~
Reprod. toxicity (mg/kg bw/d)	14.8	quotient	12.25 ~~6.68~~
R234886			
Effective application rate (g/ha)	27.95		
Acute toxicity (mg/kg bw)	377.6 ^{b)}	quotient	0.074
Reprod. toxicity (mg/kg bw/d)	>20.0 ^{b)}	quotient	1.397
R401553			
Effective application rate (g/ha)	7.53		
Acute toxicity (mg/kg bw)	377.6 ^{b)}	quotient	0.020
Reprod. toxicity (mg/kg bw/d)	>20.0 ^{b)}	quotient	< 0.376
R402173			
Effective application rate (g/ha)	3.17		
Acute toxicity (mg/kg bw)	377.6 ^{b)}	quotient	0.008
Reprod. toxicity (mg/kg bw/d)	>20.0 ^{b)}	quotient	< 0.158
Prothioconazole-S-methyl			
Effective application rate (g/ha)	26.44		
Acute toxicity (mg/kg bw)	>200 ^{b)}	quotient	< 0.131
Reprod. toxicity (mg/kg bw/d)	7.8 ^{b)}	quotient	3.39
1,2,4-triazole			
Effective application rate (g/ha)	16.78		
Acute toxicity (mg/kg bw)	>200 ^{b)}	quotient	< 0.084
Reprod. toxicity (mg/kg bw/d)	7.8 ^{b)}	quotient	2.15
Prothioconazole-thiazocine			
Effective application rate (g/ha)	25.21		
Acute toxicity (mg/kg bw)	>200 ^{b)}	quotient	< 0.126
Reprod. toxicity (mg/kg bw/d)	7.8 ^{b)}	quotient	3.232
Prothioconazole-triazolylketone			
Effective application rate (g/ha)	9.82		
Acute toxicity (mg/kg bw)	>200 ^{b)}	quotient	< 0.049
Reprod. toxicity (mg/kg bw/d)	7.8 ^{b)}	quotient	1.258

a) Effective application rate was calculated considering 100% transformation from prothioconazole into prothioconazole-desthio and taking into account the molecular ratio between parent and degradation product.

b) Due to lack of effect data, a ten times higher toxicity compared to the parent was assumed to provide a worst-case estimate.

* Application rate x MAFm

All acute and chronic ratios are below the relevant threshold for all substances, demonstrating that the risk to birds from contaminated drinking water is acceptable following the proposed use of FGF11.

zRMS comments:

The leaf scenario does not have to be considered taking into account the proposed uses.

All acute and chronic ratios are below the relevant threshold for all substances, demonstrating that the risk to birds from contaminated drinking water is acceptable following the proposed use of FGF11.

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

Not relevant.

9.2.4 Overall conclusions

The acute and chronic risk from the two active substances (azoxystrobin and prothioconazole) was acceptable at the screening level for the intended uses in cereals and oilseed rape. The acute risk from the metabolite prothioconazole-desthio was acceptable at the screening level whereas the reproductive risk was acceptable at Tier 1.

The risk to the mixture was calculated using the Finney's equation for acute toxicity and was acceptable at the screening level.

The risk from exposure to contaminated drinking water and secondary poisoning was also indicated to be acceptable following the proposed use of FGF11 according to GAP submitted.

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

9.3.1 Toxicity data

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

Species	Substance	Exposure System	Results	Reference	
Azoxystrobin					
Rat	Azoxystrobin	Oral, 1 d, Acute	LD ₅₀ > 5000 mg/kg bw	EFSA 2010	
Rat	Azoxystrobin	Oral, Developmental toxicity	NOAEL = 32 mg/kg bw/d		
Prothioconazole					
Rat	Prothioconazole	Oral, 1 d, Acute	LD ₅₀ > 6200 mg/kg bw	EFSA 2007	
Rat	Prothioconazole	Oral, Developmental toxicity	NOAEL = 9.7 (parental), 95.6 (reproduction) mg/kg bw/d		
Prothioconazole metabolites					
Rat	Prothioconazole - desthio	Oral, 1 d, Acute	LD ₅₀ = 2506 (females), 2806 (males) 2652 (geomean) mg/kg bw		
Mouse	Prothioconazole - desthio	Oral, 1 d, Acute	LD ₅₀ = 3459 (females), 2235 (males) mg/kg bw		
Rat	Prothioconazole-desthio	Oral, Developmental toxicity	NOAEL = 2.5 (parental), 10 (reproduction) mg/kg bw/d		

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

zRMS comments:

Mammalian toxicity data for azoxystrobin, prothioconazole and prothioconazole metabolite JAU 6476-desthio provided in Table 9.3-1 above were confirmed by zRMS that they are in line with EU agreed endpoints reported in EFSA-Journal 2010; 8(4):1542 and EFSA Scientific Report (2007) 106, respectively.

9.3.1.1 Justification for new endpoints

The endpoints used in the risk assessment are those reported in the EFSA conclusion (2007 and 2010) for prothioconazole and azoxystrobin, respectively.

9.3.2 Risk assessment for spray applications

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

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the surrogate use on cereals, AR 1 x 1 kg formulation (160 g azoxystrobin/ha + 200 g prothioconazole/ha) considering a BBCH interval 30-69 covers uses N.1,2,3,5,6 according to GAP.

For application on oilseed rape, patterns reported in the GAP: AR 1 x 1 kg formulation /ha, BBCH 61-73 have been addressed.

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

Screening Step

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

Table 9.3-2: First-tier assessment of the acute and long-term/reproductive risk for mammals from azoxystrobin due to the use of FGF11 in cereals/oilseed rape

Intended use		Cereal / Oilseed rape				
Active substance/product		azoxystrobin				
Application rate (g/ha)		1 x 160 a.s./ha				
Acute toxicity (mg/kg bw)		>5000				
TER criterion		10				
Crop scenario	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a	
Growth stage						
Cereal / Oilseed rape	Small herbivorous mammal	118.4	1	18.94	263.4	
Reprod. toxicity (mg/kg bw/d)		32				
TER criterion		5				
Crop scenario	Indicator/generic focal species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{lt}	
Growth stage						
Cereal / Oilseed rape	Small herbivorous mammal	48.3	1	4.07	7.9	

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

Table 9.3-3: First-tier assessment of the acute and long-term/reproductive risk for mammals from prothioconazole due to the use of FGF11 in cereals/oilseed rape

Intended use		Cereal / Oilseed rape				
Active substance/product		prothioconazole				
Application rate (g/ha)		1 x 200 a.s./ha				
Acute toxicity (mg/kg bw)		>6200				
TER criterion		10				
Crop scenario	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a	
Growth stage						
Cereal / Oilseed rape	Small herbivorous mammal	118.4	1	23.67	261.9	
Reprod. toxicity (mg/kg bw/d)		95.6				
TER criterion		5				
Crop scenario	Indicator/generic focal species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{lt}	
Growth stage						
Cereal / Oilseed rape	Small herbivorous mammal	48.3	1	5.09	18.8	

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

Table 9.3-4: First-tier assessment of the acute and long-term/reproductive risk for mammals from prothioconazole-desthio due to the use of FGF11 in cereals/oilseed rape

Prothioconazole-desthio due to the use of FGR 11 in cereals/oilseed rape						
Intended use		Cereal / Oilseed rape				
Active substance/product		Prothioconazole-desthio				
Application rate (g/ha)		1 x 181.4 a.s./ha ^a				
Acute toxicity (mg/kg bw)		2235				
TER criterion		10				
Crop scenario	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a	
Growth stage						
Cereal / Oilseed rape	Small herbivorous mammal	118.4	1	21.47	104.1	
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 _{lt}	
Growth stage						
Cereal / Oilseed rape	Small herbivorous mammal	48.3	1	4.62	2.2	

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

^a The rates are calculated based on the molecular weight ratio (312.194 g/mol : 344.26 g/mol = 0.907) with a 100% conversion from Prothioconazole to Prothioconazole-desthio

First tier assessment

Table 9.3-5: First-tier assessment of long-term/reproductive risk for mammals from prothioconazole-desthio due to the use of FGF11 in cereals and oilseed rape

Intended use		Cereal (BBCH 30-69) and Oilseed rape (BBCH 61-73)				
Active substance/product		Prothioconazole-desthio				
Application rate (g/ha)		1 x 181.4 a.s./ha ^a				
Reprod. toxicity (mg/kg bw/d)		10				
TER criterion		5				
Crop scenario Growth stage	Indicator/generic focal species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{lt}	
Cereals						
BBCH ≥ 20	Small insectivorous mammal	1.9	0.53	0.18	55.1	
BBCH ≥ 40	Small herbivorous mammal	21.7	0.53	2.07	4.82	
BBCH 30 -39	Small omnivorous mammal	3.9	0.53	0.37	26.8	
BBCH ≥ 40	Small omnivorous mammal	2.3	0.53	0.22	45.5	
Oilseed rape						
BBCH ≥ 20	Small insectivorous mammal	1.9	0.53	0.18	55.1	
BBCH ≥ 40	Small herbivorous mammal	18.1	0.53	1.73	5.8	
All season	Large herbivorous mammal	14.3	0.53	1.37	7.3	
BBCH ≥ 40	Small omnivorous mammal	1.9	0.53	0.18	55.1	

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

^a The rates are calculated based on the molecular weight ratio (312.194 g/mol : 344.26 g/mol = 0.907) with a 100% conversion from Prothioconazole to Prothioconazole-desthio

For application on oilseed rape, all acute and chronic ratios are below the relevant threshold for all substances, demonstrating that the risk to mammals from dietary exposure is acceptable following the proposed use of FGF11.

For application on cereals, assessments on azoxystrobin and prothioconazole indicate a low risk, however for metabolite prothioconazole-desthio, TER_A is above the trigger of 10, whilst TER_{LT} is below the trigger value of 5, showing an unacceptable risk for exposure to small herbivorous mammal (vole), at BBCH ≥ 40. Thus, a higher tier assessment is required for chronic exposure to metabolite prothioconazole-desthio.

zRMS comments:

The calculations of the acute and long-term risk assessment for both active substances and metabolite prothioconazole-desthio performed in the Tables above has been accepted by zRMS.

For application on cereals, TER_A and TER_{LT} values for azoxystrobin and prothioconazole are above triggers of 10 and 5, respectively, indicated a low risk, however for metabolite prothioconazole-desthio, TER_A is above the trigger of 10, whilst TER_{LT} is below the trigger value of 5, showing an unacceptable risk for exposure to small herbivorous mammal (vole), at BBCH ≥ 40.

Therefore, a higher tier assessment is required for chronic exposure to metabolite prothioconazole-desthio.

For application on oilseed rape, all acute and chronic ratios are above the relevant trigger value for all substances, demonstrating that the risk to mammals from dietary exposure is acceptable following the proposed use of FGF11.

Mixture toxicity

As FGF11 contains two active substances, a mixture risk assessment is required. For acute risk assessment the Finney's equation is used to calculate a mixture toxicity (LD_{50, mix}) based on the toxicity of each substance and their proportions within the product. Due to the differences in toxicity between prothioconazole and its metabolite prothioconazole-desthio and the simple assumption that the metabolite forms rapidly at a rate of 1, the calculations are performed for both azoxystrobin + prothioconazole and azoxystrobin + prothioconazole-desthio.

For the acute exposure, according to the EFSA 2009, 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

Table 9.3-6: Mammalian LD₅₀ (mix) for azoxystrobin and prothioconazole when combined as FGF11

Active substance	Concentration a.s. in mixture [g/L]	Fraction a.s. in mixture	LD ₅₀ a.s. [mg/kg b.w.]	Fraction a.s./ LD ₅₀ a.s.	Surrogate LD ₅₀ [mg/kg b.w.]	Tox per fraction (a.s.)	Contribution to predicted toxicity (mix) [%]
Azoxystrobin	160	0.44	>5000	0.000088	5602.4	11250.0	49.8
Prothioconazole	200	0.56	>6200	0.000090		11160.0	50.2

Following Appendix B (step 1) in B&M guidance a surrogate LD₅₀ = 5602.4 mg/kg b.w. is calculated based on the assumption of dose additivity. A combined acute risk assessment is not required if for one active substance the deviation between ‘tox per fraction (a.s.)’ and ‘tox per fraction (mix)’ is ≤ 10% as in that case the risk is covered by the assessment for that active substance. For FGF11 this does not apply because the deviation for both active substances is more than 10%. Azoxystrobin contributes to 49.8 % to mixture toxicity, while the prothioconazole have an impact on the predicted risk of 50.2 %, therefore, surrogate LD₅₀ was used in the acute risk assessment.

Table 9.3-7: Screening assessment and Tier 1 of the acute risk for mammals due to the use of FGF11 in cereals and oilseed rape

Intended use		Cereals / Oilseed rape, 1.0 L _{PPP} /ha [equivalent to 160 g azoxystrobin + 200 g prothi-conazole]			
Active substance/product		FGF11			
Application rate (g/ha)		1 x 360			
LD ₅₀ (mix) (mg/kg bw)		5602.4			
TER criterion		10			
Screening step					
Crop scenario Growth stage	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a
Cereals / Oilseed rape	Small herbivorous mammal	118.4	1	42.61	131.5

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 + prothioconazole-desthio

Table 9.3-8: Mammalian LD₅₀ (mix) for azoxystrobin and prothioconazole-desthio when combined as FGF11

Active substance	Concentration a.s. in mixture [g/L]	Fraction a.s. in mixture	LD ₅₀ a.s. [mg/kg b.w.]	Fraction a.s./ LD ₅₀ a.s.	Surrogate LD ₅₀ [mg/kg b.w.]	Tox per fraction (a.s.)	Contribution to predicted toxicity (mix) [%]
Azoxystrobin	160	0.47	5000	0.000094	3016.9	10668.8	28.3
Prothioconazole-desthio	181.4	0.53	2235	0.000237		4206.3	71.7

Following Appendix B (step 1) in B&M guidance a surrogate LD₅₀ = 3016.9 mg/kg b.w. is calculated based on the assumption of dose additivity. A combined acute risk assessment is not required if for one active substance the deviation between ‘tox per fraction (a.s.)’ and ‘tox per fraction (mix)’ is ≤ 10% as in that case

the risk is covered by the assessment for that active substance. For FGF11 this does not apply because the deviation for both active substances is more than 10%. Azoxystrobin contributes to 28.3 % to mixture toxicity, while the prothioconazole have an impact on the predicted risk of 71.7 %, therefore, surrogate LD₅₀ was used in the acute risk assessment.

Table 9.3-9: Screening assessment and Tier 1 of the acute risk for birds due to the use of FGF11 in cereals and oilseed rape

Intended use		Cereals / Oilseed rape , 1.0 L _{PPP} /ha [equivalent to 160 g azoxystrobin + 181.4 g prothioconazole-desthio]			
Active substance/product		FGF11			
Application rate (g/ha)		1 x 341.4			
LD ₅₀ (mix) (mg/kg bw)		3016.9			
TER criterion		10			
Screening step					
Crop scenario Growth stage	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a
Cereals / Oilseed rape	Small herbivorous mammal	118.4	1	40.41	74.7

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

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

As regards the risk to reproduction from exposure to more than one active substance, it is currently not recommended to consider the use of predicted toxicity values as surrogates in the risk assessment. The chronic combined risk is considered to be addressed by the risk assessment of the individual substances.

zRMS comments:

Acute combined risk assessment:

LD₀ mix values calculated with consideration of relevant toxicity endpoints for both of active substances and active substance – azoxystrobin and metabolite prothioconazole-desthio has been validated by zRMS.

Overall, based on calculations provided in the Tables from 9.3-6 to 9.3-9 an acceptable acute risk to mammals is concluded for the combined effects of simultaneous exposure to prothioconazole and azoxystrobin or metabolite prothioconazole-desthio and azoxystrobin at the screening step for intended uses of on cereals and oilseed rape.

Higher-tier risk assessment

Chronic risk for exposure to prothioconazole-desthio (JAU-desthio) was not acceptable at Tier 1 for the scenario for small herbivorous mammal (vole) at BBCH $\geq$ 40, resulting in a TER = 4.82.

A higher tier risk assessment is needed therefore to demonstrate a safe risk.

Two refinement options for the “vole” scenario are presented in the following table:

- *Refinement of the deposition factor*

The EFSA (2009) value for deposition factor is 0.3, however, since the publication of this guidance document the crop interception values have been revised (EFSA 2014). In this revision the crop interception of cereals relevant to the proposed GAP (BBCH 30-69) was increased from 70% to 80%. As such, the deposition factor can be reduced from 0.3 to 0.2. Recalculation of the shortcut value using this reduced deposition factor results in a TER > 5 and the risk to mammals represented by this scenario is acceptable. This

refinement is possible because i) the crop is suitably established so there is confidence in the crop interception values at these growth stages and ii) the grasses on which the voles feed is below the level of the cereal crop, so interception occurs before contact with the foodstuff relevant to the scenario.

- Residue in foliage

The risk assessment can be refined by using a DT₅₀ value for prothioconazole-desthio in wheat, this means a DT₅₀ value of 3.2 days, which originates from 8 residue trials submitted during the EU review of prothioconazole (EFSA Journal (2007) 106, 1-98 and DAR). Both, the residue trials as well as the DT₅₀ were evaluated and accepted during the EU review of prothioconazole and this DT₅₀ value can therefore be used in support of this application. Thus, it would be deemed acceptable to use this refined DT₅₀ for the re-calculation of the Time-Weighted Average Factor (f_{twa}). Using the DT₅₀ of 3.2 days and an averaging time of 21 days, it gives a twa-factor of 21.8%, thus, the use of a f_{twa} of 0.22 instead of the default value of 0.53 would be deemed appropriate.

Table 9.3-10: JAU-desthio – Higher-tier assessment of the long-term/reproductive risk for the “vole” due to the use of FGF11 in cereals – refined parameters

Intended use		Cereals						
Active substance/product		Prothioconazole-desthio						
Application rate (g/ha)		1 x 181.4 ^a						
Reprod. toxicity (mg/kg bw/d)		10						
TER criterion		5						
Crop scenario	Generic focal species	FIR/bw	Dep. factor	Mean RUD	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{LT}
Growth stage								
Cereals BBCH ≥ 40	Small herbivorous mammal “vole”	1.33	0.2 ^b 0.1	54.2	14.42	0.22	0.575 0.287	17.39 34.84

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

^a The rates are calculated based on the molecular weight ratio (312.194 g/mol : 344.26 g/mol = 0.907) with a 100% conversion from prothioconazole to prothioconazole-desthio

^b Refinement of deposition factor from 0.3 to 0.2 in line with updated crop interception from FOCUS (2015)

As indicated by TER higher than the trigger of 5, the long-term risk to mammals exposed to prothioconazole-desthio for the intended uses of FGF11 is indicated to be acceptable based on a higher tier risk assessment.

zRMS comments:

The refined long-term risk assessment for the “vole” scenario at BBCH > 40 in cereals has been amended by zRMS considering DF of 0.1. It should be noted that Deposition factor (DF) of 0.1 (based on an interception value of 90% in spring and winter cereals, at BBCH 40-69, (Table 1.5 of “Generic Guidance for Tier 1 FOCUS Ground Water Assessments” (Version 2.2; May 2014) is considered instead of 0.2 value.

Based on the calculations in the Table 9.3-10 the TER_{LT} value is above trigger of 5, indicating an acceptable long-term risk to this species.

Overall, acceptable long-term risk could be concluded for mammals from exposure of Prothioconazole-desthio in FGF11.

9.3.2.2 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 ($K_{oc} \geq 500$ L/kg).

With a geometric K_{oc} of 391.5 and 1765 mL/g for azoxystrobin and prothioconazole, respectively, azoxystrobin belongs to the groups of less sorptive substances whilst prothioconazole belongs to the group of more sorptive substances. The relevant metabolites for surface water belonging to the group of less sorptive substances are R234886 ($K_{oc} = 21$), R401553 ($K_{oc} = 188$ mL/g), R402173 ($K_{oc} = 25$ mL/g), prothioconazole-thiazocine ($K_{oc} = 165$ mL/g), prothioconazole-triazolylketone ($K_{oc} = 1$) and 1,2,4-triazole ($K_{oc} = 83$). The relevant metabolites for surface water belonging to the group of more sorptive substances are prothioconazole-desthio ($K_{oc} = 574$ mL/g) and prothioconazole-S-methyl ($K_{oc} = 2526$ mL/g). To achieve a concise risk assessment, the risk envelope approach is applied using the highest application rate on cereals (1×1000 g_{PPP}/ha). Here, the assessment for the use on cereals covers the risk for birds from application on oilseed rape also.

For the metabolites the effective application rate is calculated based on the parental application rate taking into account the molecular weight ratio and the maximum occurrence in surface water. In addition, for those metabolites for which no measured effect endpoints are available, a ten times higher toxicity was assumed compared to the parent substance.

Table 9.3-11: Assessment of the risk for mammals due to exposure via contaminated drinking water in puddles: consideration of ratio between application rate and toxicity endpoint

Azoxystrobin			
Effective application rate (g/ha)	160		
Acute toxicity (mg/kg bw)	>5000	quotient	<0.032
Reprod. toxicity (mg/kg bw/d)	32	quotient	5
Prothioconazole			
Effective application rate (g/ha)	200		
Acute toxicity (mg/kg bw)	>6200	quotient	<0.032
Reprod. toxicity (mg/kg bw/d)	95.6	quotient	2.092
Prothioconazole-desthio			
Effective application rate (g/ha)	181.4 ~~98.85~~		
Acute toxicity (mg/kg bw)	2235	quotient	0.08 ~~0.044~~
Reprod. toxicity (mg/kg bw/d)	10	quotient	18.14 ~~9.885~~
R234886			
Effective application rate (g/ha)	27.95		
Acute toxicity (mg/kg bw)	>500 ^{b)}	quotient	<0.056
Reprod. toxicity (mg/kg bw/d)	3.2 ^{b)}	quotient	8.736
R401553			
Effective application rate (g/ha)	7.53		
Acute toxicity (mg/kg bw)	>500 ^{b)}	quotient	<0.015
Reprod. toxicity (mg/kg bw/d)	3.2 ^{b)}	quotient	2.352
R402173			
Effective application rate (g/ha)	3.17		
Acute toxicity (mg/kg bw)	>500 ^{b)}	quotient	0.006
Reprod. toxicity (mg/kg bw/d)	3.2 ^{b)}	quotient	0.991
Prothioconazole-S-methyl			
Effective application rate (g/ha)	26.44		
Acute toxicity (mg/kg bw)	>620 ^{b)}	quotient	<0.042
Reprod. toxicity (mg/kg bw/d)	9.56	quotient	2.765
1,2,4-triazole			
Effective application rate (g/ha)	16.78		
Acute toxicity (mg/kg bw)	>620 ^{b)}	quotient	< 0.027
Reprod. toxicity (mg/kg bw/d)	9.56	quotient	1.755
Prothioconazole-thiazocine			

Effective application rate (g/ha)	25.21		
Acute toxicity (mg/kg bw)	>620 ^{b)}	quotient	< 0.040
Reprod. toxicity (mg/kg bw/d)	9.56	quotient	2.637
Prothioconazole-triazolylketone			
Effective application rate (g/ha)	9.82		
Acute toxicity (mg/kg bw)	>620 ^{b)}	quotient	< 0.015
Reprod. toxicity (mg/kg bw/d)	9.56	quotient	1.027

^{a)} Effective application rate was calculated considering 100% transformation from prothioconazole into prothioconazole-desthio and taking into account the molecular ratio between parent and degradation product.

^{b)} Due to lack of effect data, a ten times higher toxicity compared to the parent was assumed to provide a worst-case estimate.

All acute and chronic ratios are below the relevant threshold for all substances, demonstrating that the risk to mammals from contaminated drinking water is acceptable following the proposed use of FGF11.

zRMS comments:

The leaf scenario does not have to be considered taking into account the proposed uses.

All acute and chronic ratios are below the relevant threshold for all substances, demonstrating that the risk to from contaminated drinking water is acceptable following the proposed use of FGF11.

9.3.2.3 Effects of secondary poisoning

Active substances

Based on a $\log P_{ow} > 3$ for the active substance prothioconazole (i.e. $\log P_{ow}$ of 3.82 at pH 7; for details, see EFSA Scientific report (2007) 106, 1- 98), a potential for bioaccumulation has to be considered for this compound on a hypothetical basis. By contrast, azoxystrobin indicate no potential for bioaccumulation with respect to a $\log P_{ow} < 3$ (i.e. 2.5; for details, see EFSA Journal 2010; 8(4):1542). Thus, only for prothioconazole a risk assessment was performed for exposure from accumulation in food chains in agreement with the current guidance document. No mixture toxicity risk assessment is considered to be required.

Prothioconazole metabolites

The $\log P_{ow}$ values of prothioconazole-desthio and prothioconazole-S-methyl amount to 3.04 and 4.19, respectively, and thus do exceed the trigger value of 3. The remaining metabolites of prothioconazole do not show $\log P_{ow}$ values above 3 with -0.71 for 1,2,4-triazole, 1.9 for prothioconazole-thiazocine and 0.33 for prothioconazole-triazolylketone. In conclusion, a potential for bioaccumulation may be expected for the active substance prothioconazole and its metabolites prothioconazole-desthio (M04) and prothioconazole - S-methyl (M01). Consequently, a deterministic risk assessment by calculating TER values was performed only for these compounds of concern.

Azoxystrobin metabolites

As outlined in the underlying residue definitions in the EFSA Journal 2010; 8(4):1542, the metabolites of azoxystrobin, i.e. R234886, R401553 and R402173 have estimated $\log P_{ow}$ of 1.29, 0.97 and 1.46, respectively. Based on a $\log P_{ow} < 3$ no potential for bioaccumulation has to be considered for these compounds

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 measured/predicted concentrations in soil/porewater / is based on experimental data.

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

Table 9.3-12: Assessment of the risk for earthworm-eating mammals due to exposure to prothioconazole and prothioconazole metabolites via bioaccumulation in earthworms (secondary poisoning) for the intended use cereals (worst case)

Parameter	Prothioconazole	Prothioconazole-desthio (M04)	Prothioconazole - S-methyl (M01)	Comments
PEC _{soil} (mg/kg soil)	0.053	0.028	0.008	Part B, Section 8 (worst case)
log P _{ow} / P _{ow}	3.82/6607 3.4 / 2511.9	3.04 / 1096.5	4.19 / 15448.2	EFSA Scientific Report (2007) 106, 1-98
K _{oc}	1765	575.4	2556.3	Geomean (M04 and M01) EFSA Scientific Report (2007) 106, 1-98
f _{oc}	0.02	0.02	0.02	Default
BCF _{worm}	2.27 0.878	1.216	3.642	BCF _{worm/soil} = (0.84 + 0.12 × P _{ow}) / f _{oc} × K _{oc}
PEC _{worm}	0.12 0.047	0.034	0.029	PEC _{worm} = PEC _{soil} × BCF _{worm/soil}
Daily dietary dose (mg/kg bw/d)	0.153 0.060	0.044	0.037	DDD = PEC _{worm} × 1.28
NOEL (mg/kg bw/d)	95.6	10	9.56	EFSA Scientific Report (2007) 106, 1-98. NOEL of the parent compound prothioconazole was divided by a factor of 10 (worst-case assumption) for M01
TER _{lt}	624.9 1605.6	229.4	256.3	

TER values shown in bold fall below the relevant trigger.

As outlined in the table above, the long-term TER value is above 5. Thus, an acceptable risk for earthworm-eating mammals can be concluded. No further considerations have to be taken into account.

zRMS comments:

The Applicants' approach in evaluation of the risk of secondary poisoning is in line with EFSA (2009).

Compounds selected for this assessment are agreed by the zRMS. Evaluation was not triggered for remaining metabolites of active substance due to their log P_{ow} <3.

Overall, acceptable risk for earthworms - eating mammals via secondary poisoning for all relevant compounds could be concluded for mammals.

Risk assessment for fish-eating mammals via secondary poisoning

According to EFSA/2009/1438, the risk for piscivorous 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 and the regulatory acceptable concentration for aquatic organisms as limit value for admissible concentrations of prothioconazole and prothioconazole-desthio in water.

No risk assessment is considered required for prothioconazole-S-methyl, as predicted surface water concentrations are low and therefore the risk from secondary poisoning for fish-eating birds is considered low as well. According to degradation pathway, as reported in Part B8, prothioconazole is readily degraded into the metabolite prothioconazole-S-methyl (M01) which then is promptly converted into metabolite prothioconazole-desthio (M04). Due to its low concentration and considering that a.s. and metabolite prothioconazole-desthio are already assessed for secondary poisoning, the risk assessment for metabolite M01 is considered to be covered.

Table 9.3-13: Assessment of the risk for fish-eating mammals due to exposure to Prothioconazole and Prothioconazole-desthio (M04) via bioaccumulation in fish (secondary poisoning) cereals (worst case)

Parameter	Prothioconazole	Prothioconazole-desthio (M04)	comments
PEC _{sw} (mg/L)	0.0217	0.0391	Part B – Section 8 (Step 1 as a worst case)
BCF _{fish}	19.7	65	EFSA Scientific report (2007) 106, 1-98
BMF	-	-	biomagnification factor (relevant for BCF ≥ 2000)
PEC _{fish}	0.428 ~~0.227~~	2.54 ~~1.347~~	PEC _{fish} = PEC _{water} × BCF _{fish}
Daily dietary dose (mg/kg bw/d)	0.06 ~~0.03212~~	0.36 ~~0.191~~	DDD = PEC _{fish} × 0.142
NOEL (mg/kg bw/d)	95.6	10	EFSA Scientific Report (2007) 106, 1-98
TER _{lt}	1593.3 ~~2971.4~~	27.8 ~~52.3~~	

TER values shown in bold fall below the relevant trigger.

As outlined in the table above, the long-term TER value is above 5. Thus, an acceptable risk for fish-eating mammals can be concluded. No further considerations have to be taken into account.

zRMS comments:

The Applicants' approach in evaluation of the risk of secondary poisoning is in line with EFSA (2009).

Compounds selected for this assessment are agreed by the zRMS. Evaluation was not triggered for remaining metabolites of active substance due to their log Pow <3.

Overall, acceptable risk for fish - eating mammals via secondary poisoning for all relevant compounds could be concluded for mammals.

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

Not relevant.

9.3.4 Overall conclusions

The acute and long-term risk for prothioconazole and azoxystrobin as well as the acute risk for metabolite prothioconazole-desthio were indicated to be acceptable for mammals at the screening level for the intended uses in cereals and oilseed rape. The long-term risks for the metabolite prothioconazole-desthio were solved in the Tier 1 assessment, except for the small herbivorous mammal "vole" scenario following application to cereals of prothioconazole-desthio. As such, additional refinement options were required for the long-term risk from the metabolite prothioconazole-desthio in cereals: refinement of the deposition factor together with refinement of DT₅₀ in food items resulted in a TER > 5 and an acceptable risk to mammals is presented for this scenario.

The TERa for mixture toxicities were above the trigger value of 10 indicating low risk for mammals for applications on cereals and oilseed rape at the screening level.

The risk from exposure to contaminated drinking water and secondary poisoning was considered acceptable following the proposed use of FGF11.

In conclusion, an acceptable overall risk for mammals is indicated for the intended GAP uses of FGF11.

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

No data available regarding the potential effects of FGF11 to reptiles and amphibians and no data considered necessary.

9.5 Effects on aquatic organisms (KCP 10.2)

9.5.1 Toxicity data

Studies on the toxicity to aquatic organisms have been carried out with azoxystrobin, prothioconazole and their relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents (EFSA Conclusion 2007, EFSA conclusion 2010, Addendum 2014).

Effects on aquatic organisms of FGF11 were not evaluated as part of the EU assessment of the active substances. Two aquatic studies with the product FGF11 on *Daphnia magna* and green algae *Pseudokirchneriella subcapitata* have been provided.

Table 9.5-1: Relevant endpoints for the risk assessment for aquatic organisms – azoxystrobin and relevant metabolites

Relevant metabolites					
Species	Substance	Exposure system	End point	Toxicity (mg/L)	Reference
Fish					
<i>Oncorhynchus mykiss</i>	Azoxystrobin	96 hr (flow-through)	Mortality, EC ₅₀	0.47 (m)	EFSA 2010
<i>Lepomis macrochirus</i>	Azoxystrobin	96 hr (flow-through)	Mortality, EC ₅₀	1.1 (m)	
<i>Pimephales promelas</i>	Azoxystrobin	33 d (flow-through)	Growth NOEC	0.147 (m)	
<i>Oncorhynchus mykiss</i>	R234886	96 hr (flow-through)	Mortality, EC ₅₀	> 150 (m)	
<i>Oncorhynchus mykiss</i>	R401553	96 hr (static)	Mortality, EC ₅₀	> 120 (n)	
<i>Oncorhynchus mykiss</i>	R402173	96 hr (static)	Mortality, EC ₅₀	62 (n)	
Aquatic invertebrate					
<i>Daphnia magna</i>	Azoxystrobin	48 h (static)	Mortality, EC ₅₀	0.23 (m)	EFSA 2010
<i>Macrocyclus fuscus</i>	Azoxystrobin	48 h (static)	Mortality, EC ₅₀	0.13 (n)	
<i>Mysidopsis bahia</i>	Azoxystrobin	96 h (static)	Mortality, EC ₅₀	0.055 (n)	
<i>Mysidopsis bahia</i>	Azoxystrobin	48 h (static)	Mortality, EC ₅₀	0.068 (n)	
<i>Crassostrea gigas</i>	Azoxystrobin	48 hr static)	Mortality, EC ₅₀	1.3 (n)	
<i>Mysidopsis bahia</i>	Azoxystrobin	28-day	Reproduction (endpoint based on adult mortality)	0.00954 (mm)	
<i>Daphnia magna</i>	Azoxystrobin	21 d (static)	Reproduction, NOEC	0.044 (m)	
<i>Daphnia magna</i>	R234886	48 h (static)	Mortality, EC ₅₀	> 180 (n)	
<i>Daphnia magna</i>	R401553	48 h (static)	Mortality, EC ₅₀	> 120 (n)	
<i>Daphnia magna</i>	R402173	48 h (static)	Mortality, EC ₅₀	> 100 (n)	
Sediment dwelling organisms					
<i>Chironomus riparius</i>	Azoxystrobin	28 d (static)	NOEC	0.8 (n)	EFSA 2010
Algae					
<i>Raphidocelis subcapitata</i>	Azoxystrobin	72 h (static)	EC ₅₀	0.36 (m)	EFSA 2010
<i>Skeletonema costatum</i>	Azoxystrobin	72 h (static)	Biomass: EbC ₅₀ Growth rate: ErC ₅₀	0.098 0.3 (n)	

<i>Navicula pelliculosa</i>	Azoxystrobin	120 h (static)	72h Biomass: EbC ₅₀ 72h Growth rate: ErC ₅₀	0.014 0.146 (n)	
<i>Anabaena flos-aquae</i>	Azoxystrobin	120 h (static)	72h Biomass: EbC ₅₀ 72h Growth rate: ErC ₅₀	9.5 13.9 (m)	
<i>Raphidocelis subcapitata</i>	R234886	72 h (static)	EC ₅₀	47.0 (m)	
<i>Raphidocelis subcapitata</i>	R402173	72 h (static)	Biomass: EbC ₅₀ Growth rate: ErC ₅₀	67 67 (n)	
<i>Raphidocelis subcapitata</i>	R401553	72 h (static)	Biomass: EbC ₅₀ Growth rate: ErC ₅₀	>120 >120 (n)	
Higher plant					
<i>Lemna gibba</i>	Azoxystrobin	14 d (static)	Dry weight, EC ₅₀ Fronds, ErC ₅₀	>6.4 3.2 (n)	EFSA 2010

Mesocosm studies

The mesocosm study is considered to be a well-conducted mesocosm with an appropriate diversity and abundance of species. It should be noted that azoxystrobin was only applied once, and concentrations were only measured 21 hours after application and not throughout the course of the study. Species/groups were present in sufficient numbers to allow appropriate statistical analysis.

The Notifier proposed that the no observed ecologically adverse effects concentration (NOEAEC) is 10 µg/L. No uncertainty or assessment factor was proposed.

From the summary above it can be concluded that 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.
- 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:

- Chydorus* – significantly greater numbers on study day 10 and 28
- Pompholyx* sp – significantly greater numbers than the control on day 14 only
- Testudinella* 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.

Weight-of-evidence approach as agreed on EU level

The complete data base on azoxystrobin for aquatic invertebrates was discussed during the renewal process of azoxystrobin, including a geomean approach, an SSD approach and the mesocosm study above. From the overall data set a weight-of-evidence approach was derived and a **RAC of 3.3 µg a.s./L** was decided for aquatic invertebrates during expert consultation (EFSA conclusion, 2010).

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

n: based on nominal concentrations; mm: based on mean measured concentrations

zRMS comments:

Aquatic toxicity data for provided in Tables 9.5-1 above are in line with EU agreed endpoints reported in EFSA Scientific Report 2010; 8(4):1542.

Table 9.5-2: Relevant endpoints for the risk assessment for aquatic organisms – prothioconazole and relevant metabolites

Relevant metabolites					
Species	Test substance	Exposure system	Endpoint	Toxicity (mg/L)	Reference
Fish					
<i>Oncorhynchus mykiss</i>	Prothioconazole	Acute 96 hr static	Mortality, LC ₅₀	1.83 (mm)	EFSA 2007
<i>Lepomis macrochirus</i>	Prothioconazole	Acute 96 hr static	Mortality, LC ₅₀	4.59 (mm)	
<i>Cyprinus carpio</i>	Prothioconazole	Acute 96 hr static	Mortality, LC ₅₀	6.91 (n)	
<i>Oncorhynchus mykiss</i>	Prothioconazole	97 day early-life stage (ELS)	NOEC (Reduction in swim-up and increase in time to swim up)	0.308 (mm)	
<i>Oncorhynchus mykiss</i>	Prothioconazole-desthio	Acute 96 hr static	Mortality, LC ₅₀	6.63 (n)	
<i>Leuciscus idus melanotus</i>	Prothioconazole-desthio	Acute 96 hr static	Mortality, LC ₅₀	13.2 (mm)	
<i>Oncorhynchus mykiss</i>	Prothioconazole-desthio	96 day ELS	NOEC (deformities)	0.00334 (mm)	
<i>Oncorhynchus mykiss</i>	Prothioconazole-S-methyl	Acute 96 hr static-renewal	Mortality, LC ₅₀	1.79 (mm)	
<i>Oncorhynchus mykiss</i>	1,2,4-triazole	Acute 96 hr static	Mortality, LC ₅₀	498 (mm)	
<i>Oncorhynchus mykiss</i>	1,2,4-triazole	97 day ELS	NOEC	3.2 (n)	
Aquatic invertebrates					
<i>Daphnia magna</i>	Prothioconazole	Acute 48 hr static	Immobility, EC ₅₀	1.3 (n)	EFSA 2007
<i>Daphnia magna</i>	Prothioconazole	21 day static-renewal	NOEC (reduction of off-spring)	0.56 (n)	
<i>Daphnia magna</i>	Prothioconazole-desthio	Acute 48 hr static	Immobility, EC ₅₀	>10 (n)	
<i>Daphnia magna</i>	Prothioconazole-desthio	21 day static-renewal	NOEC (reduction of off-spring)	0.1 (n)	
<i>Daphnia magna</i>	Prothioconazole-S-methyl	Acute 48 hr static	Immobility, EC ₅₀	2.8 (n)	
<i>Daphnia magna</i>	1,2,4-triazole	Acute 48 hr static	Immobility, EC ₅₀	900 (n)	
Sediment-dwelling organisms					
<i>Chironomus riparius</i>	Prothioconazole	28 d static spiked water	NOEC (emergence and development rate)	9.14 (n)	EFSA 2007
<i>Chironomus riparius</i>	Prothioconazole-desthio	28 d static spiked water	NOEC (emergence)	2.0 (n)	
Algae					
<i>Pseudokirchneriella subcapitata</i>	Prothioconazole	96 h static	Growth rate 72 hour ErC ₅₀	2.18 (m)	EFSA 2007
<i>Scenedesmus subspicatus</i>	Prothioconazole-desthio	96 h static	Growth rate 96 hour ErC ₅₀	0.55 (n)	
<i>Pseudokirchneriella subcapitata</i>	Prothioconazole-S-methyl	72 h static	Growth rate 72 hour ErC ₅₀	47.4 (m)	
<i>Pseudokirchneriella subcapitata</i>	1,2,4-triazole	96 h static	Growth rate 96 hour ErC ₅₀	22.5 (mm)	

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

n: based on nominal concentrations; mm: based on mean measured concentrations

zRMS comments:

Aquatic toxicity data for prothioconazole and prothioconazole -desthio provided in Tables 9.5-2 above are in line with EU agreed endpoints reported in EFSA Scientific Report (2007) 106.

No studies on effects of prothioconazole and metabolite JAU 6476-desthio to *Lemna gibba* were available during the first EU review. It is noted that testing of aquatic macrophytes was not required for prothioconazole being a fungicide.

Table 9.5-3: Endpoints and effect values relevant for the risk assessment for aquatic organisms – FGF11 (Azoxystrobin 160 + Prothioconazole 200 g/L SC)

Species	Substance	Exposure System	Results	Reference
<i>Daphnia magna</i>	Azoxystrobin 160 + Prothioconazole 200 g/L SC	48 hour, Semi-static	EC ₅₀ =0.120 mg test item/L (nom)	Bauer J., Mollandin, G., 2024, 179211220, KCP 10.2.1/01
<i>Pseudokirchneriella subcapitata</i>	Azoxystrobin 160 + Prothioconazole 200 g/L SC	72 hours, Static	ErC ₅₀ =2.79 mg test item/L (nom) EyC ₅₀ = 0.679mg test item/L (nom)	Bauer J., Mollandin, G., 2024, 179211210, KCP 10.2.1/02

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

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

zRMS comments:

Studies on effects of the formulated product on aquatic organisms listed in Table 9.5-3 were evaluated by the zRMS and considered acceptable. Summaries of the performed studies together with zRMS evaluation may be found in Appendix 2.

9.5.1.1 Justification for new endpoints

In addition to the EU agreed endpoints, the applicant provides further effect data of the formulated product FGF11 to aquatic invertebrates (acute exposure) and algae. Final reports are provided in Appendix 2.

Aquatic degradation products

Data on the relevant aquatic degradation products of azoxystrobin (R401553, R402173 and R234886) and of prothioconazole (prothioconazole-S-methyl, prothioconazole-desthio, and 1,2,4-triazole) are available from the EFSA conclusion (2007).

9.5.2 Risk assessment

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

The relevant global maximum FOCUS Step 1, 2 and 3 to 4 PEC_{SW} for risk assessments covering the proposed use pattern and the resulting PEC/RAC ratios are presented in the following tables.

9.5.2.1 Individual substance risk assessment

Azoxystrobin and relevant 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.

Azoxystrobin - a.s.

Table 9.5-4: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for azoxystrobin for each organism group based on FOCUS Steps 1, 2 calculations for the use of FGF11 in winter and spring cereals (1 × 160 g a.s./ha, BBCH 30-69)

Group		Fish acute	Fish chronic	Inverteb. acute	Inverteb. chronic	Higher tier, invertebrates	Algae	Sediment dweller chronic (spiked water)	Higher plant
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>M. bahia</i>	<i>M. bahia</i>	EU-agreed weight-of-evidence approach	<i>N. pelliculosa</i>	<i>C. riparius</i>	<i>L. gibba</i>
Endpoint		LC ₅₀	NOEC	EC ₅₀	NOEC		ErC ₅₀	NOEC	ErC ₅₀
(µg/L)		470	147	55	9.54		146	800	3200
AF		100	10	100	10		10	10	10
RAC (µg/L)		4.7	14.7	0.55	0.954	3.3	14.6	80	320
FOCUS Scenario	PEC _{sw-max} (µg/L)								
Step 1		PEC/RAC ratios							
	35.6 ~~36.5~~	7.57 ~~7.77~~	2.42 ~~2.48~~	64.72 ~~66.36~~	37.3 ~~38.26~~	10.8 ~~11.06~~	2.43 ~~2.50~~	0.44 ~~0.46~~	0.11 ~~0.11~~
Step 2 - N-EU									
Mar-Sep	6.49	1.38	0.44	11.80	6.80	1.967	0.44	0.081	0.020
Step 2 - S-EU									
Oct-May	11.9	2.53	0.81	21.64	12.47	3.61	0.82	0.15	0.04
Jun-Sep	9.19	1.96	0.63	16.71	9.63	2.78	0.63	0.11	0.03

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 9.5-5: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for azoxystrobin for each organism group based on FOCUS Steps 1, 2 calculations for the use of FGF11 in winter and spring oilseed rape (1 × 160 g a.s./ha, BBCH 30-69)

Group		Fish acute	Fish chronic	Inverteb. acute	Inverteb. chronic	Higher tier, invertebrates	Algae	Sediment dweller chronic (spiked water)	Higher plant
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>M. bahia</i>	<i>M. bahia</i>	EU-agreed weight-of-evidence approach	<i>N. pelliculosa</i>	<i>C. riparius</i>	<i>L. gibba</i>
Endpoint		LC ₅₀	NOEC	EC ₅₀	NOEC		ErC ₅₀	NOEC	ErC ₅₀
(µg/L)		470	147	55	9.54		146	800	3200
AF		100	10	100	10		10	10	10
RAC (µg/L)		4.7	14.7	0.55	0.954	3.3	14.6	80	320
FOCUS Scenario	PEC _{sw-max} (µg/L)								
Step 1		PEC/RAC ratios							
	36.5	7.77	2.48	66.36	38.26	11.06	2.50	0.46	0.11
Step 2 - N-EU									
Mar-Sep	2.77	0.59	0.19	5.04	2.90	0.84	0.19	0.03	0.01
Step 2 - S-EU									
Oct-May	4.46	0.95	0.30	8.11	4.68	1.35	0.31	0.06	0.01
Jun-Sep	3.61	0.77	0.25	6.56	3.78	1.09	0.25	0.05	0.01

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

Table 9.5-6: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for azoxystrobin for relevant organisms based on FOCUS Step 3 calculations for the use of FGF11 in winter cereals (1 × 160 g a.s./ha, BBCH 30-69)

Group		Fish acute	Inverteb. acute	Inverteb. chronic	Higher tier
Test species		<i>O. mykiss</i>	<i>M. bahia</i>	<i>M. bahia</i>	EU-agreed weight-of-evidence approach
Endpoint		LC ₅₀	EC ₅₀	NOEC	
(µg/L)		470	55	9.54	
AF		100	100	10	
RAC (µg/L)		4.7	0.55	0.954	3.3
FOCUS Scenario	PEC _{sw-max} (µg/L)				
BBCH > 30					
D1 Ditch	3.38	0.72	6.15	3.54	1.02
D1 Stream	2.11	0.45	3.84	2.21	0.64
D2 Ditch	4.11	0.87	7.47	4.31	1.25
D2 Stream	2.57	0.55	4.67	2.69	0.78
D3 Ditch	1.01	0.21	1.84	1.06	0.31
D4 Pond	0.309	0.07	0.56	0.32	0.09
D4 Stream	0.75	0.16	1.36	0.79	0.23
D5 Pond	0.104	0.02	0.19	0.11	0.03
D5 Stream	0.813	0.17	1.48	0.85	0.25
D6 Ditch	1.01	0.21	1.84	1.06	0.31
R1 Pond	0.115	0.02	0.21	0.12	0.03
R1 Stream	1.05	0.22	1.91	1.10	0.32
R3 Stream	1.46	0.31	2.65	1.53	0.44
R4 Stream	1.82	0.39	3.31	1.91	0.55
BBCH < 69					
D1 Ditch	1.69	0.36	3.07	1.77	0.51
D1 Stream	1.07	0.23	1.95	1.12	0.32
D2 Ditch	2.27	0.48	4.13	2.38	0.69
D2 Stream	1.42	0.30	2.58	1.49	0.43
D3 Ditch	1.02	0.22	1.85	1.07	0.31
D4 Pond	0.255	0.05	0.46	0.27	0.08
D4 Stream	0.876	0.19	1.59	0.92	0.27
D5 Pond	0.108	0.02	0.20	0.11	0.03
D5 Stream	0.946	0.20	1.72	0.99	0.29
D6 Ditch	1.02	0.22	1.85	1.07	0.31
R1 Pond	0.147	0.03	0.27	0.15	0.04
R1 Stream	1.21	0.26	2.20	1.27	0.37
R3 Stream	1.48	0.31	2.69	1.55	0.45
R4 Stream	1.72	0.37	3.13	1.80	0.52

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

Table 9.5-7: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for azoxystrobin for relevant organisms based on FOCUS Step 3 calculations for the use of FGF11 in spring cereals (1 × 160 g a.s./ha, BBCH 30-69)

Group		Fish acute	Inverteb. acute	Inverteb. chronic	Higher tier
Test species		<i>O. mykiss</i>	<i>M. bahia</i>	<i>M. bahia</i>	EU-agreed weight-of-evidence approach
Endpoint		LC ₅₀	EC ₅₀	NOEC	
(µg/L)		470	55	9.54	
AF		100	100	10	
RAC (µg/L)		4.7	0.55	0.954	3.3
FOCUS Scenario	PEC _{sw-max} (µg/L)				
BBCH > 30					
D1 Ditch	2.60	0.55	4.73	2.73	0.79
D1 Stream	1.63	0.35	2.96	1.71	0.49
D3 Ditch	1.01	0.21	1.84	1.06	0.31
D4 Pond	0.34	0.07	0.62	0.36	0.10
D4 Stream	0.83	0.18	1.51	0.87	0.25
D5 Pond	0.106	0.02	0.19	0.11	0.03
D5 Stream	0.854	0.18	1.55	0.90	0.26
R4 Stream	1.90	0.40	3.45	1.99	0.58
BBCH < 69					
D1 Ditch	1.87	0.40	3.40	1.96	0.57
D1 Stream	1.18	0.25	2.15	1.24	0.36
D3 Ditch	1.02	0.22	1.85	1.07	0.31
D4 Pond	0.317	0.07	0.58	0.33	0.10
D4 Stream	0.874	0.19	1.59	0.92	0.26
D5 Pond	0.111	0.02	0.20	0.12	0.03
D5 Stream	0.886	0.19	1.61	0.93	0.27
R4 Stream	1.95	0.41	3.55	2.04	0.59

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

Table 9.5-8: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for azoxystrobin for relevant organisms based on FOCUS Step 3 calculations for the use of FGF11 in winter oilseed rape (1 × 160 g a.s./ha, BBCH 61-73)

Group		Inverteb. acute	Inverteb. chronic	Higher tier
Test species		<i>M. bahia</i>	<i>M. bahia</i>	EU-agreed weight-of-evidence approach
Endpoint		EC ₅₀	NOEC	
(µg/L)		55	9.54	
AF		100	10	
RAC (µg/L)		0.55	0.954	3.3
FOCUS Scenario	PEC _{sw-max} (µg/L)			
BBCH > 61				
D2 Ditch	1.24	2.25	1.30	0.38
D2 Stream	1.06	1.93	1.11	0.32
D3 Ditch	1.02	1.85	1.07	0.31
D4 Pond	0.228	0.41	0.24	0.07
D4 Stream	0.876	1.59	0.92	0.27
D5 Pond	0.102	0.19	0.11	0.03
D5 Stream	0.946	1.72	0.99	0.29
R1 Pond	0.158	0.29	0.17	0.05
R1 Stream	1.22	2.22	1.28	0.37
R3 Stream	1.17	2.13	1.23	0.35

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

Table 9.5-9: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for azoxystrobin for relevant organisms based on FOCUS Step 3 calculations for the use of FGF11 in spring oilseed rape (1 × 160 g a.s./ha, BBCH 61-73)

Group		Inverteb. acute	Inverteb. chronic	Higher tier
Test species		<i>M. bahia</i>	<i>M. bahia</i>	EU-agreed weight-of-evidence approach
Endpoint		EC ₅₀	NOEC	
(µg/L)		55	9.54	
AF		100	10	
RAC (µg/L)		0.55	0.954	3.3
FOCUS Scenario	PEC _{sw-max} (µg/L)			
BBCH > 61				
D1 Ditch	2.62	4.76	2.75	0.79
D1 Stream	1.64	2.98	1.72	0.50
D3 Ditch	1.020	1.85	1.07	0.31
D4 Pond	0.296	0.54	0.31	0.09
D4 Stream	0.876	1.59	0.92	0.27
D5 Pond	0.246	0.45	0.26	0.07
D5 Stream	0.945	1.72	0.99	0.29
R1 Pond	0.249	0.45	0.26	0.08
R1 Stream	1.390	2.53	1.46	0.42

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 the intended uses in cereals, calculated PEC/RAC ratios for azoxystrobin did not indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for aquatic invertebrates as characterised by an EC₅₀ and NOEC for *M. bahia* of 55 and 9.54 µg a.s./L, respectively, in connection with an assessment factor of 100 and 10, respectively) in several FOCUS Steps 3 scenarios. However, the effect refinement based on the EU agreed weight-of-evidence approach with a RAC of 3.3 µg a.s./L did indicate a potential risk only in one FOCUS Step 3 scenario (D1 and D2 Ditch) for winter cereals.

It is to be noted that D1 and D2 Ditch scenarios are not relevant across Member States of Central Zone, thus no further risk assessment is conducted for this specific exposure scenario.

Also D2 Ditch is failing at Step 3 FOCUS scenario assessment for application on winter cereals. Implementation with mitigation measures should be addressed only at national level.

Further PEC/RAC ratios were calculated for scenario D2 Ditch of the EU agreed weight-of-evidence approach based on FOCUS Step 4 PEC_{SW} considering reduced exposure of surface water bodies.

For the intended uses in oilseed rape, calculated PEC/RAC ratios for azoxystrobin did not indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for aquatic invertebrates as characterised by an EC₅₀ and NOEC for *M. bahia* of 55 and 9.54 µg a.s./L, respectively, in connection with an assessment factor of 100 and 10, respectively) in several FOCUS Steps 3 scenarios. However, the effect refinement based on the EU agreed weight-of-evidence approach with a RAC of 3.3 µg a.s./L did indicate an acceptable risk in all of the FOCUS Step 3 scenarios. No further assessment is required.

Table 9.5-10: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for azoxystrobin for aquatic invertebrates based on FOCUS Step 4 mitigations for the use of FGF11 in winter cereals (1 × 160 g a.s./ha, BBCH 30-69)

VFSmod	-	-	-	-	5 m	10 m
Vegetated runoff buffer	0 m	0 m	10 m	20 m	-	-
No-spray buffer	0 m	0 m	10 m	20 m	-	-
Drift-reducing nozzles	0%	50%	0%	0%	-	-
BBCH > 30	PEC _{SW} values					
D2 Ditch	4.11	4.11	4.11	4.11	4.11	4.11
Higher tier: 3.3	PEC/RAC ratios - BBCH > 30					
D2 Ditch	1.25	1.25	1.25	1.25	1.25	1.25

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 azoxystrobin, based on the results of the risk assessments at FOCUS Step 4 with the EU agreed weight-of-evidence approach for aquatic invertebrates, a risk is indicated for the FOCUS scenarios D2 Ditch for winter cereals.

It is to be noted that D2 Ditch scenario and thus appropriate risk mitigation measures are to be considered only for France where this FOCUS Scenario is relevant. According to GAP, no other SEU MSs should require to assess FOCUS Scenario D2 Ditch.

Since the main entry route is *via* drainage a label restriction is deemed necessary according to Regulation (EU) No. 547/2011, and if this scenario is relevant at national MS level, the safety precaution phrase SPe2 shall be applied: „To protect groundwater/aquatic organisms do not apply to drained soils.“

zRMS comments:

Azoxystrobin

The risk assessment for a.s.- azoxystrobin considering acute and long-term endpoints agreed in EFSA Conclusion 2010 provided in the Tables 9.5-4 to 9.5-7 has been validated by zRMS.

For the intended uses in cereals, calculated PEC/RAC ratios for azoxystrobin did not indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for aquatic invertebrates as characterised by an EC_{50} and NOEC for *M. bahia* of 55 and 9.54 $\mu\text{g a.s./L}$, respectively, in connection with an assessment factor of 100 and 10, respectively) in several FOCUS Steps 3 scenarios.

Based on the performed calculations the following conclusions at FOCUS STEP 3 may be derived:

1. Winter cereals cereals at BBCH 30-69:

- Azoxystrobin : an unacceptable acute risk can be concluded for D1, D2, D3, and D4 (s), D5 (s), D6, R1 (s), R3 and R4 scenarios
- Azoxystrobin : an unacceptable long-term risk can be concluded for D1, D2, D3, and D4 (s), D6, R1 (s), R3 and R4 scenarios

2. Spring cereals at BBCH 30-69:

- Azoxystrobin : an unacceptable acute risk can be concluded for D1, D3, and D4 (s), D5 (s) and R4 scenarios
- Azoxystrobin : an unacceptable long-term risk can be concluded for D1, D3, and R4 scenarios

It should be noted that the risk from R scenarios not defined for spring cereals is covered by the risk assessment performed for these scenarios available for winter cereals.

3. Winter oilseed rape at BBCH 61-73

- Azoxystrobin : an unacceptable acute risk can be concluded for D2, D3, and D4 (s), D5 (s), R1 (s), R3 scenarios
- Azoxystrobin : an unacceptable chronic risk can be concluded for D2, D3, R1 (s), R3 scenarios

4. Spring oilseed rape at BBCH 61-73

- Azoxystrobin : an unacceptable acute risk can be concluded for D1, D3, and D4 (s), D5(s), R1 (s) scenarios
- Azoxystrobin : an unacceptable chronic risk can be concluded for D1, D3, R1 (s) scenarios

The refined risk based on the EU agreed weight-of-evidence approach with a RAC of 3.3 $\mu\text{g a.s./L}$ an acceptable risk for all FOCUS Step 3 scenarios except D1 and D2 Ditch scenarios for winter cereals.

Further PEC/RAC ratios were calculated for cereals for scenario D2 Ditch of the EU agreed weight-of-evidence approach-based FOCUS Step 4 PEC_{SW} considering 20-meter vegetative buffer zone to surface water bodies.

Based on the results of the risk assessments at FOCUS Step 4 with the EU agreed weight-of-evidence approach for aquatic invertebrates ($RAC = 3.3 \mu\text{g a.s./L}$) **a high risk is indicated** for the FOCUS scenarios D2 Ditch with 20-meter vegetative buffer zone for winter cereals.

It is to be noted that D2 Ditch scenario and thus appropriate risk mitigation measures are to be considered only for France where this FOCUS Scenario is relevant.

Since the main entry route is *via drainage* a label restriction is deemed necessary according to Regulation (EU) No. 547/2011, and if this scenario is relevant at national MS level, the safety precaution phrase SPe2 shall be applied: „To protect groundwater/aquatic organisms do not apply to drained soils.“

In addition, it should be noted that that D1 and D2 Ditch scenarios are not relevant across Member States of Central Zone, thus no further risk assessment is conducted for this specific exposure scenario for central zone countries.

Metabolite R401553

Table 9.5-11: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the metabolite R401553 for each organism group based on FOCUS Steps 1, 2 calculations for the use of FGF11 in winter and spring cereals (1 × 160 g a.s./ha, BBCH 30-69)

Group		Fish acute	Inverteb. acute	Algae	Sediment dweller chronic (spiked water)
Test species		<i>O. mykiss</i>	<i>D. magna</i>	<i>R. subcapitata</i>	<i>C. riparius</i> *
Endpoint		LC ₅₀	EC ₅₀	ErC ₅₀	NOEC
(µg/L)		> 120000	> 120000	> 120000	80
AF		100	100	10	10
RAC (µg/L)		1200	1200	12000	8
FOCUS Scenario	PEC _{sw-max} (µg/L)				
Step 1		PEC/RAC ratios			
	6.20	< 0.01	< 0.01	< 0.001	0.78
Step 2 - N-EU					
Mar-Sep	0.435	< 0.001	< 0.001	< 0.001	0.054
Step 2 - S-EU					
Oct-May	0.809	< 0.001	< 0.001	< 0.001	0.10
Jun-Sep	0.622	< 0.001	< 0.001	< 0.001	0.08

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

*surrogate parent endpoint

Table 9.5-12: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the metabolite R401553 for each organism group based on FOCUS Steps 1, 2 calculations for the use of FGF11 in winter and spring oilseed rape (1 × 160 g a.s./ha, BBCH 61-73)

Group		Fish acute	Inverteb. acute	Algae	Sediment dweller chronic (spiked water)
Test species		<i>O. mykiss</i>	<i>D. magna</i>	<i>R. subcapitata</i>	<i>C. riparius</i> *
Endpoint		LC ₅₀	EC ₅₀	ErC ₅₀	NOEC
(µg/L)		> 120000	> 120000	> 120000	80
AF		100	100	10	10
RAC (µg/L)		1200	1200	12000	8
FOCUS Scenario	PEC _{sw-max} (µg/L)				
Step 1		PEC/RAC ratios			
	6.20	< 0.01	< 0.01	< 0.001	0.78
Step 2 - N-EU					
Mar-Sep	0.178	< 0.001	< 0.001	< 0.001	0.02
Step 2 - S-EU					
Oct-May	0.295	< 0.001	< 0.001	< 0.001	0.04
Jun-Sep	0.237	< 0.001	< 0.001	< 0.001	0.03

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

*surrogate parent endpoint

Metabolite R402173

Table 9.5-13: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the metabolite R402173 for each organism group based on FOCUS Steps 1, 2 calculations for the use of FGF11 in winter and spring cereals (1 × 160 g a.s./ha, BBCH 30-69) - acidic soil

Group		Fish acute	Inverteb. acute	Algae	Sediment dweller chronic (spiked water)
Test species		<i>O. mykiss</i>	<i>D. magna</i>	<i>R. subcapitata</i>	<i>C. riparius</i> *
Endpoint		LC ₅₀	EC ₅₀	ErC ₅₀	NOEC
(µg/L)		62000	> 100000	67000	80
AF		100	100	10	10
RAC (µg/L)		620	1000	6700	8
FOCUS Scenario	PEC _{sw-max} (µg/L)				
Step 1		PEC/RAC ratios			
	6.78	0.01	< 0.01	0.001	0.85
Step 2 - N-EU					
Mar-Sep	0.678	0.001	< 0.001	< 0.001	0.08
Step 2 - S-EU					
Oct-May	1.33	0.002	< 0.001	< 0.001	0.17
Jun-Sep	1.00	0.002	< 0.001	< 0.001	0.13

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

*surrogate parent endpoint

Table 9.5-14: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the metabolite R402173 for each organism group based on FOCUS Steps 1, 2 calculations for the use of FGF11 in winter and spring cereals (1 × 160 g a.s./ha, BBCH 30-69) - alkaline soil

Group		Fish acute	Inverteb. acute	Algae	Sediment dweller chronic (spiked water)
Test species		<i>O. mykiss</i>	<i>D. magna</i>	<i>R. subcapitata</i>	<i>C. riparius</i> *
Endpoint		LC ₅₀	EC ₅₀	ErC ₅₀	NOEC
(µg/L)		62000	> 100000	67000	80
AF		100	100	10	10
RAC (µg/L)		620	1000	6700	8
FOCUS Scenario	PEC _{sw-max} (µg/L)				
Step 1		PEC/RAC ratios			
	8.30	0.01	< 0.01	0.001	1.04
Step 2 - N-EU					
Mar-Sep	0.830	0.001	< 0.001	< 0.001	0.10
Step 2 - S-EU					
Oct-May	1.63	0.003	< 0.002	< 0.001	0.20
Jun-Sep	1.23	0.002	< 0.001	< 0.001	0.15

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

*surrogate parent endpoint

Table 9.5-15: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the metabolite R402173 for each organism group based on FOCUS Steps 1, 2 calculations for the use of FGF11 in winter and spring oilseed rape (1 × 160 g a.s./ha, BBCH 61-73) - acidic soil

Group		Fish acute	Inverteb. acute	Algae	Sediment dweller chronic (spiked water)
Test species		<i>O. mykiss</i>	<i>D. magna</i>	<i>R. subcapitata</i>	<i>C. riparius</i> *
Endpoint		LC ₅₀	EC ₅₀	ErC ₅₀	NOEC
(µg/L)		62000	> 100000	67000	80
AF		100	100	10	10
RAC (µg/L)		620	1000	6700	8
FOCUS Scenario	PEC _{sw-max} (µg/L)				
Step 1		PEC/RAC ratios			
	6.78	0.01	< 0.01	0.001	0.85
Step 2 - N-EU					
Mar-Sep	0.229	< 0.001	< 0.001	< 0.001	0.03
Step 2 - S-EU					
Oct-May	0.433	< 0.001	< 0.001	< 0.001	0.05
Jun-Sep	0.331	< 0.001	< 0.001	< 0.001	0.04

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

*surrogate parent endpoint

Table 9.5-16: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the metabolite R402173 for each organism group based on FOCUS Steps 1, 2 calculations for the use of FGF11 in winter and spring oilseed rape (1 × 160 g a.s./ha, BBCH 61-73) - alkaline soil

Group		Fish acute	Inverteb. acute	Algae	Sediment dweller chronic (spiked water)
Test species		<i>O. mykiss</i>	<i>D. magna</i>	<i>R. subcapitata</i>	<i>C. riparius</i> *
Endpoint		LC ₅₀	EC ₅₀	ErC ₅₀	NOEC
(µg/L)		62000	> 100000	67000	80
AF		100	100	10	10
RAC (µg/L)		620	1000	6700	8
FOCUS Scenario	PEC _{sw-max} (µg/L)				
Step 1		PEC/RAC ratios			
	8.30	0.01	< 0.01	0.001	1.04
Step 2 - N-EU					
Mar-Sep	0.279	< 0.001	< 0.001	< 0.001	0.03
Step 2 - S-EU					
Oct-May	0.529	< 0.001	< 0.001	< 0.001	0.07
Jun-Sep	0.404	< 0.001	< 0.001	< 0.001	0.05

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

*surrogate parent endpoint

Metabolite R234886

Table 9.5-17: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the metabolite R234886 for each organism group based on FOCUS Steps 1, 2 calculations for the use of FGF11 in winter and spring cereals (1 × 160 g a.s./ha, BBCH 30-69) - acidic soil

Group		Fish acute	Inverteb. acute	Algae	Sediment dweller chronic (spiked water)
Test species		<i>O. mykiss</i>	<i>D. magna</i>	<i>R. subcapitata</i>	<i>C. riparius</i> *
Endpoint		LC ₅₀	EC ₅₀	ErC ₅₀	NOEC
(µg/L)		>150000	> 180000	47000	80
AF		100	100	10	10
RAC (µg/L)		>1500	>1800	4700	8
FOCUS Scenario	PEC _{sw-max} (µg/L)				
Step 1		PEC/RAC ratios			
	14.9	<0.0099	< 0.0083	0.0032	1.86
Step 2 - N-EU					
Mar-Sep	2.44	<0.0016	< 0.0013	0.0005	0.31
Step 2 - S-EU					
Oct-May	4.71	<0.003	< 0.003	0.001	0.59
Jun-Sep	3.58	<0.002	< 0.002	0.001	0.45

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

*surrogate parent endpoint

Table 9.5-18: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the metabolite R234886 for each organism group based on FOCUS Steps 1, 2 calculations for the use of FGF11 in winter and spring cereals (1 × 160 g a.s./ha, BBCH 30-69) - alkaline soil

Group		Fish acute	Inverteb. acute	Algae	Sediment dweller chronic (spiked water)
Test species		<i>O. mykiss</i>	<i>D. magna</i>	<i>R. subcapitata</i>	<i>C. riparius</i> *
Endpoint		LC ₅₀	EC ₅₀	ErC ₅₀	NOEC
(µg/L)		>150000	> 180000	47000	80
AF		100	100	10	10
RAC (µg/L)		>1500	>1800	4700	8
FOCUS Scenario	PEC _{sw-max} (µg/L)				
Step 1		PEC/RAC ratios			
	23.7	<0.016	< 0.013	0.005	2.96
Step 2 - N-EU					
Mar-Sep	3.79	<0.003	< 0.002	0.001	0.47
Step 2 - S-EU					
Oct-May	7.33	0.005	< 0.004	0.002	0.92
Jun-Sep	5.56	0.004	< 0.003	0.001	0.70

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

*surrogate parent endpoint

Table 9.5-19: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the metabolite R234886 for each organism group based on FOCUS Steps 1, 2 calculations for the use of FGF11 in winter and spring oilseed rape (1 × 160 g a.s./ha, BBCH 61-73) - acidic soil

Group		Fish acute	Inverteb. acute	Algae	Sediment dweller chronic (spiked water)
Test species		<i>O. mykiss</i>	<i>D. magna</i>	<i>R. subcapitata</i>	<i>C. riparius</i> *
Endpoint		LC ₅₀	EC ₅₀	ErC ₅₀	NOEC
(µg/L)		>150000	> 180000	47000	80
AF		100	100	10	10
RAC (µg/L)		>1500	>1800	4700	8
FOCUS Scenario	PEC _{sw-max} (µg/L)				
Step 1		PEC/RAC ratios			
	14.9	<0.01	< 0.008	0.003	1.86
Step 2 - N-EU					
Mar-Sep	0.887	<0.0006	< 0.0005	0.0002	0.11
Step 2 - S-EU					
Oct-May	1.59	<0.001	< 0.0009	0.0003	0.20
Jun-Sep	1.24	<0.0008	< 0.0007	0.0003	0.16

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

*surrogate parent endpoint

Table 9.5-20: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the metabolite R234886 for each organism group based on FOCUS Steps 1, 2 calculations for the use of FGF11 in winter and spring oilseed rape (1 × 160 g a.s./ha, BBCH 61-73) - alkaline soil

Group		Fish acute	Inverteb. acute	Algae	Sediment dweller chronic (spiked water)
Test species		<i>O. mykiss</i>	<i>D. magna</i>	<i>R. subcapitata</i>	<i>C. riparius</i> *
Endpoint		LC ₅₀	EC ₅₀	ErC ₅₀	NOEC
(µg/L)		>150000	> 180000	47000	80
AF		100	100	10	10
RAC (µg/L)		>1500	>1800	4700	8
FOCUS Scenario	PEC _{sw-max} (µg/L)				
Step 1		PEC/RAC ratios			
	23.7	<0.016	< 0.013	0.005	2.96
Step 2 - N-EU					
Mar-Sep	1.36	<0.0009	< 0.0007	0.00031	0.17
Step 2 - S-EU					
Oct-May	2.46	<0.0016	< 0.0014	0.0005	0.31
Jun-Sep	1.91	<0.0013	< 0.001	0.0004	0.24

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

*surrogate parent endpoint

For the metabolites of azoxystrobin (R401553, R402173 and R234886), an acceptable risk was indicated for the group of aquatic organisms at Focus Step 1, 2 including acidic and alkaline soils (for R402173 and R234886). Therefore, no further assessment is deemed necessary.

zRMS comments:

The calculations of the risk assessment for the aquatic organism for the metabolites of azoxystrobin (R401553, R402173 and R234886), indicated an acceptable risk at Focus Step 1, 2 including acidic and alkaline soils (for R402173 and R234886). Therefore, no further assessment is deemed necessary.

Prothioconazole and relevant metabolites

Prothioconazole - a.s.

Table 9.5-21: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prothioconazole for each organism group based on FOCUS Steps 1, 2 calculations for the use of FGF11 in cereals (BBCH 30-69) and oilseed rape (BBCH 61-73) - 1 × 200 g a.s./ha

Group		Fish acute	Fish chronic	Inverteb. acute	Inverteb. chronic	Algae	Sediment dweller chronic (spiked water)
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>P. subcapitata</i>	<i>C. riparius</i>
Endpoint		LC ₅₀	NOEC	EC ₅₀	NOEC	ErC ₅₀	NOEC
(µg/L)		1830	308	1300	560	2180	9140
AF		100	10	100	10	10	10
RAC (µg/L)		18.3	30.8	13	56	218	914
FOCUS Scenario	PEC _{sw-max} (µg/L)						
Step 1		PEC/RAC ratios					
	21.7	1.19	0.70	1.67	0.39	0.10	0.02
Step 2							
N-EU	1.84	0.10	0.06	0.14	0.03	0.01	0.002
S-EU	1.84	0.10	0.06	0.14	0.03	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**

zRMS comments:

The calculations of the risk assessment for the aquatic organism for prothioconazole indicated an acceptable risk at Focus Step 1, 2 as PEC/RAC value is below trigger of 1 for all proposed uses. No additional calculations are required.

Metabolite prothioconazole-S-methyl (M01)

Table 9.5-22: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the metabolite prothioconazole-S-methyl (M01) for each organism group based on FOCUS Steps 1, 2 calculations for the use of FGF11 in winter and spring cereals (1 × 200 g a.s./ha, BBCH 30-69)

Group		Fish acute	Inverteb. acute	Algae	Sediment dweller chronic (spiked water)
Test species		<i>O. mykiss</i>	<i>D. magna</i>	<i>P. subcapitata</i>	<i>C. riparius</i>
Endpoint		LC ₅₀	EC ₅₀	ErC ₅₀	NOEC*
(µg/L)		1800	2800	47400	914
AF		100	100	10	10
RAC (µg/L)		18	28	4740	91.4
FOCUS Scenario	PEC _{sw-max} (µg/L)				
Step 1		PEC/RAC ratios			
	4.58	0.254	0.164	< 0.001	0.050
Step 2 – N-EU					
Mar - Sept	0.419	0.023	0.015	< 0.001	0.005
Step 2 – S-EU					
Oct-May	0.762	0.042	0.027	< 0.001	0.008
Jun - Sept	0.59	0.033	0.021	< 0.001	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**

* Surrogate parent endpoint

Table 9.5-23: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the metabolite prothioconazole-S-methyl (M01) for each organism group based on FOCUS Steps 1, 2 calculations for the use of FGF11 in winter and spring oilseed rape (1 × 200 g a.s./ha, BBCH 61-73)

Group		Fish acute	Inverteb. acute	Algae	Sediment dweller chronic (spiked water)
Test species		<i>O. mykiss</i>	<i>D. magna</i>	<i>P. subcapitata</i>	<i>C. riparius</i>
Endpoint		LC ₅₀	EC ₅₀	ErC ₅₀	NOEC*
(µg/L)		1800	2800	47400	914
AF		100	100	10	10
RAC (µg/L)		18	28	4740	91.4
FOCUS Scenario	PEC _{sw-max} (µg/L)				
Step 1		PEC/RAC ratios			
	4.58	0.254	0.164	< 0.001	0.050
Step 2 – N-EU					
Mar - Sept	0.243	0.014	0.009	< 0.001	0.003
Step 2 – S-EU					
Oct-May	0.290	0.016	0.010	< 0.001	0.003
Jun - Sept	0.243	0.014	0.009	< 0.001	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** *Surrogate parent endpoint

zRMS comments:

The calculations of the risk assessment for the aquatic organism for metabolite prothioconazole-S-methyl (M01) indicated an acceptable risk at Focus Step 1, 2 as PEC/RAC value is below trigger of 1 for all proposed uses. No additional calculations are required.

Metabolite prothioconazole-desthio (M04)

Table 9.5-24: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the metabolite prothioconazole-desthio (M04) for each organism group based on FOCUS Steps 1, 2 calculations for the use of FGF11 in winter and spring cereals (1 × 200 g a.s./ha, BBCH 30-69)

Group		Fish acute	Fish chronic	Inverteb. acute	Inverteb. chronic	Algae	Sediment dweller chronic (spiked water)
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>S. subspicatus</i>	<i>C. riparius</i>
Endpoint		LC ₅₀	NOEC	EC ₅₀	NOEC	ErC ₅₀	NOEC
(µg/L)		6630	3.34	>10000	100	550	2000
AF		100	10	100	10	10	10
RAC (µg/L)		66.3	0.334	>100	10	55	200
FOCUS Scenario	PEC _{sw-max} (µg/L)						
Step 1		PEC/RAC ratios					
	39.2	0.59	117.37	<0.39	3.92	0.71	0.20
Step 2							
N-EU	3.67	0.055	10.99	<0.037	0.367	0.067	0.018
Step 2 – S-EU							
Oct - May	6.74	0.102	20.18	<0.067	0.674	0.123	0.034
Jun - Sept	5.20	0.078	15.57	<0.052	0.520	0.095	0.026

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 9.5-25: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the metabolite prothioconazole-desthio (M04) for each organism group based on FOCUS Steps 1, 2 calculations for the use of FGF11 in winter and spring oilseed rape (1 × 200 g a.s./ha, BBCH 61-73)

Group		Fish acute	Fish chronic	Inverteb. acute	Inverteb. chronic	Algae	Sediment dweller chronic (spiked water)
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>S. subspicatus</i>	<i>C. riparius</i>
Endpoint		LC ₅₀	NOEC	EC ₅₀	NOEC	ErC ₅₀	NOEC
(µg/L)		6630	3.34	>10000	100	550	2000
AF		100	10	100	10	10	10
RAC (µg/L)		66.3	0.334	>100	10	55	200
FOCUS Scenario	PEC _{sw-max} (µg/L)						
Step 1		PEC/RAC ratios					
	39.2	0.59	117.37	<0.39	3.92	0.71	0.20
Step 2 – N-EU							
N-EU	1.56	0.02	4.67	<0.02	0.16	0.03	0.008
Step 2 – S-EU							
Oct - May	2.52	0.04	7.54	<0.03	0.25	0.05	0.013
Jun - Sept	2.04	0.03	6.11	<0.02	0.20	0.04	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**

Table 9.5-26: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the metabolite prothioconazole-desthio (M04) for chronic fish based on FOCUS Steps 3 calculations for the use of FGF11 in winter cereals (1 × 200 g a.s./ha, BBCH 30-69)

Group		Fish chronic
Test species		<i>O. mykiss</i>
Endpoint		NOEC
(µg/L)		3.34
AF		10
RAC (µg/L)		0.334
FOCUS Scenario	PEC _{sw-max} (µg/L)	
BBCH > 30		
D1 Ditch	0.005	0.01
D1 Stream	0.019	0.06
D2 Ditch	0.029	0.09
D2 Stream	0.07	0.21
D3 Ditch	0.003	0.01
D4 Pond	0.007	0.02
D4 Stream	0.005	0.01
D5 Pond	0.011	0.03
D5 Stream	0.011	0.03
D6 Ditch	0.002	0.01
R1 Pond	0.043	0.13
R1 Stream	0.374	1.12
R3 Stream	0.463	1.39
R4 Stream	0.684	2.05
BBCH < 69		
D1 Ditch	0.104	0.31
D1 Stream	0.022	0.07
D2 Ditch	0.062	0.19
D2 Stream	0.094	0.28
D3 Ditch	0.01	0.03
D4 Pond	0.011	0.03
D4 Stream	0.008	0.02
D5 Pond	0.013	0.04
D5 Stream	0.014	0.04
D6 Ditch	0.015	0.04
R1 Pond	0.035	0.10
R1 Stream	0.239	0.72
R3 Stream	0.451	1.35
R4 Stream	0.485	1.45

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 9.5-27: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the metabolite prothioconazole-desthio (M04) for chronic fish based on FOCUS Steps 3 calculations for the use of FGF11 in spring cereals (1 × 200 g a.s./ha, BBCH 30-69)

Group		Fish chronic
Test species		<i>O. mykiss</i>
Endpoint		NOEC
(µg/L)		3.34
AF		10
RAC (µg/L)		0.334
FOCUS Scenario	PEC _{sw-max} (µg/L)	
BBCH > 30		
D1 Ditch	0.104	0.31
D1 Stream	0.022	0.07
D3 Ditch	0.007	0.02
D4 Pond	0.011	0.03
D4 Stream	0.006	0.02
D5 Pond	0.011	0.03
D5 Stream	0.011	0.03
R4 Stream	0.550	1.65
BBCH < 69		
D1 Ditch	0.112	0.34
D1 Stream	0.022	0.07
D3 Ditch	0.008	0.02
D4 Pond	0.01	0.03
D4 Stream	0.009	0.03
D5 Pond	0.012	0.04
D5 Stream	0.012	0.04
R4 Stream	0.566	1.69

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 9.5-28: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the metabolite prothioconazole-desthio (M04) for chronic fish based on FOCUS Steps 3 calculations for the use of FGF11 in winter oilseed rape (1 × 200 g a.s./ha, BBCH 61-73)

Group		Fish chronic
Test species		<i>O. mykiss</i>
Endpoint		NOEC
(µg/L)		3.34
AF		10
RAC (µg/L)		0.334
FOCUS Scenario	PEC _{sw-max} (µg/L)	
BBCH > 61		
D2 Ditch	0.161	0.48
D2 Stream	0.13	0.39
D3 Ditch	0.009	0.03
D4 Pond	0.011	0.03
D4 Stream	0.008	0.02
D5 Pond	0.013	0.04
D5 Stream	0.014	0.04
R1 Pond	0.039	0.12
R1 Stream	0.272	0.81
R3 Stream	0.349	1.04

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 9.5-29: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the metabolite prothioconazole-desthio (M04) for chronic fish based on FOCUS Steps 3 calculations for the use of FGF11 in spring oilseed rape (1 × 200 g a.s./ha, BBCH 61-73)

Group		Fish chronic
Test species		<i>O. mykiss</i>
Endpoint		NOEC
(µg/L)		3.34
AF		10
RAC (µg/L)		0.334
FOCUS Scenario	PEC _{sw-max} (µg/L)	
BBCH > 61		
D1 Ditch	0.111	0.33
D1 Stream	0.024	0.07
D3 Ditch	0.012	0.04
D4 Pond	0.011	0.03
D4 Stream	0.009	0.03
D5 Pond	0.013	0.04
D5 Stream	0.014	0.04
R1 Pond	0.082	0.25
R1 Stream	0.321	0.96

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 the intended uses in cereals, calculated PEC/RAC ratios for the metabolite prothioconazole-desthio (M04) did not indicate an acceptable risk for chronic fish (risk characterised by NOEC for *O. mykiss* of 3.34 µg p.m./L, in connection with an assessment factor of 10) in several FOCUS Steps 3 scenarios (R1 Stream, R3 Stream, R4 Stream). Therefore, further PEC/RAC ratios were calculated based on FOCUS

Step 4 PEC_{SW} considering reduced exposure of surface water bodies.

For the intended uses in winter oilseed rape, calculated PEC/RAC ratios for the prothioconazole metabolite prothioconazole-desthio (M04) did not indicate an acceptable risk for chronic fish (risk characterised by NOEC for *O. mykiss* of 3.34 µg p.m./L, in connection with an assessment factor of 10) at FOCUS Step 3 - R3 Stream scenario.

For the use in spring oilseed rape, a safe use was indicated for the metabolite prothioconazole-desthio (M04) at FOCUS Step 3. Therefore, no further assessment is deemed necessary.

Table 9.5-30: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the metabolite prothioconazole-desthio (M04) for chronic fish based on FOCUS Step 4 mitigations for the use of FGF11 in winter cereals (1 × 200 g a.s./ha, BBCH 30-69)

VFSmod	-	-	-	-	-	-	5 m
Vegetated runoff buffer	0 m	0 m	0 m	10 m	10 m	20 m	-
No-spray buffer	0 m	0 m	0 m	10 m	10 m	20 m	-
Drift-reducing nozzles	0%	75%	90%	0%	50%	0%	-
BBCH > 30	PEC_{SW} values						
R1 Stream	0.374	0.374	0.374	0.17	0.17	0.089	0.024
R3 Stream	0.463	0.463	0.463	0.211	0.211	0.111	0.194
R4 Stream	0.684	0.684	0.684	0.311	0.311	0.163	0.084
BBCH < 69							
R3 Stream	0.451	0.451	0.451	0.203	0.203	0.106	0.006
R4 Stream	0.485	0.485	0.485	0.221	0.221	0.116	0.137
Fish chronic: 0.334	PEC/RAC ratios - BBCH > 30						
R1 Stream	1.12	1.12	1.12	0.51	0.51	0.27	0.07
R3 Stream	1.39	1.39	1.39	0.63	0.63	0.33	0.58
R4 Stream	2.05	2.05	2.05	0.93	0.93	0.49	0.25
				PEC/RAC ratios - BBCH < 69			
R3 Stream	1.35	1.35	1.35	0.61	0.61	0.32	0.02
R4 Stream	1.45	1.45	1.45	0.66	0.66	0.35	0.41

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 9.5-31: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the metabolite prothioconazole-desthio (M04) for chronic fish based on FOCUS Step 4 mitigations for the use of FGF11 in spring cereals (1 × 200 g a.s./ha, BBCH 30-69)

VFSmod	-	-	-	-	-	5 m
Vegetated runoff buffer	0 m	0 m	0 m	10 m	20 m	-
No-spray buffer	0 m	0 m	5 m	10 m	20 m	-
Drift-reducing nozzles	0%	90%	75%	50%	0%	-
BBCH > 30	PEC _{sw} values					
R4 Stream	0.55	0.55	0.55	0.25	0.131	0.15
BBCH < 69						
R4 Stream	0.566	0.566	0.566	0.258	0.135	0.156
Fish chronic: 0.334	PEC/RAC ratios - BBCH > 30					
R4 Stream	1.65	1.65	1.65	0.75	0.39	0.45
	PEC/RAC ratios - BBCH < 69					
R4 Stream	1.69	1.69	1.69	0.77	0.40	0.47

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 the metabolite prothioconazole-desthio (M04) for chronic fish based on FOCUS Step 4 mitigations for the use of FGF11 in winter oilseed rape (1 × 200 g a.s./ha, BBCH 61-73)

VFSmod	-	-	-	-	-	5 m
Vegetated runoff buffer	0 m	0 m	0 m	10 m	20 m	-
No-spray buffer	0 m	0 m	0 m	10 m	20 m	-
Drift-reducing nozzles	0%	50%	90%	50%	0%	-
BBCH > 61	PEC _{sw} values					
R3 Stream	0.349	0.349	0.349	0.156	0.081	0.006
Fish chronic: 0.334	PEC/RAC ratios - BBCH > 61					
R3 Stream	1.04	1.04	1.04	0.47	0.24	0.02

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 the intended uses in cereals and oilseed rape, calculated PEC/RAC ratios for the metabolite prothioconazole-desthio (M04) indicate a safe risk for the group of chronic fish at FOCUS Step 4 when 10 m vegetative buffer strip + 10 m no-spray buffer zone are applied in winter cereals (BBCH 30-69). For spring cereals (BBCH 30-69) and winter oilseed rape (BBCH 61-73) a 10 m vegetative buffer strip + 10 m no-spray buffer zone + 50% drift reduction are required. No mitigation measures are required for applications in spring oilseed rape.

zRMS comments:

Winter and spring cereals:

The calculations of the risk assessment for metabolite prothioconazole-desthio (M04) to aquatic organism in cereals provided from Tables 9.5-25 to 9.5-27 has been validated by zRMS.

For the intended uses in winter cereals, calculated PEC/RAC ratios for the metabolite prothioconazole-desthio (M04) did not indicate an acceptable risk for chronic fish in several FOCUS Steps 3 scenarios (R1 Stream, R3 Stream, R4 Stream). Therefore, further PEC/RAC ratios were calculated for these scenarios (R1, R3 and R4) is based on FOCUS Step 4 PEC_{sw} scenario, with 10-meter vegetative buffer zone to surface water bodies and in case of use in spring cereals for R4 scenario with 10 m vegetative buffer zone + 50% drift reduction and then acceptable risk has been concluded.

It should be noted that the risk from R scenarios not defined for spring cereals is covered by the risk assessment performed for these scenarios available for winter cereals.

For this reason, for R1 and R3 scenarios – 10-meter vegetative buffer zone to surface water bodies is applied.

Winter and spring oilseed rape:

For the intended uses in winter oilseed rape, calculated PEC/RAC ratios for the prothioconazole metabolite prothioconazole-desthio (M04) did not indicate an acceptable risk for chronic fish at FOCUS Step 3 for R3 Stream scenario. Therefore, further PEC/RAC ratios were calculated based on FOCUS Step 4 PEC_{sw} with 10 vegetative buffer zone + 50% drift reduction and then acceptable risk has been concluded.

For the use in spring oilseed rape, a safe use was indicated for the metabolite prothioconazole-desthio (M04) at FOCUS Step 3. Therefore, no further assessment is deemed necessary.

Metabolite 1,2,4-triazole (M13)

Table 9.5-33: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the metabolite 1,2,4-triazole (M13) for each organism group based on FOCUS Steps 1, 2 calculations for the use of FGF11 in winter and spring cereals (1 × 200 g a.s./ha, BBCH 30-69)

Group		Fish acute	Fish chronic	Inverteb. acute	Algae	Sediment dweller chronic (spiked water)
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>P. subcapitata</i>	<i>C. riparius</i>
Endpoint		LC ₅₀	NOEC	EC ₅₀	ErC ₅₀	NOEC*
(µg/L)		498000	3200	900000	22500	9140
AF		100	10	100	10	10
RAC (µg/L)		4980	320	9000	2250	914
FOCUS Scenario	PEC _{sw-max} (µg/L)					
Step 1		PEC/RAC ratios				
	5.43	0.00	0.02	< 0.001	0.00	0.01
Step 2 – N-EU						
Mar - Sept	0.262	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Step 2 – S-EU						
Oct-May	0.380	< 0.001	0.001	< 0.001	< 0.001	< 0.001
Jun - Sept	0.321	< 0.001	0.001	< 0.001	< 0.001	< 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**

* Surrogate parent endpoint

Table 9.5-34: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the metabolite 1,2,4-triazole (M13) for each organism group based on FOCUS Steps 1, 2 calculations for the use of FGF11 in winter and spring oilseed rape (1 × 200 g a.s./ha, BBCH 61-73)

Group		Fish acute	Fish chronic	Inverteb. acute	Algae	Sediment dweller chronic (spiked water)
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>P. subcapitata</i>	<i>C. riparius</i>
Endpoint		LC ₅₀	NOEC	EC ₅₀	ErC ₅₀	NOEC*
(µg/L)		498000	3200	900000	22500	9140
AF		100	10	100	10	10
RAC (µg/L)		4980	320	9000	2250	914
FOCUS Scenario	PEC _{sw-max} (µg/L)					
Step 1		PEC/RAC ratios				
	5.43	0.001	0.017	< 0.001	0.002	0.006
Step 2 – N-EU						
Mar - Sept	0.180	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Step 2 – S-EU						
Oct-May	0.217	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Jun - Sept	0.199	< 0.001	< 0.001	< 0.001	< 0.001	< 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**

* Surrogate parent endpoint

For the intended uses in cereals and oilseed rape, calculated PEC/RAC ratios for the metabolite 1,2,4-triazole (M13) indicate a safe risk for the group of aquatic organisms at Focus Step 1, 2. Therefore, no further assessment is deemed necessary.

zRMS comments:

The calculations of the risk assessment for the aquatic organism for metabolite 1,2,4-triazole (M13) indicated an acceptable risk at Focus Step 1, 2 as PEC/RAC value is below trigger of 1 for all proposed uses. No additional calculations are required.

9.5.2.2 Mixture toxicity risk assessment

The evaluation of the mixture toxicity risks for aquatic (and sediment-dwelling) organisms was performed in accordance with the “Guidance 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) and in EFSA Journal 2013;11(7):3290 (Aquatic Guidance Document, abbreviated as EFSA, 2013). The whole mixture assessment is based on the model of concentration addition (CA) and particularly the decision scheme as provided by EFSA (2013, section 10.3.11) was applied.

To keep the assessment concise the focus of this section of this registration report is on the parts of the assessment leading to the most critical results, i.e. application on winter cereals at 1 kg/ha, according to the FOCUS scenarios assessed in B8 (BBCH>30 and BBCH < 69).

The calculator tool AGD_AquaMix¹ (v.1.22) was adopted, and the raw excel files can be found attached to present document at the end of the mixture toxicity assessment.

Mixture toxicity assessment has been considered for combination of azoxystrobin + prothioconazole and

¹ “Aquatic MixTox Tool”. The current version can be found on the EFSA Knowledge Junction platform, DOI: [10.5281/zenodo.4593675](https://doi.org/10.5281/zenodo.4593675) (Aquatic Mixture Toxicity Tool and additional Information | Zenodo)

of azoxystrobin + prothioconazole-desthio.

Mixture toxicity assessment of azoxystrobin + prothioconazole

Mixture toxicity endpoints are assessed from active substances azoxystrobin and prothioconazole endpoints, where possible derived on the same test organisms. For azoxystrobin and prothioconazole, acute fish data are available for *Oncorhynchus mykiss*. For acute invertebrates, endpoints were applied from studies available on *Daphnia magna*. For algae data on *Selenastrum capricornutum* (formerly *Pseudokirchneriella subcapitata*) were adopted for both aa.ss..

Toxicity data on formulation provided for acute exposure to fish and effect on algae, have been also included in the mixture toxicity assessment.

Screening Step on ETR (Simplified approach)

According to EFSA 2013, if no synergistic effects are indicated and the ETR (Exposure Toxicity Ratio) values of the individual a.s. (ETR_i) contained in the formulation are below the relevant trigger value, the mixture RA can follow a simplified approach

For algae, where no synergism was detected (see table 9.5-36), this simplified approach was checked:

Table 9.5-35: Screening step 1 for Algae (BBCH>30)

Algae	ETR		Any a.s. above ETRtrigger?	ETRtrigger/n (if needed)	All ETR _i ≤ ETRtrigger/n?
	azo	prothio			
Step 1	0.099	0.01	no	0.05	no
Step 2					
N-Europe	0.018	0.001	no	0.05	yes
S-Europe	0.033	0.001	no	0.05	yes
Step 3					
D1 Ditch	0.009	0.001	no	0.05	yes
D1 Stream	0.006	0	no	0.05	yes
D2 Ditch	0.011	0.001	no	0.05	yes
D2 Stream	0.007	0.001	no	0.05	yes
D3 Ditch	0.003	0.001	no	0.05	yes
D4 Pond	0.001	0	no	0.05	yes
D4 Stream	0.002	0	no	0.05	yes
D5 Pond	0	0	no	0.05	yes
D5 Stream	0.002	0	no	0.05	yes
D6 Ditch	0.003	0.001	no	0.05	yes
R1 Pond	0	0	no	0.05	yes
R1 Stream	0.003	0	no	0.05	yes
R2 Stream					
R3 Stream	0.004	0.001	no	0.05	yes
R4 Stream	0.005	0	no	0.05	yes

Table 9.5-36: Screening step 1 for Algae (BBCH<69)

Algae	ETR		Any a.s. above ETRtrigger?	ETRtrigger/n (if needed)	All ETRi ≤ ETRtrigger/n?
	azo	prothio			
Step 1	0.099	0.01	no	0.05	no
Step 2					
N-Europe	0.018	0.001	no	0.05	yes
S-Europe	0.033	0.001	no	0.05	yes
Step 3					
D1 Ditch	0.005	0.001	no	0.05	yes
D1 Stream	0.003	0.001	no	0.05	yes
D2 Ditch	0.006	0.001	no	0.05	yes
D2 Stream	0.004	0.001	no	0.05	yes
D3 Ditch	0.003	0.001	no	0.05	yes
D4 Pond	0.001	0	no	0.05	yes
D4 Stream	0.002	0.001	no	0.05	yes
D5 Pond	0	0	no	0.05	yes
D5 Stream	0.003	0.001	no	0.05	yes
D6 Ditch	0.003	0.001	no	0.05	yes
R1 Pond	0	0	no	0.05	yes
R1 Stream	0.003	0	no	0.05	yes
R2 Stream					
R3 Stream	0.004	0.001	no	0.05	yes
R4 Stream	0.005	0	no	0.05	yes

For algae, all individual aa.ss. are below the ETR triggers, and given the synergism is excluded, it is highly unlikely that substances with $ETR_i < ETR_{trigger/n}$ will lead to high risk in a full mixture risk assessment. Thus mixture assessment for algae is concluded. For aquatic invertebrates, where a potential synergistic effect has been detected with MDR, and for fish, where no endpoint is available on formulation (thus no MDR can be checked) the assessment has been further developed.

Evaluation STEP 1.

Are measured toxicity data (ECx) available for the given endpoint (typically chronic data available only for a.s.)?

Only for the a.s. (ECxa.s.): Go to 7

For both formulation (ECxPPP) and a.s. (ECxa.s.): Go to 2

Evaluation STEP 2:

According to EFSA 2013, where data on formulation are available, measured formulation toxicity (ECxPPP) should be compared with the calculated mixture toxicity (ECXmix-CA) by mean of the model deviation ratio ($MDR = EC_{mix-CA} / EC_{xPPP}$)

If $MDR = 0.2-5$ (CA approximately holds for the mixture): Go to 3

If $MDR > 5$ (mixture more toxic than CA): Go to 10

If $MDR < 0.2$ (mixture less toxic than CA): Go to 9

Table 9.5-37: MDR calculation for the mixtures azoxystrobin + prothioconazole and azoxystrobin + prothioconazole-desthio

Toxicity endpoint	Test substance	ECxi Toxicity value µg/L	ECxmix-CA as in PPP µg sum a.s./L	ECxPPP Measured toxicity of PPP (converted to be a.s. based) µg sum a.s./L	Contribution to overall tox- icity [%]	MDR Model De- viation Ra- tio MDR = ECx mix- CA / ECx PPP)
Mixture of azoxystrobin + prothioconazole						
Fish (<i>Onchorhynchus mykiss</i>)	AZO	470		-	75.7	-
	PROTHIO	1830			24.3	
	FGF11		801			
Aquatic inverte- brates (<i>Daphnia magna</i>)	AZO	230		38.5	81.9	11.01
	PROTHIO	1300			18.1	
	FGF11	120	424			
Algae (<i>Selenastrum capricornutum</i> and <i>Pseudokirchneriella subcapitata</i>)	AZO	360		894.5	82.9	0.75
	PROTHIO	2180			17.1	
	FGF11	2790	671			
Mixture of azoxystrobin + prothioconazole-desthio						
Fish (<i>Onchorhynchus mykiss</i>)	AZO	470		-	92.6	-
	PROTHIO- DESTHIO	6630			7.4	
	FGF11		928.3			
Aquatic invertebrates (<i>Daphnia magna</i>)	AZO	230		36.5	97.5	13.11
	PROTHIO- DESTHIO	>10000			2.5	
	FGF11	120	478.3			
Algae (<i>Selenastrum capricornutum</i> and <i>Pseudokirchneriella subcapitata</i>)	AZO	360		848.3	57.4	0.52
	PROTHIO- DESTHIO	550			42.6	
	FGF11	2790	440.9			

For Algae, MDR calculations indicate values between 0.2 and 5, i.e. the observed and calculated mixture toxicities are considered in agreement and the assumption of additive toxicity can be concluded. Assessment on algae has been already concluded at Screening Step 1 (see above)

For aquatic invertebrates, more-than additive (i.e. synergistic) mixture toxicity is theoretically indicated since MDR is greater than 5 → Go to Step 10.

In Step 10, EFSA 2013 requires to check on the apparent synergism as observed in the measured mixture toxicity data. Actually, toxicity data available on single aa.ss. and formulation are heterogenous. Both studies performed on azoxystrobin and prothioconazole had a static exposure design, whilst in the study on formulation, a semi-static design was applied. Considering the fast degradation of prothioconazole in water, the semi-static design can be regarded as worst-case exposure design, and the analytical determination reported in the study with formulation, can demonstrate that recovery of prothioconazole, drastically dropped to approx. 30% within 24 hours. This can strengthen the idea that toxicity data available are not homogenous and suitable enough to address properly the MDR on aquatic invertebrates, which is the most sensitive organism when exposed to azoxystrobin or formulation. Moreover, endpoints are calculated and determined with different approaches. Endpoints for prothioconazole and formulation has been expressed as nominal concentrations, whilst for azoxystrobin endpoint is determined as mean measured concentrations. Taking under consideration the heterogeneity of the input data, no true synergistic can be confirmed, and according EFSA 2013, the assessment should proceed with STEP 3. → Go to Step 3

Evaluation STEP 3

In this step, the calculated EC_xmix-CA (as in PPP) reported in the table above, are compared with EC_xmix-CA(as in PEC_{mix}) calculated according to the relative proportions single a.s. PEC_{sw} shows in the PEC_{mix}.

Therefore, calculate EC_xmix-CA for the mixture composition of the a.s. at the PEC_{mix} and compare with the estimate calculated for the formulation (as already done in step 2 above).

As worst case scenario, FOCUS PEC_{sw} values considered for mixture toxicity assessment refer to application in winter cereals (BBCH>30 and BBCH<69).

Table 9.5-38: STEP 3: Summary table for Step 1,2,3 PEC_{sw} adopted for mixture toxicity on winter cereals (BBCH>30)

	Max PEC _{sw} (µg/L)		PEC _{mix} (µg/L)	Pi (fraction) in PEC _{mix}	
	AZO	PROTHIO		AZO	PROTHIO
Step 1	36.5	21.7	58.2	0.627	0.373
Step 2					
N-Europe	6.49	1.84	8.33	0.779	0.221
S-Europe	11.9	1.84	13.74	0.866	0.134
Step 3					
D1 Ditch	3.38	1.27	4.65	0.727	0.273
D1 Stream	2.11	0.987	3.097	0.681	0.319
D2 Ditch	4.11	1.28	5.39	0.763	0.237
D2 Stream	2.57	1.09	3.66	0.702	0.298
D3 Ditch	1.01	1.27	2.28	0.443	0.557
D4 Pond	0.309	0.044	0.353	0.875	0.125
D4 Stream	0.75	0.935	1.685	0.445	0.555
D5 Pond	0.104	0.044	0.148	0.703	0.297
D5 Stream	0.813	1.01	1.823	0.446	0.554
D6 Ditch	1.01	1.25	2.26	0.447	0.553
R1 Pond	0.115	0.044	0.159	0.723	0.277
R1 Stream	1.05	0.834	1.884	0.557	0.443
R2 Stream					
R3 Stream	1.46	1.17	2.63	0.555	0.445
R4 Stream	1.82	0.837	2.657	0.685	0.315

Table 9.5-39: STEP 3: Summary table for Step 1,2,3 PECsw adopted for mixture toxicity on winter cereals (BBCH<69)

	Max PECsw (µg/L)		PECmix (µg/L)	Pi (fraction) in PECmix	
	AZO	PROTHIO		AZO	PROTHIO
Step 1	36.50	21.70	58.20	0.627	0.373
Step 2					
N-Europe	6.49	1.84	8.33	0.779	0.221
S-Europe	11.90	1.84	13.74	0.866	0.134
Step 3					
D1 Ditch	1.69	1.28	2.97	0.569	0.431
D1 Stream	1.07	1.12	2.19	0.489	0.511
D2 Ditch	2.27	1.28	3.55	0.639	0.361
D2 Stream	1.42	1.14	2.56	0.555	0.445
D3 Ditch	1.02	1.27	2.29	0.445	0.555
D4 Pond	0.26	0.04	0.30	0.853	0.147
D4 Stream	0.88	1.10	1.98	0.443	0.557
D5 Pond	0.11	0.04	0.15	0.711	0.289
D5 Stream	0.95	1.18	2.13	0.445	0.555
D6 Ditch	1.02	1.27	2.29	0.445	0.555
R1 Pond	0.15	0.07	0.22	0.674	0.326
R1 Stream	1.21	0.84	2.05	0.591	0.409
R2 Stream					
R3 Stream	1.48	1.18	2.66	0.556	0.444
R4 Stream	1.72	0.84	2.56	0.673	0.327

Please consider that:

- PECmix is calculated by the sum of PECsw on single aa.ss. Calculated mixture toxicity (ECmix-CA a.s. in PECmix) is then equal to:

$$EC_{x \text{ mix-CA}} = \sum (p_i \text{ PEC}/EC_x i)) \text{ expressed as mg a.s./L}$$

The relative proportion of a.s. is considered sufficiently similar if the outcome of these calculations deviates less than 20 %. Hence, if ECxPPP (proportion of a.s. as contained in PPP) divided by ECxmix-CA (proportion of a.s. at PECmix) yields a value between 0.8 and 1.2, a direct comparison of PECmix with the ECxPPP is feasible. If the mixture composition differs more profoundly, the measured data cannot be used directly for calculating ETR. Instead, the calculated approach was used to perform the mixture RA.

Table 9.5-40: STEP 3: (ETRmix) between ECmix-CA (a.s. in PPP) and ECmix-CA (a.s. in PECmix) toxicity on winter cereals (BBCH>30)

Invertebrates	
ECxmix-CA (a.s. in PPP)/ ECxmix-CA (a.s. in PECmix)	
Step 1	1.28
Step 2	
N-Europe	1.51
S-Europe	1.64
Step 3	
D1 Ditch	1.43
D1 Stream	1.36
D2 Ditch	1.48
D2 Stream	1.39
D3 Ditch	1.00
D4 Pond	1.65
D4 Stream	1.00
D5 Pond	1.39
D5 Stream	1.00
D6 Ditch	1.00
R1 Pond	1.42
R1 Stream	1.17
R2 Stream	
R3 Stream	1.17
R4 Stream	1.36

Table 9.5-41: STEP 3: (ETRmix) between ECmix-CA (a.s. in PPP) and ECmix-CA (a.s. in PECmix) toxicity on winter cereals (BBCH<69)

Invertebrates	
ECxmix-CA (a.s. in PPP)/ ECxmix-CA (a.s. in PECmix)	
Step 1	1.28
Step 2	
N-Europe	1.51
S-Europe	1.64
Step 3	
D1 Ditch	1.19
D1 Stream	1.07
D2 Ditch	1.30
D2 Stream	1.17
D3 Ditch	1.00
D4 Pond	1.62
D4 Stream	1.00
D5 Pond	1.40
D5 Stream	1.00
D6 Ditch	1.00
R1 Pond	1.35

R1 Stream	1.22
R2 Stream	
R3 Stream	1.17
R4 Stream	1.35

For the exposure scenario in which the mixture composition expressed as ECxPPP is similar to the relative proportions of the aa.ss. as in the PECmix, (outcome is highlighted in yellow in the table above), the risk assessment should proceed with a mixture RA based on measured mixture toxicity, where PECmix is divided by ECxPPP, . → Go to Step 4

For other exposure scenario, where no similarity between composition in ECxPPP and in PECmix was identified, the mixture risk assessment should proceed with the analysis on the driver, according to Step 5
→ Go to Step 5

Evaluation Step 4 and Step 5

Table 9.5-42: STEP 4 and 5: ETRmixPPP (PECmix / ECxPPP expressed as sum aa.ss./L) toxicity on winter cereals (BBCH>30) and toxicity drivers

Invertebrates			
ETRmix-PPP (Step 4)		Toxicity driver (Step 5)	
		Azo	Prothio
Step 1	Go to Step 5/8	Driver	
Step 2			
N-Europe	Go to Step 5/8	Driver	
S-Europe	Go to Step 5/8	Driver	
Step 3			
D1 Ditch	Go to Step 5/8	Driver	
D1 Stream	Go to Step 5/8	Driver	
D2 Ditch	Go to Step 5/8	Driver	
D2 Stream	Go to Step 5/8	Driver	
D3 Ditch	0.06		
D4 Pond	Go to Step 5/8	Driver	
D4 Stream	0.04		
D5 Pond	Go to Step 5/8	Driver	
D5 Stream	0.05		
D6 Ditch	0.06		
R1 Pond	Go to Step 5/8	Driver	
R1 Stream	0.05		
R2 Stream			
R3 Stream	0.07		
R4 Stream	Go to Step 5/8	Driver	

Table 9.5-43: STEP 4 and 5: ETRmixPPP (PECmix / ECxPPP expressed as sum aa.ss./L) toxicity on winter cereals (BBCH<69) and toxicity drivers

Invertebrates			
ETRmix-PPP		Toxicity driver	
		Azo	Prothio
Step 1	Go to Step 5/8	Driver	
Step 2			
N-Europe	Go to Step 5/8	Driver	
S-Europe	Go to Step 5/8	Driver	
Step 3			
D1 Ditch	0.08		
D1 Stream	0.06		
D2 Ditch	Go to Step 5/8	Driver	
D2 Stream	0.07		
D3 Ditch	0.06		
D4 Pond	Go to Step 5/8	Driver	
D4 Stream	0.05		
D5 Pond	Go to Step 5/8	Driver	
D5 Stream	0.06		
D6 Ditch	0.06		
R1 Pond	Go to Step 5/8	Driver	
R1 Stream	Go to Step 5/8		
R2 Stream			
R3 Stream	0.07		
R4 Stream	Go to Step 5/8	Driver	

As outlined in the tables above, for the maximum application to winter cereals at BBCH 30-69, all ETRmixPPP values for mixture toxicity to invertebrate (acute) are below the relevant trigger value at FO-CUS Step 3 at the latest. Thus, further considerations have to be taken into account and risk assessment for these scenarios is developed through Step 8b. For the other exposure scenarios, azoxystrobin results to be always driving the toxicity of the formulation, thus the risk assessment can rely one performed with a.s. azoxystrobin.

Evaluation Step 8b

RQmix is finally applied to address the risk assessment of the mixture, considering higher tier endpoint for azoxystrobin (RAC= 3.3 µg a.s./L). It is to be noted that in the excel files attached, endpoint used for all mixture assessment on acute exposure to aquatic invertebrate has been derived from studies on *Daphnia magna*, for sake of comparability within different aa.ss. in the mixture. In the RQmix approach, endpoint on the most sensitive organisms should be adopted, thus initially an assessment with $RAC_{\text{azoxystrobin}} = 0.55 \mu\text{g a.s./l}$ should be considered. For simplicity, only the RQmix approach with refined endpoint has been displayed.

Table 9.5-44: STEP 8b: RQmix considering higher tier endpoint for azoxystrobin

Acute invertebrate		Scenario	Azoxystrobin [µg a.s./L]		Prothioconazole [µg a.s./L]		RQmix
			PEC _{sw}	RAC	PEC _{sw}	RAC	
		BBCH >30					
		D3 Ditch	1.01	3.3 (Higher tier)	1.27	13	0.404
		D4 Stream	0.750		0.935		0.299
		D5 Stream	0.813		1.01		0.324

	BBCH <69	D6 Ditch	1.01		1.25		0.402
		R1 Stream	1.05		0.834		0.382
		R3 Stream	1.46		1.17		0.532
		D1 Ditch	1.69		1.28		0.611
		D1 Stream	1.07		1.12		0.410
		D2 Ditch	2.27		1.28		0.786
		D3 Ditch	1.02		1.14		0.397
		D4 Stream	0.876		1.10		0.350
		D5 Stream	0.946		1.18		0.377
		D6 Ditch	1.02		1.27		0.407
		R1 Stream	1.21		0.836		0.431
		R3 Stream	1.48		1.18		0.539

As outlined in the table above, for the maximum application to winter cereals at BBCH 30-69, all RQmix values for mixture toxicity to invertebrates (acute) are below the relevant trigger value at FOCUS Step 3 at the latest. Thus, no further considerations have to be taken into account.

zRMS comments:

zRMS agrees with the calculations of mixture toxicity of prothioconazole +azoxystrobin.

Algae

For algae, all individual aa.ss. are below the ETR triggers, and given the synergism is excluded, it is highly unlikely that substances with $ETR_i < ETR_{trigger/n}$ will lead to high risk in a full mixture risk assessment. Thus, mixture assessment for algae is concluded.

Aquatic invertebrates:

For aquatic invertebrates, where a potential synergistic effect has been detected with MDR, and for fish, where no endpoint is available on formulation (thus no MDR can be checked) the assessment has been developed.

For the maximum application to winter cereals at BBCH 30-69, all ETR_{mixPPP} values for mixture toxicity to invertebrate (acute) are below the relevant trigger value at FOCUS Step 3 at the latest. Further considerations have to be taken into account and risk assessment for these scenarios is developed through Step 8b. For the other exposure scenarios, azoxystrobin results to be always driving the toxicity of the formulation, thus the risk assessment can rely one performed with a.s. azoxystrobin.

RQmix is finally applied to address the risk assessment of the mixture, considering higher tier endpoint for azoxystrobin ($RAC = 3.3 \mu g \text{ a.s./L}$). It is to be noted that in the excel files attached, endpoint used for all mixture assessment on acute exposure to aquatic invertebrate has been derived from studies on *Daphnia magna*, for sake of comparability within different aa.ss. in the mixture. In the RQmix approach, endpoint on the most sensitive organisms should be adopted, thus initially an assessment with $RAC_{azoxystrobin} = 0.55 \mu g \text{ a.s./l}$ should be considered. For simplicity, only the RQmix approach with refined endpoint has been displayed.

As outlined in the table above, for the maximum application to winter cereals at BBCH 30-69, all RQmix values for mixture toxicity to invertebrates (acute) are below the relevant trigger value at FOCUS Step 3 at the latest. Thus, no further considerations have to be taken into account.

As far as regards the mixture assessment on acute exposure to fish, according to EFSA 2013, in case measured toxicity data are available only on aa.ss. (point 1) and if there are no evidence that synergistic interactions between mixture components might occur (point 7), risk assessment for the formulation should be conducted with the calculated mixture toxicity endpoints, which are hereunder reported:

Table 9.5-45: Calculated mixture toxicity for the formulation FGF11

Species	Substance	Concentration (C _i) in formulation (g a.s./L)	P _i	EC _{xi} (mg a.s./L)	ECX _{mix-CA} (mg sum a.s. /L)
<i>Oncorhynchus mykiss</i>	azo	160	0.44	0.47	0.801
	prothio	200	0.56	1.83	

Step 7: Is there evidence that 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) which cannot be ruled out for the given species with sufficient certainty?

Yes (mixture toxicity calculation not feasible): Measured mixture toxicity data required for RA (if becoming available: Go to 2)

No (mixture toxicity calculation feasible): Go to 8

Answer: For the time being, no mixture toxicity data is available, and there is no evidence of potential synergistic effects between two aa.ss., thus Step 8 will be addressed.

Step 8: Conduct a mixture RA based on calculated mixture toxicity according to ETR_{mix-CA} approach:

If ETR_{mix-CA} < trigger: Low risk

If ETR_{mix-CA} > trigger: Low risk not demonstrated, check single-substance refinement options

Table 9.5-46: Acceptability of risk (PEC/RAC<1) for the formulation product FGF11 for each organism group based on initial ETR_{mix-CA} for winter cereals application (BBCH <30)

Fish	
ETR _{mix-CA}	
Step 1	0.090
Step 2	
N-Europe	0.010
S-Europe	0.030
Step 3	
D1 Ditch	0.008
D1 Stream	0.005
D2 Ditch	0.009
D2 Stream	0.006
D3 Ditch	0.003
D4 Pond	0.001
D4 Stream	0.002
D5 Pond	0.000
D5 Stream	0.002
D6 Ditch	0.003
R1 Pond	0.000
R1 Stream	0.003
R2 Stream	
R3 Stream	0.004
R4 Stream	0.004

Table 9.5-47: Acceptability of risk (PEC/RAC<1) for the formulation product FGF11 for each organism group based on initial ETRmix-CA for winter cereals application (BBCH <69)

Fish	
ETRmix-CA	
Step 1	0.09
Step 2	
N-Europe	0.01
S-Europe	0.03
Step 3	
D1 Ditch	0.004
D1 Stream	0.003
D2 Ditch	0.006
D2 Stream	0.004
D3 Ditch	0.003
D4 Pond	0.001
D4 Stream	0.002
D5 Pond	0.000
D5 Stream	0.003
D6 Ditch	0.003
R1 Pond	0.000
R1 Stream	0.003
R2 Stream	
R3 Stream	0.004
R4 Stream	0.004

According to ETRmix-CA approach, at Step 8, an acceptable risk is demonstrated for fish, thus no further assessment is needed.

In conclusion, according to ETRmix-CA approach, at Step 8, an acceptable risk is demonstrated for fish.

zRMS comments:

zRMS agrees with the calculations of mixture toxicity of prothioconazole +azoxystrobin for fish

Based on the calculation performed above according to ETRmix-CA approach, at Step 8, an acceptable risk is demonstrated for fish.

Mixture toxicity endpoints are assessed from active substances azoxystrobin and prothioconazole-desthio endpoints, where possible derived on the same test organisms. For azoxystrobin and prothioconazole-desthio, acute fish data are available for *Oncorhynchus mykiss*. For acute invertebrates, endpoints were applied from studies available on *Daphnia magna*. For algae data on *Selenastrum capricornutum* (formerly *Pseudokirchneriella subcapitata*) were adopted for azoxystrobin and *Scenedesmus subspicatus* for prothioconazole-desthio.

Toxicity data on formulation provided for acute exposure to fish and effect on algae, have been also included in the mixture toxicity assessment.

Screening Step on ETR (Simplified approach)

According to EFSA 2013, if no synergistic effects are indicated and the ETR (Exposure Toxicity Ratio) values of the individual a.s. (ETR_i) contained in the formulation are below the relevant trigger value, the mixture RA can follow a simplified approach

For algae, where no synergism was detected (see table 9.5-36), this simplified approach was checked:

Table 9.5-48: Screening step 1 for Algae (BBCH>30)

Algae	ETR		Any a.s. above ETRtrigger?	ETRtrigger/n (if needed)	All ETR _i ≤ ETRtrigger/n?
	azo	prothio-desthio			
Step 1	0.099	0.071	no	0.05	no
Step 2					
N-Europe	0.018	0.007	no	0.05	yes
S-Europe	0.033	0.012	no	0.05	yes
Step 3					
D1 Ditch	0.009	0	no	0.05	yes
D1 Stream	0.006	0	no	0.05	yes
D2 Ditch	0.011	0	no	0.05	yes
D2 Stream	0.007	0	no	0.05	yes
D3 Ditch	0.003	0	no	0.05	yes
D4 Pond	0.001	0	no	0.05	yes
D4 Stream	0.002	0	no	0.05	yes
D5 Pond	0	0	no	0.05	yes
D5 Stream	0.002	0	no	0.05	yes
D6 Ditch	0.003	0	no	0.05	yes
R1 Pond	0	0	no	0.05	yes
R1 Stream	0.003	0.001	no	0.05	yes
R2 Stream					
R3 Stream	0.004	0.001	no	0.05	yes
R4 Stream	0.005	0.001	no	0.05	yes

Table 9.5-49: Screening step 1 for Algae (BBCH<69)

Algae	ETR		Any a.s. above ETRtrigger?	ETRtrigger/n (if needed)	All ETRi ≤ ETRtrigger/n?
	azo	prothio-desthio			
Step 1	0.099	0.071	no	0.05	no
Step 2					
N-Europe	0.018	0.007	no	0.05	yes
S-Europe	0.033	0.012	no	0.05	yes
Step 3					
D1 Ditch	0.005	0	no	0.05	yes
D1 Stream	0.003	0	no	0.05	yes
D2 Ditch	0.006	0	no	0.05	yes
D2 Stream	0.004	0	no	0.05	yes
D3 Ditch	0.003	0	no	0.05	yes
D4 Pond	0.001	0	no	0.05	yes
D4 Stream	0.002	0	no	0.05	yes
D5 Pond	0	0	no	0.05	yes
D5 Stream	0.003	0	no	0.05	yes
D6 Ditch	0.003	0	no	0.05	yes
R1 Pond	0	0	no	0.05	yes
R1 Stream	0.003	0	no	0.05	yes
R2 Stream					
R3 Stream	0.004	0.001	no	0.05	yes
R4 Stream	0.005	0.001	no	0.05	yes

For algae, all individual aa.ss. are below the ETR triggers, and given the synergism is excluded, it is highly unlikely that substances with $ETR_i < ETR_{trigger/n}$ will lead to high risk in a full mixture risk assessment. Thus mixture assessment for algae is concluded. For aquatic invertebrate, where a potential synergistic effect has been detected with MDR, and for fish, where no endpoint is available on formulation (thus no MDR can be checked) the assessment has been further developed.

Similarly to assessment conducted for azoxystrobin + prothioconazole, MDR calculations is 0.52 for Algae and 13.11 for aquatic invertebrates (refer to Table 9.5-37).

For Algae, MDR calculations indicate values between 0.2 and 5, i.e. the observed and calculated mixture toxicities are considered in agreement and the assumption of additive toxicity can be concluded. Assessment on algae has been already concluded at Screening Step 1 (see above).

For aquatic invertebrates, more-than additive (i.e. synergistic) mixture toxicity is theoretically indicated since MDR is greater than 5 → Go to Step 10

In Step 10, EFSA 2013 requires to check on the apparent synergism as observed in the measured mixture toxicity data. Actually, toxicity data available on single aa.ss. and formulation are heterogenous. Both studies performed on azoxystrobin and prothioconazole-desthio had a static exposure design, whilst in the study on formulation, a semi-static design was applied. Semi-static design with one test item renewal can be regarded as worst case exposure design, when comparing to static one. This can strengthen the idea that toxicity data available are not homogenous and suitable enough to address properly the MDR on aquatic invertebrates, which is the most sensitive organism when exposed to azoxystrobin or formulation. Moreover, endpoints are calculated and determined with different approaches. Endpoints for prothioconazole-desthio and formulation has been expressed as nominal concentrations, whilst for azoxystrobin endpoint is determined as mean measured concentrations. Taking under consideration the heterogeneity of the input

data, no true synergistic can be confirmed, and according EFSA 2013, the assessment should proceed with STEP 3. → Go to Step 3

Evaluation STEP 3

In this step, the calculated EC_xmix-CA (as in PPP) reported in the table above, are compared with EC_xmix-CA(as in PEC_{mix}) calculated according to the relative proportions single a.s. PEC_{sw} shows in the PEC_{mix}.

Therefore, calculate EC_xmix-CA for the mixture composition of the a.s. at the PEC_{mix} and compare with the estimate calculated for the formulation (as already done in step 2 above).

As worst case scenario, FOCUS PEC_{sw} values considered for mixture toxicity assessment refer to application in winter cereals (BBCH>30 and BBCH<69).

Table 9.5-50: STEP 3: Summary table for Step 1,2,3 PEC_{sw} adopted for mixture toxicity on winter cereals (BBCH>30)

	Max PEC _{sw} (µg/L)		PEC _{mix} (µg/L)	Pi (fraction) in PEC _{mix}	
	AZO	PROTHIO-DES-THIO		AZO	PROTHIO-DES-THIO
Step 1	35.6	39.2	74.8	0.476	0.524
Step 2					
N-Europe	6.49	3.67	10.16	0.639	0.361
S-Europe	11.9	6.74	18.64	0.638	0.362
Step 3					
D1 Ditch	3.38	0.005	3.385	0.999	0.001
D1 Stream	2.11	0.019	2.129	0.991	0.009
D2 Ditch	4.11	0.029	4.139	0.993	0.007
D2 Stream	2.57	0.07	2.64	0.973	0.027
D3 Ditch	1.01	0.003	1.013	0.997	0.003
D4 Pond	0.309	0.007	0.316	0.978	0.022
D4 Stream	0.75	0.005	0.755	0.993	0.007
D5 Pond	0.104	0.011	0.115	0.904	0.096
D5 Stream	0.813	0.011	0.824	0.987	0.013
D6 Ditch	1.01	0.002	1.012	0.998	0.002
R1 Pond	0.115	0.043	0.158	0.728	0.272
R1 Stream	1.05	0.374	1.424	0.737	0.263
R2 Stream					
R3 Stream	1.46	0.463	1.923	0.759	0.241
R4 Stream	1.820	0.684	2.504	0.727	0.273

Table 9.5-51: STEP 3: Summary table for Step 1,2,3 PECsw adopted for mixture toxicity on winter cereals (BBCH<69)

	Max PECsw (µg/L)		PECmix (µg/L)	Pi (fraction) in PECmix	
	AZO	PROTHIO-DES-THIO		AZO	PROTHIO-DES-THIO
Step 1	35.6	39.2	74.8	0.476	0.524
Step 2					
N-Europe	6.49	3.67	10.16	0.639	0.361
S-Europe	11.9	6.74	18.64	0.638	0.362
Step 3					
D1 Ditch	1.69	0.104	1.794	0.942	0.058
D1 Stream	1.07	0.022	1.092	0.980	0.020
D2 Ditch	2.27	0.062	2.332	0.973	0.027
D2 Stream	1.42	0.094	1.514	0.938	0.062
D3 Ditch	1.02	0.01	1.03	0.990	0.010
D4 Pond	0.255	0.011	0.266	0.959	0.041
D4 Stream	0.876	0.008	0.884	0.991	0.009
D5 Pond	0.108	0.013	0.121	0.893	0.107
D5 Stream	0.946	0.014	0.96	0.985	0.015
D6 Ditch	1.02	0.015	1.035	0.986	0.014
R1 Pond	0.147	0.035	0.182	0.808	0.192
R1 Stream	1.21	0.239	1.449	0.835	0.165
R2 Stream					
R3 Stream	1.48	0.451	1.931	0.766	0.234
R4 Stream	1.720	0.485	2.205	0.780	0.220

Please consider that:

- PECmix is calculated by the sum of PECsw on single aa.ss. Calculated mixture toxicity (ECmix-CA a.s. in PECmix) is then equal to:

$$EC_{x \text{ mix-CA}} = \sum (p_i \text{ PEC} / EC_x i) \text{ expressed as mg a.s./L}$$

The relative proportion of a.s. is considered sufficiently similar if the outcome of these calculations deviates less than 20 %. Hence, if ECxPPP (proportion of a.s. as contained in PPP) divided by ECxmix-CA (proportion of a.s. at PECmix) yields a value between 0.8 and 1.2, a direct comparison of PECmix with the ECxPPP is feasible. If the mixture composition differs more profoundly, the measured data cannot be used directly for calculating ETR. Instead, the calculated approach was used to perform the mixture RA.

Table 9.5-52: STEP 3: (ETRmix) between ECmix-CA (a.s. in PPP) and ECmix-CA (a.s. in PECmix) toxicity on winter cereals (BBCH>30)

Invertebrates	
ECmix-CA (a.s. in PPP)/ ECmix-CA (a.s. in PECmix)	
Step 1	1.01
Step 2	
N-Europe	1.35
S-Europe	1.34
Step 3	
D1 Ditch	2.08
D1 Stream	2.06
D2 Ditch	2.07
D2 Stream	2.03
D3 Ditch	2.07
D4 Pond	2.03
D4 Stream	2.07
D5 Pond	1.89
D5 Stream	2.05
D6 Ditch	2.08
R1 Pond	1.53
R1 Stream	1.55
R2 Stream	
R3 Stream	1.59
R4 Stream	1.52

Table 9.5-53: STEP 3: (ETRmix) between ECmix-CA (a.s. in PPP) and ECmix-CA (a.s. in PECmix) toxicity on winter cereals (BBCH<69)

Invertebrates	
ECmix-CA (a.s. in PPP)/ ECmix-CA (a.s. in PECmix)	
Step 1	1.01
Step 2	
N-Europe	1.35
S-Europe	1.34
Step 3	
D1 Ditch	1.96
D1 Stream	2.04
D2 Ditch	2.03
D2 Stream	1.95
D3 Ditch	2.06
D4 Pond	2.00
D4 Stream	2.06
D5 Pond	1.86
D5 Stream	2.05
D6 Ditch	2.05
R1 Pond	1.69
R1 Stream	1.74

R2 Stream	
R3 Stream	1.61
R4 Stream	1.63

For the exposure scenario in which the mixture composition expressed as ECxPPP is similar to the relative proportions of the aa.ss. as in the PECmix, (outcome is highlighted in yellow in the table above), the risk assessment should proceed with a mixture RA based on measured mixture toxicity, where PECmix is divided by ECxPPP. Similarity was determined only at FOCUS Step 1, thus this is not taken into account, since at Step 3 FOCUS assessment no similarity on any scenarios could be found.

For these exposure scenarios, where no similarity between composition in ECxPPP and in PECmix was identified, the mixture risk assessment should proceed with the analysis on the driver, according to Step 5 → Go to Step 5

Evaluation Step 5

Table 9.5-54: STEP 5: toxicity drivers on winter cereals (BBCH>30 and BBCH<69)

Next Step for:	Invertebrates		
FOCUS	azo	prothio-desthio	Conclusion
Step 1	Driver		Go to Step 6
Step 2			
N-Europe	Driver		Go to Step 6
S-Europe	Driver		Go to Step 6
Step 3			
D1 Ditch	Driver		Go to Step 6
D1 Stream	Driver		Go to Step 6
D2 Ditch	Driver		Go to Step 6
D2 Stream	Driver		Go to Step 6
D3 Ditch	Driver		Go to Step 6
D4 Pond	Driver		Go to Step 6
D4 Stream	Driver		Go to Step 6
D5 Pond	Driver		Go to Step 6
D5 Stream	Driver		Go to Step 6
D6 Ditch	Driver		Go to Step 6
R1 Pond	Driver		Go to Step 6
R1 Stream	Driver		Go to Step 6
R2 Stream			
R3 Stream	Driver		Go to Step 6
R4 Stream	Driver		Go to Step 6

As outlined in the tables above, for the maximum application to winter cereals at BBCH 30-69, for all exposure scenarios, azoxystrobin results to be always driving the toxicity of the formulation, thus the risk assessment can rely one performed with a.s. azoxystrobin.

As far as regards the mixture assessment on acute exposure to fish, according to EFSA 2013, in case measured toxicity data are available only on aa.ss. (point 1) and if there is no evidence that synergistic interactions between mixture components might occur (point 7), risk assessment for the formulation should be

conducted with the calculated mixture toxicity endpoints, which are hereunder reported:

Table 9.5-55: Calculated mixture toxicity for the formulation FGF11

Species	Substance	Concentration (C _i) in formulation (g a.s./L)	P _i	EC _{xi} (mg a.s./L)	EC _{Xmix-CA} (mg sum a.s. /L)
<i>Oncorhynchus mykiss</i>	azo	160	0.47	0.47	0.928
	prothio-desthio	200	0.53	6.63	

Step 7: Is there evidence that 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) which cannot be ruled out for the given species with sufficient certainty?

Yes (mixture toxicity calculation not feasible): Measured mixture toxicity data required for RA (if becoming available: Go to 2)

No (mixture toxicity calculation feasible): Go to 8

Answer: For the time being, no mixture toxicity data is available, and there is no evidence of potential synergistic effects between two aa.ss., thus Step 8 will be addressed.

Step 8: Conduct a mixture RA based on calculated mixture toxicity according to ETRmix-CA approach:

If ETRmix-CA < trigger: Low risk

If ETRmix-CA > trigger: Low risk not demonstrated, check single-substance refinement options

Table 9.5-56: Acceptability of risk (PEC/RAC<1) for the formulation product FGF11 for each organism group based on initial ETRmix-CA for winter cereals application (BBCH <30)

Fish	
ETRmix-CA	
Step 1	0.082
Step 2	
N-Europe	0.014
S-Europe	0.026
Step 3	
D1 Ditch	0.007
D1 Stream	0.004
D2 Ditch	0.009
D2 Stream	0.005
D3 Ditch	0.002
D4 Pond	0.001
D4 Stream	0.002
D5 Pond	0.000
D5 Stream	0.002
D6 Ditch	0.002
R1 Pond	0.000
R1 Stream	0.002
R2 Stream	

R3 Stream	0.003
R4 Stream	0.004

Table 9.5-57: Acceptability of risk (PEC/RAC<1) for the formulation product FGF11 for each organism group based on initial ETRmix-CA for winter cereals application (BBCH <69)

Fish	
ETRmix-CA	
Step 1	0.082
Step 2	
N-Europe	0.014
S-Europe	0.026
Step 3	
D1 Ditch	0.004
D1 Stream	0.002
D2 Ditch	0.005
D2 Stream	0.003
D3 Ditch	0.002
D4 Pond	0.001
D4 Stream	0.002
D5 Pond	0.000
D5 Stream	0.002
D6 Ditch	0.002
R1 Pond	0.000
R1 Stream	0.003
R2 Stream	
R3 Stream	0.003
R4 Stream	0.004

According to ETRmix-CA approach, at Step 8, an acceptable risk is demonstrated for fish, thus no further assessment is needed.

In conclusion, according to ETRmix-CA approach, at Step 8, an acceptable risk is demonstrated for fish.

Nested EXCEL files:

Aquatic mixture toxicity for azoxystrobin + prothioconazole for application in winter cereals (FOCUS Scenario BBCH>30):



AGD_AquaMix_SEU_a
zoprothio_bbch30.xlsr

Aquatic mixture toxicity for azoxystrobin + prothioconazole for application in winter cereals (FOCUS Scenario BBCH<69):



AGD_AquaMix_SEU_a
zoprothio_bbch69.xlsr

Aquatic mixture toxicity for azoxystrobin + prothioconazole-desthio for application in winter cereals (FOCUS Scenario BBCH>30):



AGD_AquaMix_SEU_a
zoprothiodesthio_bbc

Aquatic mixture toxicity for azoxystrobin + prothioconazole-desthio for application in winter cereals (FOCUS Scenario BBCH<69):



AGD_AquaMix_SEU_a
zoprothiodesthio_bbc

zRMS comments:

The calculations of the mixture toxicity assessment for azoxystrobin + prothioconazole-desthio for all proposed uses have been validated by zRMS.

For algae, all individual a.s. are below the ETR triggers, and given the synergism is excluded, it is highly unlikely that substances with $ETR_i < ETR_{trigger}/n$ will lead to high risk in a full mixture risk assessment. Thus, mixture assessment for algae is concluded. For aquatic invertebrate, where a potential synergistic effect has been detected with MDR, and for fish, where no endpoint is available on formula-tion (thus no MDR can be checked) the assessment has been developed.

For the maximum application to winter cereals at BBCH 30-69, for all exposure scenarios, azoxystrobin results to be always driving the toxicity of the formulation in case of aquatic invertebrates , thus the risk assessment can rely one performed with a.s. azoxystrobin.

ETRmix-CA approach, at Step 8, an acceptable risk is demonstrated for fish.

Formulation FGF11

Measured toxicity endpoints are used for the risk assessment of FGF11. Aquatic toxicity risk assessment for the formulated product based on the observed toxicity is reported in the table below.

Table 9.5-58: Acceptability of risk (PEC/RAC<1) for the formulation product FGF11 for each organism group based on PEC_{sw} following spray application (Rautmann drift values) for field crops (cereals and oilseed rape)

Group			Aquatic invertebrates	Algae
Test species			<i>D. magna</i>	<i>R. subcapitata</i>
Endpoint (µg/L)			EC ₅₀	ErC ₅₀
AF			120	2790
RAC (µg/L)			100	10
			1.2	279
Spray drift buffer (m)	Drift rate (%)	Initial PEC _{sw} (µg product/L)		
1	2.77	10.4	8.67	0.04
3	0.95	3.56	2.97	0.01
5	0.59	2.21	1.84	0.01
10	0.29	1.09	0.91	0.00

zRMS comments:

The risk assessment for formulation FGF11 based on measured endpoints for formulation and PEC_{sw} values calculated in Section 8 with consideration of non-spray drift buffer has been accepted by zRMS.

Risk assessment performed with formulation toxicity data on aquatic invertebrates demonstrate an acceptable risk when application of formulation FGF11 is implemented with mitigation measure as 10 m buffer zone for all proposed uses.

It should be noted that mixture toxicity assessment is available and calculations performed in the Table 9.5 58 is additional only.

9.5.3 Overall conclusions

Azoxystrobin and its metabolites

For azoxystrobin, based on the results of the risk assessments at FOCUS Step 3, the risk was acceptable for all the FOCUS exposure scenario other than D2 Ditch when considering the EU agreed weight-of-evidence approach for aquatic invertebrates.

It is to be noted that according to GAP D2 Ditch scenario is only relevant for France. As far as regards Step 4 FOCUS assessment, even when considering the highest mitigations available for D2 Ditch scenario, no risk is deemed acceptable. Since the main entry route for D2 Ditch is *via* drainage a label restriction is deemed necessary according to Regulation (EU) No. 547/2011, and if these scenarios are relevant at national MS level, the safety precaution phrase SPe2 shall be applied: „*To protect aquatic organisms do not apply to drained soils.*“

For the metabolites R401553, R402173 and R234886 of azoxystrobin an acceptable risk was indicated at FOCUS Step 1, 2 without applying any mitigation measures.

Prothioconazole and its metabolites

For prothioconazole and its metabolites prothioconazole-S-methyl, and 1,2,4-triazole, an acceptable risk was indicated at FOCUS Step 1, 2 for application to cereals and oilseed rape without applying any mitigation measures.

For the metabolite prothioconazole-desthio (M04), an acceptable risk was indicated based on FOCUS Step 4 PEC_{SW} for all scenarios when including the following risk mitigation measures:

- *Winter cereals* (BBCH > 30 < 69): 10 m vegetative buffer strip + 10 m no-spray buffer zone (R1, R3 and R4 scenarios)
- *Spring cereals* (BBCH > 30 < 69): 10 m vegetative buffer strip + 10 m no-spray buffer zone + 50% drift reduction (R4 scenario) and 10 m vegetative buffer strip + 10 m no-spray buffer zone (R1, R3 scenarios)
- *Winter oilseed rape* (BBCH 61-73): 10 m vegetative buffer strip + 10 m no-spray buffer zone + 50% drift reduction (R3 scenario)
- *Spring oilseed rape* (BBCH 61-73): None

Mixture assessment

Mixture toxicity assessment has been performed considering EFSA 2013 and the calculation sheet AGD_AquaMix (v. 1.22).

In line with the argumentation already provided earlier, mixture toxicity has been presented for azoxystrobin + prothioconazole and for azoxystrobin + prothioconazole-desthio

Azoxystrobin results to be the toxicity driver for the majority of the exposure scenario on fish, aquatic invertebrates and algae.

For Algae, MDR calculations indicate values between 0.2 and 5, i.e. the observed and calculated mixture toxicities are considered in agreement with the assumption of additive toxicity. Since synergism could be excluded, assessment for Algae has been finalised at the Screening Step, according to the simplified approach according to EFSA 2013.

For aquatic invertebrates, more-than additive (i.e. synergistic) mixture toxicity is theoretically indicated since MDR is greater than 5. But input data are very heterogenous, considering the exposure designs and the calculation of the endpoints thus not suitable enough to address properly the MDR on aquatic invertebrates, which is the most sensitive organism when exposed to azoxystrobin or formulation.

Finally, through ETRmix-CA approach an acceptable risk can be demonstrated for aquatic invertebrates and for fish.

Formulation assessment

Risk assessment performed with formulation toxicity data on aquatic invertebrates ~~and fish~~ demonstrated an acceptable risk when application of formulation FGF11 is implemented with mitigation measure as 10 m buffer strip for all proposed uses.

zRMS comments:

Conclusions above were amended accordingly with consideration of the outcome of the performed risk assessment.

Please note that Additional calculations may be required by cMS that do not accept surface water exposure derived using FOCUS models.

The acceptability and applicability of the indicated risk mitigation measures has to be confirmed at the cMS level.

The following text is added due to agreements during the Central Zone harmonisation meetings.

It should be noted that this text has no impact on the outcome of zonal evaluation of formulation FGF11 which was performed in line with the EU agreed methodology.

“The endpoint E_rC_{50} is selected in this Core Assessment but there are some uncertainties regarding the level of protection reached for primary producers. This is indicated for macrophytes in the aquatic Guidance Document (EFSA Journal 2013;11(7):3290) that recommends: “... a proper calibration between different tiers (higher and lower tier data) for macrophytes should be performed in the future”. Such calibration should be extended to algae. Until available relevant information on the level of protection reached is considered at EU level, it is recommended to address this uncertainty at each Member State level in the National Addendum if considered necessary, although it would be highly appreciated to have a harmonised approach in the Central zone.”

9.6 Effects on bees (KCP 10.3.1)

9.6.1 Toxicity data

Acute toxicity tests on honeybees with Azoxystrobin and Prothioconazole are available in the respective EU related documents (EFSA conclusion 2010 and EFSA conclusion 2007, respectively).

Acute and chronic toxicity studies have been carried out with the formulation FGF11 (Azoxystrobin 160 + Prothioconazole 200 g/L SC). New studies with the formulation FGF11 were not evaluated as part of the EU assessment of the active substances and endpoints are shown in the table below and are listed in Appendix 1 and summarized in Appendix 2.

Table 9.6-1: Relevant endpoints for the risk assessment for bees

Species	Test item	Exposure System	Results	Reference
Active substances				
Apis mellifera adults	Azoxystrobin	Acute, oral, 48h	LD ₅₀ > 25 µg a.s./bee	EFSA 2010
		Acute, contact, 48h	LD ₅₀ > 200 µg a.s./bee	
Apis mellifera adults	Prothioconazole	Acute, oral, 48h	LD ₅₀ > 71 µg a.s./bee	EFSA 2007
		Acute, contact, 48h	LD ₅₀ > 200 µg a.s./bee	
Product FGF11 (Azoxystrobin 160 + Prothioconazole 200)				
Apis mellifera	Azoxystrobin 160 + Prothioconazole 200 g/L SC	Acute contact, limit test - 48h	LD ₅₀ > 400 µg/bee NOED ≥ 400.0 µg/bee	Schabio, S., Horstmann, C., 2024, 179211035, KCP 10.3.1.1.1/01 KCP 10.3.1.1.2/01
		Acute oral, limit test - 48h	LD ₅₀ > 454.8 µg/bee NOED ≥ 454.8 µg/bee	
Apis mellifera	Azoxystrobin 160 + Prothioconazole 200 g/L SC	Chronic, oral (adult) - 10 d	LDD ₅₀ = 20.9 µg product/bee/day NOEDD = 15.5 µg prod./bee/day	Schabio, S., Horstmann, C., 2024, 179211136, KCP 10.3.1.2/01
Apis mellifera	Azoxystrobin 160 + Prothioconazole 200 g/L SC	Chronic, Larvae - 22 d	NOEDD 29.16 µg prod./larva/dev. period ED ₅₀ = 93.88 µg prod./larva ED ₁₀ = 20.66 µg prod./larva	Venturi, S., 2024, BT212/23, KCP 10.3.1.3/01
Bombus terrestris	Azoxystrobin 160 + Prothioconazole 200 g/L SC	Acute contact, limit test - 48h	LD ₅₀ > 650 µg/bee NOED ≥ 650 µg/bee	Schabio, S., Horstmann, C., 2024, 179211105, KCP 10.3.1.1.1/02 KCP 10.3.1.1.2/02
		Acute oral, limit test - 48h	LD ₅₀ > 722.2 µg/bee NOED ≥ 722.2 µg/bee	
Apis mellifera	Azoxystrobin 160 + Prothioconazole 200 g/L SC	Semi-field study (tunnel test) according to EPPO Guideline No. 170(4), 2010	Overall, a foliar application of Azoxystrobin 160 g/L + Prothioconazole 200 g/L SC during active foraging of honeybees under semi-field conditions did not result in any significant adverse effects.	Portail, B., 2025, S24-102790 KCP 10.3.1.5/01
Apis mellifera	Azoxystrobin 160 + Prothioconazole 200 g/L SC	A Semi-Field Study to Evaluate Side Effects of Azoxystrobin 160 + Prothioconazole 200 SC on the Honey Bee (Apis mellifera L.) on Phacelia tanacetifolia according to CEB 230 in France in 2024,	Overall, a foliar application of Azoxystrobin 160 g/L + Prothioconazole 200 g/L SC during active foraging of honeybees or in the evening after bee flight under semi-field conditions did not result in adverse effects.	Roig, J. (2024), Study number S24-102576, UPL Study number UPL/2024/1020

Values in **bold** are used for the risk assessment

zRMS comments:

Acute bee toxicity data for azoxystrobin and prothioconazole provided in Table 9.6-1 are in line with EU agreed endpoints reported in EFSA Journal 2010; 8(4):1542 and EFSA Scientific Report (2007) 106, respectively.

To fulfil the data requirements as set by Commission Regulation (EU) No 284/2013, studies on acute toxicity to adult bees and bumble bees and chronic and larvae toxicity to bees were submitted with the formulated product. Studies on effects of the formulated product to bees listed in Table above were evaluated by the zRMS and considered acceptable. The reported endpoints are confirmed.

In addition, two semi-fields studies for bees have been conducted for formulation Azoxystrobin 160 + Prothioconazole 200 SC.

The first one the semi-field study by Portail, B (2025) with FGF11 performed to determine the potential effects of FGF11 on the honeybee (*Apis mellifera* L.) after one foliar application at a rate of 160.02 g a.i./ha Azoxystrobin and 204.07 g a.i./ha Prothioconazole (13.10 g product/ha or 1 L product/ha) during bee flight under semi-field conditions in *Phacelia tanacetifolia* in Germany in 2024, following the OEPP/EPPO Guideline No. 170(4) (2010) with partial integration of the EFSA Guidance Document No. 11(7): 3295 (2013) and the EU Guideline 7029/VI/95 rev. 4 (1997).

The second one by Roig, J. (2024) was performed for Azoxystrobin 160 + Prothioconazole 200 SC on the honeybee (*Apis mellifera* L.) in two test item application scenarios, T1 (outside of active bee foraging) and T2 (during active bee foraging), under semi-field conditions in full flowering *Phacelia tanacetifolia* at BBCH 65-66 according to CEB 230 in France. The target application rate of the test item Azoxystrobin 160 + Prothioconazole 200 SC was 1 L product/ha.

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

9.6.1.1 Justification for new endpoints

Risk assessments are based on the available data for azoxystrobin (acute oral and contact for honey bees), prothioconazole (acute oral and contact for honey bees), and data on the relevant formulation FGF11. For the formulation, acute and chronic tests with *Apis mellifera* are available together with acute tests with *Bombus terrestris* (see 9.6.3).

9.6.2 Risk assessment

The evaluation of the risk for bees was performed in accordance with the recommendations of the “Guidance Document on Terrestrial Ecotoxicology”, as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002). Chronic risk was assessed using the EPPO updated scheme (2010)².

In addition, a risk assessment according to the draft EFSA bee guidance 2013 (EFSA Journal 2013;11(7):3295) is presented. EFSA guidance is still under revision and currently the risk assessment performed according to it, is not mandatory in the regulatory context.

New EFSA guidance has been published on 11 May 2023 but currently the risk assessment performed according to it, is not mandatory in the regulatory context. Even though the EFSA guidance of 2013 was never agreed at EU level, a preliminary dietary assessment is performed for exposure of honeybees for uptake of food and for acute exposure of bumble bees. No assessment to exposure via guttation water or surface water should be performed, as in the updated guidance it is indicated that the water scenario is no longer considered for the risk assessment. This is due to the lack of sufficient data to quantify the water consumption, frequency, and magnitude of water collection by bees.

Risk assessment according to EFSA 2013 is to be generally provided just for demonstrative purpose. Its use is to be decided at Member State level.

² OEPP/EPPO (2010) EPPO Standards PP3/10(3) Environmental risk assessment scheme for plant protection products. Chapter 10: honeybees. Bulletin OEPP/EPPO Bulletin 40, 323–331.

9.6.2.1 Hazard quotients for bees

Risk assessment according to SANCO/10329/2002

To achieve a concise risk assessment, the risk envelope approach is applied. As the application rate is identical for all intended crops, the risk in winter wheat (use XXX) is the only crop to be considered in the risk assessment and covers the risk for bees from all other intended uses.

The hazard quotient for acute contact and oral toxicity (HQ) is calculated using the following equation:

$$HQ = \frac{AR}{\text{Acute LD}_{50}}$$

With:

AR = Application rate in g product/ha or g a.s./ha

Acute LD₅₀ expressed in µg product/bee or µg a.s./bee

The risk assessments for the active substances azoxystrobin and prothioconazole and the formulation FGF11 are presented in the following tables.

Table 9.6-2: First-tier assessment of the risk for bees for azoxystrobin due to the use of FGF11 in cereals (1-3, 5-6)

Intended use	Cereals (1-3, 5-6)		
Active substance	Azoxystrobin		
Application rate (g/ha)	160		
Test design	LD ₅₀ (lab.) (µg/bee)	Single application rate (g/ha)	Q _{HO} , Q _{HC} criterion: Q _H ≤ 50
Oral toxicity	> 25	160	< 6.4
Contact toxicity	> 200		< 0.8

Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure. Q_H values shown in **bold** breach the relevant trigger.

Table 9.6-3: First-tier assessment of the risk for bees for prothioconazole due to the use of FGF11 in cereals (1-3, 5-6)

Intended use	Cereals (1-3, 5-6)		
Active substance	Prothioconazole		
Application rate (g/ha)	200		
Test design	LD ₅₀ (lab.) (µg/bee)	Single application rate (g/ha)	Q _{HO} , Q _{HC} criterion: Q _H ≤ 50
Oral toxicity	> 71	200	< 2.8
Contact toxicity	> 200		< 1.0

Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure. Q_H values shown in **bold** breach the relevant trigger.

Table 9.6-4: First-tier assessment of the risk for bees due to the use of FGF11 in cereals (1-3, 5-6)

Intended use	Cereals (1-3, 5-6)		
Product	FGF11 (Azoxystrobin 160 + Prothioconazole 200 g/L S)		
Application rate (g/ha)	1000		
Test design	LD ₅₀ (lab.) (µg prod./bee)	Single application rate (g/ha)	Q _{HO} , Q _{HC} criterion: Q _H ≤ 50
Oral toxicity	> 454.8	1000	< 2.2
Contact toxicity	> 400		< 2.5

Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure. Q_H values shown in **bold** breach the relevant trigger.

The acute hazard quotients (HQs) are lower than the trigger value of 50, indicating that both active substances (azoxystrobin and prothioconazole), and the formulation FGF11 pose a low risk to bees following the application of the formulation according to the use pattern proposed.

zRMS comments:

The acute risk assessment for bees presented in Tables 9.6-2-9.6-4 is validated by the zRMS. HQ_{oral}, contact and oral values for the active substances and the formulated product FGF11 are below the trigger of 50, indicating a low acute risk for bees.

Please note that the evaluation has been performed in line with SANCO/10329/2002 rev 2 final.

Overall, acceptable risk to bees may be concluded from the intended uses of FGF11 in cereals for oilseed rape.

Risk assessment for chronic oral toxicity of honeybees according to EPPO (2010)

Adult assessment according to EPPO 2010

The effects are assessed quantitatively by calculation of the ratio between the no observed effect level (NOED) and the exposure, with a safety factor set to 1. Exposure is assessed by estimating the amount of residues that may be ingested by a bee in 1 day. For adult honeybees, only nectar consumption is relevant.

As data on residues in plant material is not available, the chronic risk assessment for adult honeybees is based on the generic worst-case residue value of 1 mg a.s./kg plant matrix in nectar as given in the revised EPPO scheme. The daily exposure dose is a generic worst-case exposure of 0.128 µg a.s./bee/day, considering a worst-case sugar intakes of bee foragers of 128 mg/nectar/bee.

The ratio between the NOEL (= NOED in µg a.s./bee/day) and the exposure (also in µg a.s./bee/day) was calculated.

Table 9.6-5: Chronic risk assessment to adult honey bees following the intended uses of FGF11 in cereals and oilseed rape

Exposure route	Test substance	NOEDD	Generic worst-case residue intake	TER	Trigger value
Oral	FGF11	15.5 µg./bee/day	0.128µg a.s./bee/day	121.09	1

EPPO (2010) suggests a chronic TER trigger (NOEDD/daily dose) of 1. The calculated TER values indicate a high margin of safety above the trigger of 1. Thus, a chronic risk for adult bees following the use of FGF11 can be excluded.

Larval assessment according to EPPO 2010

The potential risk for honeybee larvae and larvae development is assessed through the amount of residues that may be ingested by the bee larva during its whole development. The ratio between the NOED (µg a.s./bee larvae) and the exposure (in µg a.s./bee larvae) is calculated (TER_{chronic}).

Exposure levels are estimated in the first instance as for adults based on the concentration in real parts of the plants as a surrogate for residual concentrations in nectar and pollen. Thus, a generic worst-case residue value of 1 mg a.s./kg plant matrix in pollen and nectar as given in the revised EPPO scheme (2010) is used.

Data for consumption of the overall sugar by honeybee larvae is set to 15.1 sugar/larva/day. Given that the developmental period of bee larvae is 5 days, the intake is given as $5 \times 15.1 = 75.5 \mu\text{g/larva/developmental period}$.

Table 9.6-6: Chronic risk assessment to larvae following the intended uses of FGF11 in cereals and oilseed rape

Exposure route	ED ₁₀	Generic worst-case residue intake	TER	Trigger value
Oral	20.66 $\mu\text{g/larva/developmental period}$	0.0755 $\mu\text{g/larvae/developmental period}$	273.6	1

The EPPO 2010 scheme proposes a trigger of 1 for assessment of the risk to honey bees, considering oral exposure to adult and larvae. For chronic oral assessment to adult and larvae, an acceptable risk is determined.

zRMS comments:

The chronic risk for bees according to EPPO 2010 is not required and has not been validated by zRMS.

Risk assessment according to EFSA 2013

Screening Step, Acute and Chronic Risk Assessment

It is noted that for cereals, only exposure via pollen is considered relevant for bees in agreement with the EFSA guidance document (2013). Consequently, for oral exposure, nurse bees instead of foragers have been considered for honey bees in the assessment below. For oilseed rape, both nectar and pollen exposure were considered.

Consideration of acute and chronic mixture toxicity

According to the EFSA Guidance Document on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bee” (2013)) mixture toxicity and toxicity of formulated products with two or more active substances must be specifically considered in the risk assessment.

For the assessment of toxicity, a surrogate LD₅₀/LDD₅₀/NOED can be calculated. The EFSA Guidance Document indicates that the following equation should be used for deriving a surrogate LD₅₀/LDD₅₀/NOED for a mixture of active substances with known toxicity assuming dose additivity.

Example LD₅₀ mixtox calculation:

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

where:

X (a.s.i) = fraction of active substance (i) in the mixture

LD₅₀ (a.s.i) = acute toxicity value for the active substance (i)

LD₅₀ (mix) = surrogate LD50 of the mixture

Since for the active substances azoxystrobin and prothioconazole only acute honey bee toxicity data is available, only the surrogate honey bee LD₅₀ (oral and contact) can be calculated. The surrogate (predicted) LD₅₀ of the mixture (oral and contact) is provided in the tables below.

Where the measured toxicity value of a formulated product with more than one active substance is available, which is the case for FGF11 F, this value should be compared with the predicted mixture toxicity assuming dose additivity.

In the tables below, the measured and predicted LD₅₀ values are compared.

Table 9.6-7: Mixture toxicity assessment for LD₅₀ (oral) - predicted and measured LD₅₀ mix

Test substance	Concentration of a.s. in formulation (g/L)	Fraction of a.s. in formulation ^a	Acute oral LD ₅₀ (µg/bee)	Surrogate (predicted) acute oral LD ₅₀ mix (µg/bee)	Toxicity per fraction	Contribution to the overall toxicity
Azoxystrobin	160	0.444	> 25	> 39.1	56.3	69.4
Prothioconazole	200	0.556	> 71		127.8	30.6
FGF11	360	1	> 454.8 / 145.8 ^b		n.a.	n.a.

Values in bold provide the measured and predicted mixture toxicity endpoint

^a Concentration of an active substance in the formulation, divided by the total concentration of all active substances in the formulation.

^b Product endpoint recalculated to total a.s. content and considering product density

Table 9.6-8: Mixture toxicity assessment for LD₅₀ (contact) - predicted and measured LD₅₀ mix

Test substance	Concentration of a.s. in formulation (g/L)	Fraction of a.s. in formulation ^a	Acute contact LD ₅₀ (µg/bee)	Surrogate (predicted) acute contact LD ₅₀ mix (µg/bee)	Toxicity per fraction	Contribution to the overall toxicity
Azoxystrobin	160	0.444	> 200	> 200	450.0	44.4
Prothioconazole	200	0.556	> 200		360.0	55.6
FGF11	360	1	> 400 / 128.3 ^b		n.a.	n.a.

Values in bold provide the measured and predicted mixture toxicity endpoint

^a Concentration of an active substance in the formulation, divided by the total concentration of all active substances in the formulation.

^b Product endpoint recalculated to total a.s. content and considering product density

Based on the mixture toxicity assessment provided above, it can be stated that the measured LD₅₀mix is higher compared to the predicted LD₅₀mix for both acute oral and contact exposure. However, the calculations are based on unbound endpoints, thus the calculated mix tox cannot be considered fully reliable since data does not fully reflect toxicity of the active substances. When assessing the underlying studies in further detail, reliability may be questioned. The acute oral and contact study on the active substance azoxystrobin was initially submitted for the DAR (1997), however, no details on the tested concentrations or behavioural abnormalities were reported, but only the final unbound LD₅₀ for acute and contact toxicity, with >25 µg a.s./bee and > 200 µg a.s./bee, respectively. Regarding the active substance prothioconazole, the original study was initially submitted in the DAR (2004), with study details being presented in the initial RAR (2018). In the oral testing, no mortalities were observed in the tested concentrations. However, for contact testing, no clear dose-response was observed as mortalities of 25%, 6% and 22% occurred at 50 µg a.s./bee, 100 µg a.s./bee and 200 µg a.s./bee after 48h, respectively.

Therefore, it is considered more suitable to rely on the available data with the formulation FGF11.

It is noted that prothioconazole is transformed into prothioconazole-desthio very quickly and to 100 % (see argumentation already provided for birds and mammals risk assessment). Therefore, mixture toxicity of azoxystrobin and prothioconazole-desthio also needs to be considered. However, no acute toxicity data on prothioconazole-desthio is available from the EFSA conclusion (2007) In any case, toxicity of prothioconazole-desthio is comparable to prothioconazole and since a transformation factor of 0.907 from prothioconazole to prothioconazole-desthio is to be considered, the mixture toxicity assessment azoxystrobin + prothioconazole covers the risk from mixture toxicity of azoxystrobin + prothioconazole-desthio. Consequently, the conclusion as taken above from the mixture assessment on azoxystrobin + prothioconazole is likewise applicable for azoxystrobin + prothioconazole-desthio, i.e. measured product data should be used for the risk assessment.

Even though the EFSA guidance of 2013 was never noted at EU level, a dietary assessment is performed for exposure of honeybees for uptake of food and for acute exposure of bumble bees. No assessment to exposure via guttation water or surface water should be performed, as in the updated guidance it is indicated that the water scenario is no longer considered for the risk assessment. This is due to the lack of sufficient data to quantify the water consumption, frequency, and magnitude of water collection by bees.

Table 9.6-9: Screening and first Tier assessment of the risk for bees due to the use of FGF11 in cereals (1.0 L product/ha)

Intended use	Cereals						
Product	FGF11 (Azoxystrobin 160 + Prothioconazole 200 g/L)						
Application rate (g/ha)	1 x 160 azoxystrobin + 200 prothioconazole (total a.s.)						
Scenario	Downward spraying; crop attractive for pollen only (nurse bees); BBCH 30-69						
Screening dietary assessment							
Test design	LD ₅₀ / NOED [µg total a.s./bee/(day)]				HQ / ETR	HQ / ETR trigger	
Acute oral - honey bee	> 454.8				0.02	> 0.2	
Acute contact - honey bee	> 400				2.5	> 42	
Chronic oral - honey bee	20.9				0.364	> 0.03	
Larvae - honey bee	20.66				0.21	> 0.2	
Acute oral - bumble bee	> 722.2				0.02	> 0.036	
Acute contact - bumble bee	> 650				1.5	> 7	
Tier 1 dietary assessment							
Test design	L(D)D ₅₀ /NOED	Foraging area	BBCH	Ef	SV * twa	ETR	ETR trigger
Chronic oral - Honey bees	20.9	Treated crop	30-69	1	0.92 * 0.72	0.032	> 0.03
		Weeds - post emergence	30-39	0.5	2.9*0.72	0.036	
			40-69	0.3		0.022	
		Field margin	30-69	0.0092	2.9*0.72	0.001	
		Adjacent crop	30-69	0.0033	5.8*0.72	0.001	
		Succeeding crop	30-69	1	0.54* 0.72	0.019	
Chronic Larvae - Honey bees	20.66	Treated crop	30-69	1	0.15*0.85	0.01	> 0.2
		Weeds - post emergence	30-39	0.5	2.2*0.85	0.05	
			40-69	0.3		0.03	
		Field margin	30-69	0.0092	2.2*0.85	< 0.01	
		Adjacent crop	30-69	0.0033	4.4*0.85	< 0.01	
		Succeeding crop	30-69	1	0.4*0.85	0.02	

Ef: Exposure factor; SV: Shortcut value

Values in **bold** exceed the relevant ETR or HQ trigger.

Table 9.6-10: Screening and first Tier assessment of the risk for bees due to the use of FGF11 in oilseed rape (1.0 L product/ha)

Intended use	Oilseed rape							
Product	FGF11 (Azoxystrobin 160 + Prothioconazole 200 g/L)							
Application rate (g/ha)	1 x 160 azoxystrobin + 200 prothioconazole (total a.s.)							
Scenario	Downward spraying; crop attractive for pollen and nectar (forager bees); BBCH 61-73							
Screening dietary assessment								
Test design		LD ₅₀ / NOED [µg total a.s./bee/(day)] a)			HQ / ETR		HQ / ETR trigger	
Acute oral - honey bee		> 454.8			0.02		> 0.2	
Acute contact - honey bee		> 400			2.5		> 42	
Chronic oral - honey bee		20.9			0.364		> 0.03	
Larvae - honey bee		20.66			0.21		> 0.2	
Acute oral - bumble bee		> 722.2			0.02		> 0.036	
Acute contact - bumble bee		> 650			1.5		> 7	
Tier 1 dietary assessment								
Test design	LD ₅₀ /NOED [µg/bee/day]	Foraging area	BBCH	Ef	SV * twa	ETR	ETR trigger	
Chronic oral - Honey bees	20.9	Treated crop	40-69	1	5.8 * 0.72	0.200	> 0.03	
			≥70	1	0 * 0.72	0.000		
		Weeds - post emergence	40≥70	0.25	2.9 * 0.72	0.025		
		Field margin	40≥70	0.0092	2.9 * 0.72	0.001		
		Adjacent crop	40≥70	0.0033	5.8 * 0.72	0.001		
		Succeeding crop	40≥70	1	0.54 * 0.72	0.019		
Chronic Lar- vae - Honey bees	20.66	Treated crop	40-69	1	4.4*0.85	0.18	> 0.2	
			≥70	1	0*0.85	< 0.01		
		Weeds - post emergence	40≥70	0.25	2.2*0.85	0.02		
		Field margin	40≥70	0.0092	2.2*0.85	< 0.01		
		Adjacent crop	40≥70	0.0033	4.4*0.85	< 0.01		
		Succeeding crop	40≥70	1	0.4*0.85	0.02		

Ef: Exposure factor; SV: Shortcut value
Values in **bold** exceed the relevant ETR or HQ trigger.

As can be seen from the assessments above, the risk for acute oral and contact exposure to honey bees and bumble bees is indicated to be acceptable for the use in cereals and oilseed rape based on Tier 1 risk assessments.

At screening step in cereals, a risk was indicated for chronic adult exposure and larval honey bees. At tier 1 assessment, a risk was still indicated for chronic exposure to adult honeybees, for the treated crop and weeds (post-emergence) at early BBCH stage scenarios.

For the use in oilseed rape, at screening a potential risk is indicated for oral chronic exposure of adult honey bees and larval honey bees. However, at tier 1 assessment, a risk was indicated for chronic exposure to adult honey bees for the the treated crop only. The remaining scenarios indicate an acceptable risk to adult honey bees and honey bee larvae.

Actually, semi-field tests are ongoing with formulation FGF11, in order to demonstrate with higher tier study that exposure to treated crop and treated weeds in the fields are not posing an unacceptable risk to adult honeybees. The studies will be provided as soon as they will be finalized, and the risk assessment according to higher tier data will be implemented.

zRMS comments:

zRMS is aware that the new EFSA bee GD is not yet noted by the MSs, nonetheless several MS (partially) use it. This is in line with PRAS 133 (2015), stating that it is recommended to perform the first tier of the honeybee risk assessment following the EFSA (2013) Bee GD with available data. In the same time during Harmonisation Meeting in Warsaw in 2023 it was decided that chronic risk to bees should be performed in Core Dossier according to EFSA GD 2013.

Therefore, following cMSs requests, the screening and first Tier of EFSA risk assessment according to the guidance document (2013) was included by the Applicant and evaluated by zRMS.

Based on the results the risk for acute oral and contact exposure to honey bees and bumble bees is indicated to be acceptable for the use in cereals and oilseed rape based on Tier 1 risk assessments.

At screening step in cereals, a risk was indicated for chronic adult exposure and larval honey bees. At tier 1 assessment, a risk was still indicated for chronic exposure to adult honeybees, for the treated crop and weeds (post-emergence) at early BBCH stage scenarios.

For the use in oilseed rape, at screening a potential risk is indicated for oral chronic exposure of adult honey bees and larval honey bees. However, at tier 1 assessment, a risk was indicated for chronic exposure to adult honey bees for the treated crop only. The remaining scenarios indicate an acceptable risk to adult honey bees and honey bee larvae.

Two higher tier studies (semi-field ~~and field~~) with formulation FGF11 have been provided in Germany and France, in order to demonstrate an acceptable risk to honeybees.

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

~~Higher tier studies are ongoing and higher tier risk assessment according to EFSA 2013 will be provided as soon as results from the semi field studies will be available. For study details please refer to KCP 10.3.5.1/01 and KCP 10.3.1.5/02 in Appendix 2.~~

A semi-field study with FGF11 on honey bees was performed. This study was performed to determine the potential effects of FGF11 on the honeybee (*Apis mellifera* L.) after one foliar application at a rate of 160.02 g a.i./ha Azoxystrobin and 204.07 g a.i./ha Prothioconazole (13.10 g product/ha or 1 L product/ha) during bee flight under semi-field conditions in *Phacelia tanacetifolia* in Germany in 2024, following the OEPP/EPPG Guideline No. 170(4) (2010) with partial integration of the EFSA Guidance Document No. 11(7): 3295 (2013) and the EU Guideline 7029/VI/95 rev. 4 (1997). Potential effects of the test item were evaluated against those observed in honeybees treated with tap water (control) and a toxic reference item (Dimethoate, 400 g a.i. dimethoate/ha).

There was no effect of the test item on adult worker, or worker larvae and pupae mortality, neither during the exposure period in the tunnel tents nor during the monitoring period.

Overall, a transient reduction in flight activity was observed in the test item group T on the day of application (0DAA) and the following day (1DAA). Afterwards, honeybee flight activity was similar in control and test item treatments.

No biological relevant test item effect was detected on honeybee behaviour.

There was no test item related effect neither on colony strength nor on the development of the brood for all stages and food storage area.

Overall, a foliar application of Azoxystrobin 160 g/L + Prothioconazole 200 g/L SC during active foraging of honeybees under semi-field conditions did not result in any significant adverse effects. For study details please refer to KCP 10.3.1.5/01 in Appendix 2.

~~In addition~~

The second study by Roig, J. (2024) was performed in France for Azoxystrobin 160 + Prothioconazole 200 SC formulation on the honeybee (*Apis mellifera* L.) in two test item application scenarios, T1 (outside of active bee foraging) and T2 (during active bee foraging), under semi-field conditions in full flowering *Phacelia tanacetifolia* at BBCH 65-66 according to CEB 230 in France. The target application rate of the test item Azoxystrobin 160 + Prothioconazole 200 SC was 1 L product/ha.

Based on the results of two higher studies from exposure from foliar application of Azoxystrobin 160 g/L + Prothioconazole 200 g/L SC during active foraging of honeybees under semi-field conditions did not result in any significant adverse effects and a safe risk can be granted.

It should be noted that refinement of chronic risk assessment based on semi-field studies is left for final decision of MSs according to recommendation given in CZHM, in Ecotoxicology, 2023.

9.6.3 Effects on bumble bees

The hazard quotient for acute toxicity (HQ) is calculated using the following equation:

$$HQ = \frac{AR}{\text{Acute LD}_{50}}$$

With:

AR = Application rate in g product/ha or g a.s./ha

Acute LD₅₀ expressed in µg product/bee or µg a.s./bee

Table 9.6.3-1: First-tier assessment of the risk for *Bombus terrestris* due to the use of FGF11 in cereals and oilseed rape

Product	FGF11 (Azoxystrobin 160 + Prothioconazole 200 g/L S)		
Application rate (g a.s./ha)	1 × 1000		
Intended use	Cereals and oilseed rape		
Test design	LD₅₀ (lab.) (µg prod./bee)	Single application rate (g prod./ha)	Q_{HO}, Q_{HC} criterion: Q_H ≤ 50
Oral toxicity	> 722.2	1000	< 1.39
Contact toxicity	> 650		< 1.54

Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure. Q_H values shown in **bold** breach the relevant trigger.

The acute hazard quotients (HQs) are lower than the trigger value of 50, indicating that the formulation FGF11 pose a low risk to bumble bees following the application of the formulation according to the use pattern proposed.

For the EFSA risk assessment based on bumble bee studies, please refer to the honey bee section above.

zRMS comments:

The acute risk assessment for bees presented in Tables 9.6-2-9.6-4 is validated by the zRMS.

HQ_{oral}, contact and oral values for the active substances and the formulated product FGF11 are below the trigger of 50, indicating a low acute risk for bumble bees.

Please note that the evaluation has been performed in line with SANCO/10329/2002 rev 2 final.

Overall, acceptable risk to bumble bees may be concluded from the intended uses of FGF11 in cereals and for oilseed rape.

9.6.4 Effects on solitary bees

No data available and considered necessary.

9.6.5 Overall conclusions

Risk assessment based on SANCO and EPPO:

The acute hazard quotients (HQs) are lower than the trigger value of 50 for both honey bees and bumble bees, indicating that both active substances (azoxystrobin and prothioconazole) and the formulation FGF11 pose a low risk to bees following the application of the formulation according to the use pattern proposed.

The chronic risk assessment provided TER values above the triggers. Thus, a chronic risk for adult bees and honeybee larvae following the use of FGF11 must be excluded at the National level of each Member state that requires that evaluation.

Risk assessment based on EFSA 2013:

The acute risk assessment for honey bees and bumble bees based on the available data set indicated an acceptable risk for acute oral and contact exposure of honey bees and bumble bees based on the screening Tier assessment.

For the remaining scenarios chronic exposure of honey bee adults and larvae, a risk was indicated for chronic exposure of adult honey bees for the exposure on treated crop and weeds in the field for cereals and on treated crop for application on oilseed rape. ~~Higher tier studies (semi-field studies) are currently ongoing and higher tier refinements will be addressed as soon final reports will be available.~~

To refine the chronic risk assessment two higher two higher studies to bees has been evaluated by zRMS. Based on the results of foliar application of Azoxystrobin 160 g/L + Prothioconazole 200 g/L SC during active foraging of honeybees under semi-field conditions did not result in any significant adverse effects and a safe risk can be granted.

In the same time, it should be noted that refinement of chronic risk assessment based on semi-field studies is left for final decision of MSs according to recommendation given in CZHM, in Ecotoxicology, 2023.

~~Relevance of the risk assessment performed according to EFSA 2013 should be considered at Member State level.~~

9.7 Effects on arthropods other than bees (KCP 10.3.2)

9.7.1 Toxicity data

No laboratory studies with glass plates are available for the active substances azoxystrobin and prothioconazole. However, for the risk assessment of FGF11 (Azoxystrobin 160 + Prothioconazole 200 g/L SC), it is considered most appropriate to use data on FGF11. Extended laboratory studies are available with the formulation FGF11 for *Aphidius rhopalosiphi*, *Typhlodromus pyri*, *Coccinella septempunctata* and *Chrysoperla carnea*.

New studies with the formulation FGF11 were not evaluated as part of the EU assessment of the active substances. Endpoints are shown in the table below and are listed in Appendix 1 and summarized in Appendix 2.

Table 9.7-1: Relevant endpoints for the risk assessment for non-target arthropods

Species	Substance	Exposure System	Results	Reference
<i>Aphidius rhopalosiphi</i>	Azoxystrobin 160 + Prothioconazole 200 g/L SC	Extended lab study, dried residues on treated bean plants (3D), limit test	Mortality - LR ₅₀ > 1911 mL prod./ha Repro - ER₅₀ > 1911 mL prod./ha	Leopold J., 2024, 179211002, KCP 10.3.2.2/01
<i>Typhlodromus pyri</i>	Azoxystrobin 160 + Prothioconazole 200 g/L SC	Extended lab study, dried residues on treated bean leaf disks (2D), limit test	Mortality - LR ₅₀ > 1902 mL prod./ha Repro - ER₅₀ > 1902 mL prod./ha	Leopold J., 2024, 179211060, KCP 10.3.2.2/02
<i>Coccinella septempunctata</i>	Azoxystrobin 160 + Prothioconazole 200 g/L SC	Extended lab study, dried residues on treated bean leaf disks (2D), limit test	LR₅₀ > 1904 mL prod./ha	Leopold J., 2024, 179211012, KCP 10.3.2.2/03
<i>Chrysoperla carnea</i>	Azoxystrobin 160 + Prothioconazole 200 g/L SC	Extended lab study, dried residues on treated bean leaf disks (2D), limit test	LR₅₀ > 1904 mL prod./ha	Leopold J., 2024 179211047 KCP 10.3.2.2/04
Field or semi-field tests				
Not required				

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

zRMS comments:

Studies on effects of the formulated product to NTA listed in Table above were evaluated by the zRMS and considered acceptable. The reported endpoints are confirmed.
Summary of the performed studies together with zRMS evaluation may be found in Appendix 2.

9.7.1.1 Justification for new endpoints

Risk assessments are most adequately conducted based on the available data for FGF11 (Azoxystrobin 160 + Prothioconazole 200 g/L SC), since contact exposure of non-target arthropod is considered to be mainly to the formulation (mixture) rather than to single active substances. Data are available on extended laboratory tests on the standard species *Aphidius rhopalosiphi*, *Typhlodromus pyri* and the additional species *Coccinella septempunctata*, and *Chrysoperla carnea*. No Tier 1 glass plate studies have been conducted since toxicity to non-target organisms had to be expected based on the available data from the EU review of prothioconazole.

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 II.

9.7.2.1 Risk assessment for in-field exposure

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use groups cereals also covers the risk for non-target arthropods from all other intended uses (see Table 9.1-1).

Table 9.7-2: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of FGF11 in cereals (1-3, 5-6)

Intended use	Cereals		
Product	FGF11		
Application rate (ml/ha)	1 × 1000		
MAF (foliar)	1		
Test species Tier II	LR₅₀/ER₅₀ (lab.) (mL/ha)	PER_{in-field} (mL/ha)	HQ_{in-field} criterion: HQ ≤ 1
<i>Typhlodromus pyri</i>	> 1902	1000	< 0.53
<i>Aphidius rhopalosiphi</i>	> 1911		< 0.52
<i>Coccinella septempunctata</i>	> 1904		< 0.53
<i>Chrysoperla carnea</i>	> 1904		< 0.53

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

An acceptable in-field risk was indicated for the intended use in cereals for *Aphidius rhopalosiphi*, *Typhlodromus pyri*, *Coccinella septempunctata* and *Chrysoperla carnea*. No further assessment is deemed necessary.

zRMS comments:

Acceptable in-field risk were demonstrated for the indicator species *T. pyri* and *A. rhopalosiphi* and two additional species using mortality and reproduction data from extended laboratory studies for product FGF11.

Based on the calculations performed in the Table 9.7-2 an acceptable in-field risk was indicated for the intended use in cereals (worst case scenario) for *Aphidius rhopalosiphi*, *Typhlodromus pyri*, *Coccinella septempunctata* and *Chrysoperla carnea*. No further assessment is required.

9.7.2.2 Risk assessment for off-field exposure

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group cereals also covers the risk for non-target arthropods from all other intended uses (see 9.1.2).

Table 9.7-3: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of FGF11 in cereals (1-3, 5-6)

Intended use		Cereals			
Active substance/product		FGF11			
Application rate (ml/ha)		1 × 1000			
MAF (foliar)		1			
Vdf		2D studies: 5; 3D studies: none			
Test species Tier II	LR ₅₀ (lab.) (mL/ha)	Drift rate	PER _{off-field} (mL/ha)	CF	HQ _{off-field} criterion: HQ ≤ 1
<i>Typhlodromus pyri</i> (3D)	> 1902	2.77	138.50	5	< 0.073
<i>Aphidius rhopalosiphi</i> (2D)	> 1911		27.70		< 0.014
<i>Coccinella septempunctata</i> (2D)	> 1904				< 0.015
<i>Chrysoperla carnea</i> (2D)	> 1904				< 0.015

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.

Accordingly, an acceptable off-field risk was indicated for the intended use in cereals for *Aphidius rhopalosiphi*, *Typhlodromus pyri*, *Coccinella septempunctata* and *Chrysoperla carnea*.

zRMS comments:

The risk assessment presented in Table 9.7-3 is validated by the zRMS.

As a worst case the VDF of 5 has been considered by the Applicant according to recommendation given in Central Zone since available investigations indicate that VDF of 10 recommended by ESCORT 2 guidance document may lead to underestimation of the exposure.

It should be, however, noted that according to EFSA Supporting publication 2019: EN-1673, VDF of 5 should be considered as the interim solution that will be reflected in the SANCO/10329/2002 rev 2 final with its implementation considered further. Since use of VDF of 5 was not reflected in the current SANCO terrestrial guidance, its use is not yet mandatory.

Based on the calculations performed above an acceptable off-field risk was indicated for the intended use in cereals for *Aphidius rhopalosiphi*, *Typhlodromus pyri*, *Coccinella septempunctata* and *Chrysoperla carnea*.

9.7.2.3 Additional higher-tier risk assessment

Not relevant.

9.7.2.4 Risk mitigation measures

No risk mitigation needed.

9.7.3 Overall conclusions

Toxicity data from studies with FGF11 were used to perform risk assessment for the formulated product.

An acceptable in-field and off-field risk was shown for the intended use in cereals for *Aphidius rhopalosiphi*, *Typhlodromus pyri*, *Coccinella septempunctata* and *Chrysoperla carnea*, thereby also covering the use in oilseed rape. The risk to non-target arthropods from exposure to FGF11 is considered safe.

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

9.8.1 Toxicity data

Studies on the toxicity to earthworms and other non-target soil organisms have been carried out with azoxystrobin, prothioconazole and their relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents (EFSA Conclusion 2007, EFSA conclusion 2010).

Effects on earthworms and other non-target soil organisms of FGF11 were not evaluated as part of the EU assessment of the active substances. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

Table 9.8-1: Relevant endpoints for the risk assessment for earthworms and other non-target soil organisms

Species	Substance	Exposure System	Results	Reference
Azoxystrobin				
<i>Eisenia fetida</i>	Azoxystrobin 250 g/L SC (YF10537)	56d, chronic 10 % peat content	NOEC = 20 mg a.s./kg d.w. soil ¹ NOEC_{corr.} = 10 mg/kg dw soil*	EFSA (2010)
<i>Folsomia candida</i>	Azoxystrobin 250 g/L SC (YF10537)	28 d, chronic	NOEC = 50 mg a.s./kg d.w. soil ¹ NOEC_{corr.} = 25 mg/kg dw soil*	
Azoxystrobin metabolites				
<i>Eisenia fetida</i>	R234886	56d, chronic 10 % peat content	NOEC _{parent compound} = 20 mg a.s./kg dw soil NOEC _{corr.} = 10 mg/kg dw soil* NOEC_{10x} = 1 mg/kg dw soil ²	EFSA (2010)
<i>Eisenia fetida</i>	R401553 (SYN501657)	56d, chronic 10 % peat content	NOEC _{parent compound} = 20 mg a.s./kg d.w. soil NOEC _{corr.} = 10 mg/kg dw soil* NOEC_{10x} = 1 mg/kg dw soil ²	
<i>Eisenia fetida</i>	R402173 (SYN501114)	56d, chronic 10 % peat content	NOEC _{parent compound} = 20 mg a.s./kg d.w. soil NOEC _{corr.} = 10 mg/kg dw soil* NOEC_{10x} = 1 mg/kg dw soil ²	
<i>Folsomia candida</i>	R234886	28 d, chronic	NOEC _{parent compound} = 50 mg a.s./kg d.w. soil NOEC _{corr.} = 25 mg/kg dw soil* NOEC_{10x} = 2.5 mg/kg dw soil ²	
<i>Folsomia candida</i>	R401553 (SYN501657)	28 d, chronic	NOEC _{parent compound} = 50 mg a.s./kg d.w. soil NOEC _{corr.} = 25 mg/kg dw soil* NOEC_{10x} = 2.5 mg/kg dw soil ²	
<i>Folsomia candida</i>	R402173 (SYN501114)	28 d, chronic	NOEC _{parent compound} = 50 mg a.s./kg d.w. soil NOEC _{corr.} = 25 mg/kg dw soil* NOEC_{10x} = 2.5 mg/kg dw soil ²	
Prothioconazole				
<i>Eisenia fetida</i>	Prothioconazole 250 g/L EC	56 d, chronic 10 % peat content	NOEC = 1.33 mg a.s./kg d.w. soil ¹ NOEC_{corr.} = 0.665 mg/kg dw*	EFSA (2007)
<i>Folsomia candida</i>	Prothioconazole	28 d, chronic 10 % peat content	NOEC ≥ 64 mg a.s./kg dw soil NOEC_{corr.} ≥ 32 mg/kg dw soil*	
<i>Hypoaspis aculeifer</i>	Prothioconazole	34 d, chronic 10 % peat content	NOEC ≥ 100 mg/kg dw soil NOEC_{corr.} ≥ 50 mg/kg dw soil*	
Prothioconazole metabolites				

<i>Eisenia fetida</i>	Prothioconazole-desthio (M04)	56 d, chronic 10 % peat content	NOEC _{repro} = 1 mg p.m./kg dw soil NOEC_{corr} = 0.5 mg p.m./kg dw soil*	EFSA (2007)
<i>Folsomia can- dida</i>	Prothioconazole-desthio (M04)	28 d, chronic 10 % peat content	NOEC = 62.5 mg p.m./kg dw soil NOEC_{corr} = 31.25 mg/kg dw soil*	
<i>Eisenia fetida</i>	Prothioconazole-S-me- thyl (M01)	56 d, chronic 10 % peat content	NOEC = 100 mg p.m./kg dw soil NOEC_{corr} = 50 mg/kg dw soil*	
<i>Folsomia can- dida</i>	Prothioconazole-S-me- thyl (M01)	28 d, chronic 10 % peat content	NOEC ≥ 31.6 mg p.m./kg dw NOEC_{corr} ≥ 15.8 mg/kg dw soil*	
Product FGF11 (Azoxystrobin 160 + Prothioconazole 200)				
<i>Eisenia andrei</i>	Azoxystrobin 160 + Prothioconazole 200 g/L SC	56 d, chronic 10 % peat content	NOEC _{repro} = 55.6 mg prod/kg dw soil EC ₁₀ = 22.4 mg prod/kg dw soil EC ₂₀ = 54.2 mg prod./kg dw soil NOEC _{mortality} ≥100 mg prod./kg dw soil EC_{10 corr} = 11.2 mg prod/kg dw soil*	Hübner, S. (2024), 179211022, KCP 10.4.1.1/01
<i>Folsomia can- dida</i>	Azoxystrobin 160 + Prothioconazole 200 g/L SC	Mixed into soil 14 d, chronic 5 % peat content	NOEC _{repro} = 171 mg prod./kg dw soil EC ₁₀ = 211.9 mg prod./kg dw soil NOEC _{mortality} ≥1000 mg prod./kg dw soil NOEC_{corr} = 85.5 mg prod/kg dw soil*	Hübner, S. (2024), 179211016, KCP 10.4.2.1/02
<i>Hypoaspis acu- leifer</i>	Azoxystrobin 160 + Prothioconazole 200 g/L SC	Mixed into soil 14 d, chronic 5 % peat content	NOEC _{repro} ≥1000 mg prod./kg dw soil EC ₁₀ = n.d. EC _{20/50} >1000 mg prod./kg dw soil NOEC_{repro} ≥ 500 mg prod./kg dw soil*	Hübner, S. (2024), 179211089, KCP 10.4.2.1/01
Field studies				
Not available for Azoxystrobin 160 + Prothioconazole 200 g/L SC				
Litter bag test				
No litter bag study was conducted with Azoxystrobin, Prothioconazole, their metabolites or Azoxystrobin 160 + Prothioconazole 200 g/L SC				

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

* Corrected value derived by dividing the endpoint by a factor of 2 in accordance with the EPPO earthworm scheme 2002, since both active substances Azoxystrobin and Prothioconazole presented a log Pow > 2.

¹ No long-term studies are available with active substances. Endpoints for active substances derived from formulations (Azoxystrobin 250 g/L SC (YF10537) and Prothioconazole 250 g/L EC), presented in EFSA conclusion 2010 and 2007, respectively were used to conduct the risk assessment.

² Derived from parental endpoints using the 10x approach for metabolites (NOEC_{parental compound}/10).

zRMS comments:

Data for earthworms for n and prothioconazole and azoxystrobin provided in Table 9.8-1 are in line with EU agreed endpoints reported in EFSA Journal 2010; 8(4):1542 and EFSA Scientific Report (2007) 106, respectively.

Studies on effects of the formulated product to earthworm and other soil macro-organism listed above were evaluated by the zRMS and considered acceptable. The reported endpoints are confirmed.

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

9.8.1.1 Justification for new endpoints

New studies were conducted with FGF11 on non-target soil organisms in the laboratory (*Eisenia andrei*, *Hypoaspis aculeifer* and *Folsomia candida*). New data are listed in Appendix 1 and summarised in Appendix 2.

Considering EFSA (2010), no toxicity data on predatory mites are available on azoxystrobin or on any representative formulation. Nonetheless, the lack of data for *Hypoaspis* is not considered critical, since the full data set is submitted for formulation FGF11 and from an overview of toxicity data available, the most

sensitive soil organism is earthworm and predatory mite is deemed to be the less sensitive following exposure to FGF11.

9.8.2 Risk assessment

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

9.8.2.1 First-tier risk assessment

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

According to the assessment of environmental-fate data, multi-annual accumulation (PEC_{S-accu}) in soil had to be considered for azoxystrobin and the metabolite R234886 but was not relevant for the metabolites R401553 and R402173 of azoxystrobin, prothioconazole and the respective metabolites.

Azoxystrobin and relevant metabolites

Table 9.8-2: First-tier assessment of the chronic risk for earthworms and other non-target soil organisms for azoxystrobin due to the use of FGF11 in cereals and oilseed rape

Intended use		Cereals & oilseed rape	
Chronic effects on earthworms			
Product/active substance	NOEC _{corr.} (mg/kg dw)	PEC _{S-accu} ** (mg/kg dw)	TER _{lt} (criterion TER ≥ 5)
Azoxystrobin	10*	0.069	144.9
Chronic effects on other soil organism			
Product/active substance	NOEC _{corr.} (mg/kg dw)	PEC _{S-accu} ** (mg/kg dw)	TER _{lt} (criterion TER ≥ 5)
Azoxystrobin (<i>Folsomia candida</i>)	25*	0.069	362.3

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

* Endpoint for active substance azoxystrobin derived from study with formulation 250 g/L SC (YF10537).

** $PEC_{S,accumulation} = PEC_{S,ini} + PEC_{S,plateau}$

Table 9.8-3: First-tier assessment of the chronic risk for earthworms and other non-target soil organisms for the metabolites of azoxystrobin R234886, R401553 and R402173 due to the use of FGF11 in cereals and oilseed rape

Intended use		Cereals & oilseed rape	
Chronic effects on earthworms			
Product/active substance	NOEC _{10x} (mg/kg dw)*	PEC _{soil} ¹ (mg/kg dw)	TER _{lt} (criterion TER ≥ 5)
R234886	1	0.013 ²	76.9
R401553	1	0.004	250
R402173	1	0.006	166.7
Chronic effects on other soil organisms			
Product/active substance	NOEC _{10x} (mg/kg dw)*	PEC _{soil} ¹ (mg/kg dw)	TER _{lt} (criterion TER ≥ 5)
R234886 (<i>Folsomia candida</i>)	2.5	0.013 ²	192.3
R401553 (<i>Folsomia candida</i>)	2.5	0.004	625
R402173 (<i>Folsomia candida</i>)	2.5	0.006	416.7

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

* Endpoints for azoxystrobin metabolites derived from parental compound (ten times higher toxicity assumed).

¹ If not indicated otherwise, PEC_{soil-initial} was used for risk assessment

² PEC_{S,accumulation} = PEC_{S,ini} + PEC_{S,plateau}

Prothioconazole and relevant metabolites

Table 9.8-4: First-tier assessment of the acute and chronic risk for earthworms and other non-target soil organisms for prothioconazole due to the use of FGF11 in cereals and oilseed rape

Intended use	Cereals & oilseed rape		
Chronic effects on earthworms			
Product/active substance	NOEC _{corr.} (mg/kg dw)	PEC _{soil} ¹ (mg/kg dw)	TER _{it} (criterion TER ≥ 5)
Prothioconazole	0.665*	0.053	12.5
Chronic effects on other soil organisms			
Product/active substance	NOEC _{corr.} (mg/kg dw)	PEC _{soil} ¹ (mg/kg dw)	TER _{it} (criterion TER ≥ 5)
Prothioconazole (<i>Folsomia candida</i>)	≥ 32	0.053	≥ 603.8
Prothioconazole (<i>Hypoaspis aculeifer</i>)	≥ 50		≥ 943.4

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

* Endpoint for active substance prothioconazole derived from study with formulation 250 g/L EC

¹ If not indicated otherwise, PEC_{soil-initial} was used for risk assessment

Table 9.8-5: First-tier assessment of the chronic risk for earthworms and other non-target soil organisms for the metabolites of prothioconazole prothioconazole-desthio (M04) and prothioconazole-S-methyl (M01) due to the use of FGF11 in cereals and oilseed rape

Intended use		Cereals & oilseed rape	
Chronic effects on earthworms			
Product/active substance	NOEC _{corr.} (mg/kg dw)	PEC _{soil} ¹ (mg/kg dw)	TER _{it} (criterion TER ≥ 5)
Prothioconazole-desthio (M04)	0.5	0.028	17.9
Prothioconazole-S-methyl (M01)	50	0.008	6250
Chronic effects on other soil organisms			

Product/active substance	NOEC _{corr.} (mg/kg dw)	PEC _{soil} ¹ (mg/kg dw)	TER _{it} (criterion TER ≥ 5)
Prothioconazole-desthio (M04) (<i>Folsomia candida</i>)	31.25	0.028	1116.1
Prothioconazole-S-methyl (M01) (<i>Folsomia candida</i>)	≥ 15.8	0.008	≥ 1975

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

¹ If not indicated otherwise, PEC_{soil}-initial was used for risk assessment

Formulation FGF11

Table 9.8-6: First-tier assessment of the chronic risk for earthworms and other non-target soil organisms due to the use of FGF11 in cereals and oilseed rape

Organisms due to the use of FGF11 in cereals and oilseed rape			
Intended use	Cereals & oilseed rape		
Chronic effects on earthworms			
Product/active substance	EC ₁₀ /NOEC _{corr.} (mg/kg dw)	PEC _{soil} * (mg/kg dw)	TER _{lt} (criterion TER ≥ 5)
FGF11 (Azoxystrobin 160 + Prothioconazole 200 g/L SC)	11.2	0.299	37.5
Chronic effects on other soil organisms			
Product/active substance	NOEC _{corr.} (mg/kg dw)	PEC _{soil} * (mg/kg dw)	TER _{lt} (criterion TER ≥ 5)
FGF11(Azoxystrobin 160 + Prothioconazole 200 g/L SC) (<i>Folsomia candida</i>)	85.5	0.299	286.0
FGF11(Azoxystrobin 160 + Prothioconazole 200 g/L SC) (<i>Hypoaspis aculeifer</i>)	≥ 500		≥ 1672

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

*The formulation components are considered to dissipate rapidly after application, therefore only one application is taken into consideration. The rate of formulation was based on a specific density of 1.228 g/mL with a maximum application of 1.0 L/ha assuming 80% crop interception

All TER values for earthworms and other soil non-target organisms for the active substances azoxystrobin, prothioconazole and their relevant metabolites, and the formulated product FGF11 are above the trigger value of 5. Thus, the risk of FGF11 following the intended uses in cereals and oilseed rape is considered acceptable.

zRMS comments:

The calculations of the risk assessment for earthworms and other soil non-target organisms for the active substances azoxystrobin, prothioconazole and their relevant metabolites, and the formulated product FGF11 are above the trigger value of 5. Thus, the risk of FGF11 following the intended uses in cereals and oilseed rape is considered acceptable.

9.8.2.2 Higher-tier risk assessment

Not relevant.

9.8.3 Overall conclusions

Exposure to azoxystrobin, prothioconazole and their metabolites indicated an acceptable risk for earthworms and other non-target soil organisms for the intended uses in cereals and oilseed rape using Tier 1 assessments.

The risk to non-target soil meso- and macrofauna from the intended uses of the formulated product FGF11

was indicated to be acceptable at Tier 1 assessment.

9.9 Effects on soil microbial activity (KCP 10.5)

9.9.1 Toxicity data

Studies on effects to soil microorganisms have been carried out with azoxystrobin metabolites, prothioconazole and relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents (EFSA Conclusion 2007 and EFSA conclusion 2010).

Effects on soil microorganisms of the formulation FGF11 were not evaluated as part of the EU assessment of the active substances. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

Table 9.9-1: Relevant endpoints for the risk assessment of soil microorganisms

Table 5.5-1: Relevant endpoints for the risk assessment of soil microorganisms				
Endpoint	Substance	Exposure System	Results	Reference
Azoxystrobin + metabolites				
N-mineralisation	Azoxystrobin 250 EC ¹	28 d	No effect (< 25%) at 2500 g a.s./ha, i.e. 2 mg a.s./kg dry soil*	EFSA 2010
N-mineralisation	R234886	28 d	No effect (< 25%) at 10 mg/ kg dry soil	
N-mineralisation	R401553 (SYN501657)	28 d	No effect (< 25%) at 2.643 mg test item/kg dry soil	
N-mineralisation	R402173 (SYN501114)	28 d	No effect (< 25%) at 4.131 mg test item/kg dry soil	
Prothioconazole + metabolites				
N-mineralisation	Prothioconazole	28 d	No effect (< 25%) at 2.71 mg a.s./kg dry soil	EFSA 2007
N-mineralisation	Prothioconazole-des-thio (M04)	28 d	No effect (< 25%) at 0.27 mg p.m./kg dry soil	
N-mineralisation	Prothioconazole-des-thio (M04)	28 d	No effect (< 25%) at 1.37 mg p.m./kg dry soil	
N-mineralisation	Prothioconazole-S-methyl (M01)	28 d	No effect (< 25%) at 2.69 mg p.m./kg dry soil	
Product FGF11 (Azoxystrobin 160 + Prothioconazole 200)				
N-mineralisation	Azoxystrobin 160 + Prothioconazole 200 g/L SC	28 d	No effect (< 25%) at 1.50 mg and 15.0 mg prod./kg dry soil	Hammesfahr, U. (2024), 179211080, KCP 10.5/01

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

* Assuming soil depth of 5 cm and soil bulk density of 1.5 g/cm³

¹ No long-term studies are available with active substances. Endpoints for active substances derived from formulations (Azoxystrobin 250 g/L SC (YF10537) and Prothioconazole 250 g/L EC), presented in EFSA conclusion 2010 and 2007, respectively were used to conduct the risk assessment.

zRMS comments:

Soil micro-organism toxicity data for azoxystrobin, prothioconazole and their relevant metabolites provided in Tables 9.9-1 above were confirmed by zRMS that they are in line with EU agreed endpoints reported in EFSA-Journal 2010; 8(4):1542, Azoxystrobin Review Report for first inclusion; 7581/VI/97-Final; 1998#d and EFSA Scientific Report (2007) 106, respectively. Studies on effects of the formulated product to earthworm and other soil macro-organism listed above were evaluated by the zRMS and considered acceptable. The reported endpoints are confirmed. Summary of the performed studies together with zRMS evaluation may be found in Appendix 2.

9.9.1.1 Justification for new endpoints

Information on the toxicity of azoxystrobin (surrogate endpoint from solo formulation), azoxystrobin metabolites and prothioconazole and relevant metabolites (EFSA Conclusion 2007 and EFSA conclusion 2010) were used to perform the risk assessment.

Moreover, a new study on the toxicity of FGF11 on soil micro-organisms was provided and is used to assess the risk.

9.9.2 Risk assessment

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

The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2, and were already used in the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) (see 0).

Table 9.9-2: Assessment of the risk for effects on soil micro-organisms due to the use of FGF11 in cereals and oilseed rape

Intended use	Cereals & oilseed rape		
N-mineralisation			
Product/active substance	Max. conc. with effects ≤ 25 % (mg/kg dw)	PEC _{soil} * (mg/kg dw)	Risk acceptable (margin of safety)?
Azoxystrobin ³	2.0	0.069	Yes (29.0)
R234886	10.0	0.013 ¹	Yes (769)
R401553	2.643	0.004	Yes (661)
R402173	4.131	0.006	Yes (689)
Prothioconazole	2.71	0.053	Yes (51.1)
Prothioconazole-S-methyl (M01)	2.69	0.008	Yes (336)
Prothioconazole-desthio (M04)	1.37	0.028	Yes (48.9)
FGF11	15.0	0.299 ²	Yes (50.2)

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

* If not indicated otherwise, $PEC_{soil-initial}$ was used for risk assessment

¹ $PEC_{S,accumulation} = PEC_{S,ini} + PEC_{S,plateau}$

² The formulation components are considered to dissipate rapidly after application, therefore only one application is taken into consideration. The rate of formulation was based on a specific density of 1.228 g/mL with a maximum application of 1.0 L/ha assuming 80% crop interception

³ The endpoint was recalculated based on the solo-formulation data from the EU review.

The margins of safety shown in the above table indicate that PEC_{soil} values do not exceed the trigger value for acceptable functional effects (adverse effects $\leq 25\%$ on N-transformation). Thus, it can be concluded that the use of FGF11 at the proposed rates does not pose a risk to soil micro-organisms.

zRMS comments:

The risk assessment presented in Table 9.9-2 above is validated by zRMS.

The effects on the nitrogen transformations are acceptable ($<25\%$) at concentration which is higher than the maximum relevant PECs for the maximum application rate of active substance prothioconazole and azoxystrobin and its metabolites.

9.9.3 Overall conclusions

An acceptable risk for soil microorganisms (N-transformation) is indicated for azoxystrobin, prothioconazole and their metabolites as well as the formulation FGF11 for the intended uses in cereals and oilseed rape.

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 active substances azoxystrobin and prothioconazole. Full details of these studies are provided in the respective EU DAR and related documents (EFSA 2007, EFSA 2010).

Effects on non-target terrestrial plants of FGF11 were not evaluated as part of the EU assessment of the active substances (EFSA 2007, EFSA 2010). New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

Species (most sensitive)	Substance	Exposure System	Results	Reference
Azoxystrobin				
Lettuce, radish, Wheat (most sensitive species)	Azoxystrobin	Seedling emergence	LC ₅₀ > 20 mg/kg EC ₅₀ > 20 mg/kg NOEC > 20 mg/kg	EFSA 2010
Prothioconazole				
<i>Amaranthus retro- flexus</i>	Prothioconazole	Pre-emergence /Seed- ling emergence	At 250 g a.s./ha there was 10% max. phytotoxic effect.	EFSA 2007
<i>Amaranthus retro- flexus</i> , <i>Beta vulgaris</i>	Prothioconazole	Post-emergence /Seed- ling growth	At 200 g a.s./ha there was 5% max. phytotoxic effect.	
Product FGF11 (Azoxystrobin 160 + Prothioconazole 200)				
4 dicots + 2 monocots, Limit test	Azoxystrobin 160 + Prothioconazole 200 g/L SC	21 d Seedling emergence	ER ₅₀ ≥ 1.9 L/ha NOER ≥ 1.9 L/ha No effect ≥ 50% at 1.9 L/ha	Horstmann, C. (2024), 179211086, KCP 10.6.2/01
4 dicots + 2 monocots, Limit test	Azoxystrobin 160 + Prothioconazole 200 g/L SC	21 d Vegetative vigour	ER ₅₀ ≥ 1.9 L/ha NOER ≥ 1.9 L/ha No effect ≥ 50% at 1.9 L/ha	Horstmann, C. (2024), 179211087, KCP 10.6.2/02

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

zRMS comments:

Studies on effects of the formulated product to non target terrestrial plants listed above were evaluated by the zRMS and considered acceptable. The reported endpoints are confirmed.
Summary of the performed studies together with zRMS evaluation may be found in Appendix 2.

9.10.1.1 Justification for new endpoints

New studies were conducted in order to assess the risk of FGF11 to non-target terrestrial plants.

9.10.2 Risk assessment

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

Risk assessments are presented based on Tier 2 data for seedling emergence and growth as well as vegetative vigour.

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

The risk assessment is based on the “Guidance Document on Terrestrial Ecotoxicology”, (SANCO/10329/2002 rev.2 final, 2002)³. It is restricted to off-field situations, as non-target plants are non-crop plants located outside the treated area, where they may be exposed to spray drift. The amount of spray drift reaching off-crop habitats is calculated using the 90th percentile estimates derived by the BBA (2000)⁴ from the spray-drift predictions of Ganzelmeier & Rautmann (2000)⁵.

For the use pattern proposed for FGF11 the risk assessment of non-target terrestrial plants is presented in the tables below.

Table 9.10-2: Assessment of the risk for non-target plants due to the use of FGF11 in cereals and oilseed rape

Intended use	Cereals & oilseed rape			
Active substance/product	FGF11			
Application rate (L/ha)	1 × 1.0			
Test design	ER₅₀ (L prod. /ha)	Drift percentile (%)	PER_{off-field} (L/ha)	TER criterion: TER ≥ 5
Seedling emergence test / Vegetative vigour test	> 1.9	2.77	0.0277	> 68.59

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

All TER values for non-target terrestrial plants (NTTPs) are above the trigger value of 5. Thus, the risk of FGF11 to non-target terrestrial plants following the intended uses is considered acceptable.

zRMS comments:

The calculations of the risk assessment for non-target plants have been validated by zRMS. Overall, an acceptable off-field risk is demonstrated for the proposed worst-case use 1 l/ha on cereals. The other intended uses of in the oilseed rape crop has an acceptable risk assessment to non-target terrestrial plants, using the risk envelope approach, without the need for mitigation measures.

9.10.2.3 Higher-tier risk assessment

Not relevant, based on the outcome of the Tier 2 risk assessments

9.10.2.4 Risk mitigation measures

Not relevant, based on the outcome of the Tier 2 risk assessments.

³ SANCO (2002). Guidance Document on terrestrial ecotoxicology under Council Directive 91/414/EEC. SANCO/10329/2002. 17 October 2002.

⁴ BBA (2000) Bundesanzeiger Jg. 52 (Official Gazette), Nr 100, S. 9879-9880 (25.05.2000) Bekanntmachung über die Abtrifteckwerte, die bei der Prüfung und Zulassung von Pflanzenschutzmitteln herangezogen werden. Public domain.

⁵ Ganzelmeier H., Rautmann D. (2000) Drift, drift-reducing sprayers and sprayer testing. Aspects of Applied Biology 57, 2000, Pesticide Application. Public domain.

9.10.3 Overall conclusions

Risk assessments for FGF11 demonstrated an acceptable risk to non-target terrestrial plants for the intended uses in cereals and oilseed rape.

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

No other relevant data were identified in the EU review of the active substances and are considered necessary.

9.12 Monitoring data (KCP 10.8)

Not available.

9.13 Classification and Labelling

According to the criteria given in Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008, the following classification and labelling with regard to ecotoxicological data is proposed for the preparation:

For the acute classification, a surrogate classification based on both active substances is performed based on CLP Regulation EU 1272/2008. Two co-formulants are environmentally classified and the available information for the classification of FGF11 formulation is summarised below, for sake of confidentiality, the identity of the classified co-formulant has been addressed as Substance 1 and Substance 2.

Table 9.13-1: Information on the classification of the substances in FGF11

	ACUTE		CHRONIC		C (% w/w)
	Hazard cat.	M	Hazard cat.	M	
Azoxystrobin	H400	10	H410	10	14.55
Prothioconazole	H400	10	H410	1	18.18
Substance 1	Not classified	-	H412	-	0.90
Substance 2	H400	-	H412	-	0.05

Azoxystrobin:

The ATP15 was published in August 2020 and is mandatory since March 2022.
Azoxystrobin is classified as Aquatic Chronic 1, with a M factor of 10.

Prothioconazole:

The ATP 17 has been published in March 2021 and should apply from 17 December 2022.
The classification of prothioconazole was changed from Aquatic chronic category 2 to Aquatic Chronic category 1.

Classification for Aquatic acute toxicity is based on the CLP calculation, as described below

Step 1: Classify as Acute 1 if $\Sigma (\text{Acute } 1 \times M) \geq 25$.

Using the classification of the substances contained in the formulated product, FGF11 is to be classified as H400, Category Acute 1.

Classification for Aquatic chronic toxicity is based on the CLP calculation, as described below

Step 1: Classify as Chronic 1 if $\Sigma (\text{Chronic } 1 \times M) \geq 25$.

Step 2: Classify as Chronic 2 if $\Sigma (10 \times \text{Chronic } 1 \times M) + \Sigma (\text{Chronic } 2) \geq 25$.

Step 3: Classify as Chronic 3 if $\Sigma (100 \times \text{Chronic 1} \times M) + \Sigma (10 \times \text{Chronic 2}) + \Sigma (\text{Chronic 3}) \geq 25$.

Step 4: Classify as Chronic 4 if $\Sigma (\text{Chronic 1}) + \Sigma (\text{Chronic 2}) + \Sigma (\text{Chronic 3}) + \Sigma (\text{Chronic 4}) \geq 25$.

Summation method:

Step 1: $(14.55 \times 10) + (18.18 \times 1) + (0.90 \times 1) + (0.05 \times 1) > 25\% \Rightarrow$ classify as Category Chronic 1

Using the classification of the substances contained in the formulated product, FGF11 is to be classified as H410, Category Chronic 1

Table 9.13-2: Labelling Classification for formulation FGF11

	ACUTE	CHRONIC
Hazard class(es), categories	Acute Category 1	Chronic category 1
Hazard pictograms or Code(s) for hazard pictogram(s)		
Signal word	Warning	Warning
Hazard statement(s)	H400: Very toxic to aquatic life	H410: Very toxic to aquatic life with long lasting effects
Precautionary statement(s)	~~P273: Avoid release to the environment—if this is not the intended use.~~ P391: Collect spillage. P501: Dispose of contents and container in accordance with national regulation.	~~P273: Avoid release to the environment—if this is not the intended use.~~ P391: Collect spillage. P501: Dispose of contents and container in accordance with national regulation.

zRMS comments:

The classification of the product H400, H410 have been validated by zRMS.

Finally, H410 is proposed on the label.

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.2.1/01	Bauer J., Mollandin, G.	2024	Azoxystrobin 160 g/L + Prothioconazole 200 g/L SC: Acute Toxicity to <i>Daphnia magna</i> in a Semi-Static 48-hour Immobilisation Study No.: 179211220 GLP, Unpublished	N	ARYSTA LifeScience S.A.S.
KCP 10.2.1/02	Bauer J., Mollandin, G.	2024	Azoxystrobin 160g/L + Prothioconazole 200g/L SC: Toxicity to <i>Pseudokirchneriella subcapitata</i> in an Algal Growth Inhibition Test Study No.: 179211210 GLP, Unpublished	N	ARYSTA LifeScience S.A.S.
KCP 10.3.1.1.1/01 KCP 10.3.1.1.2/01	Schabio, S., Horstmann, C.	2024	Azoxystrobin 160 + Prothioconazole 200 g/L SC: Effects (Acute Contact and Oral) on Honey Bees (<i>Apis mellifera</i> L.) in the Laboratory Study No.: 179211035 GLP, Unpublished	N	ARYSTA LifeScience S.A.S.
KCP 10.3.1.1.1/02 KCP 10.3.1.1.2/02	Schabio, S., Horstmann, C.	2024	Azoxystrobin 160 g/L + Prothioconazole 200 g/L SC: Acute Contact and Oral Toxicity to Bumblebees (<i>Bombus terrestris</i> L.) in the Laboratory Study No.: 179211105 GLP, Unpublished	N	UPL Europe Ltd.
KCP 10.3.1.2/01	Schabio, S., Horstmann, C.	2024	Azoxystrobin 160 + Prothioconazole 200 g/L SC: Chronic Oral Toxicity Test on the Honey Bee (<i>Apis mellifera</i> L.) in the Laboratory Study No.: 179211136 GLP, Unpublished	N	ARYSTA LifeScience S.A.S.
KCP 10.3.1.3/01	Venturi, S.	2024	Effects of AZOXYSTROBIN 160 + PROTHIOCONAZOLE 200 g/L SC on honeybees (<i>Apis mellifera</i> L.) 22-day larval toxicity test with repeated exposure Study No.: BT212/23 GLP, Unpublished	N	ARYSTA LifeScience S.A.S.
KCP 10.3.1.5/01	Portail, B.	2025	Azoxystrobin 160 g/L + Prothioconazole 200 g/L SC - A Semi-Field Study to Evaluate Side Effects on Honeybees (<i>Apis mellifera</i> L.) in <i>Phacelia tanacetifolia</i> in Germany 2024, Study number S24-102790, GLP, Unpublished	N	UPL Europe Ltd.
KCP 10.3.1.5/02	Roig, J.	2024	A Semi-Field Study to Evaluate Side Effects of Azoxystrobin 160 + Prothioconazole 200 SC on the Honey Bee	N	UPL Europe

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			(Apis mellifera L.) on Phacelia tanacetifolia according to CEB 230 in France in 2024, Study number S24-102576, GLP, Unpublished		Ltd.
KCP 10.3.2.2/01	Leopold, J.	2024	Azoxystrobin 160 g/L + Prothioconazole 200 g/L SC: Effects on the Parasitoid <i>Aphidius rhopalosiphi</i> (Hymenoptera, Braconidae), Extended Laboratory Study - Limit Test Study No.: 179211002 GLP, Unpublished	N	ARYSTA LifeScience S.A.S
KCP 10.3.2.2/02	Leopold, J.	2024	Azoxystrobin 160 g/L + Prothioconazole 200 g/L SC: Effects on the Predatory Mite <i>Typhlodromus pyri</i> (Acari: Phytoseiidae), Extended Laboratory Study - Aged Residue Test Study No.: 179211060 GLP, Unpublished	N	ARYSTA LifeScience S.A.S
KCP 10.3.2.2/03	Leopold, J.	2024	Azoxystrobin 160 g/L + Prothioconazole 200 g/L SC: Effects on the Ladybird Beetle <i>Coccinella septempunctata</i> (Coleoptera, Coccinellidae), Extended Laboratory Study - Limit Test Study No.: 179211012 GLP, Unpublished	N	ARYSTA LifeScience S.A.S.
KCP 10.3.2.2/04	Leopold, J.	2024	Azoxystrobin 160 g/L + Prothioconazole 200 g/L SC: Effects on the Lacewing <i>Chrysoperla carnea</i> (Neuroptera: Chrysopidae), Extended Laboratory Study - Limit Test Study No.: 179211047 GLP, Unpublished	N	ARYSTA LifeScience S.A.S.
KCP 10.4.1.1/01	Hübner, S.	2024	Azoxystrobin 160 + Prothioconazole 200 g/L SC: Effects on Reproduction and Growth of Earthworms <i>Eisenia andrei</i> in Artificial Soil Study No.: 179211022 GLP, Unpublished	N	ARYSTA LifeScience S.A.S.
KCP 10.4.2.1/01	Hübner, S.	2024	Azoxystrobin 160 + Prothioconazole 200 g/L SC: Effects on Reproduction of the Predatory Mite <i>Hypoaspis aculeifer</i> in Artificial Soil Study No.: 179211089 GLP, Unpublished	N	ARYSTA LifeScience S.A.S.
KCP 10.4.2.1/02	Hübner, S.	2024	Azoxystrobin 160 + Prothioconazole 200 g/L SC: Effects on Reproduction of Collembola (<i>Folsomia candida</i>) in Artificial Soil Study No.: 179211016 GLP, Unpublished	N	ARYSTA LifeScience S.A.S.
KCP 10.5/01	Hammesfahr, U.	2024	Azoxystrobin 160 + Prothioconazole 200 g/L SC: Effects on the Activity of the Soil Microflora in the Laboratory (Nitrogen Transformation)	N	ARYSTA LifeScience

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
			Study No.: 179211080 GLP, Unpublished		S.A.S.
KCP 10.6.2/01	Stürtz, S., Horstmann, C.	2024	Azoxystrobin 160 + Prothioconazole 200 g/L SC: Effects on Terrestrial (Non-Target) Plants: Seedling Emergence and Seedling Growth Test Study No.: 179211086 GLP, Unpublished	N	ARYSTA LifeScience S.A.S.
KCP 10.6.2/02	Stürtz, S., Horstmann, C.	2024	Azoxystrobin 160 + Prothioconazole 200 g/L SC: Effects on Terrestrial (Non-Target) Plants: Vegetative Vigour Test Study No.: 179211087 GLP, Unpublished	N	ARYSTA LifeScience S.A.S.

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

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

List of data submitted by the applicant and not relied on

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

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

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

Appendix 2 Detailed evaluation of the new studies

A 2.1 KCP 10.1 Effects on birds and other terrestrial vertebrates

A 2.1.1 KCP 10.1.1 Effects on birds

A 2.1.1.1 KCP 10.1.1.1 Acute oral toxicity

No additional data submitted.

A 2.1.1.2 KCP 10.1.1.2 Higher tier data on birds

No additional data submitted.

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

No additional data submitted.

A 2.1.2.2 KCP 10.1.2.2 Higher tier data on mammals

No additional data submitted.

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

No additional data submitted.

A 2.2 KCP 10.2 Effects on aquatic organisms

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

A 2.2.1.1 Study 1

Comment from zRMS:	The study was conducted in line with OECD 202 with no minor deviations. Measured initial concentrations of Azoxystrobin ranged from 103% to 117% of nominal. In the aged samples the measured concentrations were between 95% and 113% of nominal and hence all results were expressed based on nominal concentrations (Table 1). However, for Prothioconazole the initial concentrations ranged from 101% to 111% of nominal. In the aged samples the measured concentrations were between 13% and 53% of nominal, and hence all results were expressed based on time weighted average concentrations. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: 48 EC₅₀ = 0.120 mg test item/L nom 48 EC₅₀ = 0.02989 mg sum of a.s./L (0.01730 mg Azoxystrobin/L + 0.01259 mg Prothioconazole/L).
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Reference: KCP 10.2.1/01

Report Azoxystrobin 160 g/L + Prothioconazole 200 g/L SC: Acute Toxicity to *Daphnia*

	<i>magna</i> in a Semi-Static 48-hour Immobilisation Test, Bauer, J. and Mollandin, G. (2024), Study no.179211220
Guideline(s):	OECD guideline 202 (2004)
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Executive summary

Daphnia magna (< 24 h old) were exposed in a semi-static 48 h acute immobilisation test to Azoxystrobin 160 + Prothioconazole 200 SC in semi-static concentration-response test at six test concentrations and water control (test solution renewed at 24h). The reference item potassium dichromate was tested in a separate test. The recorded effects were immobility and behavioural changes of the daphnids after 24 and 48 h.

The 48-hour NOEC was determined to be < 0.019 mg test item/L (< 0.00275 mg Azoxystrobin/L, < 0.00162 mg Prothioconazole/L). The 48-hour LOEC was determined to be ≤ 0.019 mg test item/L (≤ 0.00275 mg Azoxystrobin/L, ≤ 0.00162 mg Prothioconazole/L) and the 48-hour EC₅₀ value was calculated to be 0.120 mg test item/L (0.01730 mg Azoxystrobin/L, 0.01259 mg Prothioconazole/L). The reported results refer to nominal test item concentrations and additionally to nominal concentrations of Azoxystrobin and to time weighted average concentrations of Prothioconazole. The validity criteria of OECD 202 (2004) were met in the study.

Material and Methods

A. MATERIALS

1. Test material	Azoxystrobin 160 + Prothioconazole 200 SC
Description	Liquid, light brown, SC (suspension concentrate)
Lot/Batch #	ARD/AP/360/SC/36
Content of a.s.	Azoxystrobin: 162.20 ± 0.48 g/L (14.45 ± 0.04% w/w) Prothioconazole: 200.54 ± 0.65 g/L (17.86 ± 0.06% w/w) Density: 1.1228 ± 0.0012 g/mL
Stability of test material	Stable under storage conditions (20 ± 5 °C, in the dark) Expiry date: 23 May 2025
2. Vehicle and/or positive control	Vehicle control: Test medium Positive control: Potassium dichromate, facility routine testing (Jan 2024). The result of this test indicates a 24 h EC ₅₀ = 1.74 mg/L (CI 95%: 1.34 – 1.63 mg/L) which is within the required range of OECD 202 (24 h EC ₅₀ = 0.6 – 2.1 mg/L) and within the historical range of the test facility.
3. Test organism	
Species	<i>Daphnia magna</i> Straus
Strain	Clone V
Source	In-house laboratory culture
Age	Freshly hatched daphnids, less than 24 h
Acclimation period	Not required, bred in test medium under test conditions
Feeding	During holding, daphnids were fed with <i>Desmodesmus subspicatus</i> at least on all workdays. No feeding during the test.

Test units	100-mL glass vessels filled with ~ 60 mL test solution, covered with a lid, loading rate of daphnids $\geq$ 10 mL per organism
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4. Environmental conditions

Test water	Elendt M4 medium
Hardness	250 mg/L as CaCO ₃
Water temperature	Nominal: 18 – 22°C and constant within $\pm$ 1°C; actual: 20.0 - 20.5 °C in the freshly prepared media; 20.1 - 20.3 °C in the aged test media
Oxygen saturation	Nominal: $\geq$ 3 mg/L; actual: 8.7 - 9.2 mg/L in the freshly prepared media; 8.6 - 8.8 mg/L in the aged test media
pH	Nominal: 6 – 9; actual: 8.1 in the freshly prepared media; 7.9 - 8.0 in the aged test media
Lighting	16 h photoperiod/8 h darkness
Aeration	None

B. STUDY DESIGN AND METHODS

1. In-life dates	23 May 2024 to 24 Jun 2024 (experimental period)
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2. Experimental conditions

Test design

Daphnia magna (< 24 h old) were exposed in a semi-static 48 h test design to Azoxystrobin 160 + Prothioconazole 200 SC at six test concentrations and a water control (test solution renewed at 24h). The reference item potassium dichromate was tested in a separate test. The recorded effects were immobility and behavioural changes of the daphnids after 24 and 48 h.

Number of animals per treatment

20 daphnids per treatment: five daphnids/replicate; four replicates/test substance treatment and control

Test concentrations

Azoxystrobin 160 + Prothioconazole 200 SC was tested at nominal 1, 0.455, 0.207, 0.094, 0.043, and 0.019 mg test item/L (spacing factor 2.2) corresponding to nominal concentrations of 0.145, 0.0657, 0.0299, 0.0136, 0.00621, 0.00275 mg Azoxystrobin/L and to time weighted average concentrations of 0.138, 0.0517, 0.0232, 0.00972, 0.00417, 0.00162 mg Prothioconazole/L, and a control. The reference item was tested in a separate study.

Treatment/Application

A stock solution of 10 mg test item/L was prepared by dissolving 9.5 and 9.4 mg test item into 950 mL and 940 mL test water by intense stirring for 15 minutes each. Adequate volumes of this stock solution were diluted with test water to prepare the test media of the desired test concentrations. The test media were prepared just before introduction of the daphnids (= start of the test) and test medium renewal on Day 1.

Analytics

The purpose of the analytical part was to perform the analysis of the concentrations of Azoxystrobin and Prothioconazole in the test medium samples by HPLC LC-MS/MS. For the determination of the stability of the test item under the test conditions and of the maintenance of the test item concentrations during the

test period, duplicate samples from the aged test media of all test concentrations and the control were collected at day 1 (after 24 hours of exposure) and at the end of the test by pouring together the contents of the test beakers of each treatment and stored at ≤ 20 °C. The suitability and validity of the applied methodology was verified by means of fortified sample recovery experiments and the method was validated according to SANTE/2020/12830 rev. 2 guideline.

3. Sampling and measurements

The mobility of the daphnids was determined by visual observation after 24 and 48 hours. Those animals that were not able to swim within 15 seconds after gentle agitation of the test beaker were considered to be immobile (even if they could still move their antennae). Any signs of disease or stress or unusual behaviour were documented in the raw data.

4. Calculation of toxicity

Immobility and behavioural abnormalities were determined for each treatment and observation time.

5. Statistics

The 24-hour and 48-hour EC₅₀, EC₂₀ and EC₁₀ and the 95 % confidence limits were calculated by probit analysis.

The NOEC and LOEC after 24 and 48 hours were calculated by Step-down Cochran Armitage test procedure.

The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ToxRat Solutions GmbH.

Results and Discussion

A. ANALYTICAL RESULTS

Measured initial concentrations of Azoxystrobin ranged from 103% to 117% of nominal. In the aged samples the measured concentrations were between 95% and 113% of nominal and hence all results were expressed based on nominal concentrations (Table 1). However, for Prothioconazole the initial concentrations ranged from 101% to 111% of nominal. In the aged samples the measured concentrations were between 13% and 53% of nominal, and hence all results were expressed based on time weighted average concentrations (Table 2).

Table 1. Summary of Analytical Results – Azoxystrobin

Sample Description		Test start (0h) % of nominal ¹	Day 1 aged (24h) % of nominal ¹	Day 1 aged (0h) % of nominal ¹	Day 2 aged (24h) % of nominal ¹
Nominal concentration [mg test item/L]	Nominal concentration [mg a.s./L]				
Control	0	n.a.	n.a.	n.a.	n.a.
0.019	0.00275	117	120	115	112
0.043	0.00621	103	115	113	113
0.094	0.0136	111	115	102	95
0.207	0.0299	108	113	106	105
0.455	0.0657	106	98	97	98
1	0.145	109	109	103	110

¹ mean value of all measured samples per treatment group

n.a.: not applicable

Table 2. Summary of Analytical Results – Prothioconazole

Sample Description		Test start (0h) % of nominal ¹	Day 1 aged (24h) % of nominal ¹	Day 1 aged (0h) % of nominal ¹	Day 2 aged (24h) % of nominal ¹	Time Weighted Average ²	
Nominal concentration [mg test item/L]	Fresh (0h) concentration [mg a.s./L]					[%]	[mg a.s./L]
Control	0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
0.019	0.00339	110	19	104	13	48	0.00162
0.043	0.00768	101	26	108	21	54	0.00417
0.094	0.0168	106	30	102	26	58	0.00972
0.207	0.0370	107	35	102	32	63	0.0232
0.455	0.0813	107	38	96	35	64	0.0517
1	0.179	111	55	103	53	78	0.138

¹ mean value of all measured samples per treatment group

² time weighted average calculated according to OECD Guidance Document No. 23, Annex 2

n.a.: not applicable

B. BIOLOGICAL RESULTS

After 48 hours of exposure no immobilisation of the test daphnids was observed in the control. At the test concentrations of 0.019, 0.043, 0.094, 0.207, 0.455, and 1 mg test item/L, 3, 7, 8, 13, 14, and 18 daphnids were immobile, respectively and the respective EC_{10,20,50} and NOEC and LOEC are presented in the Table 3.

At the test concentration 0.455 mg/L two daphnids were moving slower compared to the control.

Table 3. Influence of Azoxystrobin 160 g/L + Prothioconazole 200 g/L SC on the Mobility of *Daphnia magna*

Immobility	24 hours [mg test item/L]	48 hours [mg test item/L]
EC ₅₀ 95 % CI	1.155 0.653-4.183	0.120 0.071-0.194
EC ₂₀ 95 % CI	0.315 0.176-0.532	0.024 0.008-0.045
EC ₁₀ 95 % CI	0.159 0.061-0.263	0.011 0.002-0.023
NOEC	0.094	< 0.019
LOEC	0.207	≤ 0.019

Values refer to nominal test concentrations CI: Confidence interval

C. VALIDITY CRITERIA

In the control the immobility was 0% at test end (required: ≤ 10% in the control). Dissolved oxygen concentrations were ≥ 8.7 mg/L (required: ≥ 3 mg/L.) Thus, the study fulfils all validity criteria of OECD test guideline 202 (2004).

Conclusion

The toxic effect of the test item Azoxystrobin 160 g/L + Prothioconazole 200 g/L SC to *Daphnia magna* was assessed in a semi-static concentration-response test. The 48-hour NOEC was determined to be < 0.019 mg test item/L (< 0.00275 mg Azoxystrobin/L, < 0.00162 mg Prothioconazole/L). The 48-hour LOEC was determined to be ≤ 0.019 mg test item/L (≤ 0.00275 mg Azoxystrobin/L, ≤ 0.00162 mg Prothioconazole/L) and the 48-hour EC₅₀ value was calculated to be 0.120 mg test item/L (0.01730 mg Azoxystrobin/L, 0.01259 mg Prothioconazole/L).

The reported results refer to nominal test item concentrations and additionally to nominal concentrations of Azoxystrobin and to time weighted average concentrations of Prothioconazole. The validity criteria of OECD 202 (2004) were fulfilled in the study.

Study 2

Comment from zRMS:	The study was conducted in line with OECD 201. Measured initial concentrations of Azoxystrobin ranged from 83% to 105% of nominal. In the aged samples the measured concentrations were between 89% and 102% of nominal and hence all results were expressed based on nominal concentrations. However, for Prothioconazole the initial concentrations ranged from 76% to 95% of nominal. In the aged samples the measured concentrations were between 12% and 73% of nominal and hence all results were expressed based on geometric mean measured concentrations. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: ErC₅₀=2.79 mg test item/L (nom) EyC₅₀= 0.679 mg test item/L (nom)
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Reference:	KCP 10.2.1/02
Report	Azoxystrobin 160 g/L + Prothioconazole 200 g/L SC: Toxicity to <i>Pseudokirchneriella subcapitata</i> in an Algal Growth Inhibition Test, Bauer, J. and Mollandin, G. (2024), Study no. 179211210
Guideline(s):	OECD guideline 201 (2011)
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Executive Summary

The effects of Azoxystrobin 160 + Prothioconazole 200 SC technical on the growth of the alga *Pseudokirchneriella subcapitata* were investigated in a 72h study under static conditions. Algae were exposed at 10, 3.16, 1, 0.32 and 0.1 mg test item/L, and a control, corresponding to nominal concentrations of 0.0145, 0.0462, 0.145, 0.457 and 1.45 mg Azoxystrobin/L and to geometric mean measured concentrations of 0.00212, 0.0138, 0.0891, 0.411 and 1.65 mg Prothioconazole/L. A reference item was tested in a separate study. The cell density, which had been adjusted to 5000 cells/mL at the start of the test, was measured daily by fluorescence measurement. The recorded effect was inhibition of algal growth based on yield and growth rate

Measured initial concentrations of Azoxystrobin ranged from 83% to 105% of nominal. In the aged samples the measured concentrations were between 89% and 102% of nominal, and hence all results were expressed based on nominal concentrations. However, for Prothioconazole the initial concentrations ranged from 76% to 95% of nominal. In the aged samples the measured concentrations were between 12% and 73% of nominal, and hence all results were expressed based on geometric mean measured concentrations.

For Azoxystrobin 160 + Prothioconazole 200 SC the 72-hour EyC₅₀ was calculated to be 0.679 mg test item/L and the 72-hour ErC₅₀ value was calculated to be 2.79 mg test item/L. Azoxystrobin (based on nominal concentrations): The 72-hour EyC₅₀ was calculated to be 0.098 mg test item/L and the 72-hour ErC₅₀ value was calculated to be 0.402 mg test item/L Prothioconazole (based on geometric mean measured

concentrations): The 72-hour $E_{yC_{50}}$ was calculated to be 0.047 mg test item/L and the 72-hour $E_{rC_{50}}$ value was calculated to be 0.322 mg test item/L. The validity criteria of OECD 201 (2011) were met in the study.

Materials and Methods

A. MATERIALS

1. Test material

Azoxystrobin 160 + Prothioconazole 200 SC

Description

Liquid, light brown, SC (suspension concentrate)

Lot/Batch

ARD/AP/360/SC/36

Content of a.s.

Azoxystrobin: 162.20 ± 0.48 g/L ($14.45 \pm 0.04\%$ w/w)

Prothioconazole: 200.54 ± 0.65 g/L ($17.86 \pm 0.06\%$ w/w)

Density: 1.1228 ± 0.0012 g/mL

Stability of test material

Stable under storage conditions (20 ± 5 °C, in the dark)

Expiry date: 23 May 2025

2. Vehicle and/or positive control

Vehicle control: OECD TG 201 test water

Positive control: Potassium dichromate is tested as reference item twice a year. In the latest test in Jan 2024 the results were as follows: 72 h $E_{yC_{50}}$: 0.612 mg/L; 72 h $E_{rC_{50}}$: 1.53 mg/L, 72 h $E_{bC_{50}}$: 0.661 mg/L.

3. Test organism

Species

Pseudokirchneriella subcapitata (Korshikov) Hindák (formerly known as *Selenastrum capricornutum* and renamed as *Raphidocelis subcapitata* Korshikov)

Strain

SAG 61.81

Source

Culture Collection of Algae at Goettingen University (SAG), 37073 Göttingen, Germany

Age

Algae cells for inoculation were taken from an exponentially growing pre-culture which was set up 4 days prior to the test start under similar conditions as in the test.

Acclimation period

Algae had been grown semi-continuously at the test site under sterile culture conditions.

Test units

50 mL Erlenmeyer flasks containing ~50 mL of test medium, covered with watch glasses

4. Environmental conditions

Test water

Algae were cultivated and tested in OECD TG 201 culture medium according to the OECD guideline 201 (2011). The composition of the culture medium was:

Macro-nutrients:

NaHCO₃ 50.0 mg/L

K₂HPO₄ 1.6 mg/L

MgSO₄ · 7 H₂O 15.0 mg/L

NH₄Cl 15.0 mg/L

CaCl₂ · 2 H₂O 18.0 mg/L

MgCl₂ · 6 H₂O 12.0 mg/L

Trace elements:

H₃BO₃ 185 µg/L

MnCl ₂ · 4 H ₂ O	415 µg/L
ZnCl ₂	3.0 µg/L
CoCl ₂ · 6 H ₂ O	1.5 µg/L
CuCl ₂ · 2 H ₂ O	0.01 µg/L
Na ₂ MoO ₄ · 2 H ₂ O	7.0 µg/L
FeCl ₃ · 6 H ₂ O	64 µg/L
Na ₂ EDTA · 2 H ₂ O	100 µg/L

The test water was prepared 8 days before test start to allow the pH to stabilize.

**Water temperature
pH**

Nominal: 21 – 24 °C; actual: 22.1 – 22.6°C
Nominal: increase by ≤ 1.5 units in the control; actual: 7.7 - 8.0 at test start and 7.9 – 8.0 at test end in all treatments

Lighting

Continuous illumination at a light intensity of nominal: 4440 – 8800 Lux < ± 15% variation; actual: continuously illuminated at a mean of 8328 Lux (range: 7800 – 8750 lux)

Shaking

The test units were continuously stirred by magnetic stirrers. The flasks were incubated in a water bath, placed in a random order and were repositioned each day to minimize differences in test conditions.

B. STUDY DESIGN AND METHODS

1. In-life dates

16 Apr 2024 to 18 Jun 2024 (experimental period)

2. Experimental conditions

Test design

The green alga *Pseudokirchneriella subcapitata* was exposed in a static 72 h test to Azoxystrobin 160 + Prothioconazole 200 SC at five concentrations, each with three replicates, and six replicates of a test water control. A reference item was tested in a separate study. The recorded effects were algal growth based on growth rate and yield.

Inoculum at test start

The target cell density at start of the exposure period was 10⁴ cells/mL. Measured mean cell density in the control was 5000 cells/mL which was used as initial cell density for all treatments. The test was performed with three replicates per test item concentration and six replicates for the control.

Test concentrations

Test substance concentrations were 10, 3.16, 1, 0.32 and 0.1 mg test item/L (spacing factor 3.16), corresponding to nominal concentrations of 0.0145, 0.0462, 0.145, 0.457 and 1.45 mg Azoxystrobin/L and to geometric mean measured concentrations of 0.00212, 0.0138, 0.0891, 0.411 and 1.65 mg Prothioconazole/L. In addition, a control group with untreated test water was used.

Treatment/Application

A stock solution of 10 mg test item/L was prepared by dissolving 9.2 mg test item into 920 mL test water by intense stirring for 25 minutes. Adequate volumes of this stock solution were diluted with test water to prepare the test media of the desired test concentrations. The test media were prepared just before introduction of the algae (= start of the test).

Analytics

The purpose of the analytical part was to perform the analysis of the concentrations of Azoxystrobin and Prothioconazole in the test medium samples by HPLC LC-MS/MS. For the determination of the stability of the test item under the test conditions and of the maintenance of the test item concentrations during the test period, duplicate samples from the test media of all test concentrations and the control (containing algae) were taken after 24 hours, after 48 hours and at the end of the test (after the 72 hours test period) by pooling aliquots of the contents of the test beakers of each treatment and stored at ≤ 20 °C. The suitability and validity of the applied methodology was verified by means of fortified sample recovery experiments and the method was validated according to SANTE/2020/12830 rev. 2 guideline.

3. Sampling and measurements

The cell density on each observation time was determined by spectrophotometric measurement. Therefore, defined volumes of the algal suspensions from all replicates and from the blanks were sampled after 24, 48 and 72 hours of exposure, and were not replaced. The algal cell densities were calculated by subtracting the absorption of the blanks, from each of the measured absorption of the test media (with algae) at a wavelength of 685 nm.

Based on the counted cell densities and the absorption from an algal suspension and its dilutions, a linear regression was performed for the calculation of the cell densities of the replicates during the test.

To check for any effect of the test item on the morphology of the algal cells, at least one sample was taken after the test period of 72 hours. The shape of the treated algal cells compared to the control was examined microscopically.

4. Calculation of toxicity

From the cell density, the average specific growth rate (r) and yield (y) together with their inhibition in the test concentrations compared to the control were calculated based on the equations given in the guideline.

5. Statistics

Based on the calculated cell densities, the 72-hour ErC_{50} and the 72-hour EyC_{50} , the corresponding EC_{20} and EC_{10} values and where possible their 95 %-confidence limits were calculated by a three parametric normal cumulative distribution function (CDF) or a three parametric logistic cumulative distribution function (CDF).

For the determination of the 72-hour LOEC and the 72-hour NOEC, the calculated growth rates and yields at each test concentration were tested for significant differences compared to the control values by Williams t-test (yield and growth rate).

The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ToxRat® Solutions GmbH.

Results and Discussion

A. ANALYTICAL RESULTS

Measured initial concentrations of Azoxystrobin ranged from 83% to 105% of nominal. In the aged samples the measured concentrations were between 89% and 102% of nominal and hence all results were expressed based on nominal concentrations (Table 1). However, for Prothioconazole the initial concentrations ranged from 76% to 95% of nominal. In the aged samples the measured concentrations were between 12% and 73% of nominal and hence all results were expressed based on geometric mean measured concentrations (Table 2).

Table 1. Summary of Analytical Results – Azoxystrobin

Sample Description		Test start (0h) % of nominal ¹	Day 1 aged (24h) % of nominal ¹	Day 2 aged (48h) % of nominal ¹	Test end aged (72h) % of nominal ¹
Nominal concentration [mg test item/L]	Nominal concentration [mg a.s./L]				
Control	0	n.a.	n.a.	n.a.	n.a.
0.1	0.0145	92	88	85	89
0.32	0.0462	83	92	88	96
1.00	0.145	102	99	101	102
3.16	0.457	97	101	92	89
10	1.45	105	-*	103	100

¹ mean value of all measured samples per treatment group

* measured result not considered since regarded to be not plausible

n.a.: not applicable

Table 2. Summary of Analytical Results – Prothioconazole

Sample Description		Test start (0h) % of nominal ¹	Day 1 aged (24h) % of nominal ¹	Day 2 aged (48h) % of nominal ¹	Test end aged (24h) % of nominal ¹	Geometric mean measured ²	
Nominal concentration [mg test item/L]	Fresh (0h) concentration [mg a.s./L]					[%]	[mg a.s./L] ³
Control	0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
0.1	0.0179	84	13	6	5	12	0.00212
0.32	0.0572	76	33	14	12	24	0.0138
1.00	0.179	88	62	43	24	50	0.0891
3.16	0.564	91	81	69	52	73	0.411
10	1.79	95	-*	95	82	92**	1.65**

¹ mean value of all measured samples per treatment group

² geometric mean calculated according to OECD Guidance Document No. 23, Annex 2

³ tabulated results represent results rounded to three significant digits

* measured result not considered for calculation of geometric mean

** geometric mean calculated without 24h result using only 0h, 48h and 72h results, since 24 h result regarded to be not plausible

n.a.: not applicable

B. BIOLOGICAL RESULTS

A significant concentration-response relationship was determined. After 72 hours the inhibition of yield ranged from 1.6 to 97.1 % and the inhibition of growth rate ranged from 0.2 to 73.6 %. A negative value indicates an increase in growth relative to the control.

The 72-hour E_yC_{50} was calculated to be 0.679 mg test item/L and the E_rC_{50} 2.79 mg test item/L. The 72-hour E_yC_{10} was calculated to be 0.231 mg test item/L and the E_rC_{10} 0.309 mg test item/L. The 72-hour NOE_yC was determined to be 0.1 mg test item/L and the associated 72-hour LOE_yC of 0.32 mg test item/L. The 72-hour NOE_rC was determined to be 0.32 mg test item/L and the associated 72-hour LOE_rC is 1 mg test item/L.

Based on nominal Azoxystrobin concentrations

The 72-hour E_yC_{50} was calculated to be 0.098 mg test item/L and the 72-hour E_rC_{50} value was calculated to be 0.402 mg test item/L. The 72-hour NOE_yC was determined to be 0.0145 mg test item/L and the associated 72-hour LOE_yC was 0.0462 mg test item/L. The 72-hour NOE_rC was determined to be 0.0462 mg test item/L and the associated 72-hour LOE_rC was 0.145 mg test item/L.

Based on geometric mean measured Prothioconazole concentrations

The 72-hour $E_{yC_{50}}$ was calculated to be 0.047 mg test item/L and the 72-hour $E_{rC_{50}}$ value was calculated to be 0.322 mg test item/L. The 72-hour NOE_{yC} was determined to be 0.00212 mg test item/L and the associated 72-hour LOE_{yC} was 0.0138 mg test item/L. The 72-hour NOE_{rC} was determined to be 0.0138 mg test item/L and the associated 72-hour LOE_{rC} was 0.0891 mg test item/L.

Table 3. Overview of Inhibition of Yield and Growth Rate at 72 h

Nominal concentration [mg test item/L]	0.1	0.32	1	3.16	10
% Inhibition yield	1.6	17.0*	69.5*	92.5*	97.1*
% Inhibition growth rate	0.2	4.1	26.4*	55.9*	73.6*

Negative values in ‘% inhibition’ indicate an increase in growth relative to that of the control

* mean value significantly different from the control (tested with Williams t-test, $\alpha = 0.05$, one-sided smaller)

Table 4. Influence of Azoxystrobin 160g/L + Prothioconazole 200g/L SC on the Growth of *Pseudokirchneriella subcapitata*

Parameter	Yield [mg test item/L]	Growth rate [mg test item/L]
72-hour EC_{50}	0.679	2.79
95 % conf. interval	0.554 - 0.832	2.33 - 3.26
72-hour EC_{20}	0.334	0.696
95 % conf. interval	0.251 - 0.449	0.511 - 0.879
72-hour EC_{10}	0.231	0.309
95 % conf. interval	0.157 - 0.340	0.199 - 0.427
72-hour NOEC	0.1	0.32
72-hour LOEC	0.32	1

Values refer to nominal test item concentrations

The microscopic examination did not show any effects of the algae exposed up to a nominal test concentration of 10 mg test item/L and the algal cells in the control.

C. VALIDITY CRITERIA

In the control, the cell number increased by a factor of 79.1 over 72 h (required: ≥ 16). The mean coefficient of variation of the daily growth rates in the control (section-by-section growth rates) during 72 h was 8.3% (required: $\leq 35\%$). The coefficient of variation of the average specific growth rates in the replicates of the control after 72 h was 3.1 % (required: $\leq 7\%$). Thus, the validity criteria of OECD 201 (2011) were fulfilled.

Conclusion

The influence of Azoxystrobin 160g/L + Prothioconazole 200g/L SC on the growth of the freshwater green algae *Pseudokirchneriella subcapitata* was assessed in a static concentration-response test. The 72-hour $E_{yC_{50}}$ was calculated to be 0.679 mg test item/L and the 72-hour $E_{rC_{50}}$ value was calculated to be 2.79 mg test item/L. The 72-hour NOE_{yC} was determined to be 0.1 mg test item/L and the associated 72-hour LOE_{yC} was 0.32 mg test item/L. The 72-hour NOE_{rC} was determined to be 0.32 mg test item/L and the associated 72-hour LOE_{rC} was 1 mg test item/L.

Azoxystrobin (based on nominal concentrations)

The 72-hour $E_{yC_{50}}$ was calculated to be 0.098 mg test item/L and the 72-hour $E_{rC_{50}}$ value was calculated to be 0.402 mg test item/L. The 72-hour NOE_{yC} was determined to be 0.0145 mg test item/L and the associated 72-hour LOE_{yC} was 0.0462 mg test item/L. The 72-hour NOE_{rC} was determined to be 0.0462 mg test item/L and the associated 72-hour LOE_{rC} was 0.145 mg test item/L.

Prothioconazole (based on geometric mean measured concentration)

The 72-hour E_yC_{50} was calculated to be 0.047 mg test item/L and the 72-hour E_rC_{50} value was calculated to be 0.322 mg test item/L. The 72-hour NOE_yC was determined to be 0.00212 mg test item/L and the associated 72-hour LOE_yC was 0.0138 mg test item/L. The 72-hour NOE_rC was determined to be 0.0138 mg test item/L and the associated 72-hour LOE_rC was 0.0891 mg test item/L.

The initial concentrations and the maintenance of the exposure concentrations during the test were determined in the analytical part. All reported results refer to nominal test item concentrations and additionally to nominal concentrations of Azoxystrobin and to geometric mean concentrations of Prothioconazole. The validity criteria of OECD 201 (2011) were met in the study

A 2.2.2 KCP 10.2.2 Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms

No additional data submitted.

A 2.2.3 KCP 10.2.3 Further testing on aquatic organisms

No additional data submitted.

A 2.3 KCP 10.3 Effects on arthropods

A 2.3.1 KCP 10.3.1 Effects on bees

A 2.3.1.1 KCP 10.3.1.1 Acute toxicity to bees

A 2.3.1.1.1 KCP 10.3.1.1.1 Acute oral toxicity to bees

A 2.3.1.1.1.1 Study 3

Comment from zRMS:	The study was conducted in line with OECD 213 and 214 with minor deviations. All the validity criteria were met and minor deviations are considered to have no impact on the validity of the study is considered acceptable with the following endpoints relevant for the risk assessment: The contact LD_{50} (48 h) >400 µg /bee. The oral LD_{50} (48 h) = 454.8 µg /bee.
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Reference:	KCP 10.3.1.1.1/01 KCP 10.3.1.1.2/01
Report:	Azoxystrobin 160 + Prothioconazole 200 g/L SC: Effects (Acute Contact and Oral) on Honey Bees (<i>Apis mellifera</i> L.) in the Laboratory, Schabio, S., Horstmann, C. (2024), Study no. 179211035
Guideline(s):	OECD 213 (1998), OECD 214 (1998)
Deviations:	OECD 214 (1998): Application volume was increased to 5 µL compared to 1 µL as recommended in the TG. However, a higher volume ensures a more reliable dispersion of the test item; and no adverse effects on the outcome of the study are to be expected.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Executive summary

Oral and contact toxicity of Azoxystrobin 160 + Prothioconazole 200 g/L SC on the honeybee, *Apis mellifera*, were assessed in a limit test design under laboratory conditions. Bees were exposed to a dose of 400 µg product/bee by feeding (oral test) and by topical application (contact test). One control (sucrose solution) and four concentrations of a reference item (dimethoate) were tested in parallel. Bee mortality and condition were assessed after 4, 24 and 48 h.

As no mortality occurred above 50%, the 48-h oral LD₅₀ of Azoxystrobin 160 + Prothioconazole 200 g/L SC was determined to be > 454.8 µg product/bee. The oral NOED was determined to be ≥ 454.8 µg product/bee.

The contact LD₅₀ was estimated to be > 400.0 µg product/bee. The contact NOED was determined to be ≥ 400.0 µg product/bee. The validity criteria of OECD 213 and OECD 214 (1998) were fulfilled.

Material and Methods

A. MATERIALS

1. Test material	Azoxystrobin 160 + Prothioconazole 200 g/L SC
Description	Liquid, light brown, SC (suspension concentrate)
Lot/Batch #	ARD/AP/360/SC/36
Content of aa.ss.	Azoxystrobin: 162.20 ± 0.48 g/L (14.45 ± 0.04% w/w) Prothioconazole: 200.54 ± 0.65 g/L (17.86 ± 0.06% w/w) Density: 1.1228 ± 0.0012 g/mL
Stability of test material	Stable under storage conditions (20 ± 5 °C, in the dark) Expiry date: May 23, 2025
2. Vehicle and/or positive control	Vehicle: Aqueous sucrose solution (50% w/v) Positive control: Reference item
Reference item	Danadim Progress (a.s.: dimethoate)
Description	Liquid, colourless to light yellow, EC (emulsion concentrate)
Lot/Batch #	0001138506
Content of active substance	Dimethoate: 400 g/L
Stability of reference item	At +25 °C or cooler, in the dark Expiry date: Nov 2023
3. Test organism	
Species	Worker honey bees, <i>Apis mellifera</i> L.
Source	Honey bee colonies, disease-free and queen-right, bred by the test facility.
Acclimation period	Oral test: Prior to the test initiation, the honeycomb with the bees was transferred from the apiary to the laboratory and the bees were removed from the comb and starved for 20 minutes for all treatment groups, prior to application.
Diet	Contact test: 50 % w/v sucrose solution <i>ad libitum</i> was given directly after treatment. Oral test: During the exposure phase, the bees were provided with a 50% w/v sucrose solution containing either no test substance (con-

trol) or the test substance or the reference substance. During the subsequent observation period, the bees were supplied *ad libitum* with 50% w/v sugar solution.

Test units

Stainless steel chambers (8.2 cm length x 5.9 cm height x 4.2 cm width) with removable glass sheet as front side and perforated bottom to allow for ventilation, lined with filter paper.

4. Environmental conditions

Temperature

Nominal: $25 \pm 2^{\circ}\text{C}$; actual: $25 - 27^{\circ}\text{C}$

Relative humidity

Nominal: 50 - 70%; actual: 60 - 66%

Photoperiod

Darkness

B. STUDY DESIGN AND METHODS

1. In-life dates

21 Aug 2023 to 24 Aug 2023 (experimental period)

2. Experimental conditions

Test design

Acute oral and contact effects of Azoxystrobin 160 + Prothioconazole 200 g/L SC on the honey bee, *Apis mellifera*, were assessed in a limit test design under laboratory conditions. One control (sucrose solution) and four doses of a reference item were tested in parallel; the application was done either orally *via* feeding solutions (oral test) or *via* topical application to the dorsal part of the thorax (contact test). Bee mortality and condition were assessed after 4, 24 and 48 h.

Number of animals per treatment

Ten bees/test unit; five replicates/test item dose level and control, and three replicates /reference item treatment.

Test doses

Azoxystrobin 160 + Prothioconazole 200 g/L SC was tested in a limit test design at nominally 400.0 μg product/bee (contact and oral test) corresponding to 454.8 μg consumed product/bee (oral test). The nominal dose was chosen based on range finding tests. The control group for the oral test received untreated 50% aqueous sugar solution, and for the contact test honey bees were treated with a 5 μL droplet of tap water containing 0.1% Triton X-100. Controls were always tested in parallel.

The reference item Danadim Progress (a.s. dimethoate) was tested at nominally at 0.10, 0.15, 0.20, 0.30 μg a.s./bee (contact test) and at 0.05, 0.08, 0.15, and 0.30 μg a.s./bee (oral test). Corresponding to the consumed oral dose of 0.06, 0.08, 0.17, and 0.33 μg dimethoate/bee.

Treatment/Application

Oral test: The treated sucrose solution was offered in syringes, which were weighed before and after introduction into the cages (duration of uptake approx. 4 hours). Afterwards, the syringes containing the treated sucrose solution were removed, weighed and replaced by ones containing fresh, untreated food. The mean target dose levels (e.g. 400 μg product/bee nominal) would have been obtained if exactly 20 mg/bee of the treated food were ingested. In practice, uptake of the treated sugar solutions differed slightly from the nominal 20 mg/bee and results are given based on the measured consumption.

Contact test: The bees were anaesthetized for approx. 20 seconds with carbon dioxide. Subsequently, a 5 µL droplet of the test solution was placed on the dorsal bee thorax using a calibrated pipette. Afterwards the bees were returned to the cages.

3. Observations and assessments

Mortality was recorded 4, 24 and 48 h after treatment. Behavioural abnormalities such as symptoms of poisoning in comparison to the control were recorded as well at each observation.

For determination of the actual uptake in the oral toxicity test, the amount of application solutions consumed was determined by weighing the syringes before and after introduction into the cages.

The test temperature and relative humidity were recorded.

4. Calculation of toxicity

The numbers of dead and affected bees were determined and the percentage mortality was calculated.

5. Statistics

As no test item treatment group showed mortality above 50.0 %, no statistical evaluation on the LD₅₀ has been carried out.

The contact LD₅₀ values of the reference item were determined according to Probit Analysis (according to Finney 1971).

The oral LD₅₀ values of the reference item were determined according to Moving Averages (Thompson & Weil, 1952).

The contact and oral NOED values of the test item were determined using Fisher's Exact Binomial Test (one-sided greater, $\alpha = 0.05$), which is a distribution-free test and does not require testing for normality or homogeneity prior to analysis.

The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ® ToxRat Solutions GmbH.

Results and Discussion

A. BIOLOGICAL RESULTS

Oral test: Actual oral consumed doses of 454.8 µg product/bee (target dose: 400 µg product/bee) led to mortality of 2.0% at test termination (48 hours). No mortality occurred in the water control (50 % w/v sucrose solution) and no abnormal behaviour was observed during the test duration.

As no mortality occurred above 50%, the oral LD₅₀ was estimated to be > 454.8 µg product/bee. The oral NOED was determined to be ≥ 454.8 µg product/bee.

Contact test: The dose of 400.0 µg product/bee led to mortality of 2.0% at test termination (48 h). No mortality occurred in the water control group (water + 0.1% Triton X-100). One moribund bee occurred after 24 h in the test item group and was considered as behavioural abnormality.

As no mortality occurred above 50%, the contact LD₅₀ was estimated to be > 400.0 µg product/bee. The contact NOED was determined to be ≥ 400.0 µg product/bee.

Table 1: Acute oral toxicity of Azoxystrobin 160 + Prothioconazole 200 g/L SC to honey bees (*Apis mellifera*)

Test doses		Mean mortality [%]		
Nominal [µg product/bee]	Consumed [µg product/bee]	4 h	24 h	48 h
Control: 50 % (w/v) aqueous sucrose solution				
Control		0	0	0
Test item: Azoxystrobin 160 + Prothioconazole 200 g/L SC				
400.0	454.8	2	2	2
Reference item: Danadim Progress				
Nominal test doses		Mean mortality [%]		
[µg product/bee]	[µg dimethoate/bee]	4 h	24 h	48 h
0.05	0.06	0	3.3	
0.08	0.08	0	13.3	
0.15	0.17	0	93.3	
0.30	0.33	6.7	96.7	
Acute oral endpoints [µg product/bee]				
		24 h	48 h	
LD ₅₀ (95% CI)		> 454.8 (n.d.)	> 454.8 (n.d.)	
NOED		≥ 454.8	≥ 454.8	

n.d. = not determined due to mathematical reasons

Table 2: Acute contact toxicity of Azoxystrobin 160 + Prothioconazole 200 g/L SC to honey bees (*Apis mellifera*)

Test doses [µg product/bee]	Mean mortality [%]		
	4 h	24 h	48 h
Control: 50 % (w/v) aqueous sucrose solution			
Control	0	0	0
Test item: Test item: Azoxystrobin 160 + Prothioconazole 200 g/L SC			
400.0	0	0	2.0
Reference item: Danadim Progress			
Nominal test doses [µg product/bee]	Mean mortality [%]		
	4 h	24 h	48 h
0.10	0	6.7	10.0
0.15	0	46.7	63.3
0.20	3.3	80.0	80.0
0.30	3.3	93.3	93.3
Acute contact endpoints [µg product/bee]			
		24 h	48 h
LD ₅₀ (95% CI)		> 400.0 (n.d.)	> 400.0 (n.d.)
NOED		≥ 400.0	≥ 400.0

n.d. = not determined due to mathematical reasons

B. VALIDITY CRITERIA

The mortality in the control was 0.0% for both oral and contact tests after 48 h (required: $\leq 10\%$) and the 24 h LD₅₀ of the toxic standard was determined to be 0.14 µg and 0.16 µg a.s./bee (required: 0.10 - 0.35 µg a.s./bee) for the oral and contact test, respectively. Therefore, the validity criteria of OECD 213 and OECD 214 (1998) were fulfilled.

Conclusion

In this acute toxicity test with the honeybee *Apis mellifera*, the 48-h oral LD₅₀ of Azoxystrobin 160 + Prothioconazole 200 g/L SC was determined to be > 454.8 µg product/bee. The oral NOED was determined to be ≥ 454.8 µg product/bee.

The contact LD₅₀ was estimated to be > 400.0 µg product/bee. The contact NOED was determined to be ≥ 400.0 µg product/bee. The validity criteria of OECD 213 and OECD 214 (1998) were fulfilled.

A 2.3.1.1.2 Study

Comment from zRMS:	The study was conducted in line with OECD 246 with minor deviations. It was noted that the analytical recovery of azoxystrobin in the low-dose test item solution was 63% in one retention sample. The sample was analysed twice to confirm the result. In addition, the second retention sample was analysed. However, the recovery of the stock solution and the second retention sample confirmed the correct dosing of the test item and recovery was ranging between 93 - 94% of duplicate measurements. Therefore, results were based on nominal concentrations. All the validity criteria were met and minor deviations are considered to have no impact on the validity of the study is considered acceptable with the following endpoints relevant for the risk assessment: The contact LD₅₀ (48 h) > 650.5 µg /bee The oral LD₅₀ (48 h) > 722.2 µg /bee
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Reference:	KCP 10.3.1.1.1/02 KCP 10.3.1.1.2/02
Report:	Azoxystrobin 160 g/L + Prothioconazole 200 g/L SC: Acute Contact and Oral Toxicity to Bumble bees (<i>Bombus terrestris</i> L.) in the Laboratory, Schabio, S., Horstmann, C. (2024), Study no. 179211105
Guideline(s):	OECD 246 (2017), OECD 247 (2017)
Deviations:	OECD 246 (2017): It was noted that the analytical recovery of azoxystrobin in the low-dose test item solution was 63% in one retention sample. The sample was analysed twice to confirm the result. In addition, the second retention sample was analysed. However, the recovery of the stock solution and the second retention sample confirmed the correct dosing of the test item and recovery was ranging between 93 - 94% of duplicate measurements. Therefore, results were based on nominal concentrations. OECD 247 (2017): None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Executive summary

Oral and contact toxicity of Azoxystrobin 160 + Prothioconazole 200 g/L SC on the bumble bee, *Bombus terrestris* was assessed at five test item concentrations under laboratory conditions. Bumble bees were exposed via feeding solutions (oral test) and by topical application (contact test). One control (contact test: sucrose solution + 0.1% Triton-X, oral test: sucrose solution) and one concentration of a reference item (dimethoate) were tested in parallel. Bumble bee mortality and condition were assessed after 4, 24 and 48 h.

As no mortality occurred above 50%, the 48-h oral LD₅₀ of Azoxystrobin 160 + Prothioconazole 200 g/L SC was determined to be > 722.2 µg product/bee. The oral NOED was determined to be ≥ 722.2 µg product/bee.

The contact LD₅₀ was estimated to be > 650 µg product/bee. The contact NOED was determined to be ≥ 650 µg product/bee. The validity criteria of OECD 246 and OECD 247 (2017) were fulfilled.

Material and Methods

A. MATERIALS

1. Test material	Azoxystrobin 160 + Prothioconazole 200 g/L SC
Description	Liquid, light brown, SC (suspension concentrate)
Lot/Batch #	ARD/AP/360/SC/36
Content of aa.ss.	Azoxystrobin: 162.20 ± 0.48 g/L (14.45 ± 0.04% w/w) Prothioconazole: 200.54 ± 0.65 g/L (17.86 ± 0.06% w/w) Density: 1.1228 ± 0.0012 g/mL
Stability of test material	Stable under storage conditions (20 ± 5 °C, in the dark) Expiry date: May 23, 2025
2. Vehicle and/or positive control	Contact Test: Water control = Tap water with 0.1 % v/v Triton X-100 (applied after anaesthetization) Oral Test: Water control = 50 % w/v sucrose solution (500 g sucrose/L tap water) Positive control: Reference item
Reference item	Danadim Progress (a.s.: dimethoate)
Description	Liquid, colourless to light yellow, EC (emulsion concentrate)
Lot/Batch #	0001138506
Content of active substance	Dimethoate: 400 g/L
Stability of reference item	At +25 °C or cooler, in the dark Expiry date: Nov 2023
3. Test organism	
Species	Worker bumble bees, <i>Bombus terrestris</i> L.
Source	Commercial bumble bee breeding company (Koppert Deutschland GmbH, Germany).
Acclimation period	The bumble bees were acclimatised to the test conditions prior to application with <i>ad libitum</i> access to an untreated 50% w/v sucrose solution. Contact Test: 22 h 20 minutes Oral Test: 44 h 15 minutes including a starvation period of 4 h for all treatment groups, prior to application.

Diet Contact test: 50 % w/v sucrose solution *ad libitum* was given directly after treatment.
Oral test: During the exposure phase, the bumble bees were provided with a 50% w/v sucrose solution containing either no test substance (control) or the test substance or the reference substance.

Test units Nicot cages (cylindrical, latticed plastic cages, length of ~ 7.3 cm and a diameter of 2.2 cm).

4. Environmental conditions

Temperature Nominal: $25 \pm 2^{\circ}\text{C}$; actual: 24 - 26°C (contact and oral test)
Relative humidity Nominal: $60 \pm 20\%$; actual: 56 - 63% (contact test), 56 - 64% (oral test)
Photoperiod Continuous darkness except during observations (day light)

B. STUDY DESIGN AND METHODS

1. In-life dates 17 Oct 2023 to 20 Oct 2023 (experimental period)

2. Experimental conditions

Test design

Acute oral and contact effects of Azoxystrobin 160 + Prothioconazole 200 g/L SC on the bumble bee, *Bombus terrestris*, were assessed at five dose levels under laboratory conditions. One control (oral test: sucrose solution, contact test: sucrose solution + 0.1% v/v Triton-X 100) and one dose of a reference item were tested in parallel; the application was done either orally *via* feeding solutions (oral test) or *via* topical application to the dorsal part of the thorax (contact test). Bee mortality and condition were assessed after 4, 24 and 48 h.

Number of animals per treatment

Contact test: One bumble bee/test unit; 30 replicates/test item dose level and control and reference item.

Oral test: One bumble bee/test unit; 35 replicates/test item dose level and control, and reference item.

Test doses

Contact test: Azoxystrobin 160 + Prothioconazole 200 g/L SC was tested at 40.6, 81.3, 162.5, 325, and 650 µg product/bumble bee.

Oral test: Dose levels were set to nominally 27.7, 61.0, 134.3, 295.5, and 650 µg product/bumble bee, corresponding to 32.3, 73.0, 129.0, 292.2, and 722.2 µg consumed product/bee.

The nominal doses were chosen based on a range finding tests. The control group for the oral test received untreated 50% aqueous sugar solution, and for the contact test bumble bees were treated with a 5µL droplet of tap water containing 0.1% Triton X-100. Controls were always tested in parallel.

The reference item Danadim Progress (a.s. dimethoate) was tested at a single dose of 10 µg dimethoate/bumble bee in the contact test and at 4.0 µg dimethoate/bumble bee in the oral test, corresponding to the consumed oral dose of 3.5 µg dimethoate/bumble bee.

Treatment/Application

Contact test: The bumble bees were anaesthetized with carbon dioxide. Subsequently, a 5 µL droplet of the test solution/control/reference item was placed on the dorsal thorax using a calibrated pipette. Afterwards the bumble bees were returned to the cages.

Oral test: Approximately 40 µL of food solution per bumble bee was provided in syringes, which were weighed before and after introduction into the cages (duration of uptake approx. 4 hours). Afterwards, the syringes containing the treated sucrose solution were removed, weighed and replaced by ones containing fresh, untreated food. The nominal target dose levels would have been obtained if exactly 40 mg/bumble bee of the treated food were ingested. In practice, uptake of the treated sugar solutions differed slightly from the nominal 40 mg/bumble bee and results are given based on the measured consumption.

Analytics

For the determination of actual test concentrations of the contact and oral test, duplicate samples of 10 mL aliquots were taken from freshly prepared test item solutions of the highest and lowest dose, and the controls. Actual concentrations of azoxystrobin and prothioconazole were determined by using HPLC-UV detection. The analytical method was fully validated in accordance with the requirements of Guidance Document SANTE/2020/12830 Rev. 2 (2023).

3. Observations and assessments

Mortality was recorded 4, 24 and 48 h after treatment. Behavioural abnormalities such as symptoms of poisoning in comparison to the control were recorded as well at each observation.

For determination of the actual uptake in the oral toxicity test, the amount of application solutions consumed was determined by weighing the syringes before and after introduction into the cages.

The test temperature and relative humidity were recorded.

4. Calculation of toxicity

The numbers of dead and affected bumble bees were determined, and the percentage mortality was calculated.

5. Statistics

As no test item treatment group showed mortality above 50.0 %, no statistical evaluation on the LD₅₀ has been carried out.

The contact and oral NOED values of the test item were determined with the Chi² 2x2 test with Bonferroni Correction (one-sided greater, $\alpha = 0.05$).

The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, (ToxRat Solutions GmbH).

Results and Discussion

A. ANALYTICAL RESULTS

No residues of azoxystrobin and prothioconazole at or above the limit of detection (LOD: 2 µg /L in 50% (w/v) aqueous sucrose solution for azoxystrobin and prothioconazole) were detected in any of the oral and contact control samples.

The measured concentrations of azoxystrobin in the oral and contact application solutions of the lowest and the highest test item group ranged from 93 to 100% of nominal, respectively.

Because of low recovery of azoxystrobin in the low-dose test item solution of 63%, sample A was analysed twice to confirm the result. In addition, sample B was analysed. The application solution was prepared using the stock solution containing 130 g product/L. The recovery of the stock solution and sample B confirmed the correct dosing of the test item and recovery was ranging between 93 - 94% of duplicate measurements. It is therefore assumed that results from duplicate measurements of sample B are correct.

For prothioconazole, recovery ranged from 84 to 97% of nominal. Since the recovery azoxystrobin and prothioconazole in the test item solutions were within the range of 80 to 120% of nominal, endpoints are based on nominal doses.

B. BIOLOGICAL RESULTS

Contact test: No mortality occurred in the water control group (containing 0.1 % v/v Triton X-100). After 48 h after application, 0.0, 3.3, 3.3, 3.3 and 6.7 % mortality occurred in the 650, 325, 162.5, 81.3 and 40.6 µg product/bumble bee test item treatment groups, respectively. One moribund bumble bee occurred during the 4 h assessment in the lowest test concentration, and during the 24 h assessment one affected bumble bee was observed in the 650 µg product/bumble bee test item group.

As no mortality occurred above 50%, the contact LD₅₀ was estimated to be > 650 µg product/bumble bee. The contact NOED was determined to be ≥ 650 µg product/bumble bee.

Oral test: No mortality occurred in the water control (50 % w/v sucrose solution) and no abnormal behaviour was observed during the test duration. Actual oral consumed doses of the test item were 722.2, 292.2, 129.0, 73.0 and 32.3 µg product/bumble bee. No test item induced behavioural effects were observed at any time in all test item treated groups.

As no mortality occurred above 50%, the oral LD₅₀ was estimated to be > 722.2 µg product/bumble bee. The oral NOED was determined to be ≥ 722.2 µg product/bumble bee.

Table 1: Acute contact toxicity of Azoxystrobin 160 + Prothioconazole 200 g/L SC to bumble bees (*Bombus terrestris*)

Test doses [µg product/bumble bee]	Mean mortality [%]		
	4 h	24 h	48 h
Control: 50% (w/v) sucrose solution + 0.1% Triton-X			
Control	0	0	0
Test item: Azoxystrobin 160 + Prothioconazole 200 g/L SC			
40.6	0	3.3	6.7
81.3	0	0	3.3
162.5	0	0	3.3
325	0	3.3	3.3
650	0	0	0
Reference item: Danadim Progress			
Nominal test doses [µg product/bumble bee]	Mean mortality [%]		
	4 h	24 h	48 h
10	0	76.7	90.0
Acute contact endpoints [µg product/bee]			
	24 h	48 h	
LD₅₀ (95% CI)	> 650 (n.d.)	> 650 (n.d.)	
NOED	≥ 650	≥ 650	

n.d. = not determined due to mathematical reasons

Table 2: Acute oral toxicity of Azoxystrobin 160 + Prothioconazole 200 g/L SC to bumble bees (*Bombus terrestris*)

Test doses		Mean mortality [%]		
Nominal [µg product/bumble bee]	Consumed [µg product/bumble bee]	4 h	24 h	48 h
Control: 50% (w/v) sucrose solution				
Control		0	0	0
Test item: Azoxystrobin 160 + Prothioconazole 200 g/L SC				
27.7	32.3	0	0	0
61.0	73.0	0	0	0
134.3	129.0	0	0	3.6
295.5	292.2	0	0	3.4
650	722.2	0	0	0
Reference item: Danadim Progress				
Nominal test doses		Mean mortality [%]		
[µg product/bumble bee]	[µg dimethoate/bumble bee]	4 h	24 h	
4.0	3.5	69.6	100.0	
Acute oral endpoints [µg product/bee]				
		24 h	48 h	
LD₅₀ (95% CI)		> 722.2 (n.d.)	> 722.2 (n.d.)	
NOED		≥ 722.2	≥ 722.2	

n.d. = not determined due to mathematical reasons

C. VALIDITY CRITERIA

The mortality in the control was 0.0% for both oral and contact tests after 48 h (required: $\leq 10\%$) and in the reference item 90.0% (contact) and 100% (oral) after 48 h (required: $\geq 50\%$). Therefore, the validity criteria of OECD 246 and OECD 247 (2017) were fulfilled.

Conclusion

In this acute toxicity test with the bumble bee *Bombus terrestris*, the 48-h oral LD₅₀ of Azoxystrobin 160 + Prothioconazole 200 g/L SC was determined to be $> 722.2 \mu\text{g product/bee}$. The oral NOED was determined to be $\geq 722.2 \mu\text{g product/bee}$.

The contact LD₅₀ was estimated to be $> 650 \mu\text{g product/bee}$. The contact NOED was determined to be $\geq 650 \mu\text{g product/bee}$. The validity criteria of OECD 246 and OECD 247 (2017) were fulfilled.

A 2.3.1.1.2 KCP 10.3.1.1.2 Acute contact toxicity to bees

Refer to studies listed under KCP 10.31.1.1, above.

A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

A 2.3.1.2.1 Study 5

Comment from zRMS:	The study was conducted in line with OECD 245 with no deviation. The concentrations of the active ingredients in the applied test item feeding solutions were within the required range of $\pm 20\%$ of the nominal concentrations. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: LDD₅₀ = $20.9 \mu\text{g product/bee/day}$ NOEDD = $15.5 \mu\text{g product/bee/day}$
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Reference:	KCP 10.3.1.2/01
Report:	Azoxystrobin 160 + Prothioconazole 200 g/L SC: Chronic Oral Toxicity Test on the Honey Bee (<i>Apis mellifera</i> L.) in the Laboratory, Schabio, S., Horstmann, C. (2024), Study no. 179211136
Guideline(s):	OECD 245 (2017)
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Executive summary

In a 10-d chronic test, young female adults of *Apis mellifera* L. were daily exposed to the test item Azoxystrobin 160 + Prothioconazole 200 g/L SC at five doses in 50% (w/v) sucrose solution. In parallel, an untreated control group, a vehicle control group (containing 0.1% xanthan) and one dose of the reference item were tested. Mortality and behavioural abnormalities were assessed daily during the 10-day exposure period.

The LDD₅₀ was calculated to be 20.9 µg product/bee/day, and the NOEDD was calculated to be 15.5 µg product/bee/day. The validity criteria of OECD 245 (2017) were fulfilled.

Materials and Methods

A. MATERIALS

1. Test material	Azoxystrobin 160 + Prothioconazole 200 g/L SC
Description	Liquid, light brown, SC (suspension concentrate)
Lot/Batch #	ARD/AP/360/SC/36
Content of aa.ss.	Azoxystrobin: 162.20 ± 0.48 g/L (14.45 ± 0.04% w/w) Prothioconazole: 200.54 ± 0.65 g/L (17.86 ± 0.06% w/w) Density: 1.1228 ± 0.0012 g/mL
Stability of test material	Stable under storage conditions (20 ± 5 °C, in the dark) Expiry date: May 23, 2025
2. Vehicle and/or positive control	Untreated control: 50% w/v sucrose solution (500 g sucrose/L de-ionised water) Vehicle control: 50% w/v sucrose solution + 0.1% w/v xanthan Positive control: Reference item
Reference item	Danadim Progress (a.s.: dimethoate)
Description	Liquid, colourless to light yellow, EC (emulsion concentrate)
Lot/Batch #	10214034
Content of active substance	Dimethoate: 400 g/L
Stability of reference item	At +25 °C or cooler, in the dark Expiry date: Nov 2023
3. Test organism	
Species	Freshly emerged worker bees, <i>Apis mellifera</i> L.
Source	Honey bee colonies, disease-free and queen-right, bred by the test facility.
Age	Adult worker bees (max 2 d old)
Pre-treatment culturing conditions	Two days prior to test start, brood combs were transferred to the experimental room and placed in hatching boxes in an incubator. After hatching, the bees were acclimated to the test conditions for ~ 1 d.
Diet	Acclimatisation: Not specified Exposure period: 50% (w/v) sucrose solution with either test item and 0.1% w/v xanthan (test item treatment group), reference item or pure solution (control group), or 50% w/v sucrose solution containing 0.1% w/v xanthan (xanthan control group) <i>ad libitum</i> .
Test units	Stainless steel chambers (8.2 cm length x 5.9 cm height x 4.2 cm width) with removable glass front and perforated bottom to allow for ventilation, lined with filter paper. Feeding syringes were introduced through the hole in the upper wall.
4. Environmental conditions	
Temperature	Nominal: 33 ± 2°C, actual: 33 - 34°C
Relative humidity	Nominal: 50 - 70%, actual: 53 - 69%

Photoperiod

Darkness (except during observation).

B. STUDY DESIGN AND METHODS

1. In-life dates

08 Aug 2023 to 18 Aug 2023 (experimental period)

2. Experimental conditions

Test design

In a 10-d chronic test, young female adults of *Apis mellifera* L. were daily exposed to the test item Azoxystrobin 160 + Prothioconazole 200 g/L SC at five doses in 50% (w/v) sucrose solution. In parallel, one untreated control group, one vehicle control group (including 0.1% w/v xanthan), and one dose of the reference item were tested. Additionally, 3 test units without bees but with full food syringes containing diet of untreated control feeding solution or vehicle control feeding solution (+ 0.1% xanthan), respectively, were used for the evaluation of evaporation. Assessments of bee mortality and sub-lethal effects were done daily during the study.

Number of animals per treatment

Three replicates per treatment group and reference substance treatment and untreated control, as well as vehicle control were used with 10 bees per replicate (i.e. 30 bees per treatment group or control). In addition, three test units for the evaporation control were used without bees.

Test doses

Azoxystrobin 160 + Prothioconazole 200 g/L SC was tested at nominal concentrations of 384, 960, 2400, 6000, and 15000 mg product/kg diet corresponding to nominal daily dietary doses of 7.68, 19.2, 48.0, 120, and 300 µg product/bee/d. The untreated control group received 50% (w/v) sucrose solution, and the vehicle control received 50% (w/v) sucrose solution containing 0.1% w/v xanthan and were tested in parallel.

Reference item

The reference item Danadim Progress (a.s.: dimethoate) was tested at a single nominal daily dietary dose of 0.02 µg dimethoate/bee/d.

Treatment/Application

The application took place for a period of 10 d. The control feeding solution(s) were prepared on day -1, 0, 3 and 7 and were stored under cool conditions in the dark. Stock and feeding solutions were freshly prepared on a daily basis. The treated and untreated feeding solutions were offered *ad libitum* to the bees. Every day feeding solutions were replaced by fresh solutions. Syringes were weighed daily before introduction into the cages and after the feeding interval.

For the test item, a stock solution was prepared by dissolving 300 mg product in 20 g of 50% w/v sucrose solution + 0.1% xanthan. The remaining test item solutions were prepared by serial dilution with 50% (w/v) sucrose solution.

The untreated control solutions contained 50% w/v sucrose solution only. The vehicle control solution contained 50% w/v sucrose solution + 0.1% xanthan. Evaporation controls were included for the untreated control as well as the vehicle control.

Analytics

For verification of the exposure concentration, freshly prepared feeding solutions of the highest test item concentration and lowest test item concentration were analysed in duplicates from day 9. Samples were

analysed using HPLC-UV. The analytical method was validated according to SANTE/2020/12830 Rev.2. (2023) .

3. Observations and assessments

Mortality and sublethal effects were assessed every day for ten days following start of exposure.

Food consumption per bee was calculated by the number of surviving bees per assessment and the amount of food consumed on the following assessment day, subtracting evaporation. The evaporation out of the food syringes was determined by daily weighing of the syringes in the additional test cages without bees. The determined mean evaporation values were taken into account for calculation of the food consumption of the untreated control, vehicle control, the test item and reference item treatments.

4. Calculation of toxicity

The mean percentage cumulative mortality was determined. In addition, the daily and overall mean food consumption was determined.

5. Statistics

In order to determine the LC_{50} and LDD_{50} values regression analysis using Probit Analysis (according to Finney 1971) was used.

Mortalities in the water control and the xanthan control group were compared with a Fisher's Exact Binomial Test. The NOEDD/NOEC of the test item was determined using Multiple sequentially-rejective Fisher Test after Bonferroni-Holm (one-sided greater, $\alpha = 0.05$).

The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0. (ToxRat Solutions GmbH).

Results and Discussion

A. ANALYTICAL RESULTS

No residues of azoxystrobin and prothioconazole were detected at or above the limit of detection (LOD: 0.5 mg azoxystrobin/L, LOD: 0.5 mg prothioconazole/L) in any of the control samples. The recoveries of azoxystrobin in the feeding solutions ranged from 95 - 99% and for prothioconazole the recovery ranged from 92 - 97% of nominal. Since the recovery per treatment group was within the range of 80 to 120% of nominal, the concentrations were confirmed, and further evaluations were done with nominal concentrations.

B. BIOLOGICAL RESULTS

In the test item groups mortality occurred in the upper four test item treated dose levels (960 to 15000 μg product/kg diet) ranging from 6.7 to 100.0% at test end (10 days following the start of chronic exposure). There was no mortality in both, the untreated control (50% w/v sucrose solution) and the vehicle control (containing 0.1% xanthan). In the reference item group mortality was 100% after 4 d.

Test item related behavioural abnormalities were observed in the highest test item treatment (15000 μg product/kg feeding solution) on day 3 and 4 in form of 1 moribund and 3 affected bees, as well as in the 6000 mg product/kg feeding solution treatment on day 5 in the form of 2 moribund bees.

Therefore, the LDD_{50} was calculated to be 20.9 μg product/bee/day, the LC_{50} was calculated to be 1675 mg product/kg diet. The respective NOEDD was calculated to be 15.5 μg product/bee/day and the respective NOEC was determined to be 960 mg product/kg diet.

Table 1: Mortality of bees in the chronic toxicity feeding test after 10 d

Nominal test concentration [mg product/kg diet]	Nominal test dose [µg product/bee/d]	Actual dose [µg product/bee/d]	Cumulative mortality after 10 d [%]
Control: 50 % (w/v) sucrose solution			
Untreated control			0
Vehicle control (+ 0.1% xanthan)			0
Test item: Azoxystrobin 160 + Prothioconazole 200 g/L SC			
384	7.68	8.65	0.0
960	19.2	15.5	6.7
2400	48.0	25.3	83.3*
6000	120	63.6	100.0*
15000	300	162.3	100.0*
Reference item: Dimethoate technical			
1.0 [mg a.s./kg diet]	0.02 [µg a.s./bee/d]	0.013 [µg a.s./bee/d]	100*
10 d endpoints based on nominal and actually consumed dose/concentration			
	[mg product/kg diet]	[µg product/bee/d]	
LC₅₀ / LDD₅₀	1675	20.9	
NOEC / NOEDD	960	15.5	

* Statistically significant difference compared to the control

C. VALIDITY CRITERIA

The mean mortality in the untreated control as well as in the vehicle control was 0.0% (required: < 15%) and the average mortality in the reference item treatment was 100.0% on day 4 (required: ≥ 50%). Consequently, the validity criteria of OECD 245 (2017) were fulfilled.

Conclusion

In a 10-d chronic toxicity feeding study with young female adults of *Apis mellifera* exposed to Azoxystrobin 160 + Prothioconazole 200 g/L SC the LDD₅₀ was calculated to be 20.9 µg product/bee/day, and the NOEDD was calculated to be 15.5 µg product/bee/day. The validity criteria of OECD 245 (2017) were fulfilled.

A 2.3.1.3 KCP 10.3.1.3 Effects on honey bee development and other honey bee life stages

A 2.3.1.3.1 Study 6

Comment from zRMS:	The study was conducted in line with OECD 239 with minor deviations. No assessment was performed on day 7 of the test. This voluntary deviation to the OECD Guidance Document no. 239 was chosen to avoid stress to the larvae in a very delicate stage of their growth such as the transition to prepupa: On day 7 there are no treatments and there is no feeding of the larvae, and performing a mortality assessment would involve opening the dryer with a consequent decrease of temperature and humidity. However, lacking the assessment on day 7 is not expected to affect the study results. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: EC₅₀ (D22) = 134.16 mg test item/kg food NOEC = 189.34 mg test item/kg food ED₁₀ = 20.66 µg test item/larva NOEDD (D22) = 29.16 µg test item/larva
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Reference:	KCP 10.3.1.3/01
Report:	Effects of AZOXYSTROBIN 160 + PROTHIOCONAZOLE 200 g/L SC on honeybees (<i>Apis mellifera</i> L.) 22-day larval toxicity test with repeated exposure, Venturi, S., (2024), Study no. BT212/23
Guideline(s):	OECD Guideline No. 239 (2021)
Deviations:	No assessment was performed on day 7 of the test. This voluntary deviation to the OECD Guidance Document no. 239 was chosen to avoid stress to the larvae in a very delicate stage of their growth such as the transition to prepupa: On day 7 there are no treatments and there is no feeding of the larvae, and performing a mortality assessment would involve opening the dryer with a consequent decrease of temperature and humidity. However, lacking the assessment on day 7 is not expected to affect both the integrity and the outcome of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Executive summary

The toxicity of the test substance Azoxystrobin 160 + Prothioconazole 200 g/L SC to honey bee larvae (*Apis mellifera*) was assessed in a 22 day chronic toxicity test. Honey bee larvae were either treated with the test item at five concentrations at nominally 99.65, 189.34, 359.75, 683.53, and 1298.70 mg product/kg diet (equivalent to 15.35, 29.16, 55.40, 105.26, and 200.00 µg/larvae), the reference item dimethoate at a single concentration or remained untreated (control). Effects on larval and pupal mortality as well as on adult emergence rate were assessed at the end of the test.

In this chronic larval toxicity study with Azoxystrobin 160 + Prothioconazole 200 g/L SC, the 22 day NOEDD was determined to be 29.16 µg product/larva/developmental period (4.21 µg azoxystrobin/larva and 5.21 µg prothioconazole/larva) equivalent to a NOEC of 189.34 mg product/kg diet (27.36 mg azoxystrobin/kg diet and 33.82 mg prothioconazole/kg diet).

The ED₁₀ was determined as 20.66 µg product/larva/developmental period (95% CI: 13.43-31.78). The ED₂₀ was determined as 34.74 µg product/larva/developmental period (95% CI: 25.33-47.64), and the NOEDD was calculated to be 29.16 µg product/ larva/developmental period. The validity criteria of OECD guidance document No. 239 (2016) were fulfilled.

Materials and Methods

A. MATERIALS

- 1. Test material** Azoxystrobin 160 + Prothioconazole 200 g/L SC

Description	Liquid, light brown, SC (suspension concentrate)
Lot/Batch #	ARD/AP/360/SC/36
Content of aa.ss.	Azoxystrobin: 162.20 ± 0.48 g/L (14.45 ± 0.04% w/w) Prothioconazole: 200.54 ± 0.65 g/L (17.86 ± 0.06% w/w) Density: 1.1228 ± 0.0012 g/mL
Stability of test material	Stable under storage conditions (20 ± 5 °C, in the dark) Expiry date: May 23, 2025
- 2. Vehicle and/or positive control** Vehicle control: Untreated diet
 Positive control: Reference item

Reference item	Dimethoate
Description	White crystals
Lot/Batch #	BCCF3993
Purity	99.4% w/w analysed Density: n.a.
Stability of reference item	Cool, dark storage conditions (2 - 8 °C) Expiry date: Dec 2025

3. Test organism

Species	Larvae of the honey bee, <i>Apis mellifera</i> L. (Hymenoptera, Apidea)
Source	Healthy colonies of the test facility's own stock, apparently disease-free and with a known history and physiological status
Age	3 days old, synchronized
Method of producing L1 larvae:	Each of the three colonies used in the test was treated in parallel in the same way: Four days prior to grafting, the queens of several colonies were confined, each one in her own colony in an excluder cage containing an empty comb. The caging time was max. 30 hours. Three days prior to grafting, the queen was released from the cage. The combs containing the eggs were left in the cage, near the brood during the incubation stage and until hatching (day 1). On day 1, combs were transferred to the laboratory using an insulated container.
Diet	The food was composed of three different artificial diets which were adapted to the needs of the larvae at different stages of development: - Diet A (day 1): 50% royal jelly + 50% aqueous solution containing 2% yeast extract, 12% glucose and 12% fructose - Diet B (day 3): 50% royal jelly + 50% aqueous solution containing 3% yeast extract, 15% glucose and 15% fructose - Diet C (day 4-6): 50% royal jelly + 50% aqueous solution containing 4% yeast extract, 18% glucose and 18% fructose
Test units	Crystal polystyrene grafting cells (diameter 9 mm) were sterilised with 70% ethanol solution for 30 minutes, then dried in a laminar-flow hood for 24 hours. Plates were placed into a hermetically sealed desiccator containing a dish with saturated potassium sulphate solution from day 1 until day 15. On day 8, the relative humidity was reduced to $80 \pm 5\%$ by replacing the potassium sulphate solution with a saturated sodium chloride solution. On day 15 the plates were removed from the desiccator and transferred into an emergence box with an aerated cover (humidity 50 - 80%). Emergence boxes were supplied with 50% (w/v) aqueous sucrose solution <i>ad libitum</i> .

4. Environmental conditions

Temperature	Preferably: 34 - 35°C, but not below 23°C or above 40°C; actual: 30.9 - 36.8°C (average measured: 34.3°C)
Relative humidity	Nominal: $95 \pm 5\%$ from day 1 to 8; actual: 89.7 - 97.6% (average measured: 96.5°C) Nominal: $80 \pm 5\%$ from day 8 to 15; actual: 84.1 - 88.4% (average measured: 84.9°C)

Photoperiod

Nominal: 50 - 80% from day 15 to 22; actual: 35.9 - 80.7% (average measured: 56.9°C)
During the test, the bees were kept in darkness except during feeding and observations.

B. STUDY DESIGN AND METHODS

1. In-life dates

12 Sep 2023 - 02 Oct 2023 (experimental period)

2. Experimental conditions

Test design

The toxicity of the test substance Azoxystrobin 160 + Prothioconazole 200 g/L SC to honey bee larvae (*Apis mellifera*) were assessed in a 22 day chronic toxicity test. Honey bee larvae were either treated with the test item at five concentrations, the reference item dimethoate at a single concentration or remained untreated (control). Effects on larval and pupal mortality as well as adult emergence rate were assessed at the end of the test.

Number of animals per treatment

16 larvae/replicate; 3 replicates/test and reference substance treatment and control

Test doses

Azoxystrobin 160 + Prothioconazole 200 g/L SC was tested at nominally 99.65, 189.34, 359.75, 683.53, and 1298.70 mg product/kg diet corresponding to the cumulative dose of 15.35, 29.16, 55.40, 105.26, and 200.00 µg/larvae at the end of the feeding period. A control group, receiving untreated artificial diet, and a reference item group, receiving one concentration of 48.0 mg dimethoate/kg diet (i.e. 7.39 µg a.s./larva/developmental period) were tested in parallel.

Treatment/Application

A stock solution (= highest test item concentration) was prepared by dissolving 289 mg test item in 20 mL ultrapure water, followed by 10 min sonication. The lower test item concentrations were prepared by serial dilution.

For the reference item 100 mg were dissolved in ultrapure water and diluted once to obtain the reference item solution.

For the control, diets were treated with ultrapure water only. The test item stock solutions were prepared freshly at each application day.

At test start, 20 µL of diet A (untreated) was dropped into each cell, and then one larva was grafted from the comb to the cell, onto the surface of the diet, using a grafting tool. All larvae were fed once a day (except at day 2) by dropping the food next to the larvae along the wall of the grafting cell. On day 3, 20 µL of diet B (including either water only, the test item or the reference item) were administered to each larva. On days 4, 5 and 6, larvae were fed with 30, 40 and 50 µL of diet C (including either water only, the test item or the reference item), respectively.

Analytics

Analytical samples of highest and lowest concentration were taken from day 3 to day 6 for dose verification. Samples of the control and stock solution were taken on day 6. The samples were analysed in double replicates using HPLC/DAD. The analytical method was validated in accordance with SANTE/2020/12830, rev.2 (2023).

3. Observations and assessments

Assessments of mortality and any developmental/behavioural abnormality were performed daily from day 4 to day 8 (except on day 7) and on day 15 and on day 22. On day 15 larvae that have not transformed into pupae were recorded as dead and removed. Pupal mortality and emergence rate of adults were also assessed on day 22.

On day 8 the presence of uneaten food was qualitatively recorded. Other observations (e.g. larval appearance and size, behaviour, morphological differences) and any other adverse effects were qualitatively recorded to aid in the interpretation of mortality in comparison to the control group.

Temperature and humidity were recorded continuously over the test period.

4. Calculation of toxicity

Larval mortality was evaluated from day 4 to day 8. The cumulative percentage of mortality for each treatment group was calculated from the number of dead larvae in relation to the total number of larvae per treatment group across all replicates after re-grafting on day 3. Mortality during pupation was evaluated on days 15 and 22. The cumulative percentage of pupal mortality for each treatment group was calculated from the number of larvae that had not transformed into pupae on day 15 and bees that had failed to emerge until day 22 in relation to the total number of larvae that entered pupation on day 8. The cumulative mortalities were corrected for control mortality according to the formula of Abbott (1925), modified by Schneider-Orelli (1947).

The percentage adult emergence rate for each treatment group was evaluated on day 22 and was calculated from the number of adult emerged bees on day 22 in relation to the total number of larvae per treatment group on day 3. The inhibition of emergence was calculated compared to the control.

For each test item group, the cumulative dose per larva was calculated based on the given test item concentration, the cumulative feeding volume per larva and the density of the diet (1.1228 g/mL).

5. Statistics

The Step-down Cochran-Armitage Test Procedure (one-sided greater, $\alpha = 0.05$) was used to determine the NOED/NOEC values both at D8 and D22. The LD_x/LC_x values on D8 and D22 (mortality) and the ED_x values on D22 (emergence) were calculated with the Probit analysis using linear max. likelihood regression.

For the statistical evaluation the statistics program ToxRat Pro, Version 3.3.0 was used.

Results and Discussion

A. ANALYTICAL RESULTS

No residues of azoxystrobin and prothioconazole were detected at or above the lowest calibration concentration (azoxystrobin: 2.483 mg/L, prothioconazole: 3.084 mg/L) in the control sample. The content of azoxystrobin and prothioconazole in the water solutions (at highest and lowest test item concentration) sampled on day 3 were ranging between 100.34 - 104.55%, and 93.13 - 98.50%, respectively. Thus, measured concentrations were in the range of $\pm 20\%$ of nominal, and therefore the concentrations of the larval diet were confirmed, and the endpoints are based on nominal concentrations.

B. BIOLOGICAL RESULTS

The adult emergence on day 22 was affected by the administration of the test item Azoxystrobin 160 + Prothioconazole 200 g/L SC to the larvae at dose levels of 55.40 μg product/larva and above. At day 8 the results of the control group showed mortality $< 15\%$ (8.33%), thus no statistically significant dose-response was found for larval mortality. The results of the reference item group indicated that the test system was sensitive (corrected mortality: 100% on day 8).

On day 22 the results of the control group showed an adults' emergence of 83.33%. No behavioural effects on emerged adults were observed in the control and in all treated groups. The NOED on day 22 was determined to be 29.16 µg product/larva (4.21 µg azoxystrobin/larva and 5.21 µg prothioconazole/larva) equivalent to a NOEC of 189.34 mg product/kg diet (27.36 mg azoxystrobin/kg diet and 33.82 mg prothioconazole/kg diet).

Table 1: Cumulative mortality of larvae exposed to Azoxystrobin 160 + Prothioconazole 200 g/L SC in a chronic toxicity test

Test concentra- tion [mg product/kg diet]	Cumulative dosage [µg product/ larva/dev. period]	Cumulative mean mortality [%]				Emergence on day 22 [%]
		Day 8	Day 8-15	Day 8-22	Day 22, cor- rected	
Control		8.33	3.33	9.14	-	83.33
Test item: Azoxystrobin 160 + Prothioconazole 200 g/L SC						
1298.70	200.00	60.61*	9.52	49.52	70.00*	25.00
683.53	105.26	6.06	6.06	61.62	60.00*	33.33
359.75	55.40	0.00	16.11	40.76	33.33*	55.56
189.34	29.16	3.03	0.00	18.10	13.33	72.22
99.65	15.35	0.00	8.33	22.22	6.67	77.78
Reference item: Dimethoate						
48.0 ^{a)}	7.39 ^{a)}	100.00	-	-	-	0.00
22-day endpoints						
	[mg product/kg diet]			[µg product/larva/developmental period]		
NOEC / NOEDD	189.34			29.16		
EC ₅₀ / ED ₅₀ (95% CL)	609.61 (468.12-793.83)			93.88 (72.09-122.25)		
EC ₂₀ / ED ₂₀ (95% CL)	225.58 (164.48-309.35)			34.74 (25.33-47.64)		
EC ₁₀ / ED ₁₀ (95% CL)	134.16 (87.21-206.36)			20.66 (13.43-31.78)		

* Statistically significantly increased compared to control (Step-Down Cochran-Armitage test, one-sided greater, $\alpha = 0.05$)

^{a)} [mg a.s./kg diet] or [µg a.s./larva/developmental period]

C. VALIDITY CRITERIA

Cumulative larval mortality on day 8 was 8.33% in the control (required: $\leq 15\%$) and 100% in the reference item group (required: $\geq 50\%$). Adult emergence was 83.33% in the control (required: $\geq 70\%$). Therefore, the study was considered valid in accordance with OECD guidance document No. 239 (2016).

Conclusion

In this chronic larval toxicity study with Azoxystrobin 160 + Prothioconazole 200 g/L SC, the 22 day NOEDD was determined to be 29.16 µg product/larva/developmental period (4.21 µg azoxystrobin/larva and 5.21 µg prothioconazole/larva) equivalent to a NOEC of 189.34 mg product/kg diet (27.36 mg azoxystrobin/kg diet and 33.82 mg prothioconazole/kg diet).

The ED₁₀ was determined as 20.66 µg product/larva/developmental period (95% CI: 13.43-31.78). The ED₂₀ was determined as 34.74 µg product/larva/developmental period (95% CI: 25.33-47.64), and the NOEDD was calculated to be 29.16 µg product/larva/developmental period. The validity criteria of OECD guidance document No. 239 (2016) were fulfilled.

A 2.3.1.4 KCP 10.3.1.4 Sub-lethal effects

No additional data submitted.

A 2.3.1.5 KCP 10.3.1.5 Cage and tunnel tests

Semi-field studies ongoing, please find the references already below

A 2.3.1.5.1 Study 7

Comment from zRMS:	The study was conducted to determine the potential effects of Azoxystrobin 160 g/L + Prothioconazole 200 g/L SC on the honeybee (<i>Apis mellifera</i> L.) after one foliar application of 1 L product/ha (equivalent to 160.02 g azoxystrobin/ha + 204.07 g prothioconazole/ha) during flowering (BBCH 63-65), during honeybee flight under semi-field conditions in <i>Phacelia tanacetifolia</i> in Germany. Potential effects of the test item were evaluated against those observed in honeybees treated with tap water (control) and a toxic reference item (Dimethoate, 400 g a.i. dimethoate /ha). There was no effect of the test item on adult worker, or worker larvae and pupae mortality, neither during the exposure period in the tunnel tents nor during the monitoring period. A reduction in flight activity was observed in the test item group T on the day of application (0DAA) and the following day (1DAA). Afterwards, honeybee flight activity was similar in C and T. No biological relevant test item effect was detected on honeybee behaviour. There was no test item related effect neither on colony strength nor on the development of the brood for all stages and food storage area. No residues of azoxystrobin, prothioconazole and prothioconazole-desthio were detected in any of the samples taken prior to the application and all samples taken from the control replicates were uncontaminated. The highest residue levels were detected shortly after the application of the test item (S3/0DAA). Residues detected in pollen loads were 66.4 mg/kg for azoxystrobin, 80.6 mg/kg for prothioconazole, and 2.63 mg/kg for prothioconazole-desthio. At the next sampling day (6DAA), residue levels were 1.18 mg/kg for azoxystrobin, 0.268 mg/kg for prothioconazole, and 0.320 mg/kg for prothioconazole-desthio. Residues detected in nectar from forager bees were much lower compared to residues detected in pollen loads. On 0DAA, residues detected in nectar were 0.800 mg/kg for azoxystrobin, 0.376 mg/kg for prothioconazole, and 0.197 mg/kg for prothioconazole-desthio. At the next sampling day (6DAA) residues of azoxystrobin were < LOQ (0.00309 mg/kg) and no residues of prothioconazole and prothioconazole-desthio were longer detected. Overall, a foliar application of Azoxystrobin 160 g/L + Prothioconazole 200 g/L SC during active foraging of honeybees under semi-field conditions did not result in any significant adverse effects.
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Reference:	KCP 10.3.1.5/01
Report	Azoxystrobin 160 g/L + Prothioconazole 200 g/L - A Semi-Field Study to Evaluate Side Effects on Honeybees (<i>Apis mellifera</i> L.) in <i>Phacelia tanacetifolia</i> in Germany 2024; Portail, B. 2025, study no. S24-102790
Guideline(s):	OEPP/EPPO Guideline No. 170(4), 2010, and partial integration of: EFSA Guidance Document No. 11(7): 3295 (2013), EU Guideline 7029/VI/95 rev. 4 (1997), SANTE/2020/12830 rev. 2 (2023)
Deviations:	None relevant
GLP:	Yes

Acceptability: Yes
Duplication: Not applicable
(if vertebrate study)

Executive summary

This study was conducted to determine the potential effects of Azoxystrobin 160 g/L + Prothioconazole 200 g/L SC on the honeybee (*Apis mellifera* L.) after one foliar application of 1 L product/ha (equivalent to 160.02 g azoxystrobin/ha + 204.07 g prothioconazole/ha) during flowering (BBCH 63-65), during honeybee flight under semi-field conditions in *Phacelia tanacetifolia* in Germany. Potential effects of the test item were evaluated against those observed in honeybees treated with tap water (control) and a toxic reference item (Dimethoate, 400 g a.i. dimethoate /ha).

There was no effect of the test item treatment T on adult worker bee mortality or on worker pupae/larvae mortality during the entire study period, whereas there was a clear impact of the reference item treatment R on worker bee mortality during the exposure period (0DAA to 7 DAA). There was a slight, transient reduction of honeybee flight activity at the assessments on the day of the application (4 of the 5 assessments) and on the following day. There was no effect on honeybee behaviour after exposure to the treated crop for the entire study period. There was no negative effect of the test item treatment T on the colony strength (mean number of bees per hive) nor on the number of brood cells (eggs, larvae, pupae) or food cells (pollen, nectar).

Overall, a foliar application of Azoxystrobin 160 g/L + Prothioconazole 200 g/L SC during active foraging of honeybees under semi-field conditions did not result in any significant adverse effects.

Materials and methods

A. Materials

- | | |
|--------------------|--|
| 1. Test substance: | Azoxystrobin 160 + Prothioconazole 200 g/L SC |
| Lot/Batch no.: | ARD/AP/360/SC/40 |
| Content/Purity: | Azoxystrobin nominal: 160 g/L; analysed: 160.02 g/L (14.32% w/w)
Prothioconazole: 200 g/L; analysed 204.07 g/L (18.26% w/w) |
| Control: | tap water |
| Toxic reference: | BAS 152 65 I (37.7 % w/w dimethoate) |
2. Test organisms / test system: *Apis mellifera* L. (Hymenoptera, Apoidea);

Honeybee colonies were queen-right as judged by direct observation of the queen or the presence of freshly laid eggs and had not received any medical treatments within four weeks prior to the start of the test. All hives were free of symptoms of nose-mosis, varroa-tosis or other bee diseases. At the first assessment of colony condition inside the tunnels (1DBA = BFD0), the colonies for biological assessments (replicates a-d) contained between 6045 and 8580 honeybees per colony, 4-6 combs with brood and 4-8 combs with food (nectar and/or pollen). Hives used for residue samplings (Cs and Ts) contained 5 combs containing brood and 4-6 combs containing food (nectar and/or pollen).

Before the start of mortality assessments, dead bee traps were fixed in front of the hives to record the number of dead honeybees which were carried out of the hives. One honeybee colony per tunnel was placed in the tunnels shortly before *Phacelia*

reached full bloom (BBCH 61-63) in the evening after bee flight (evening/night from 4DBA). During the exposure period in the tunnels, the bees were supplied with water from a water tray inside the hives. The water dispensers were removed from the tunnel during the application. The honeybee colonies were relocated from the tunnels to the monitoring location after the last assessment of mortality, foraging activity and behaviour in the tunnels (in the evening after daily bee flight on 7DAA).

3. Field site

The field site was located near Maulbronn, Baden-Württemberg, Germany (for details, see study report). The trial was performed in a field of *Phacelia tanacetifolia*. Shortly prior to the onset of *Phacelia* flowering, 14 tunnel tents were set up to accommodate five control replicates (four biological replicates Ca to Cd and one sampling replicate Cs), five test item treatment replicates (four biological replicates Ta to Td and one sampling replicate Ts) and four reference item replicates (Ra to Rd, no sampling replicate). Daily temperature, precipitation and humidity were measured hourly using a calibrated data logger (GLP record) located at the field site.

4. Monitoring Site

Honeybee colonies were relocated from the field site to the monitoring site in the night of 7DAA to 8 DAA in the evening after daily bee flight and monitored further until 28DAA. The monitoring site used after tunnel exposure was located near Bretten (distance to field site: ~6 km) and comprised an area which provided sufficient food sources for the bees to forage on (e.g. wild flowers). Daily temperature, precipitation and humidity were measured hourly using a calibrated data logger (GLP record) located next to the colonies. From 8DAA to 13DAA weather data were obtained from an official weather station located 5.5 km away from the monitoring site.

5. Plot Design

Each of the tunnel tents covered an area of 100 m² (20 m length x 5 m width) and had a height of approximately 3.5 m at the tunnel centre. Each tunnel frame was covered with light plastic gauze (mesh size: 1.5 mm). Before the start of the trial, paths (0.6 m) were created in the centre along the tunnel length and across the tunnel at both ends by removing the plants and smoothing ground. These paths were covered with water permeable linen sheets to enable the counting of dead bees during mortality assessments. The treated crop area (i.e. tunnel area minus path area) was 94 m².

B. Study design and method

1. In-life dates (field phase): 25 June 2024 – 30 July 2024
2. Test design:

The potential effects of Azoxystrobin 160 g/L + Prothioconazole 200 g/L SC on the honeybee (*Apis mellifera* L.) when applied once at 1 L product/ha (equivalent to 160.02 g azoxystrobin/ha + 204.07 g prothioconazole/ha) to *Phacelia tanacetifolia* in Germany 2024 following the OEPP/EPPO Guideline No. 170(4) (2010) with partial integration of the EFSA Guidance Document No. 11(7): 3295 (2013)

and the EU Guideline 7029/VI/95 rev. 4 (1997). The field phase of this study was conducted in Germany from June 2024 to July 2024 and included a total of three treatment groups:

- Test item treatment T: one application during flowering of *P. tanacetifolia* (BBCH 65) and during honeybee flight activity at a target rate of 1 L prod./ha in 300 L/ha (equivalent to 160.04 g azoxystrobin and 204.07 g prothioconazole/ha in 300 L/ha, based on analysed content of a.i.),
- Reference item group R: one application of dimethoate during flowering of *P. tanacetifolia* (BBCH 65) and during honeybee flight at a target rate of 400 g a.i./ha,
- Control group C with one application of tap water during flowering of *P. tanacetifolia* (BBCH 63-65) during daily honeybee flight activity, on the same day as the applications in the test item treatment group T and the reference item group R.

All applications were carried out at a target spray volume of 300 L water/ha.

The honeybee colonies were placed in the tunnels in the evening of 28 Jun 2024 (4DBA; DBA=Days before the application) at the flowering stage of *Phacelia tanacetifolia* = BBCH 63-65 (recorded during the assessment on 2 July 2024).

The effects of the test item treatment were examined on honeybee colonies in tunnels (5.0 m x 20.0 m and a height of approx. 3.5 m in the centre) placed over plots of flowering *Phacelia tanacetifolia*. The semi-field test comprised 5 replicates (tunnels) in the treatment group T and the control C (= 4 replicates for biological assessments plus 1 replicate “s” that was used for residue sampling only) and 4 replicates in the reference item treatment group R (no sampling).

Samples of the spray solutions for dose verification were taken from spray solutions intended for all replicates of the test item treatment group before application. Samples of forager bees for the preparation of nectar and pollen from forager bees were collected on three occasions (1DBA, 0DAA and 6DAA) in the sampling tunnels Cs and Ts. Samples were analysed for residues of azoxystrobin, prothioconazole and prothioconazole-desthio by LC/MS/MS with an LOQ (limit of quantification) of 0.01 mg/kg for all analytes in nectar and pollen. sulphur/kg (flowers), 38.1 mg sulphur/kg (nectar) and 2204 mg sulphur/kg (pollen).

The influence of the test item treatment was evaluated by comparing the results in the test item treatment group T and reference item group R to the control group C before and after the application.

3. Assessments

Mortality

Mortality of the honeybees were recorded by counting the number of dead honeybees in the dead bee traps in front of the hives and on the linen sheets which were spread out in the tunnels. At the monitoring site, only dead bee traps were used to record honeybee mortality. The dead bees found were differentiated into adult worker bees, pupae and larvae during each assessment. The exact number was recorded. Dead male bees (drones) and male brood were also recorded, but excluded from the evaluation. After each assessment, the dead bees, pupae and larvae found on the linen sheets and in the dead bee traps were removed. Mortality assessments were not performed in the sampling replicates Cs and Ts.

Flight Activity of the Honeybees

The flight activity of honeybees over the crop was assessed. At each assessment the number of bees that were both foraging on flowers and flying over the crop within three areas of 1 m² were counted for 10 to 15 seconds. Assessment areas were chosen randomly prior to each assessment.

Behaviour of the Honeybees

During the assessments of mortality and flight activity, the behaviour of the honeybees in the crop and around the hive was observed with respect to the following behavioural categories:

- Intensive cleaning,
- Trembling,
- Cramping,
- Locomotion problems,
- Inactive bees,
- Bees hanging on the crop,
- Filtering bees (Guard bees attacking and/or preventing returning bees from entering the hive),
- Clustering of large numbers of bees at the hive entrance.

Any other observations regarding unusual behaviour of the honeybees including the number of bees displaying the unusual behaviour were recorded in the raw data as well.

Assessments of honeybee behaviour were not performed in the replicates intended for residue sampling (Cs and Ts) after the application.

Condition of the Colonies and Colony Assessments

The following parameters were assessed during each colony assessment:

- Colony strength (number of bees, estimation adapted from IMDORF & GERIG, 1999, and IMDORF et al., 1987)
- Presence of a healthy queen (e.g., presence of eggs)
- Pollen storage area and area with nectar or honey (estimation adapted from IMDORF & GERIG, 1999, and IMDORF et al., 1987)
- Area containing cells with eggs, larvae and capped cells (estimation adapted from IMDORF & GERIG, 1999, and IMDORF et al., 1987)

At each assessment, the comb area covered with bees and cells with nectar, pollen, eggs, larvae and capped cells were estimated per comb side and the total number of bees and cells containing the single brood stages, pollen and nectar will be calculated for each hive. Afterwards the mean values were calculated for each treatment and assessment date.

At each colony assessment, each comb of the colonies was assessed visually for symptoms of bee diseases by a beekeeper according to standard beekeeping practice. Accordingly, any unusual observations and clear symptoms of disease (e.g., chalk brood, sacbrood, nosemosis, American or European foulbrood) or pests (e.g., *Varroa* sp., *Aethina tumida*, *Tropilaelaps* spp.) were recorded.

Assessments of the condition of the colonies was not performed during adverse weather conditions which might affect the colonies. The condition of the residue sampling colonies Cs and Ts was assessed only once prior to the application (1DBA) to record the status of the colonies.

C. Evaluation and statistics

Data evaluation

The possible influence of the test item was evaluated by comparing the results in the test item treatment group T and reference item group R to the corresponding data in the control separately, under consideration of the results of:

- Mortality of adult worker bees and worker bee larvae and pupae on the linen sheets and in the dead bee traps (mean number of dead bees);
- Flight activity (mean number of forager bees / m² / 10-15 s);
- Behaviour of the bees in the crop and around the hive;
- Condition of the colonies: Number of bees (colony strength) and food storage
- Residue analysis

Statistics

The statistical analysis of mortality data, flight data, colony condition data and bee brood development data was performed using SAS® (SAS Institute Inc., Version 9.4).

A significance level of 0.05 was chosen for all comparisons. Hypothesis testing for data obtained before the application was performed in two-sided mode. Hypothesis testing for data obtained after the application was performed using appropriate tests in their one-sided mode, i.e. only test results indicating a negative impact of the test or reference item on honeybee mortality (higher), flight activity (lower), condition of the colonies (less bees, brood and food) after the application when compared to the control group are reported.

As the reference item group was included into the study to evaluate sensitivity of the method and not for comparisons to the test item treatment group, comparisons of either treatment group to the control group were considered to be independent from each other and no corrections for multiple comparisons to the control group were made during hypothesis testing.

Original and log₁₀-transformed data were tested for normality using the Shapiro-Wilks test and for homoscedasticity using a two-sample F-test. If log₁₀-transformation allowed for use of hypothesis tests of higher statistical power, the log₁₀-transformed data were used in the analysis. Otherwise, hypothesis testing was performed using the original observations.

Normally distributed, homoscedastic data were analysed using the pooled t-Test. Normally distributed data with significantly different variances were further analysed using Satterthwaite's t-Test. Non-normally distributed data were further analysed using the Mann-Whitney Exact test.

E. Sampling & Analytics

Spray Solution Samples for Residue Analysis

Samples of the spray solutions were taken once 0DBA directly after preparation and mixing of the test item spray solution before filling the boom sprayer shortly prior to the application. Two sub-samples of 10 mL each (A for the analysis, and R as retained sample) were taken from the spray solution applied to plots Ta-Td and Ts.

Samples were frozen on dry ice directly after sampling and stored deep frozen in the freezer (< -18°C) within 12 hours after sampling.

Forager Bee Samples for Preparation of Nectar and Pollen

Forager bees for nectar and pollen preparation were collected from colonies Cs and Ts. On each sampling day ≥ 400 forager bees per tunnel tent were taken. The hive entrances were sealed before sampling and the forager bees were subsequently collected as they return to the hive by using a modified hoover ("bee vac") containing dry ice to immobilise/ kill the bees. After each sampling interval the hive was re-opened to allow honeybees to return to and leave the hive.

Directly after the sampling each sample was divided into two sub-samples with at least 200 bees each. One for preparation (A-sample) and one as a retained sample (R-sample).

Forager bee samples were collected on three sampling dates, 1DBA, 0DAA and 6DAA.

Samples were frozen on dry ice directly after sampling and stored deep frozen in the freezer (< -18°C) within 6 hours of sampling.

Sample Preparation of Pollen Loads and Honey Stomachs from Forager Bees for Residue Analysis

At least 75 forager bees from the A-sample were prepared, to achieve the required amount in all samples.

The duration of any samples remaining outside the freezer did not exceed two hours. Bees from the untreated replicates were processed first, followed by the bees from the treated replicates. After the preparation, prepared nectar and pollen samples as well as any remaining forager bee samples were stored deep frozen (< -18°C).

Pollen loads were sampled by detaching them from the legs of forager bees and transferring them into a vial.

For the preparation of nectar (honey stomach content), forager bees were fixed at the thorax and the abdomen was separated from the thorax using a pair of forceps. The contents of the honey stomach were transferred into a vial.

Directly after preparation of the nectar samples for residue analysis an additional measurement of sugar content was conducted (see deviation section). The sugar content was measured with a digital refractometer.

Sample Residue Analysis

Analysis and quantification of test item residues in spray solutions, nectar and pollen prepared from forager bees was performed at the analytical laboratories of Eurofins Agrosience Services EcoChem GmbH within 30 days after the first sampling.

Results and discussions

A. Weather conditions

During the application, the cloud coverage was 70 %, air temperature ranged between 16.9°C and 20.1°C and relative humidity ranged between 56.8 % and 74.1 %. Main wind direction was southeast and wind speeds measured inside the tunnels during the spray applications ranged between 0.5 m/s and 2.0 m/s.

Rain gauge record was 2.7 mm on the day of application. However, there was no rainfall within 3 h after application.

During the tunnel period (3DBA to 7DAA), temperatures ranged between 10.6 °C and 33.6 °C and relative humidity ranged between 41.8 % and 98.1 %. During this period rain was recorded on six days with a total of 37.5 mm, with daily precipitation amounts ranging from 1.2 mm to 12.3 mm.

During the monitoring period (8DAA to 28DAA), daily temperature ranged between 10.1 °C and 32.8 °C and relative humidity ranged between 31.8 % and 100 %. During this period rain was recorded on nine days with a total of 51.8 mm, with precipitation amounts ranging from 0.2 mm to 16.1 mm.

B. Residue Analysis

Residue Analysis of Spray Solution Samples

Azoxystrobin concentration in samples of the spray solutions applied to test item treatment group replicates Ta – Td and Ts ranged between 0.576 g/L and 0.602 g/L, which corresponds to 107 % to 112 % of the target concentration. Prothioconazole concentration ranged between 0.629 g/L and 0.682 g/L, which corresponds to 92 % to 100 % of the target concentration.

Table A 1: Results of Dose Verification (Spray Solution)

Replicate (tunnel)	Azoxystrobin nominal (g/L)	Azoxystrobin analysed (g/L)	Azoxystrobin recovery (%)	Prothioconazole nominal (g/L)	Prothioconazole analysed (g/L)	Prothioconazole Recovery (%)
Ta	0.540	0.590	109	0.682	0.629	92
Tb	0.540	0.577	107	0.682	0.672	98
Tc	0.540	0.602	112	0.682	0.686	101
Td	0.540	0.576	107	0.682	0.682	100
Ts	0.540	0.589	109	0.682	0.682	100

Residue Analysis of Field Samples

No residues of azoxystrobin, prothioconazole and prothioconazole-desthio were detected in any of the samples taken prior to the application and all samples taken from the control replicates were uncontaminated.

The highest residue levels were detected shortly after the application of the test item (S3/0DAA). Residues detected in pollen loads were 66.4 mg/kg for azoxystrobin, 80.6 mg/kg for prothioconazole, and 2.63 mg/kg for prothioconazole-desthio. At the next sampling day (6DAA), residue levels were 1.18 mg/kg for azoxystrobin, 0.268 mg/kg for prothioconazole, and 0.320 mg/kg for prothioconazole-desthio.

Residues detected in nectar from forager bees were much lower compared to residues detected in pollen loads. On 0DAA, residues detected in nectar were 0.800 mg/kg for azoxystrobin, 0.376 mg/kg for prothioconazole, and 0.197 mg/kg for prothioconazole-desthio. At the next sampling day (6DAA) residues of azoxystrobin were < LOQ (0.00309 mg/kg) and no residues of prothioconazole and prothioconazole-desthio were longer detected.

Table A 2: Results of Residue Analysis (Nectar, Pollen)

Sample Type	Sampling Interval	Timing	Tunnel	Residue of Azoxystrobin (mg/kg)	Residue of Prothioconazole (mg/kg)	Residue of Prothioconazole-desethio (mg/kg)
Nectar from bees	S1	1DBA	Cs	n.d.	n.d.	n.d.
	S1	1DBA	Ts	n.d.	n.d.	n.d.
	S3	0DAA	Cs	n.d.	n.d.	n.d.
	S3	0DAA	Ts	0.8	0.376	0.197
	S4	6DAA	Cs	n.d.	n.d.	n.d.
	S4	6DAA	Ts	<LOQ (0.00309)	n.d.	n.d.
Pollen from bees	S1	1DBA	Cs	n.d.	n.d.	n.d.
	S1	1DBA	Ts	n.d.	n.d.	n.d.
	S3	0DAA	Cs	n.d.	n.d.	n.d.
	S3	0DAA	Ts	66.4	80.6	2.63
	S4	6DAA	Cs	n.d.	n.d.	n.d.
	S4	6DAA	Ts	1.18	0.268	0.320

S Sampling
DBA/DAA days before / after the application
n.d. not detected

C. Mortality of Adult Worker Bees

During the pre-exposure period (period during confinement in the tunnel, before application) worker bee mortality was comparable between treatment groups. The mean ($\pm$ STD) mortality of bees in the period from 3DBA to 0DBA was 82.4 ± 54.7 worker bees/ day in the control, 28.6 ± 35.1 worker bees/ day in the treatment group T and 78.9 ± 58.7 worker bees/ day in the reference item treatment group R. Unusual high mortality (> 100 dead bees) was recorded on the linen sheets in the tunnels Ca, Cc, Cd (1DBA - 0DBA), Tb (1DBA - 0DBA) and Rc, Rd (1DBA - 0DBA) and is probably due to poor accommodation of the hives to the confinement situation inside the tunnels. No statistically significant differences between treatment groups were observed during the pre-exposure period.

The mean sums of dead adult bees observed during the multiple assessments on the day of application were similar between the control group C and the test item group T. The mean sums of dead adult bees observed on the day of application after the application (0DAA2) were 23.5 ± 16.0 in the control group, 10.3 ± 8.7 in the test item group T and 727.8 ± 436.0 in the reference item group R. In the reference item group, the number of dead bees was statistically significant higher ($p \leq 0.05$, right-sided pooled t-Test) in comparison to the mortality observed in the control group. There were no statistically significant differences between treatment groups C and T in summed mortalities on the day of application.

At the next day (1DAA), mean worker bee mortality was on a very low level in C and T with 9.00 ± 6.2 worker bees/ day in the control group C and 4.3 ± 4.0 worker bees/ day in the test item group T. In the reference item group R a statistically significant higher number of dead worker bees was still observed with 131.3 ± 39.2 worker bees/ day ($p \leq 0.05$, right-sided Satterthwaite t-Test).

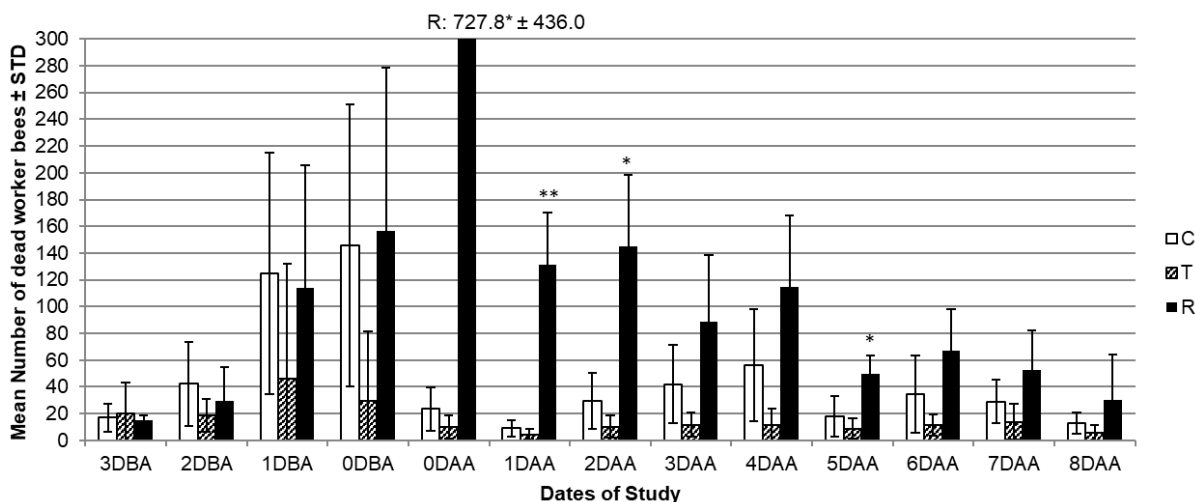
On 2DAA, mean worker bee mortality increased in all treatment groups. The mean number of dead bees observed in the reference item treatment group was statistically significantly higher in comparison to the control mortality observed in the control group ($p \leq 0.05$, right-sided pooled t-Test). The mortality of the test item treatment group was lower to the mortality observed in the control group with no significant difference.

During the exposure period in the tunnel tents (0DAA to 7DAA), no statistically significant difference was observed between the control group C and the test item treatment group T. On 5DAA, adult worker bee mortality in the reference item treatment group R was significantly higher than that of the control group C ($p \leq 0.05$, right-sided pooled t-Test). The mean worker bee mortality was statistically significant higher for the post application period in the tunnel from 0DAA to 7DAA ($p \leq 0.05$, right-sided pooled t-Test).

During the monitoring period (8DAA to 28DAA), daily mortality of adult worker bees was comparable between the treatment groups C, T and R. On 18DAA the mortality in the reference item treatment group was statistically significant higher in comparison to the control mortality observed at this day ($p \leq 0.05$, right-sided pooled t-Test). There was no significant difference for the mean mortality over the monitoring period (8DAA to 28DAA).

Mean adult honeybee mortality over the entire post-exposure period (0DAA to 28DAA) was not statistically significant different in T and R when compared to C.

Based on the evaluation outlined in the 2023 EFSA Guidance document, the risk is considered low, as the total mortality in each test item tunnel does not exceed 10% of the initial colony size.

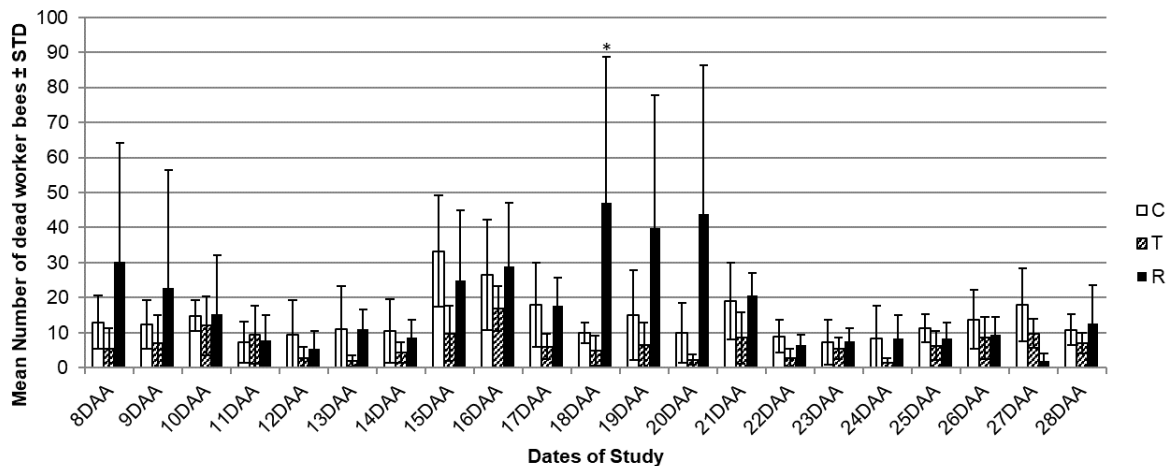


DBA/DAA days before / after application; STD standard deviation

* significantly higher than the control group C ($p \leq 0.05$, right-sided pooled t-Test)

** significantly higher than the control group C ($p \leq 0.05$, right-sided Satterthwaite t-Test)

Figure 1 Mean Adult Worker Bee Mortality (3DBA to 8DAA)



DAA = Days after the application during flowering and honeybee-flight
STD = Standard deviation;

Figure 2 Mean Adult Worker Bee Mortality at the Monitoring Site (8DAA to 28DAA)

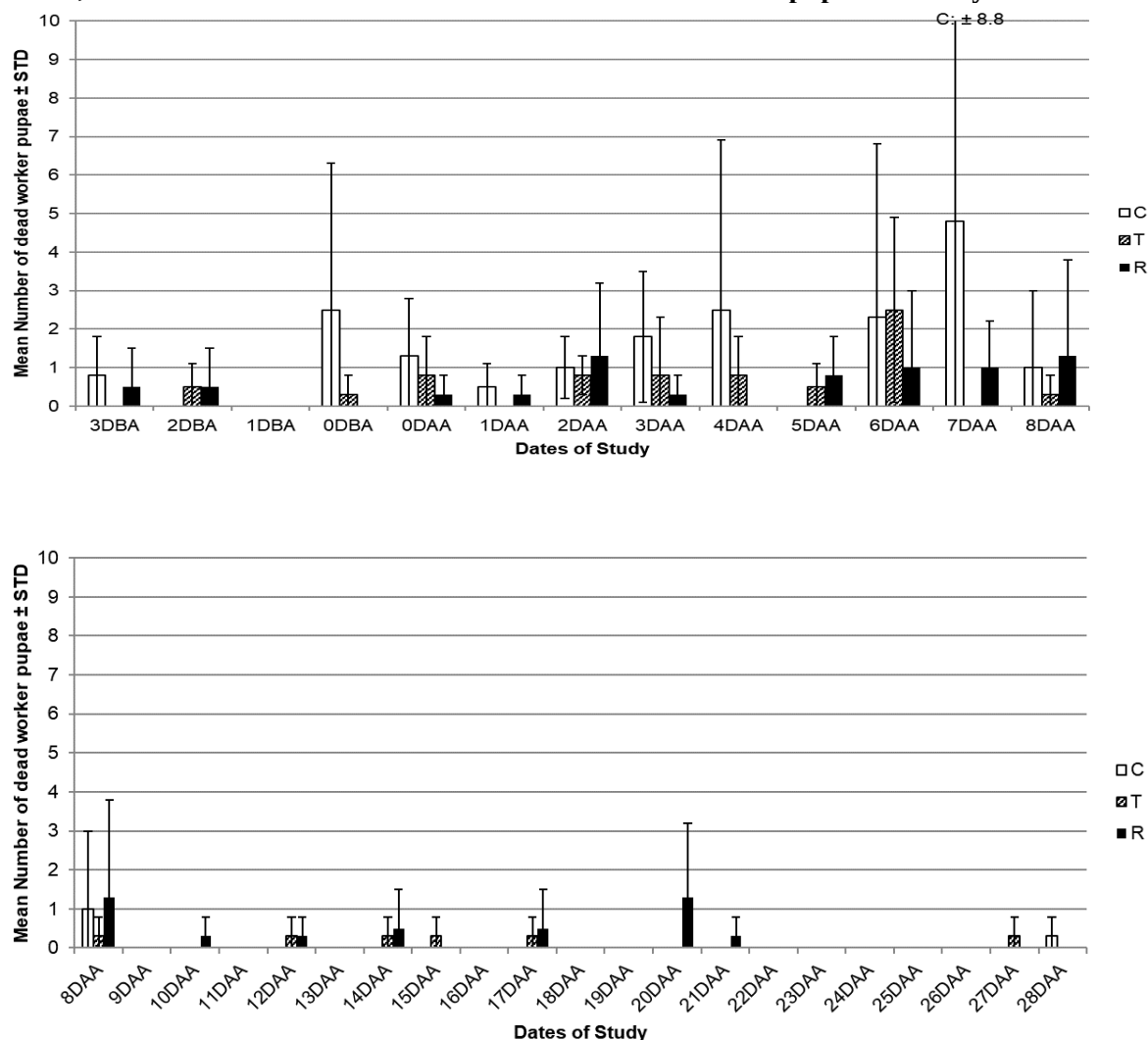
D. Mortality of Worker Bee Larvae and Pupae

During the pre-exposure period, mean pupae mortality was at a very low level in all treatment groups. No statistically significant differences between treatment groups were observed during this period. During the multiple assessments on the day of application, the sums of dead worker larvae and pupae remained at a low level in all treatment groups. No statistically significant differences between the control

group C and the treatment groups T and R were observed in summed mortalities on the day of application.

During the exposure, and the monitoring period worker larvae and pupae mortality was comparable between treatment groups. No statistically significant differences between treatment groups were observed during the exposure, and the monitoring period.

Overall, there was no effect of the test item on worker larvae and pupae mortality.



DBA/DAA = Days before/after the application during flowering and honeybee-flight There were no statistically significant differences to the control.
STD = Standard deviation;

Figure 3: Mean Worker Larvae and Pupae Mortality (3DBA to 28DAA)

E. Flight Activity of the Honeybees

During the pre-exposure period, mean (±STD) flight activity in the tunnel tents was comparable between the treatment groups. From 3DBA to 0DBA, the mean number of foraging bees/m² was 8.7 ± 2.7 in the control group C, 4.4 ± 0.9 in the test item treatment group T, and 7.2 ± 2.2 in the reference item treatment group R. Mean flight activity in T was slightly lower than in the control on 3DBA and 2DBA (Student's t-Test (pooled), left-sided, $p \leq 0.05$).

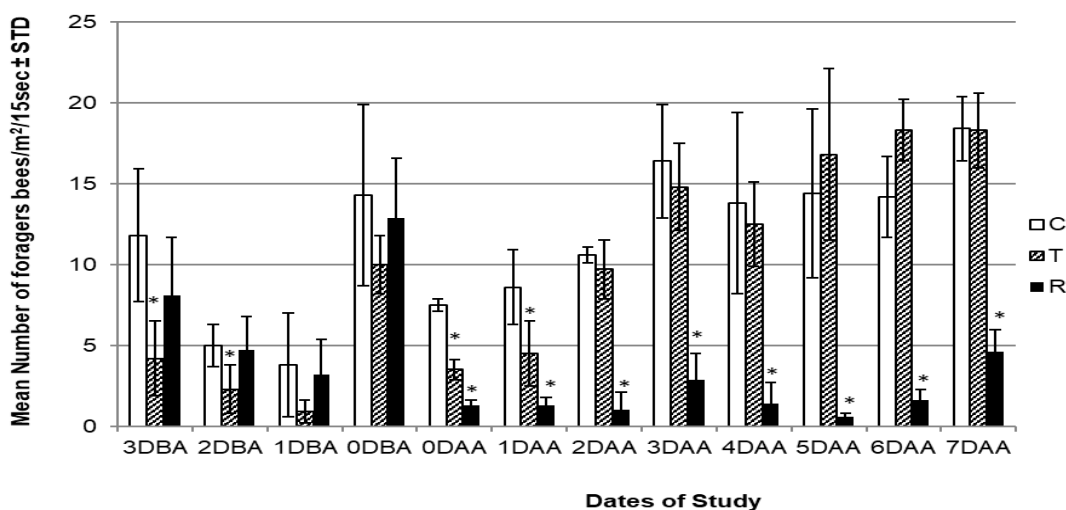
Multiple assessments of flight activity were performed after the application on 0DAA. The mean flight activity across the five assessments on this day was 9.0 ± 0.5 forager bees/m² in C, 4.2 ± 0.7 in T, and 1.4 ± 0.4 in R. The differences in T and R compared to the control were statistically significantly lower ($p \leq 0.05$, left-sided pooled t-Test). In T and R, flight activity of four of the five individual assessments on that day (30 min, 1h, 2h, and 4h after the application) was statistically significantly lower than the control values ($p \leq 0.05$, left-sided pooled t-Test).

On the day after the application (1DAA), flight activity was assessed three times (in the morning, at noon and in the afternoon). On that day, the mean flight activity across the three assessments was 8.6 ± 2.3 forager bees/m² in the control group C, 5.1 ± 1.5 in T, and 1.3 ± 0.5 in R. The flight activity in T and R was statistically significantly lower than the control values ($p \leq 0.05$, left-sided pooled t-Test).

On the subsequent days (2DAA to 7DAA), honeybee flight activity was higher in both C and T compared to the earlier days. There was no statistically significant differences between C and T during this period. Very low flight activity was observed in the reference item treatment group R during the days after the application. The flight activity was statistically significantly lower in comparison to the control at all days from 2DAA to 7DAA ($p \leq 0.05$, left-sided pooled t-Test).

During the entire exposure period (0DAA to 7DAA) the mean flight activity was approximately on the same level in C and T with 13.3 ± 1.4 forager bees/m² in the control group C and 12.5 ± 1.4 forager bees/m² in the test item treatment T. In the reference item group (R), mean flight activity was statistically significantly lower compared to C (1.9 ± 0.5 forager bees/m²; $p \leq 0.05$, left-sided pooled t-Test).

Overall, a reduction in flight activity was observed in the test item group T on the day of application (0DAA) and the following day (1DAA). Afterwards, honeybee flight activity was similar in C and T.



DBA/DAA days before / after application

STD standard deviation

* statistically lower flight activity than in the control group C ($p \leq 0.05$, two-tailed pooled t-Test before application, left-sided pooled t-Test after application)

Figure 3 Mean Flight Activity in the Treatment Groups C, T, R

F. Behaviour of the Honeybees

During the assessments of mortality and flight activity, the behaviour of the honeybees in the crop and around the hive was observed and any unusual behaviour was recorded.

During the pre-exposure period (3DBA to 0DBA), no unusual behaviour was observed in any of the treatment groups.

In the control group C and in the test item treatment group T a few bees showing unusual behaviour were recorded throughout the exposure (0DAA to 7DAA) and post (8DAA to 28DAA) exposure period. In the

reference item treatment group R an increased number of bees showing unusual behaviour were recorded directly after the application (0DAA).

Overall, no biologically relevant test item effect on behaviour was observed during the assessments. Very low levels of unusual behaviours were displayed in the treatment group T and the control group C.

G. Condition of the Colonies

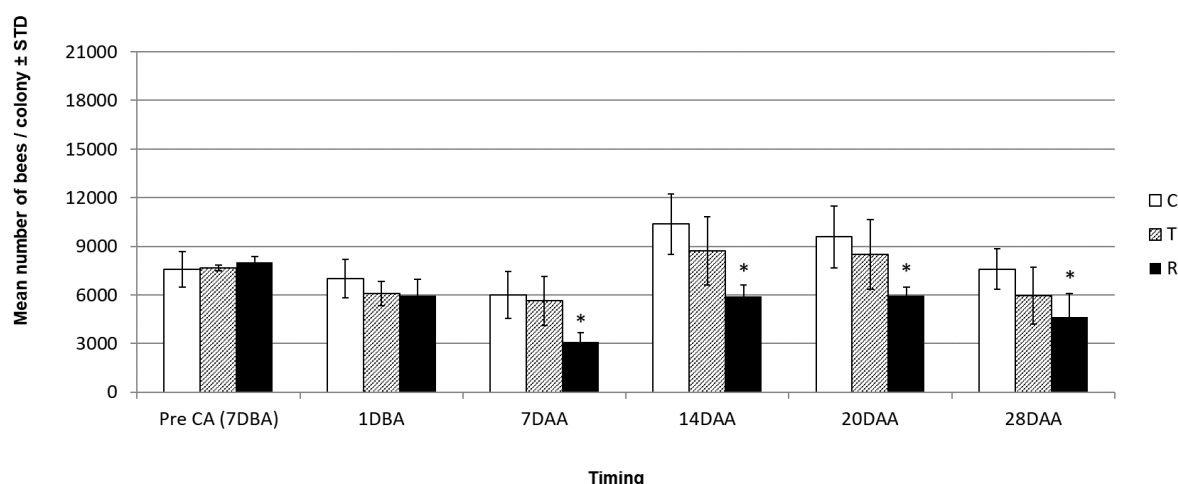
Colony Strength (Number of Bees Per Colony):

At the first colony assessment (7DBA), the mean ($\pm$ STD) number of bees was similar between the control group C, the test item treatment group T, and the reference item treatment group R, with 7573 ± 1086 bees in C, 7686 ± 179 bees in T, and 8011 ± 354 bees in R.

During the tunnel period, the mean ($\pm$ STD) number of bees first decreased in all treatment groups, reaching minima of 5996 ± 1460 in the control group C, 5639 ± 1523 in the test item group T and 3088 ± 604 in the reference item group R (statistically significant, $p \leq 0.05$, left-sided pooled t-Test) on 7DAA.

After relocation of the bee hives at the monitoring site in the evening 7DAA to 8DAA, the mean number of bees increased in all treatment groups, reaching maxima of 10368 ± 1857 in the control group C, 8726 ± 2109 in the test item group T and 5964 ± 529 in the reference item group R (statistically significant, $p \leq 0.05$, left-sided pooled t-Test) on 14DAA. After these maxima of colony strength, the total number of bees declined in all treatment groups until the last assessment 28DAA. At the last colony assessment (28DAA) the mean number of bees was 7589 ± 1253 in the control group C, 5964 ± 1745 in the test item group T, and 4648 ± 1456 in the reference item group R (statistically significant, $p \leq 0.05$, left-sided pooled t-Test). Statistically significant differences between the reference item treatment group and the control group were observed over the entire monitoring period.

Overall, there was no test item effect on colony strength.



DBA/DAA = Days before/after the application during flowering and honeybee-flight,
* Statistically significant difference to the control (t-Test (pooled), left-sided, $p \leq 0.05$)

Figure 4 Colony Size (Mean Number of Honeybees per Hive)

Number of Cells Containing Brood:

At the first colony assessment (7DBA), the mean number of brood cells were similar between treatment groups with 20600 ± 3767 brood cells in the control group C, 19550 ± 2840 brood cells in test item treatment group T, and 20800 ± 4880 brood cells in the reference item treatment group R.

Afterwards, the mean number of brood cells decreased until 7DAA in all treatment groups, reaching minima of 13100 ± 6698 in the control group C, 12200 ± 4208 in the test item group T and 10703 ± 5598 in the reference item group R on 7DAA.

Over the course of the monitoring period, the mean number of brood cells increased in all treatment groups with similar levels observed in C and T. At the last colony assessment (28DAA) the mean number of brood

cells was 29800 ± 3453 in the control group C, 26950 ± 2521 in the test item group T and 15469 ± 8457 in the reference item group R (statistically significant, $p \leq 0.05$, left-sided pooled t-Test).

All brood stages were present during the entire observation period in the control group C and the test item group T, except for replicate Cb which had no larvae on 7DAA. In the reference item group R, the replicate Rc had no eggs on 14DAA, no larvae on 28DAA, and a lack of pupae on 7DAA, 14DAA, 20DAA, and on 28DAA.

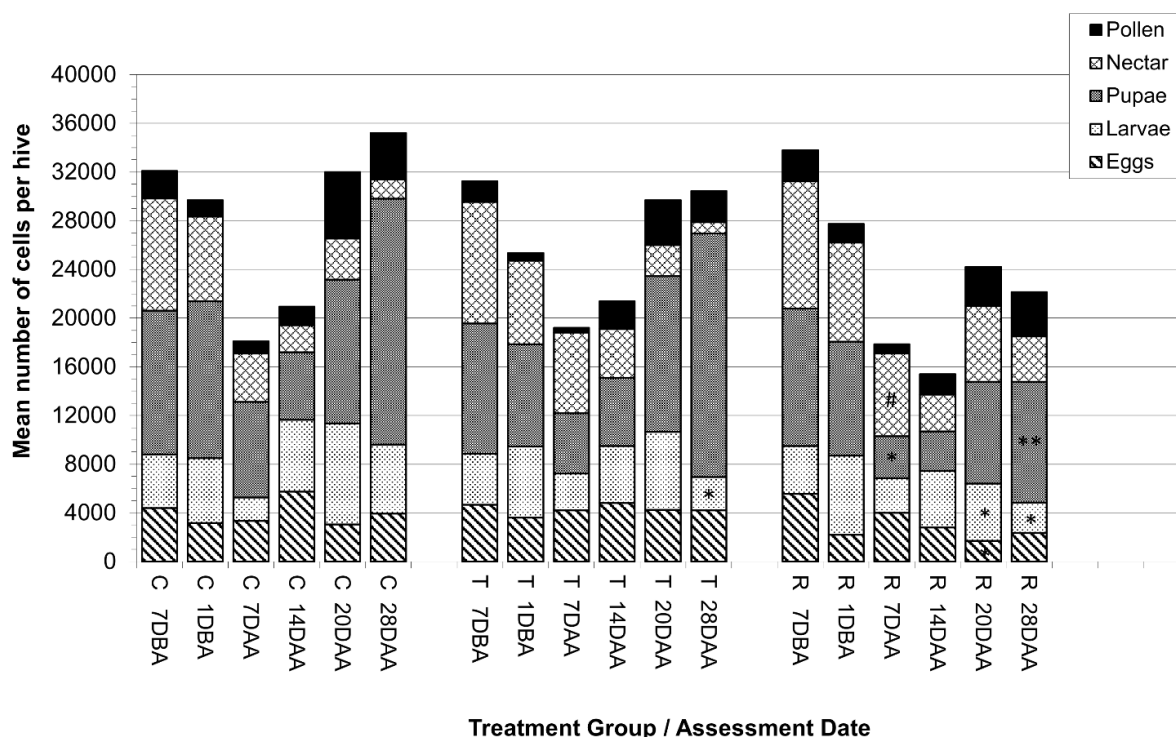
On 28DAA the mean number of larvae assessed in the test item treatment group T was statistically significant lower from that observed in the control group C ($p \leq 0.05$, left-sided pooled t-Test). It is most probably due to the low food storages observed at the end of the study. In response to this stress factor, it is common for honeybees to feed on their larval brood to help the colony survive. The mean number of eggs and capped cells was not statistically significant different between C and T.

On 7DAA and 28DAA, the number of pupae assessed in the reference item group R was statistically significant lower from that observed in the control group C ($p \leq 0.05$, left-sided pooled t-Test, and left-sided Satterthwaite t-Test).

On 20DAA the number of eggs assessed in the reference item group R were statistically significant lower from that observed in the control group C ($p \leq 0.05$, left-sided pooled t-Test).

On 20DAA and 28DAA, the number of larvae assessed in the reference item group R were statistically significant lower from that observed in the control group C ($p \leq 0.05$, left-sided pooled t-Test).

Overall, there was no test item effect on the number of cells containing brood.



DBA/DAA days before / after application

* statistically lower than in the control group C ($p \leq 0.05$, left-sided pooled t-Test)

** statistically lower than in the control group C ($p \leq 0.05$, left-sided Satterthwaite t-Test)

statistically higher than in the control group C ($p \leq 0.05$, right-sided pooled t-Test)

Figure 5 Mean Number of Brood and Food Cells per Hive

Number of Cells Containing Food:

At the first colony assessment (7DBA), the mean ($\pm$ STD) number of cells containing food (nectar and pollen) was similar between the treatment groups, with 11500 ± 1970 food cells in the control group C, 11700 ± 2369 food cells in test item treatment group T, and 13000 ± 5870 food cells in the reference item treatment group R.

The mean number of food cells decreased until 1DBA reaching 8300 ± 2745 in the control, 7500 ± 3653 in the test item treatment group T and 9700 ± 4649 in the reference item group R.

At the assessment 14DAA, the mean number of food cells was dropped to 3750 ± 4196 in the control group C, 6300 ± 663 in the test item group T and 4450 ± 2144 in the reference item group R.

On 20DAA an increase of food storage areas was observed in the control group C and the reference item group R, with 8850 ± 4652 food cells in C, and 9450 ± 2821 in the R. In the test item group T, a slight decrease to 6250 ± 2217 food cells was observed; however, this difference was not statistically significant when compared to the control group (C).

At the end of the monitoring period (28DAA), the mean number of food cells decreased again in all treatment groups, with 5400 ± 3437 in the control group C, 3500 ± 1579 in the test item group T, and 7400 ± 2597 in the reference item group R. This observation is most likely due to a lack of sufficient flowering crop in the surrounding environment. There were no statistically significant effects on the number of food cells on any of the assessment dates.

Both nectar and pollen were available to the colonies throughout the entire observation period, with following exceptions: replicates Ta and Tc had no pollen on 1DBA and 7DAA. A lack of pollen was also observed in replicate Ca and Rb on 7DAA, and in replicate Cb on 14DAA. A lack of nectar was observed in replicate Ca on 28DAA.

The mean number of food cells was not statistically significant different between the treatment groups.

Overall, there was no test item effect on food collection and/or storage.

Conclusion

The present study was performed to determine the potential effects of Azoxystrobin 160 g/L + Prothioconazole 200 g/L SC on the honeybee (*Apis mellifera* L.) after one foliar application at a rate of 160.02 g a.i./ha Azoxystrobin and 204.07 g a.i./ha Prothioconazole (13.10 g product/ha or 1 L product/ha) during bee flight under semi-field conditions in *Phacelia tanacetifolia* in Germany 2024 following the OEPP/EPPO Guideline No. 170(4) (2010) with partial integration of the EFSA Guidance Document No. 11(7): 3295 (2013) and the EU Guideline 7029/VI/95 rev. 4 (1997). Potential effects of the test item were evaluated against those observed in honeybees treated with tap water (control) and a toxic reference item (Dimethoate, 400 g a.i. dimethoate/ha).

There was no effect of the test item on adult worker, or worker larvae and pupae mortality, neither during the exposure period in the tunnel tents nor during the monitoring period.

Overall, a reduction in flight activity was observed in the test item group T on the day of application (0DAA) and the following day (1DAA). Afterwards, honeybee flight activity was similar in C and T.

No biological relevant test item effect was detected on honeybee behaviour.

There was no test item related effect neither on colony strength nor on the development of the brood for all stages and food storage area.

No residues of azoxystrobin, prothioconazole and prothioconazole-desthio were detected in any of the samples taken prior to the application and all samples taken from the control replicates were uncontaminated.

The highest residue levels were detected shortly after the application of the test item (S3/0DAA). Residues detected in pollen loads were 66.4 mg/kg for azoxystrobin, 80.6 mg/kg for prothioconazole, and 2.63 mg/kg for prothioconazole-desthio. At the next sampling day (6DAA), residue levels were 1.18 mg/kg for azoxystrobin, 0.268 mg/kg for prothioconazole, and 0.320 mg/kg for prothioconazole-desthio.

Residues detected in nectar from forager bees were much lower compared to residues detected in pollen loads. On 0DAA, residues detected in nectar were 0.800 mg/kg for azoxystrobin, 0.376 mg/kg for prothioconazole, and 0.197 mg/kg for prothioconazole-desthio. At the next sampling day (6DAA) residues of azoxystrobin were < LOQ (0.00309 mg/kg) and no residues of prothioconazole and prothioconazole-desthio were longer detected.

Overall, a foliar application of Azoxystrobin 160 g/L + Prothioconazole 200 g/L SC during active foraging of honeybees under semi-field conditions did not result in any significant adverse effects.

A 2.3.1.5.2 Study 8

Comment from zRMS:	The study was to determine the potential side effects of Azoxystrobin 160 + Prothioconazole 200 SC on the honeybee (<i>Apis mellifera</i> L.) in two test item application scenarios, T1 (outside of active bee foraging) and T2 (during active bee foraging), under semi-field conditions in full flowering <i>Phacelia tanacetifolia</i> at BBCH 65-66 according to CEB 230 in France. The target application rate of the test item Azoxystrobin 160 + Prothioconazole 200 SC was 1 L product/ha. The following requirements according to the CEB 230 guideline were met:
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	• Pre-application mortality was stabilised after installation of the colonies in the tunnels (e.g. < 300 dead bees per tunnel, depending on the colony size and total area of the sheets per tunnel) and was similar between tunnels (deviation of each tunnel from the average of all tunnels: < 60%) on the day before the application (1DBA2). • Post-application control mortality (C) did not exceed the corresponding value on 1DBA2 by >50%. • Post-application mortality in the toxic reference item R (dimethoate) was ≥ 5 times the corresponding value on 1DBA2. • Flight activity was comparable between tunnels and was > 5 forager bees/m² (equivalent to a mean value > 166 bees over all 4 flight observation areas per tunnel, based on the size of 33.3 m² per flight observation area) on 0DBA2 (shortly before the application A2 in C, T2 and R). The study included four treatment groups with one replicate (tunnel) each: one tap-water treated control group (C), one test item group treated in the evening after bee flight (T1), one test item group treated during bee flight (T2) and one reference item group (R). The effects on honeybee colonies exposed to the treated crop under confined conditions, considering mortality, flight intensity and behaviour were evaluated. Conclusion: The mortality in T1 and T2 were comparable to the C, although mortality in T2 was slightly higher as shown by the toxicity index (1.04 for T1 and 1.25 for T2). No effects on pupae and larvae were observed. The forager bee mortality index (proportion of mortality after treatment, corrected by mortality before treatment / number of bees present on the crop before treatment) was slightly reduced in T1 and T2 in comparison with C (0.84 for T1 and 0.86 for T2). The T1 treatment did not show any effect on the flight activity. The T2 treatment triggered a slight reduction of the flight activity as observed on the application day and during the following 3 days. No abnormal behaviour related to the test item treatments was observed during the study period. Colonies remained healthy during the study period and contained normal levels of bees, brood and food.
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Reference: KCP 10.3.1.5/02

Report A Semi-Field Study to Evaluate Side Effects of Azoxystrobin 160 + Prothioconazole 200 SC on the Honey Bee (*Apis mellifera* L.) on *Phacelia tanacetifolia* according to CEB 230 in France in 2024, Roig, J. (2024), Study number S24-102576, UPL Study number UPL/2024/1020

Guideline(s): CEB No. 230 (4ème éd.), 2013: Commission des Essais Biologiques - Méthode d'évaluation des effets des préparations phytopharmaceutiques sur l'abeille domestique (*Apis mellifera* L.) (part 4: Méthode d'évaluation sous tunnel des préparations phytopharmaceutiques employées en pulvérisation)

Deviations:

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study) No

Deviation: 1. Assessment of dead pupae+larvae was overlooked from 2DAA2 to 4DAA2. No impact to study expected. Based on the very low number of pupae+larvae found in all other assessments (usually zero) it is unlikely that they were present during the assessments concerned.
2. Colonies were placed in the tunnels at BBCH 63-65 instead of 61-63. Application 2 took place at BBCH 65-66 instead of 63-65 due to rapid development of the crop. No impact to study expected. *Phacelia* was at full flowering at the time of application and >5 bees/m² were observed at the application

3. Behavior of the honeybees was assessed during bee flight only, not during mortality as indicated in the Study Plan. No impact to study expected. Behavior was assessed during the bee flight, when the bees were active. Furthermore, the guideline does not specify that the behavior needs to be assessed during mortality assessments and flight assessments.

GLP: Yes

Executive Summary:

The objective of the study was to determine the potential side effects of Azoxystrobin 160 + Prothioconazole 200 SC on the honeybee (*Apis mellifera* L.) in two test item application scenarios, T1 (outside of active bee foraging) and T2 (during active bee foraging), under semi-field conditions in full flowering *Phacelia tanacetifolia* at BBCH 65-66 according to CEB 230 in France. The target application rate of the test item Azoxystrobin 160 + Prothioconazole 200 SC was 1 L product/ha.

The study included four treatment groups with one replicate (tunnel) each: one tap-water treated control group (C), one test item group treated in the evening after bee flight (T1), one test item group treated during bee flight (T2) and one reference item group (R).

The effects on honeybee colonies exposed to the treated crop under confined conditions, considering mortality, flight intensity and behaviour were evaluated.

Overall, mortality in T1 and T2 were comparable to the C, although mortality in T2 was slightly higher as shown by the toxicity index (1.04 for T1 and 1.25 for T2). No effects on pupae and larvae were observed. The forager bee mortality index (proportion of mortality after treatment, corrected by mortality before treatment / number of bees present on the crop before treatment) was slightly reduced in T1 and T2 in comparison with C (0.84 for T1 and 0.86 for T2). The T1 treatment did not show any effect on the flight activity. The T2 treatment triggered a slight reduction of the flight activity as observed on the application day and during the following 3 days. No abnormal behaviour related to the test item treatments was observed during the study period. Colonies remained healthy during the study period and contained normal levels of bees, brood and food.

MATERIALS AND METHODS

A. Materials:

- Test item:** Azoxystrobin 160 + Prothioconazole 200 g/L SC

Synonym FGF11

/Sponsor IN-code

Formulation type SC

Lot/Batch #: ARD/AP/360/SC/40

Concentration of a.i., analysed: Active 1: Azoxystrobin 160 g/L (nominal); 161.9 g/L (150.4 – 169.6 g/L), 25.72 % (w/v) (actual)
Active 2: Prothioconazole 200 g/L (nominal); 204.6 g/L (188.0 – 212.0 g/L), 20.46 % (w/v) (actual)

Description: Off-white colour suspension

CAS#: Active 1: 131860-33-8; Active 2: 178928-70-6

Expiry Date: 14 March 2026

Reference Item R1

Name: Danadim EC

Batch: 2906548

Active substance(s): Dimethoate

CAS Registry Number(s): 60-51-5

Concentration of a.i., nominal: 400 g/L (nominal)

Expiry Date: 17 March 2025

2. Test Vehicle: Tap water
3. Test Organism
Species: *Apis mellifera* L.
Age at dosing: Mixed age. hives used were single wooden box colonies with 10 combs (Langstroth type) and on average 11310, 11635, 10010 and 11895 bees per C, T1, T2 and R treatments, respectively, at the start of the test (assessed before introducing the colonies in the tunnels). The colonies were as homogeneous as possible. Healthy and queen-right bee colonies were used. Honeybee queens had the same age (around 1-year-old) at the start of the study. The queens were daughters (free mated) of carnica queens.
Source: Test colonies were obtained from GAEC de Claron, 40090 Bougue, France.
Tunnels (exposure): The study was conducted in a field belonging to the village of Poudenas, Lot-et-Garonne department, France.
The area covered by each tunnel was 200 m². The approximate dimensions of each tunnel were 40 m length, 5 m width and 3 m height in the centre. The area covered by crop was about 133 m² and the area covered by collection sheets was about 67 m². Minimum distance between treated plots and control was 4 m.
4. Environmental conditions during the exposure phase in the tunnels:
Temperature (min/max during exposure): 19.5°C during application 1 (T1) and 22.0 to 22.1°C during application 2 (C, T2, R). Recorded temperatures at the field site were between 9.1°C (minimum record) and 35.4°C (maximum record).
Wind speed: 0.0 m/s during the first application (T1 treatment) and 0.0-0.5 m/s during the second application (C, T2 and R treatments)
Relative Humidity (min/max during exposure): Recorded relative humidity at the field site was between 40% (minimum record) to 100% (maximum record).
Precipitation (min/max during exposure): There was no rainfall within 3 days after the first test item application and within 2 days after the second test item application. First rainfall recorded after the applications was 2DAA2, with 5.8 mm. From 4DAA2 onwards there was a rainy period with 19.6 mm of rain recorded 4DAA2, and 3.2 and 3.8 mm recorded on 5DAA2 and 6DAA2 respectively. During the study period there was a total of 65.6 mm of rain recorded by the on-site rain gauge. There was more rain than usual during this period since historical data show an average of 46.8 mm of rain.
Clouding (exposure): Recorded cloud cover at the field site was between 10% (minimum record) to 100% (maximum record).

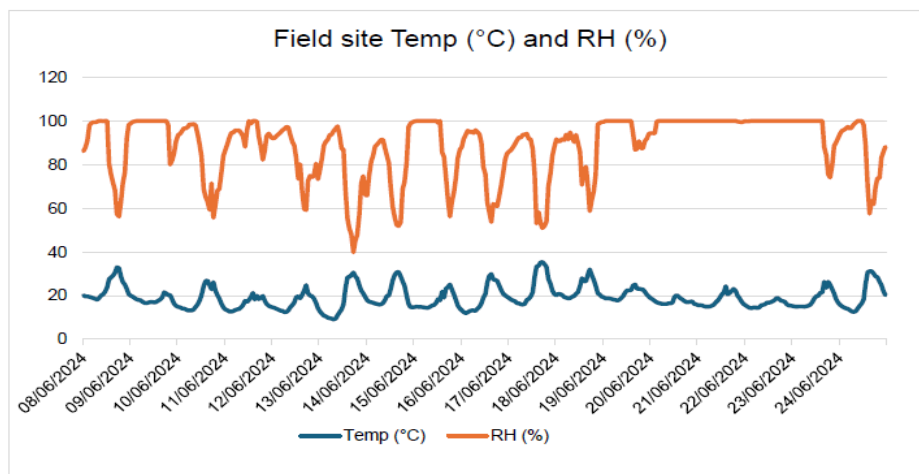


Figure 9: Temperature and relative humidity trend during the study period

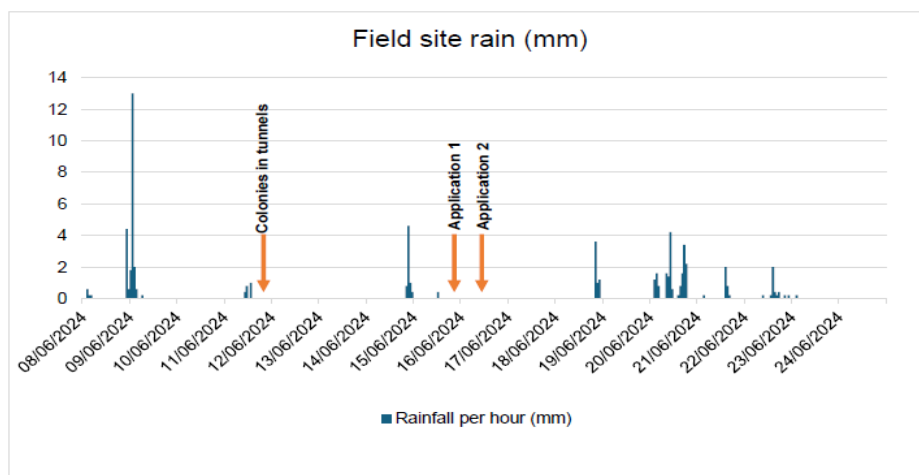


Figure 10: Rainfall during the study period

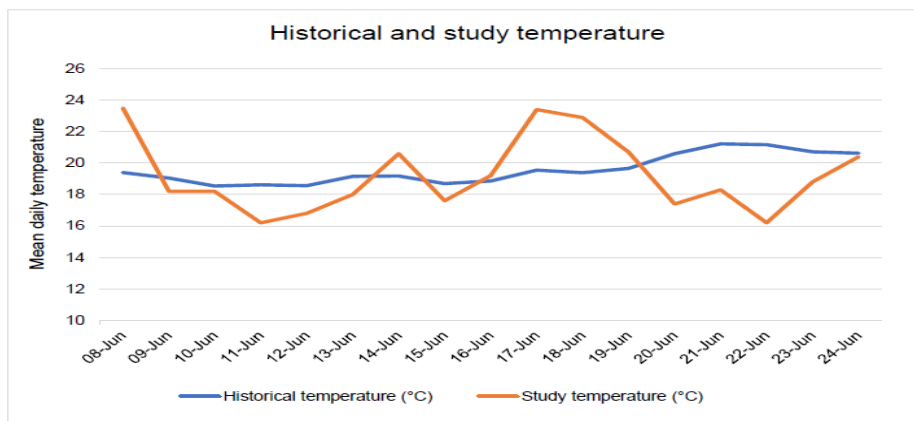


Figure 11: Historical and study mean daily temperature

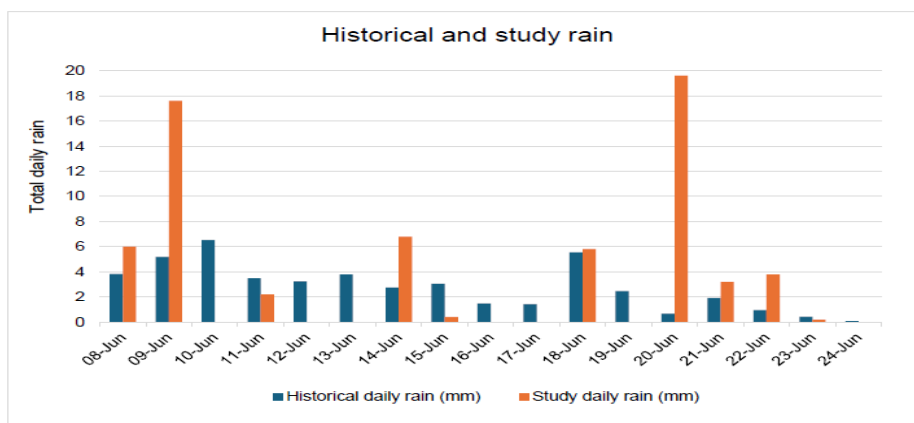


Figure 12: Historical and study total daily rain

B. Trial design and methods:

1. Experimental Dates (field phase):
08 June to 24 June 2024
Application Date:
15 June 2024 (T1) and 16 June 2024 (T2)
Exposure of the bees in the tunnels:
7 days
Exposure Units:
Phacelia tanacetifolia (full flowering at BBCH 65–66)
2. Experimental treatments
Application 1: T1, applied at full flowering of phacelia in the evening after bee flight.
Application 2: T2, R and C applied on the following day during bee flight
Target application rate of the test item Azoxystrobin 160 + Prothioconazole 200 SC was 1 L product/ha (actual rate applied 0.99 and 0.99 L product/ha at application 1 and 2, respectively). Tap water was applied in the control group and dimethoate was applied at a target rate of 1 L product/ha, i.e. 400 g active ingredient (a.i.)/ha (actual rate 0.97 L product/ha) in the reference item group. The target spray volume was 300 L/ha in all treatment groups.
3. Observations
 - Total and number of daily dead bees (adult worker bees, pupae and larvae) on the collection sheets spread out in the tunnels and total before as well as after the start of exposure in C, T1, T2 and R.
 - Flight intensity before exposure and daily during exposure (number of forager bees/m² phacelia) in C, T1, T2 and R.

- Behaviour of the bees (daily) in the crop and around the hive.
- Condition of the colonies (colony strength and area of the different brood stages and food storage per colony at each assessment date).

The summary of assessment schedule has been summarized below (Table copied from the original study):

Table 8: Assessment schedule of mortality

Time of the test	DBA/DAA	Evaluations of number of dead honey bees
Pre-exposure period (inside tunnels)	3DBA2 to 1DBA2	Once a day at the same time of day in the morning up to noon
Day of application A1 in the evening (T1)	1DBA2 (evening)	T1 was sprayed, but mortality assessment was carried out in all tunnels before the application.
Day of application A2 during bee-flight (C, T1, T2 and R)	0DBA2	Once shortly before start of application in the respective tunnel*
	0DAA2	• 2 h after application* • 4 h after application* • 6 h after application* • In the evening after daily flight activity of the bees
Exposure period (inside tunnels)	1DAA2 up to 7DAA2	Once a day if possible, at the same time in the morning up to noon

DBA2/DAA2 = Days before/after the application A2 during flowering and bee-flight

* As T1 was applied in the evening before the application 2, the assessment schedule in T1 was adapted to the timing of the application of the control.

Table 9: Assessment schedule of flight intensity and behaviour

Time of the test	DBA/DAA	Evaluations of number of forager honeybees
Pre-exposure period (inside tunnels)	4DBA2 to 1DBA2	Once a day during flight activity of the honeybees
Day of application A1 in the evening (T1)	1DBA2 (evening)	Assessment in T1 to demonstrate that flight activity had ceased, and no bees are foraging before application
Day of application A2 during bee-flight (C, T1, T2 and R)	0DBA2	Once shortly before start of application in the respective tunnels*
	0DAA2	• 15 min after application* • 1 h after application* • 3 h after application* • 5 h after application*
Exposure period	1DAA2 to 7DAA2	Once a day during flight activity of the honeybees

DBA2/DAA2 = Days before/after the application A2 during flowering and bee-flight

*As T1 was applied in the evening before the application 2, the assessment schedule in T1 was adapted to the timing of the application of the control. No assessment of T1 was done before the application 2 since this assessment was intended to guarantee exposure of the C, T2 and R treatments.

Table 10: Assessment schedule of the condition of the colonies

Timing	Evaluation of condition of colonies
8DBA2	1 st colony assessment (outside the tunnels)
2DBA2	2 nd colony assessment (inside the tunnels)
8DAA2	3 rd colony assessment (inside the tunnels)

DBA: Days before application; DAA: Days after application.

RESULTS AND DISCUSSION

Mortality of Adult Worker Bees

Throughout the period before exposure, mortality of adult bees across all future treatments was similar between C, T1 and T2. Lower mortality was observed in R for this period. One day before the second application (1DBA2, DBA = days before application) the daily mortality was stabilized in all treatments (range 40 to 127 dead bees per tunnel and per day) and the mortality between tunnels was considered similar, i.e. the deviation of each tunnel from the average of all tunnels was between -55% and 42%. The number of dead bees/tunnels on 0DBA2 was 74 in C, 58 in T1, 95 in T2 and 30 in R.

On the day the exposure started, on 0DAA2 (DAA = days after application), mortality of adult bees in C was slightly lower than T1 and comparable to T2. The number of dead bees/tunnel assessed were 80 in C, 114 in T1 and 90 in T2. A strong effect of the R treatment was observed on the day after the second application with 416 dead bees.

Over the exposure period, from 0DAA2 to 7DAA2, the daily adult bee mortality of the C, T1 and T2 treatments followed a similar trend. Mortality in R was clearly higher. Overall, during this period, the average daily mortality ($\pm$ STD) in C was 51 ± 33 , in T1 62 ± 33 , in T2 76 ± 35 and in R 148 ± 148 .

The toxicity index (proportion of change in mortality after/before treatment in each treatment tunnel compared to the same proportion in the control tunnel) was calculated as 1.04 for T1, 1.25 for T2 and 6.85 for R.

The forager bee mortality index (proportion of mortality after treatment, corrected by mortality before treatment / number of bees present on the crop before treatment) was calculated as 0.84 for T1, 0.86 for T2 and -1.43 for R.

Table 1: Summary of the mortality assessments

Treatment group		Control (C)	Test item (T1)	Test item (T2)	Reference item (R)
Mean of daily mortality per period (dead worker bees/colony) $\pm$ STD	3DBA2 to 0DBA2	214 $\pm$ 210	172 $\pm$ 117	228 $\pm$ 195	50 $\pm$ 18
	0DBA2	74	58	95	30
	0DAA2	80	114	90	416
	0DAA2 to 7DAA2	51 $\pm$ 33	62 $\pm$ 33	76 $\pm$ 35	148 $\pm$ 148
Mean of daily mortality per period (dead larvae+ pupae/colony) $\pm$ STD1)	3DBA2 to 0DBA2	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
	0DBA2	0.0	0.0	0.0	0.0
	0DAA2	0.0	0.0	0.0	0.0
	0DAA2 to 7DAA2	0.2 $\pm$ 0.4	0.6 $\pm$ 1.3	0.2 $\pm$ 0.4	0.0 $\pm$ 0.0
itox		N.A.	1.04	1.25	6.85
ifbm		N.A.	0.84	0.86	-1.43

DAA: days after application; DBA: days before application; STD: standard deviation of the daily mean mortality of each period when applicable; itox: toxicity index; ifbm: forager bees mortality index; N.A.: not applicable.

¹⁾ The assessment of dead pupae and larvae was not done 2DAA2 to 4DAA2 by accident

Overall, mortality in T1 and T2 were comparable to the C, although mortality in T2 was slightly higher as shown by the toxicity index.

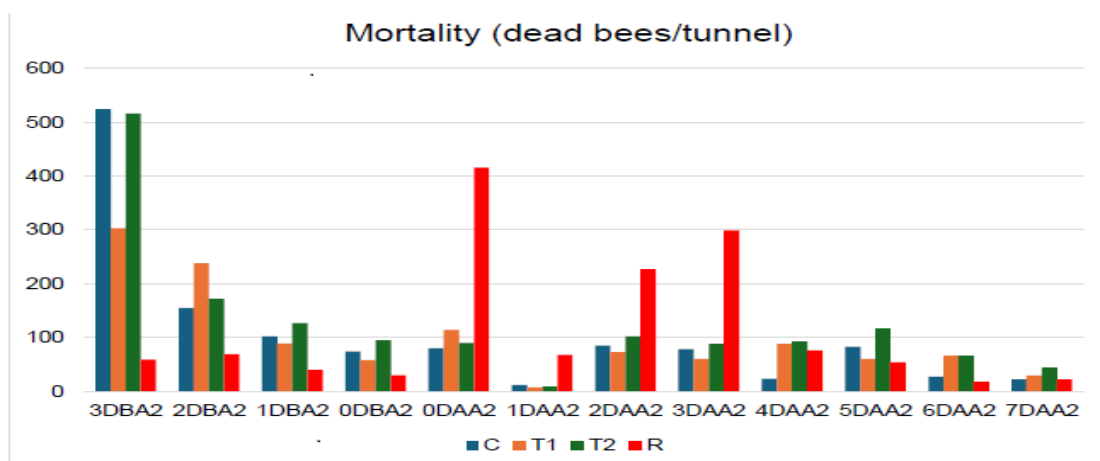
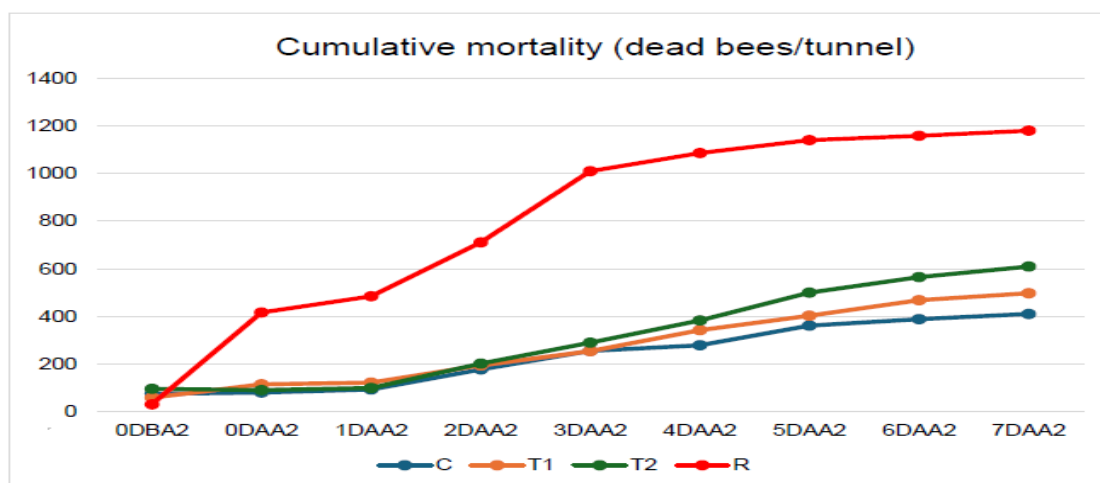


Figure 4: Total number of dead adult bees per tunnel during confinement (3DBA2 to 7DAA2)
DBA: days before application; DAA: days after application.



Mortality of Pupae

No dead pupae and larvae were observed during the pre-application period (3DBA2 to 0DBA2) in any of the future treatments nor on the day the exposure started (0DAA2). During the exposure period (0DAA2 to 7DAA2) one dead pupa was found in the C treatment, 3 in T1 and 1 in T2. These isolated observations are considered meaningless as they are not related to treatment effects.

Overall, no effects on pupae and larvae were observed.

Flight Intensity

Throughout the period before exposure, flight intensity of bees was similar across all future treatments. From 4DBA2 to 2DBA2 an increase of the flight intensity was observed in all tunnels (mean of 4 bees/m² on 4DBA2 to mean of 6.6 bees/m² on 2DBA2), showing that the bees were acclimatizing to the confined conditions. 1DBA2 the foraging activity was low due to cold and windy weather.

On 0DBA2, before the application of the C, T2 and R treatments, the flight intensity was 6.3, 6.9 and 7.5 bees/m², respectively, which ensured exposure of the bees to the treatments. T1 (applied in the evening before exposure) showed an initial flight intensity of 8.44 bees/m² at the first assessment of the exposure day. On 0DAA2, after the application, during the first exposure day, the flight intensity was comparable between the C and the T1 treatment (applied in the evening before the exposure, after bee flight) with 5.9±1.7 and 6.6±1.5 bees/m², respectively. The T2 treatment showed a slightly lower flight intensity on this day with 3.7±1.1 bees/m². The reduction in flight intensity for the T2 treatment in comparison with the flight intensity assessed before the application was 47%. The foraging activity in the R treatment had a

strong effect on the flight intensity of the bees, with a mean of 0.7 ± 0.8 bees/m² during this first exposure day, corresponding to 90% reduction in comparison with the assessment done in this tunnel before the application.

Over the exposure period, from 0DAA2 to 7DAA2, the daily bee flight intensity in C and T1 followed a very similar trend. The flight activity in the T2 treatment was slightly lower than in the C at all post-application assessments. Flight activity was intense up to 3DAA2 but due to rainy weather, the assessed number of bees decreased from this moment until the end of the study period in all tunnels. The mean flight activity for the entire post-application period was 2.5 ± 2.4 , 2.8 ± 2.7 , 1.7 ± 1.7 bees/m² for C, T1 and T2, respectively. The R treatment caused a strong reduction in flight activity with a mean of 0.1 ± 0.3 bees/m².

Thus, the T1 treatment did not show any apparent effect on the flight activity. The T2 treatment triggered a slight reduction of the flight activity as observed on the application day and during the following 3 days.

Table 2: Summary of the flight intensity assessments

Treatment group		Control (C)	Test item (T1)	Test item (T2)	Reference item (R)
Daily mean flight intensity (bees/m ²) $\pm$ STD	4DBA2 to 0DBA2	4.5 ± 2.2	4.8 ± 3.3	4.7 ± 3.1	5.0 ± 3.0
	0DBA2	6.3	NR	6.9	7.5
	0DAA2	5.9 ± 1.7	6.6 ± 1.5	3.7 ± 1.1	0.7 ± 0.8
	0DAA2 to 7DAA2	2.5 ± 2.4	2.8 ± 2.7	1.7 ± 1.7	0.1 ± 0.3

DAA: days after application; DBA: days before application; STD: standard deviation of the daily mean flight intensity of each period when applicable; NR, not recorded: T1 was applied in the evening before the start of the exposure.

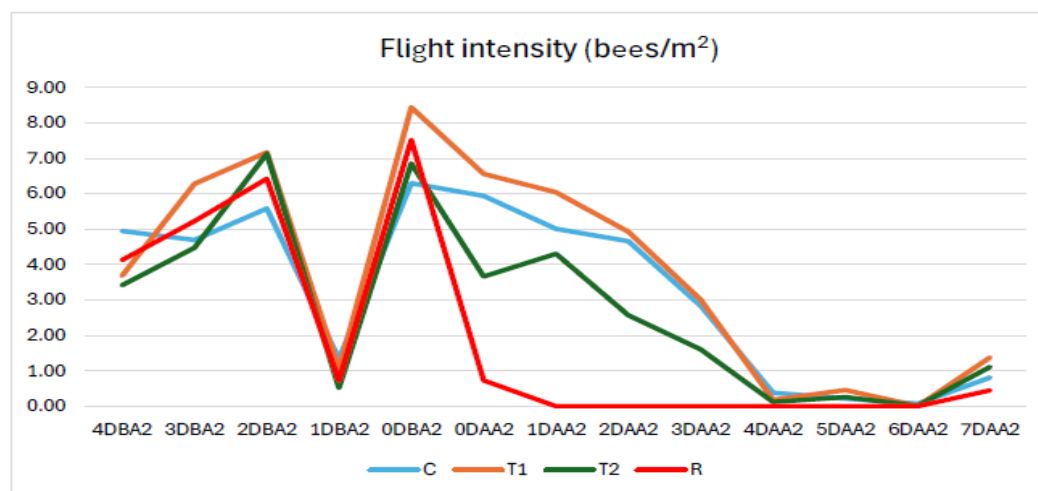


Figure 6: Total number of forager bees per tunnels during confinement (4DBA2 to 7DAA2).

DBA: days before application; DAA: days after application.

Behaviour of the Honey Bees

During the assessments of flight intensity, the behaviour of the honeybees in the crop and around the hive was assessed. No abnormal behaviour was observed in the C, T1 and T2 tunnels at any of the assessments. Trembling bees on the crop were observed one hour after the application 2 in the R tunnel, only. Overall, no abnormal behaviour related to the test item treatments was observed during the study period.

Condition of Colony

To ensure that the colonies used for the study would meet the requirements described in the guideline and to assess the health and normal development during the study period, the comb area containing bees, nectar, pollen, eggs, larvae and capped cells were estimated. Before the confinement in the tunnels (8DBA2) the colony strength was comparable between the future treatments. The mean number of bees assessed were

11310 bees/colony in the C group, 11635 bees/colony in the T1, 10010 bees/colony in the T2 group and 11895 bees/colony in the R group. Before the start of the exposure phase (2DBA2), similar number of bees were assessed with 11245, 13260, 10010 and 11570 bees/colony in the C, T1, T2 and R tunnels, respectively. On 8DAA2, the colony strength remained at similar levels, except for the R treatment, where a strong reduction of the number of bees was observed (10725, 10530, 10270, 6240 bees/colony in C, T1, T2 and R, respectively).

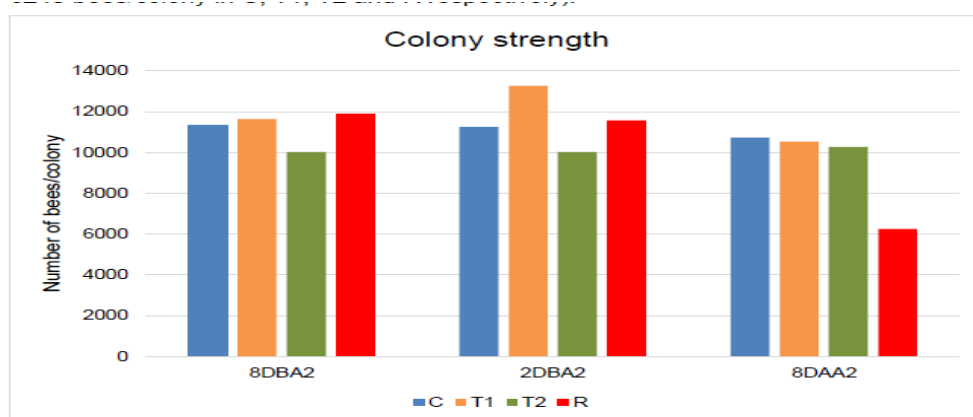


Figure 7: Number of bees per colony covered with brood (sum of cells of all brood stages) at the different assessment days

Development of the Brood Area

The amount of brood in the colonies (sum of cells containing eggs, larvae, and pupae) and the food stores in the colonies (sum of cells containing nectar and pollen) were assessed. At the start of the study all colonies were comparable and contained normal amounts of brood at all stages, nectar and pollen. There were the expected amounts of brood and food observed during the study period.

The colony development of all treatment groups showed fluctuations in a typical and normal range according to weather conditions and time of the year.

The amount of brood in the colonies (sum of cells containing eggs, larvae and pupae) assessed during the first colony assessment on 8DBA2 was 24600 cells/colony for C, 28400 cells/colony for T1, 24000 cells/colony for T2 and 28000 cells/colony for R. At the start of the test the different brood stages of all future treatment groups was comparable between the colonies (see Figure 8).

At the second colony assessment on 2DBA2 (during confinement), the abundance of brood increased slightly in all treatments. The number of cells containing brood were assessed as 34000 cells/colony for C, 32200 cells/colony for T1, 25400 cells/colony for T2 and 37200 cells/colony for R.

At the third colony assessment on 8DAA2, at the end of the exposure phase, the abundance of brood decreased in all tunnels, probably due to the influence of the confined conditions and unfavourable weather conditions. The number of cells were 7600 cells/colony for C, 8400 cells/colony for T1, 13600 cells/colony for T2 and 9400 cells/colony for R.

The number of brood stages in all treatments showed a similar trend from the first to the last assessment, with minor differences between the treatments. Overall, there was a clear reduction at the end when the colonies were confined and forced to feed on phacelia.

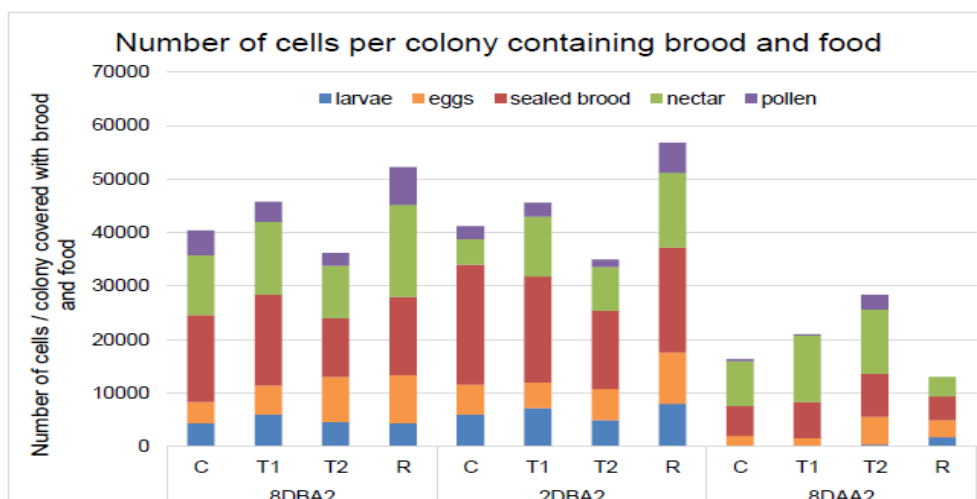


Figure 8: Number of cells per colony covered with brood and food
 DBA: days before application; DAA: days after application.

Validity criteria of the study

The application procedure resulted in precise application rates and a uniform distribution of the treatments over the tunnels. The beehives and the crop were in good condition at the start of the test, adequate for the purposes of this study as can be seen by the low background mortality. At the time of the second application the foraging intensity was high and therefore the exposure to the treatments was ensured. The reference item dimethoate produced high lasting effects on adult bee mortality and on the flight intensity during the exposure period.

The following requirements according to the CEB 230 guideline were met:

- Pre-application mortality was stabilised after installation of the colonies in the tunnels (e.g. < 300 dead bees per tunnel, depending on the colony size and total area of the sheets per tunnel) and was similar between tunnels (deviation of each tunnel from the average of all tunnels: < 60%) on the day before the application (1DBA2).
- Post-application control mortality (C) did not exceed the corresponding value on 1DBA2 by >50%.
- Post-application mortality in the toxic reference item R (dimethoate) was ≥ 5 times the corresponding value on 1DBA2.
- Flight activity was comparable between tunnels and was > 5 forager bees/m² (equivalent to a mean value > 166 bees over all 4 flight observation areas per tunnel, based on the size of 33.3 m² per flight observation area) on 0DBA2 (shortly before the application A2 in C, T2 and R).

Together these findings show that the test set-up was adequate to detect in a meaningful manner effects on the observed parameters in case these occurred.

Conclusion

Azoxystrobin 160 + Prothioconazole 200 SC was applied twice, one day before the start of the exposure after bee flight (T1) and with the bees foraging on the crop (T2) at a nominal rate of 1 L product/ha (actual rate applied 0.99 and 0.99 L product/ha for T1 and T2 respectively).

The effects on honeybee colonies exposed to the treated crop under confined conditions, considering mortality, flight intensity and behaviour were evaluated.

Overall, mortality in T1 and T2 were comparable to the C, although mortality in T2 was slightly higher as shown by the toxicity index (1.04 for T1 and 1.25 for T2). No effects on pupae and larvae were observed.

The forager bee mortality index (proportion of mortality after treatment, corrected by mortality before treatment / number of bees present on the crop before treatment) was slightly reduced in T1 and T2 in comparison with C (0.84 for T1 and 0.86 for T2).

The T1 treatment did not show any effect on the flight activity. The T2 treatment triggered a slight reduction of the flight activity as observed on the application day and during the following 3 days.

No abnormal behaviour related to the test item treatments was observed during the study period. Colonies remained healthy during the study period and contained normal levels of bees, brood and food.

A 2.3.1.6 KCP 10.3.1.6 Field tests with honeybees

A 2.3.2 KCP 10.3.2 Effects on arthropods other than bees

A 2.3.2.1 KCP 10.3.2.1 Standard laboratory testing for non-target arthropods

No additional data submitted.

A 2.3.2.2 KCP 10.3.2.2 Extended laboratory testing, aged residue studies with non-target arthropods

A 2.3.2.2.1 Study 9

Comment from zRMS:	The study was conducted in line with the respective guideline IOBC guideline Mead-Briggs et al. 2010 with no deviations. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: LR₅₀ > 1911 mL prod./ha ER₅₀ > 1911 mL prod./ha
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Reference:	KCP 10.3.2.2/01
Report	Azoxystrobin 160 g/L + Prothioconazole 200 g/L SC: Effects on the Parasitoid <i>Aphidius rhopalosiphi</i> (Hymenoptera, Braconidae), Extended Laboratory Study - Limit Test, Leopold, J. (2024), Study no. 179211002
Guideline(s):	IOBC guideline Mead-Briggs et al. 2010
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Executive summary

Effects of Azoxystrobin 160 + Prothioconazole 200 SC on survival and behaviour of the parasitoid wasp *Aphidius rhopalosiphi* were assessed in a limit test at 1911 mL product/ha under extended laboratory conditions. The parasitoids were exposed to dried residues on treated plant surfaces of barley plants (*Hordeum vulgare*). Survival was assessed after 2, 24 and 48 hours. A control and a reference substance were tested in parallel. At 48 hours, provided that the corrected mortality was ≤ 50.0 % female wasps were confined over untreated barley plants infested with cereal aphids for parasitisation. The females were removed after 24 h and the reproductive capacity was assessed after 10 d based on the numbers of aphid mummies that had developed.

The mortality of *A. rhopalosiphi* was not statistically significantly increased by a single application of 1911 mL of Azoxystrobin 160 + Prothioconazole 200 SC/ha. The corrected mortality was 6.7 % and below the trigger value of 50%. There was no statistically significant effect on reproduction at the test item treatment compared to the control. The reduction of reproduction compared to the control was 14.4% and below the trigger value of 50%. Therefore, the LR₅₀ and ER₅₀ were estimated to be > 1911 mL product/ha. The validity criteria of IOBC guideline Mead-Briggs *et al.* (2010) were fulfilled.

Materials and Methods

A. MATERIALS

1. Test material	Azoxystrobin 160 + Prothioconazole 200 SC
Description	Liquid, light brown, SC (suspension concentrate)
Lot/Batch #	ARD/AP/360/SC/36
Content of aa.ss.	Azoxystrobin: 162.20 ± 0.48 g/L (14.45 ± 0.04% w/w) Prothioconazole: 200.54 ± 0.65 g/L (17.86 ± 0.06% w/w)
	Density: 1.1228 ± 0.0012 g/mL
Stability of test material	Stable under storage conditions (20 ± 5 °C, in the dark) Expiry date: 23 May 2025
2. Vehicle and/or positive control	Vehicle control: Deionised water Positive control: Reference item
Reference item	Danadim Progress
Description	Colourless to light yellow liquid
Lot/Batch #	10214034
Content of a.s.	Dimethoate: 400 g/L nominal
Stability of reference item	Stable under storage conditions (room temperature, dark) Expiry date: Nov 2023
3. Test organism	
Species	Parasitoid (<i>Aphidius rhopalosiphi</i>),
Source	Commercial supplier, Katz Biotech AG, Germany
Age	≤ 48 hours
Acclimation period	1 - 2 days under test conditions
Diet	Fructose solution (10% (w/w) was offered during acclimatisation on a cotton wool pad, <i>ad libitum</i> . Exposure period: 1 hour and 5 minutes to 1 hours and 35 minutes before application, the seedlings were lightly sprayed with the fructose solution (10% (w/w) and were left to dry, <i>ad libitum</i> .
Test units	Exposure units: Treated pots (13 cm in diameter) with 8 - 10 barley seedlings (<i>Hordeum vulgare</i> 'Sunshine', BBCH Growth Stage 12) per pot. The plants were trimmed to a uniform height of 12 cm tall prior to the test and enclosed within a clear polyacrylic cylinder (20 cm x 10 cm), openings were covered with fine mesh gauze to prevent escape of test organisms. Ventilation was ensured via a tube. The soil surface was covered with a thin layer of quartz sand before treatment. Post-exposure units: Untreated pots (13 cm in diameter) with barley seedlings (<i>Hordeum vulgare</i> ; 12 - 24 seedlings, 10 days old) infested with 100 - 200 host aphids of all developmental stages (<i>Rhopalosiphum padi</i>) were enclosed within a clear polyacrylic cylinder (30 cm x 10 cm), openings were covered with fine mesh gauze

to prevent escape of test organisms. The soil surface was covered with a thin layer of quartz sand before treatment.

4. Environmental conditions

Temperature	Nominal: $20 \pm 2^{\circ}\text{C}$, actual: $18 - 21^{\circ}\text{C}$
Relative humidity	Nominal: 60 - 90%, actual: 74 - 79% (acclimatisation and exposure period), 78 - 79% (post-exposure period, within the test units)
Photoperiod	16/8 h light/dark photoperiod; light intensity: 830 - 900 lux (acclimatisation and exposure period), 1480 - 2030 lux (parasitisation period), 10240 - 14250 lux (post-parasitisation period)

B. STUDY DESIGN AND METHODS

1. In-life dates 17 Oct 2023 to 04 Dec 2023 (experimental phase)

2. Experimental conditions

Test design

Effects of Azoxystrobin 160 + Prothioconazole 200 SC on survival and reproduction of the parasitoid wasp *Aphidius rhopalosiphii* were assessed in a limit test under extended laboratory conditions. The parasitoids were exposed to dried residues on treated barley plant surfaces (*Hordeum vulgare*). Survival of the parasitoids was assessed after 2, 24 and 48 hours. A control and a reference substance were tested in parallel with 6 replicates each containing 5 female parasitoids. At 48 hours, provided that the corrected mortality was $\leq 50.0\%$ female wasps were confined over untreated barley plants infested with cereal aphids for parasitisation. The females were removed after 24 h and the reproductive capacity was assessed after 10 d based on the numbers of aphid mummies that had developed.

Number of animals per treatment

Exposure period: 5 test organism per treatment group; 6 replicates per group

Post-exposure period: 1 female per treatment group; 20 replicates per group

Test doses

Azoxystrobin 160 + Prothioconazole 200 SC was tested in a limit test at nominally 1911 mL product/ha, and a control group was exposed to deionised water in parallel. The test item concentration was chosen based on a non-GLP range finding test.

Reference item

Danadim Progress (a.s. dimethoate) was tested at nominally 10.0 mL product/ha.

Treatment/Application

Prior to test start, the application solution of Azoxystrobin 160 + Prothioconazole 200 SC was prepared by weighing 1341 mg of the test item and dissolving in 250 mL deionised water. The application solution of the reference substance was prepared by diluting 6.25 μL of the reference item in 250 mL of deionised water. The control test units were sprayed with deionised water only.

Appropriate volumes of the application solutions were sprayed onto barley plants by means of a Spray-Lab equipped with TeeJet 80003 EVS spraying nozzles and using a spraying pressure of 2.5 bar (Spray-lab and nozzles: Schachtner, Ludwigsburg, Germany) at $4 \text{ mg/cm}^2 \pm 10\%$ (corresponding to 400 L spray liquid/ha). After drying for 25 - 45 min, the test organisms were released into the test units.

3. Observations and assessments

The number of parasitoids alive, affected, moribund and dead was recorded at 2, 24 and 48 h after test initiation. Moribund specimens were counted as dead.

After 48 h, 20 surviving females from the control and from each test item treatment with $\leq 50\%$ mortality were confined individually over barley plants infested with host cereal aphids for 24 h. Subsequently, the females were removed, and aphid mummies were counted after 10 d for all replicates where females were alive after the 24 h parasitisation period.

Test temperature and relative humidity were recorded.

4. Calculation of toxicity

Mortality was calculated as mean percentage with standard deviation for the replicates and was corrected according to Abbott (1925) and Schneider-Orelli (1947).

The reproductive capacity was calculated as the mean number of aphid mummies per female with standard deviation. The percentage reduction of reproductive capacity was calculated based on the mean number of aphid mummies per female in the test groups relative to the control.

5. Statistics

The mortality data were analysed using the Fisher's Exact Binomial Test (one-sided greater, $\alpha = 0.05$). The percent values of wasps settled on the plants were angularly transformed (square root arcsine) prior to analysis. The transformed data, as well as reproduction data were tested for normal distribution and homogeneity using the Shapiro-Wilk's Test ($\alpha = 0.01$) and the Levene's Test ($\alpha = 0.01$). Statistically significant differences between the treatment groups and the control were analysed with the Student t-Test (one-sided smaller, $\alpha = 0.05$).

The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ® ToxRat Solutions GmbH.

Results and Discussion

A. BIOLOGICAL RESULTS

The mean mortality of *Aphidius rhopalosiphi* was 0.0% in the control and 6.7% in the test item treatment. Mortality was not statistically significantly increased in the test item treatment compared to the control (Fisher's Exact Binomial Test, one-sided greater, $\alpha = 0.05$).

The reference item applied at a rate of 10.0 mL Danadim Progress/ha produced a statistically significant corrected mortality of 96.7 % after 48 hours (Fisher's Exact Binomial Test; one-sided greater, $\alpha = 0.05$).

Reproduction rate of *A. rhopalosiphi* in the control treatment was 47.9 mummies per female. In the test item treatment, the reproduction was 41.0 mummies per female, corresponding to a reduction of 14.4 % compared to the control. Reproduction was not statistically significantly reduced compared to the control at 1911 mL product/ha (Student t-Test, one-sided smaller, $\alpha = 0.05$).

No behavioural abnormalities of parasitoids were observed in the test item treatment after 2, 24 or 48 hours.

Table 1: Mortality and parasitisation efficiency of *Aphidius rhopalosiphi* exposed to Azoxystrobin 160 + Prothioconazole 200 SC

Application rate [mL product/ha]	Mortality		Reproduction	
	Percentage mortality [%]	Percentage mortality [%] - corrected ^{a)}	Mummies per female	Effect on Reproduction [%]
Control: Deionised water				
Control	0.0	-	47.9	-
Test item: Azoxystrobin 160 + Prothioconazole 200 SC				
1911	6.7	6.7	41.0	14.4
Reference item: Danadim Progress (a.s. dimethoate)				
10.0	96.7*	96.7	-	-

- = not applicable

* Statistically significantly different compared to the control (Fisher's Exact Binomial Test, $\alpha = 0.05$)

^{a)} Corrected pre-imaginal mortality according to Abbott (1925) and improvements by Schneider-Orelli (1947).

B. VALIDITY CRITERIA

Mortality in the control was 0% (required: $\leq 10\%$) and in the reference item group 96.7% (required: $\geq 50\%$). In addition, the mean reproduction in the control was 47.9 mummies per female (required: ≥ 5 per female) and there was no parasitoid (required: ≤ 2) producing zero offspring. Thus, this study fulfils the validity criteria of the IOBC guideline Mead-Briggs et al. (2010).

Conclusion

In this limit test with the parasitoid wasp *Aphidius rhopalosiphi* under extended laboratory conditions, the mortality of *A. rhopalosiphi* was not statistically significantly increased by a single application of 1911 mL of Azoxystrobin 160 + Prothioconazole 200 SC/ha. The corrected mortality was 6.7 % and below the trigger value of 50%. There was no statistically significant effect on reproduction at the test item treatment compared to the control. The reduction of reproduction compared to the control was 14.4% and below the trigger value of 50%. Therefore, the LR₅₀ and ER₅₀ were estimated to be > 1911 mL product/ha. The validity criteria of IOBC guideline Mead-Briggs et al. (2010) were fulfilled.

A 2.3.2.2.2 Study 10

Comment from zRMS:	The study was conducted in line with the respective guideline IOBC Blümel et al. 2000, Oomen 1988 with minor deviations. The deviations from the desired target application amounts of the test item treatment were +18.0%, thus outside the recommended limit values of $\pm 10.0\%$. This was due to the fact that a plot sprayer with manual drive was used which could be less accurate if the step speed is not constant during application. However, since the deviation was relatively small, and the target value was exceeded which is considered less significant than falling below the target application amount. Therefore, this minor deviation is not expected to have an adverse effect on the outcome of the study. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: LR₅₀ > 1911 mL prod./ha ER₅₀ > 1911m L prod./ha
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Reference:	KCP 10.3.2.2/02
Report	Azoxystrobin 160 g/L + Prothioconazole 200 g/L SC: Effects on the Predatory Mite <i>Typhlodromus pyri</i> (Acari: Phytoseiidae), Extended Laboratory Study - Aged Residue Test, Leopold, J. (2024), Study no. 179211060
Guideline(s):	IOBC Blümel et al. 2000, Oomen 1988
Deviations:	The deviations from the desired target application amounts of the test item treatment were +18.0%, thus outside the recommended limit values of $\pm 10.0\%$. This was due to the fact that a plot sprayer with manual drive was used which could be less accurate if the step speed is not constant during application. However, since the deviation was relatively small, and the target value was exceeded which is considered less significant than falling below the target application amount. Therefore, this minor deviation is not expected to have an adverse effect on the outcome of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Executive summary

Effects of Azoxystrobin 160 + Prothioconazole 200 SC on survival and reproduction of the predatory mite *Typhlodromus pyri* were assessed in a limit test under extended laboratory conditions. A control and a reference substance were tested in parallel. Protonymphs of *T. pyri* were exposed to fresh residues of a single dose of the test item at 1902 mL product/ha on treated bean leaves for 14 d. Mortality was assessed after 2 and 7 d of exposure. For the control group and test item groups showing a corrected mortality < 50% after 7 d, reproductive capacity was assessed from day 7 to 14 based on the cumulative number of eggs per female.

In this extended laboratory test with the predatory mite *Typhlodromus pyri*, the LR₅₀ and ER₅₀ of Azoxystrobin 160 + Prothioconazole 200 SC were estimated to be > 1902 mL product/ha. The validity criteria of IOBC guideline Blümel et al. (2000) were fulfilled.

Materials and Methods

A. MATERIALS

1. Test material	Azoxystrobin 160 + Prothioconazole 200 SC
Description	Liquid, light brown, SC (suspension concentrate)
Lot/Batch #	ARD/AP/360/SC/36
Content of aa.ss.	Azoxystrobin: 162.20 ± 0.48 g/L ($14.45 \pm 0.04\%$ w/w) Prothioconazole: 200.54 ± 0.65 g/L ($17.86 \pm 0.06\%$ w/w) Density: 1.1228 ± 0.0012 g/mL
Stability of test material	Stable under storage conditions (20 ± 5 °C, in the dark) Expiry date: 23 May 2025
2. Vehicle and/or positive control	Vehicle control: Tap water Positive control: Reference item
Reference item	Danadim Progress
Description	Colourless to light yellow liquid
Lot/Batch #	10214034
Content of a.s.	Dimethoate: 400 g/L nominal
Stability of reference item	Stable under storage conditions (room temperature, dark) Expiry date: Nov 2023
3. Test organism	
Species	Predatory mite <i>Typhlodromus pyri</i> Scheuten, (Acari: Phytoseiidae)
Source	Commercial supplier, Katz Biotech AG, Germany.
Age	Protonymphs, not older than 24 h
Acclimation period	Under test conditions
Diet	Pollen from pine (<i>Pinus sp.</i>) and birch (<i>Betula sp.</i>) (3:1) was provided on the leaf disks approx. every four days and tap water was supplied <i>ad libitum</i> .
Test units	Treated bean leaf discs (55 mm diameter) were placed with the treated side upward on a wet cotton wool in a petri dish. The petri dish was constantly filled with tap water during the trial. A small strip of filter paper which extended onto the cotton wool pad, provides a water source for the mites. A circular glue barrier was added to prevent the mites from escaping. Set up was situated in a climate controlled chamber.
4. Environmental conditions	
Temperature	Nominal: 25 ± 2 °C; actual: 24 - 27 °C
Relative humidity	Nominal: 60 - 90%; actual: 66 - 70%
Photoperiod	16/8 h light/dark photoperiod (light intensity: actual 420 - 470 lux)

B. STUDY DESIGN AND METHODS

1. In-life dates 05 Sept 2023 to 19 Sept 2023

2. Experimental conditions

Test design

Effects of Azoxystrobin 160 + Prothioconazole 200 SC on survival and reproduction of the predatory mite *Typhlodromus pyri* were assessed in a limit test design at 1902 mL product/ha. A control and a reference substance were tested in parallel. Protonymphs of *T. pyri* were exposed to fresh residues on treated bean

leaves for 14 d. Mortality was assessed after 2 and 7 d of exposure. For the control group and test item groups showing a corrected mortality < 50% after 7 d, the reproductive capacity was assessed from day 7 to 14 based on the cumulative number of eggs per female.

Number of animals per treatment

10 protonymphs per replicate; 10 replicates per test and reference item treatment and control

Test doses

Azoxystrobin 160 + Prothioconazole 200 SC was tested at a single dose of nominally 1902 mL product/ha. A control group (tap water) and a reference item group treated with 50.0 mL Danadim Progress/ha were tested in parallel. Test rates were based on a non-GLP range finding test.

Reference item

Dimethoate was tested at nominally 50.0 mL Danadim Progress/ha.

Treatment/Application

Prior to application, the highest test item solution was prepared by dissolving 16.0 g of test item in 3000 mL of tap water. The application solution for the reference item was prepared by diluting 375 µL of reference item in 3000 mL of tap water. The control test arenas were sprayed with tap water only.

Application solutions were sprayed onto the leaf disks with a pressure of 2.0 bar and a distance of the nozzle from the leaf surface of approx. 50 cm by means of a movable plot sprayer, type “PSG-System 2” with Lechler IDK 120-04 spraying nozzles (Spray-lab and nozzles: Schachtner, Ludwigsburg, Germany). Before the application for each treatment group, the sprayer was calibrated to provide 4 mg spray solution/cm² (equivalent to 400 L/ha). The deviations from the desired target application amounts were between +6.0 and +18.0 % for the control, reference item and test item. The deviation of the test item treatment (+ 18.0 %) was outside the recommended limit values of ± 10.0 %. This was due to the fact that a plot sprayer with manual drive was used which could be less accurate if the step speed is not constant during application. However, it is expected that exceeding the target values has a less significant impact on the exposure than falling below the desired rate. Therefore, this minor deviation is not expected to have an adverse effect on the outcome of the study.

After the residues had dried, pollen was added to the leaf disks and the test organisms were transferred into the test arenas within 44 - 81 minutes after the start of the application.

3. Observations and assessments

Dead test organisms and escapees were counted on day 2 and 7 of the test period. Escapees and test organisms with abnormal appearance or failing to move after touch were considered dead.

The sex-ratio (number of males and females) was determined on day 7.

In the control group and each test item group showing a corrected mortality $\leq 50\%$ on day 7, the number of eggs per surviving female was assessed on day 10, 13, and 14 of the test period. Eggs and larvae were removed from the test units on day 7 (not included into assessment) and at each observation occasion thereafter.

4. Calculation of toxicity

For each treatment group, mortality was calculated across replicates as mean percentage $\pm$ standard deviation relative to the number of mites introduced at the beginning of the test. Correction for control mortality was performed according to Abbott's formula (1925) and to Schneider-Orelli (1947).

Reproductive capacity in the control and test item groups was calculated as mean cumulative number of eggs per female across replicates. The percentage change in reproduction in the test item groups was calculated relative to the control.

5. Statistics

Mortality data obtained from the control, test item and reference item treatment were analysed for significance using the Chi² 2x2 Table Test (one-sided greater, $\alpha = 0.05$). Reproduction data were tested for normal distribution and homogeneity of variance using the Shapiro-Wilk's Test ($\alpha = 0.01$) and the Levene's Test ($\alpha = 0.01$). Statistically significant differences between the test item groups and the control were analysed using Student t-Test (two-sample comparison, one-sided smaller, $\alpha = 0.05$).

The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ® ToxRat Solutions GmbH.

Results and Discussion

A. BIOLOGICAL RESULTS

After 7 d of exposure, mortality was 16.0% in the control and 44.0% in the test item group. Mortality was statistically significantly increased in the test item treatment group compared to the control (Chi² 2x2 Table Test; one-sided greater; $\alpha = 0.05$). Exposure to the reference item resulted in 100.0% mortality.

The mean reproductive capacity in the control at test end was 8.5 eggs per female, and 6.5 eggs per female in the test item group corresponding to a reduction of 22.8% compared to the control. Reproduction was not statistically significantly reduced compared to the control.

Since corrected mortality and the effect on reproduction was < 50% in all test item groups, the LR₅₀ and ER₅₀ were estimated to be >1902 mL product/ha.

Table 1: Effects of Azoxystrobin 160 + Prothioconazole 200 SC on mortality and reproduction of *Typhlodromus pyri* – fresh residues

Application rate [mL product/ha]	Mortality Day 7		Reproductive capacity	
	Mean ± SD [%]	Corrected mean ± SD [%]	Mean ± SD number of eggs per female	Effect on reproduction [%]
Control: Tap water				
Control	16.0 ± 12.06	-	8.5	-
Test item: Azoxystrobin 160 + Prothioconazole 200 SC				
1902	44.0 * ± 19.6	33.3	6.5	22.8
Reference item: Danadim Progress (a.s. dimethoate)				
50.0	100.0 * ± 0.0	100.0	-	-
Endpoints [mL product/ha]				
LR ₅₀ (95% CI)	>1902			
ER ₅₀ (95% CI) Reproduction	>1902			

- = not applicable; n.d. = not determined

* Statistically significantly reduced compared to the control (Chi² 2x2 Table Test, $\alpha = 0.05$, one-sided greater)

B. VALIDITY CRITERIA

The mean mortality after 7 d was 16.0% in the control (required: ≤ 20%), and the corrected mean mortality in the reference item group was 100% (required: ≥ 50%). In addition, the cumulative mean number of eggs in the control was 8.5 eggs per female (required: ≥ 4 per female). Thus, this study fulfils the validity criteria of the IOBC guideline Blümel et al. (2000).

Conclusion

In this extended laboratory test with the predatory mite *Typhlodromus pyri*, the LR₅₀ and ER₅₀ of Azoxystrobin 160 + Prothioconazole 200 SC were estimated to be > 1902 mL product/ha. The validity criteria of IOBC guideline Blümel et al. (2000) were fulfilled.

A 2.3.2.2.3 Study 11

Comment from zRMS:	The study was conducted in line with the respective guideline IOBC Schmuck et al., (2000) with no deviations. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: LR₅₀ > 1902 mL prod./ha ER₅₀ > 1902 mL prod./ha
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Reference:	KCP 10.3.2.2/03
Report	Azoxystrobin 160 g/L + Prothioconazole 200 g/L SC: Effects on the Ladybird Beetle <i>Coccinella septempunctata</i> (Coleoptera, Coccinellidae), Extended Laboratory Study - Limit Test, Leopold, J. (2024), Study no. 179211012
Guideline(s):	IOBC (Schmuck et al., 2000)
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Executive summary

Effects of Azoxystrobin 160 + Prothioconazole 200 SC on survival and behaviour of the ladybird beetle *Coccinella septempunctata* were assessed in a limit test under extended laboratory conditions. The test item was applied to the upper surface of leaf-discs of bean plants (*Phaseolus vulgaris* L.). Exposure of *C. septempunctata* larvae lasted until the hatching of the adults (11-18 days) and mortality was assessed over this period. The reproductive performance of the survivors was examined over two weeks (oviposition period) using adults from the control and from the test item concentrations.

The effect on mortality of *C. septempunctata* was below the trigger value of 50 % for higher tier studies at a test item application rate of 1904 mL Azoxystrobin 160 g/L + Prothioconazole 200 g/L SC/ha. Reproduction was reduced by 61.1 % compared to the control in the test item treatment but was >2 fertile eggs per viable female per day in the test item treatment, so the reproductive output is within the historical data base for control beetles and therefore this parameter is considered as not impacted by the treatment (Schmuck et al. 2000). The validity criteria of IOBC Schmuck et al. (2000) were fulfilled.

Materials and Methods

A. MATERIALS

1. Test material	Azoxystrobin 160 + Prothioconazole 200 SC
Description	Liquid, light brown, SC (suspension concentrate)
Lot/Batch #	ARD/AP/360/SC/36
Content of a.s.	Azoxystrobin: 162.20 ± 0.48 g/L (14.45 ± 0.04% w/w)

Stability of test material	Prothioconazole: 200.54 ± 0.65 g/L (17.86 ± 0.06% w/w) Density: 1.1228 ± 0.0012 g/mL Stable under storage conditions (20 ± 5 °C, in the dark) Expiry date: 23 May 2025
2. Vehicle and/or positive control	Vehicle control: Deionised water Positive control: Reference item
Reference item	Danadim Progress
Description	Colourless to light yellow liquid
Lot/Batch #	10214034
Content of aa.ss.	Dimethoate: 400 g/L nominal
Stability of reference item	Stable under storage conditions (room temperature, dark) Expiry date: Nov 2023
3. Test organism	
Species	<i>Coccinella septempunctata</i> L. (Coleoptera, Coccinellidae)
Source	Commercial supplier, Katz Biotech AG, Germany
Age	4 - 5 days old larvae
Acclimation period	4 - 5 days under test conditions
Diet	Larvae were fed with live aphids (<i>Acyrtosiphon pisum</i>) <i>ad libitum</i> . Aphids were replaced or added each day until larvae had entered the pupal stage. Adults were supplied with broad bean plants (<i>Vicia faba</i>) infested with aphids, <i>Acyrtosiphon pisum</i> , the infested plants were replaced once a week by fresh ones. If necessary additional aphids were added. Pollen and honey were given <i>ad libitum</i> .
Test units	Exposure units: Petri dish (60 mm diameter) with leaf discs of bean plants (<i>Phaseolus vulgaris</i> L.) were placed with their treated side upward on a wet cotton wool pad (approximately 55 mm in diameter). A Fluon treated cylinder of 30 mm height and 40 mm in diameter (approximately the lower 3 mm were not treated with Fluon to avoid contamination of the larvae) was fixed on each leaf by two elastic bands to guarantee a close position on the leaves. The cylinders were covered on the top with a plastic lid to prevent emerging beetles from escape. Pre-oviposition and oviposition period: Plastic insect rearing cages (40 cm x 40 cm x 40 cm) containing plants infested with aphids. During the oviposition period 3 folded tissue paper sheets served as egg laying surfaces for the adults. Hatching period (larvae): Plastic dishes (9 cm in diameter).
4. Environmental conditions	
Temperature	Nominal: 24 - 26°C
Relative humidity	Nominal: 60 - 72%
Photoperiod	16/8 h light/dark photoperiod; light intensity: 1040 - 1760 lux

B. STUDY DESIGN AND METHODS

- 1. In-life dates** 31 Aug 2023 to 04 Dec 2023
- 2. Experimental conditions**

Test design

Effects of Azoxystrobin 160 + Prothioconazole 200 SC on survival and reproduction of the ladybird beetle *Coccinella septempunctata* were assessed in a limit test under extended laboratory conditions. The test item was applied to the upper surface of leaf-discs of bean plants (*Phaseolus vulgaris* L.). Exposure time lasted until the hatching of the adults (11-18 days). The reproductive performance of the survivors was examined over two weeks (oviposition period) using adults from the control and from the test item concentrations.

Number of animals per treatment

Exposure period: 1 test organism per treatment group; 40 replicates per group

Post-exposure period: 11 to 16 adults per treatment group; 1 replicate per group

Test doses

Azoxystrobin 160 + Prothioconazole 200 SC was tested in a limit test at nominally 1904 mL product/ha, and a control group was exposed to deionised water in parallel. The test item concentration was chosen based on a non-GLP range finding test.

Reference item

Danadim Progress (a.s. dimethoate) was tested at nominally 8.0 mL product/ha.

Treatment/Application

Prior to test start, the application solution of Azoxystrobin 160 + Prothioconazole 200 SC was prepared by weighing 2672 mg of the test item and dissolving in 250 mL analytical grade water. The application solution of the reference substance was prepared by diluting 10.0 µL of the reference item in 250 mL of analytical grade water. The control test units were sprayed with deionised water only.

Appropriate volumes of the application solutions were sprayed onto the bean leaf discs by means of a Spray-Lab equipped with TeeJet 80015 EVS spraying nozzles (Spray-lab and nozzles: Schachtner, Ludwigsburg, Germany) at $2 \text{ mg/cm}^2 \pm 10 \%$ (corresponding to 200 L spray liquid/ha).

After application, the test units were left to dry for 40 to 60 minutes.

3. Observations and assessments

The number of living and dead larvae and pupae and number of adults hatched were counted daily except weekends.

Mortality of the adults was assessed for every day, and the sex of the dead beetles was determined. Number of eggs was counted for every day within the subsequent two weeks of oviposition.

The larval hatch is determined as difference between the total number of eggs laid and the remaining unhatched eggs.

The reproduction assessment was performed for the control and for the test item group provided that the corrected mortality was $\leq 50 \%$ (Abbott, 1925 and improvements by Schneider-Orelli, 1947).

4. Calculation of toxicity

The percentage mortality at test end was calculated for each treatment group relative to the number of beetles introduced at the beginning of the test. Correction for control mortality was performed according to Abbott (1925) and improvements by Schneider-Orelli (1947).

The average number of eggs laid per female beetle per day was determined. In addition, the percentage of fertile eggs was determined by assessing the larval hatch.

5. Statistics

For mortality data, the two-sample comparisons between the test item and the control and between the reference item and control were analysed using the Fisher's Exact Binomial Test (one-sided greater, $\alpha = 0.05$). Reproduction data were not statistically evaluated in accordance with Schmuck et al. 2000.

The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ® ToxRat Solutions GmbH.

Results and Discussion

A. BIOLOGICAL RESULTS

The mortality of *Coccinella septempunctata* was 27.5 % in the control treatment. In the test item treatment, mortality was 42.5 %, corresponding to a corrected mortality of 20.7 %, and was not statistically significantly increased compared to the control (Fisher's Exact Binomial Test, one-sided greater, $\alpha = 0.05$).

The mean number of fertile eggs per female was 7.8 in the test item treatment and was > 2 fertile eggs per viable female per day. In the control treatment, 20.1 fertile eggs per female and day were recorded. The reproduction (fertile eggs per female per day) was reduced by 61.1 % compared to the control in the test item treatment. However, in agreement with the guideline (Schmuck et al., 2000), no statistical evaluation was performed.

The larval hatching rate was 97.7 % and 92.9 % in the control and test item, respectively.

Table 1: Mortality of *C. septempunctata* exposed to Azoxystrobin 160 + Prothioconazole 200 SC

Application rate [mL product/ha]	Mortality		Reproduction	
	Percentage mortality [%]	Percentage mortality [%] - corrected ^{a)}	Fertile eggs per female per day	Reduction of Reproduction [%]
Control: Deionised water				
Control	27.5	-	20.1	-
Test item: Azoxystrobin 160 + Prothioconazole 200 SC				
1904	42.5	20.7	7.8	61.1
Reference item: Danadim Progress (a.s. dimethoate)				
8.0	100*	100	-	-

- = not applicable

* Statistically significantly different compared to the control (Fisher's Exact Binomial Test, $\alpha = 0.05$)

^{a)} Corrected pre-imaginal mortality according to Abbott (1925) and improvements by Schneider-Orelli (1947).

B. VALIDITY CRITERIA

Pre-imaginal mortality was 27.5% (required: $\leq 30\%$) in the control and 100.0% (required: $> 40\%$) in the reference item group. The reproduction rate in the control was 20.1 eggs/female per day (required: ≥ 2 fertile eggs per viable female per day), the test is considered to be valid in accordance with IOBC Schmuck et al. (2000).

Conclusion

In this limit test with the ladybird beetle *Coccinella septempunctata* under extended laboratory conditions, the mortality of *C. septempunctata* was not statistically significantly increased by a single application of 1904 mL test item/ha.

The reproductive performance of *C. septempunctata* was tested in the control and in the test item treatment. Reproduction (fecundity) was reduced by 61.1 % compared to the control in the test item treatment but was > 2 fertile eggs per viable female per day in the test item treatment, so the reproductive output is within the historical data base for control beetles and therefore this parameter is considered as not impacted by the treatment (Schmuck et al. 2000).

In addition, the reproduction in the test item treatment group is also within the range of ibacon's historical data. The mean reproduction of 56 studies conducted over the last 15 years was 8.9 eggs per female per day (median = 8.4 eggs per female per day). Overall, the mean values of the control variant ranged from 3.1 to 18.9 eggs per female per day.

With a mean value of 20.6 eggs per female per day, the control treatment is thus clearly outside the usual range, which also explains the large percentage difference to the test item treatment group.

All validity criteria were met, and the study is considered valid.

A 2.3.2.2.4 Study 12

Comment from zRMS:	The study was conducted in line with the respective guideline IOBC guideline Vogt et al. 2000 with no deviations. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: LR₅₀ > 1902 mL prod./ha ER₅₀ > 1902 mL prod./ha
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Reference:	KCP 10.3.2.2/04
Report	Azoxystrobin 160 g/L + Prothioconazole 200 g/L SC: Effects on the Lacewing <i>Chrysoperla carnea</i> (Neuroptera: Chrysopidae), Extended Laboratory Study - Limit Test, Leopold, J. (2024), 179211047 (sponsor study No. UPL/2023/1913)
Guideline(s):	IOBC guideline Vogt et al. 2000
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Executive summary

Effects of Azoxystrobin 160 + Prothioconazole 200 SC on survival and reproduction of the green lacewing *Chrysoperla carnea* were assessed in a limit test rate under extended laboratory conditions. Larvae of *C. carnea* were exposed to freshly dried residues of the test item on bean leaves (*Phaseolus vulgaris* L.). The exposure period lasted between 5 and 25 d. Mortality was assessed regularly until hatching of adult lacewings. Approximately one week after first egg laying of adult lacewings was observed, fecundity and fertility, i.e. egg deposition and hatching success, were assessed in two samplings to determine the reproductive performance of the test organisms.

Under extended laboratory conditions, a single application of 1904 mL Azoxystrobin 160 g/L + Prothioconazole 200 g/L SC/ha caused no statistically increased mortality in the test item treatment group compared to the control. The corrected mortality was 21.2 % in the test item treatment and below the trigger value of 50% for the risk assessment of extended non-target arthropod studies.

The reproductive capacity of *C. carnea* was assessed in the control and in the test item treatment. The mean hatching rate was comparable to the control in the test item application rates and was > 70 %. Reproduction

was > 15 eggs per female per day in both the control and test item treatment. This indicates that there was no negative effect of the test item on reproductive performance of *C. carnea* at an application rate of 1904 mL product/ha. The reduction of reproduction compared to the control was -7.4 % and below the trigger value of 50%. The ER₅₀ value for effects on reproduction is estimated to be higher than 1904 mL product/ha. The validity criteria of IOBC guideline Vogt et al. (2000) were fulfilled.

Materials and Methods

A. MATERIALS

1. Test material	Azoxystrobin 160 + Prothioconazole 200 SC
Description	Liquid, light brown, SC (suspension concentrate)
Lot/Batch #	ARD/AP/360/SC/36
Content of a.s.	Azoxystrobin: 162.20 ± 0.48 g/L (14.45 ± 0.04% w/w) Prothioconazole: 200.54 ± 0.65 g/L (17.86 ± 0.06% w/w) Density: 1.1228 ± 0.0012 g/mL
Stability of test material	Stable under storage conditions (20 ± 5 °C, in the dark) Expiry date: 23 May 2025
2. Vehicle and/or positive control	Vehicle control: HPLC water Positive control: Reference item
Reference item	Danadim Progress
Description	Colourless to light yellow liquid
Lot/Batch #	10214034
Content of a.s.	Dimethoate: 400 g/L nominal
Stability of reference item	Stable under storage conditions (room temperature, dark) Expiry date: Nov 2023
3. Test organism	
Species	Lacewing <i>Chrysoperla carnea</i> (Neuroptera, Chrysopidae)
Source	Katz Biotech AG, An der Birkenpfehlheide 10, D-15837 Baruth, Germany.
Age	First instar larvae (2-3 d old)
Acclimation period	Freshly hatched larvae were fed with eggs of <i>Sitotroga cerealella</i> and incubated for 2-3 d until start of exposure.
Diet	<i>Larvae</i> : UV-sterilised <i>Sitotroga cerealella</i> Oliv. eggs, <i>ad libitum</i> <i>Adults</i> : artificial diet: 1 egg, 1 egg yolk, 15 mL condensed milk, 20 g fructose, 30 g honey, 30 g brewer's yeast, 50 g wheat germ and deionised water (approximately 45 mL) mixed homogeneously, <i>ad libitum</i>
Test units	<i>Exposure units</i> : Detached primary leaves of bean plants (<i>Phaseolus vulgaris</i> L. "Maxi", 15 days old) were cut to discs of about 55 mm in diameter. These leaf cuts were treated on their upper surface. The leaf discs were placed with their treated side upwards on a wet cotton wool pad in a petri dish (60 mm in diameter). The petri dish had a hole for a wick. A Fluon treated cylinder was fixed on each leaf by two elastic bands to guarantee a close position on the leaves. The height of the cylinder was 30 mm and the diameter was 46 mm. <i>Post-exposure units</i> :

An acrylic cylinder (15 cm high and 10 cm in diameter) with a cotton net on the top for egg-laying and a hole (diameter approximately 2 cm) on the bottom to provide water through a cotton plug.

4. Environmental conditions

Temperature	Nominal: $25 \pm 2^{\circ}\text{C}$; actual: $24 - 26^{\circ}\text{C}$ during the test
Relative humidity	Nominal: 60 – 90%; actual: 60 - 71% during the tests
Photoperiod	16/8 h light/dark photoperiod; light intensity during exposure period: nominal ≥ 1000 Lux, actual: 1110 – 1410 Lux

B. STUDY DESIGN AND METHODS

1. In-life dates 02 Nov 2023 to 06 Dez 2023 (experimental period)

2. Experimental conditions

Test design

Effects of Azoxystrobin 160 + Prothioconazole 200 SC on survival and reproduction of the green lacewing *Chrysoperla carnea* were assessed in a limit test design under extended laboratory conditions. Larvae of *C. carnea* were exposed to freshly dried residues of the test item on bean leaves (*Phaseolus vulgaris* L.). The exposure period lasted as long as pupae were transferred to rearing vessels, which was approximately between 5 and 25 d after pupation. Mortality was assessed regularly until hatching of adult lacewings. Approximately one week after first egg laying of adult lacewings was observed, fecundity and fertility, i.e. egg deposition and hatching success, were assessed in two samplings to determine the reproductive performance of the test organisms.

Number of animals per treatment

Exposure phase: Four trials with 40 replicates per test/reference item treatment and control group each; one lacewing per replicate.

Reproduction phase: The surviving lacewings from the exposure phase were included in the reproduction phase.

Test doses

Azoxystrobin 160 + Prothioconazole 200 SC was tested in a limit test at nominally 1904 mL product/ha, and a control group was exposed to HPLC water in parallel. The test item concentration was chosen based on a non-GLP range finding test.

Reference item

Danadim Progress (a.s. dimethoate) was tested at nominally 170 mL Danadim Progress/ha.

Treatment/Application

For preparation of the test item solutions, a stock solution (= highest test item concentration) was prepared by diluting 2672 mg of test item in 250 mL of HPLC water. The application solution of the reference substance was prepared by diluting 213 mL of reference item in 250 mL of HPLC water. Bean plants for the controls were sprayed with HPLC water only.

Appropriate volumes of the application solutions were sprayed onto bean plants by means of a Spray-Lab equipped with TeeJet 80015 EVS spraying nozzles at 2.7 bar (Schachtner, Ludwigsburg, Germany) and left to dry. The distance of the nozzles from the top of the plants was approx. 30 cm. Before and after the

application, the sprayer was calibrated to provide 2 mg spray solution/cm² (equivalent to 200 L/ha) with a maximum deviation of 10%.

For the exposure, lacewings disks of the treated bean plants and the test organisms were transferred to the test vessels after 35 to 40 minutes after the sprayed leaves were dried. Plants for the reference groups were treated with the reference item on the day of the start of exposure.

3. Observations and assessments

For the assessment of fecundity, the number of eggs on the gauze and eggs laid on the walls of the oviposition cages was counted using two egg samples (checks) within one week. Each check covered an egg laying period of 24 h. For each check a cleaned and egg-free acrylic cylinder was used covered with a new gauze.

For the assessment of fertility, only eggs attached to the gauze were considered. Eggs of each sample (attached to the gauze) were incubated in separate plastic boxes to determine their viability. Hatched larvae were removed daily; *Sitotroga* eggs were added to avoid cannibalism. When no further hatching of larvae was observed (after 4 - 5 days), the remaining eggs where no live larva had hatched were determined as unhatched. For analysis all eggs on the gauze were counted and the percentage of hatched eggs was determined. Eggs laid on the walls of the oviposition cages were not considered for the determination of the hatching rate due to technical reasons.

The number of living and dead larvae and number of pupae developed was determined at least 3 times a week after test start and number of adults hatched was checked regularly. The number of eggs was counted after 24-hour egg-laying periods (checks) and 2 checks were done within one week. The number of larvae (from the gauze) was determined after hatching of all larvae and the hatching rate was calculated. Reproduction assessment was performed for the control and for the test item group. No reproduction testing was performed with the reference item.

4. Calculation of toxicity

The cumulative mortality was calculated based on the total number of dead larvae, pupae and adults including individuals failing to emerge or dying within 24 h after hatch. The percentage mortality was determined in relation to the number of test organisms introduced at start of exposure and was corrected based on control mortality according to Schneider-Orelli (1947).

The mean number of eggs laid per female per day was determined by dividing the total number of eggs (laid on the gauze and on the walls of the oviposition cages) by the mean number of viable females in that group (corrected for mortality during egg laying).

5. Statistics

Mortality data obtained from the control, test item and reference item treatment were analysed using the Fisher's Exact Binomial Test (one-sided greater, $\alpha = 0.05$).

The reproductive performance is evaluated only qualitatively due to the high species-inherent variability in egg-laying performance in accordance with Vogt et al. 2000.

The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ® ToxRat Solutions GmbH.

Results and Discussion

A. BIOLOGICAL RESULTS

Pre-marginal mortality

The mortality of *Chrysoperla carnea* was 17.5 % in the control treatment and was 35.0 % in the test item treatment, corresponding to a corrected mortality of 21.2 %. Mortality was not statistically significantly increased compared to the control (Fisher's Exact Binomial Test, one-sided greater, $\alpha = 0.05$).

The reference item applied at a rate of 170 mL Danadim Progress/ha produced a statistically significant mortality of 95.0 % (93.9 % corrected mortality; Fisher's Exact Binomial Test, one-sided greater, $\alpha = 0.05$) (table 1).

Table 1. Pre-imaginal mortality of *Chrysoperla carnea*

Treatment group	Mortality ^a	Corrected mortality
	[%]	[%]
Control	17.5	-
Azoxystrobin 160 + Prothioconazole 200 SC 1904 mL product/ha	35.0 n.s.	21.2
Reference item (170 mL product/ha)	95.0*	93.9

[note: test item/reference item: Fisher's Exact Binomial Test; one-sided greater; $\alpha = 0.05$; n.s. = not significant, * = significant]

^a Tabulated results represent rounded values calculated on the exact raw data

Reproduction

Reproduction of *C. carnea* was assessed in the control and in the test item treatment. The mean number of eggs per female and day was 20.0 in the control treatment. In the test item treatment, reproduction was 21.4 eggs per female and day and was > 15 eggs per female and day in both the control and test item treatment.

The mean hatching rate was 98.6 % in the control treatment and 92.1 % in the test item treatment and was higher than 70 % in both the control and in the test item treatment (table 2).

Table 2. Reproduction of *Chrysoperla carnea*

Treatment group ^a	Eggs per female per day	Effect on reproduction ^b	Larval hatching rate ^c
		[%]	[%]
Control	20.0±3.1	-	98.6 ± 0.1
Azoxystrobin 160 + Prothioconazole 200 SC (1904 mL product/ha)	21.4±1.4	-7.4	92.1 ± 2.5

[note: the tabulated results represent mean and standard deviation calculated on the exact raw data; 1 reproduction unit per treatment group with all survived lacewings]

^a Adults developed from larvae exposed to spray residues on leaves

^b Negative values indicate a better performance compared to the control

^c For the determination of the hatching rate only eggs were considered which were laid on the gauze of the oviposition cages due to technical reasons.

B. VALIDITY CRITERIA

Cumulative mortality was 17.5% in the controls (required: $\leq 20\%$) and 93.9% in the reference item groups (required: $\geq 50\%$). In addition, the mean fecundity and mean fertility in the controls were 20 eggs per female per day (required: ≥ 15 eggs per female per day) and 98.6% (required: $\geq 70\%$), respectively. Thus, this study fulfils the validity criteria of the IOBC guideline Vogt et al. (2000).

Conclusion

Under extended laboratory conditions, a single application of 1904 mL Azoxystrobin 160 g/L + Prothioconazole 200 g/L SC/ha caused no statistically increased mortality in the test item treatment group compared to the control. The corrected mortality was 21.2 % in the test item treatment and below the trigger value of 50% for the risk assessment of extended non-target arthropod studies.

The reproductive capacity of *C. carnea* was assessed in the control and in the test item treatment. The mean hatching rate was comparable to the control in the test item application rates and was > 70 %. Reproduction was > 15 eggs per female per day in both the control and test item treatment. This indicates that there was no negative effect of the test item on reproductive performance of *C. carnea* at an application rate of 1904 mL product/ha. The reduction of reproduction compared to the control was -7.4 % and below the trigger value of 50%. The ER₅₀ value for effects on reproduction is estimated to be higher than 1904 mL product/ha. The validity criteria of IOBC guideline Vogt et al. (2000) were fulfilled.

A 2.3.2.3 KCP 10.3.2.3 Semi-field studies with non-target arthropods

Not required based on the outcome of the risk assessments.

A 2.3.2.4 KCP 10.2.3.4 Field studies with non-target arthropods

Not required based on the outcome of the risk assessments.

A 2.3.2.5 KCP 10.2.3.5 Other routes of exposure for non-target arthropods

Not required based on the outcome of the risk assessments.

A 2.4 KCP 10.4 Effects on non-target soil meso- and macrofauna

A 2.4.1 KCP 10.4.1 Earthworms

A 2.4.1.1 KCP 10.4.1.1 Earthworms - sub-lethal effects

A 2.4.1.1.1 Study 13

Comment from zRMS:	The study was conducted in line with OECD 222 with no deviations. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: 56d NOEC ≥ 55.6 mg prod./kg dw soil EC₁₀ =22.4 mg product/kg dry soil (95% CI: 14.9 - 33.7) EC₂₀= 54.2 mg product/kg dry soil (95% CI: 42.0 - 70.0)
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Reference:	KCP 10.4.1.1/01
Report	Azoxystrobin 160 + Prothioconazole 200 g/L SC: Effects on Reproduction and Growth of Earthworms <i>Eisenia andrei</i> in Artificial Soil, Hübner, S. (2024), Study no. 179211022
Guideline(s):	OECD 222 (2016)
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Executive summary

Adult earthworms were exposed to artificial soil treated with the test substance Azoxystrobin 160 + Prothioconazole 200 g/L SC at eight concentrations (dose-response test) or remained untreated (control) for a

period of 28 d. After this period, the adults were removed from the test vessels and mortality, behavioural effects and biomass development (body weight change) were determined. The reproduction rate was determined after an additional period of 28 d (on day 56) based on the number of juveniles.

In this study on *Eisenia andrei* with the test item Azoxystrobin 160 + Prothioconazole 200 g/L SC, the NOEC based on reproduction was determined to be 55.6 mg product/kg dry soil. The EC₁₀ and EC₂₀ values for reproduction were determined as 22.4 mg product/kg dry soil (95% CI: 14.6 - 33.7) and 54.2 mg product/kg dry soil (95% CI: 42.0 - 70.0), respectively. All validity criteria of OECD 222 (2016) were fulfilled.

Materials and Methods

A. MATERIALS

1. Test material	Azoxystrobin 160 + Prothioconazole 200 g/L SC	
Description	Liquid, light brown, SC (suspension concentrate)	
Lot/Batch #	ARD/AP/360/SC/36	
Content of aa.ss.	Azoxystrobin: 162.20 ± 0.48 g/L (14.45 ± 0.04% w/w) Prothioconazole: 200.54 ± 0.65 g/L (17.86 ± 0.06% w/w) Density: 1.1228 ± 0.0012 g/mL	
Stability of test material	Stable under storage conditions (20 ± 5 °C, in the dark) Expiry date: May 23, 2025	
2. Vehicle and/or positive control	Vehicle control: Artificial soil mixed with deionised water Positive control: The toxic reference item carbendazim was tested in a separate study from Jan to Mar 2023 to confirm the sensitivity of the test organism. Statistically significant effects on reproduction were observed at concentrations of 1.00 mg carbendazim/kg dry soil and higher (required by OECD 222: statistically significant effects between 1 and 5 mg/kg dry soil).	
3. Test organism		
Species	Earthworm <i>Eisenia andrei</i>	
Source	In-house culture	
Age	Approximately 8 to 9 months old adults with clitellum; body weight at test start: 315 - 600 mg	
Acclimatisation	24 h in moist, untreated artificial soil.	
Diet	One day after application, 5 g of finely ground cow manure per vessel was distributed onto the soil surface and moistened. Food was replenished weekly with 5 g food per test vessel. Offspring was only fed once at day 28 with 5 g manure.	
Test units	Plastic boxes (18.3 cm x 13.6 cm x 6 cm, 189.75 cm ² area) with perforated transparent lids. Each container was filled with 616.9 g ± 1 g of the prepared soil (500 g dry weight plus deionised water). The height of the soil layer in the containers was approximately 5 cm.	
4. Environmental conditions		
Soil	Artificial soil was prepared with the following constituents:	
	Sphagnum peat	10%
	Kaolin clay	20%
	Quartz sand	69.6%
	Calcium carbonate CaCO ₃	0.46%
	CaCO ₃ was added to adjust the pH to 6.0 ± 0.5.	

	The dry components were mixed. The maximum water holding capacity (WHC) was measured to be 46%.
Temperature	Nominal: $20 \pm 2^{\circ}\text{C}$
pH	Nominal at test start: 6.0 ± 0.5 ; actual test start: 6.2 - 6.3, test end: 6.4 - 6.5
Water content	Nominal: 40 - 60% of WHC_{max} (determined as 46%), not varying more than 10% during test; actual: 23.1 - 23.7% at test start (equal to 50.2 - 51.6% of WHC_{max}) and 23.9 - 25.6% at test end (equal to 51.9 - 55.8% of WHC_{max})
Photoperiod	16 h light (light intensity: nominal: 400 - 800 Lux) to 8 h dark photoperiod

B. STUDY DESIGN AND METHODS

1. In-life dates 19 Oct 2023 to 15 Dec 2023 (experimental period)

2. Experimental conditions

Test design

Adult earthworms were exposed to artificial soil treated with the test substance at eight concentrations (dose-response test) or remained untreated (control) for a period of 28 d. After this period, the adults were removed from the test vessels and mortality, behavioural effects and biomass development (body weight change) were determined. The reproduction rate was determined after an additional period of 28 d (on day 56) based on the number of juveniles.

Number of animals per treatment

Ten earthworms/replicate; four replicates/test substance treatment and eight replicates/control

Test concentrations

Azoxystrobin 160 + Prothioconazole 200 g/L SC was tested at 1.63, 2.94, 5.29, 9.53, 17.1, 30.9, 55.6 and 100 mg product/kg dry soil. A control (receiving deionised water only) was tested in parallel. The test item concentration range was based on a non-GLP range-finding test. The reference item carbendazim was tested in a separate study.

Treatment/Application

A stock solution was prepared by weighing 525.0 mg of the test item in deionised water to reach a final net weight of 600.0 g. The stock solution was used to prepare the application solutions for the different test concentrations by dilution with an appropriate volume of deionised water. For each test item group, the respective application solution was mixed into 2100 g dry artificial soil.

While mixing the artificial soil in a laboratory mixer for approximately 5 minutes the soil of each treatment group (including the control) was moistened with deionised water. Each group was treated in one batch (two in the control) which was then split into the replicates. Thereby a water content between 40-60% of the WHC_{max} was achieved for each treatment group.

3. Sampling and measurements

After 28 d of exposure, the living adult worms were removed from the test vessels and counted and checked for any abnormal behaviour or other adverse effects.

At the beginning (prior to exposure) and at the end of the first four weeks of the test, the adult test organisms of each test vessel were weighed. Before weighing the worms were cleaned from the substrate.

At the test termination after 56 d, the number of living juveniles per test vessel was determined. The test vessels were warmed up in a water bath at 50 - 60 °C for approximately 30 min and the juvenile earthworms at the soil surface were collected and counted.

At the start and end of the test, the soil water content of the artificial soil was determined for each treatment group in an additional test vessel.

During the experiment, water evaporation losses were compensated once a week by adding deionised water up to the initial weight.

The pH of the soil was determined at the start and end of the test in an additional test vessel set up for each treatment group. Temperature and light intensity were recorded.

4. Calculation of toxicity

Adult mortality and body weight change were based on total numbers and percentage and calculated for each replicate.

The mean number of juveniles was determined for each replicate.

5. Statistics

Mortality in the test item groups was analysed for significant differences compared to the control group using Fisher's exact binomial test with Bonferroni-Holm correction ($\alpha = 0.05$, one-sided greater). The LC_{50} at day 28 was not determined by statistical analysis as no mortality above 50% was observed.

The body weight change and reproduction data were tested for normal distribution and homogeneity of variance ($\alpha = 0.01$) using the Shapiro-Wilk's test and the Levene's test, respectively. Dunnett's t-test was used to compare treatment and control values the data for body weight changes (multiple comparison, two-sided, $\alpha = 0.05$). Williams t-test (multiple comparison, $\alpha = 0.05$, one-sided smaller) was used to compare treatment and control values of the reproduction data.

The EC_{10} and the EC_{20} and their 95% confidence limits for reproduction were calculated by applying Probit-Analysis.

Statistical analysis was performed using the programme ToxRat Professional, Version 3.3.0.

Results and Discussion

A. BIOLOGICAL RESULTS

A mortality of up to 2.5% was found in the test item treated groups, which was not statistically significantly different compared to the control, where 1.3% of the earthworms died (Fisher's Exact Test, $\alpha = 0.05$, one-sided greater).

The body weight change after 28 days of adult earthworms were not statistically significantly different compared to the control up to the highest test concentration of 100 mg product/kg soil dry weight (Dunnett's t-test, $\alpha = 0.05$, two-sided).

No statistically significant effects on reproduction were observed up to concentration of 55.6 mg product/kg soil dry weight (Williams t-test, $\alpha = 0.05$, one-sided smaller). At the test concentration of 100 mg product/kg soil dry weight, reproduction was statistically significantly reduced compared to the control.

The respective endpoints are listed in the table below.

Table 1: Effects of Azoxystrobin 160 + Prothioconazole 200 g/L SC on earthworm survival, growth and reproduction.

Treatment [mg product /kg dry soil]	Mean mortal- ity after 28 d [%]	Mean change in body fresh weight after 28 d [%]	Mean reproduction after 56 d	
			mean offspring	[% of control]
Control	1.3	+15.8	95	-
Test substance: Azoxystrobin 160 + Prothioconazole 200 g/L SC				
1.63	0	+10.9	121	128
2.94	0	+12.2	101	106
5.29	0	+20.7	101	106
9.53	0	+12.9	104	110
17.1	0	+16.3	85	89
30.9	0	+17.7	80	84
55.6	2.5	+14.6	76	80
100	0	+15.7	68*	72*
Endpoints [mg product/kg dry soil]				
	NOEC	EC₁₀ (95% CI)	EC₂₀ (95% CI)	EC₅₀ (95% CI)
28 d Mortality	≥ 100	n.d.	n.d.	> 100 ¹ (n.d.)
56 d Reproduction	55.6	22.4 ² (14.9 - 33.7)	54.2 ² (42.0 - 70.0)	> 100 ¹ (n.d.)

- = not applicable; n.d. = not determined

* Statistically significantly different from the control based on William's t-test (one-sided smaller, $p \leq 0.05$)

¹ Estimated value

² Probit analysis

B. VALIDITY CRITERIA

The mean control mortality was 1.3% (required $\leq 10\%$ after 4 weeks) and the coefficient of variation of the reproduction rate per test vessel in the control was 18.3 (required $\leq 30\%$). The number of juvenile earthworms per replicate was 65 to 112 (required ≥ 45). The validity criteria of OECD 222 (2016) were therefore fulfilled.

Conclusion

In this study on *Eisenia andrei* with the product Azoxystrobin 160 + Prothioconazole 200 g/L SC, the NOEC based on reproduction was determined to be 55.6 mg product/kg dry soil. The NOEC for mortality and weight changes of the earthworm *Eisenia andrei* was determined to be ≥ 100 mg product/kg soil dry weight. The EC₁₀ and EC₂₀ values for reproduction were determined as 22.4 mg product/kg dry soil (95% CI: 14.9 - 33.7) and 54.2 mg product/kg dry soil (95% CI: 42.0 - 70.0), respectively. All validity criteria of OECD 222 (2016) were fulfilled.

A 2.4.1.2 KCP 10.4.1.2 Earthworms - field studies

No additional data submitted

A 2.4.2 KCP 10.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

A 2.4.2.1 KCP 10.4.2.1 Species level testing

A 2.4.2.1.1 Study 14

Comment from zRMS:	The study was conducted in line with OECD 226 with no deviations. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: NOEC > 1000 mg prod./kg soil dw. EC ₁₀ >10000 mg prod./kg soil dw
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Reference:	KCP 10.4.2.1/01
Report	Azoxystrobin 160 + Prothioconazole 200 g/L SC: Effects on Reproduction of the Predatory Mite <i>Hypoaspis aculeifer</i> in Artificial Soil, Hübner, S. (2024), Study no. 179211089
Guideline(s):	OECD 226 (2016)
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Executive summary

Adult female mites were exposed to soil treated with Azoxystrobin 160 + Prothioconazole 200 g/L SC at eight concentrations for a period of 14 d. Demineralized water was used as a control treatment and the reference item dimethoate was tested in parallel. At the end of the exposure period, the surviving individuals were extracted from the test units. The number of juveniles per test unit and additionally the number of surviving females were determined.

In this study on the chronic toxicity of Azoxystrobin 160 + Prothioconazole 200 g/L SC to *Hypoaspis aculeifer*, the overall NOEC was determined to be ≥ 1000 mg product/kg dry soil based on reproduction. The validity criteria of OECD 226 (2016) were fulfilled.

Materials and Methods

A. MATERIALS

1. Test material	Azoxystrobin 160 + Prothioconazole 200 g/L SC
Description	Liquid, light brown, SC (suspension concentrate)
Lot/Batch #	ARD/AP/360/SC/36
Content of aa.ss.	Azoxystrobin: 162.20 ± 0.48 g/L ($14.45 \pm 0.04\%$ w/w) Prothioconazole: 200.54 ± 0.65 g/L ($17.86 \pm 0.06\%$ w/w) Density: 1.1228 ± 0.0012 g/mL
Stability of test material	Stable under storage conditions (20 ± 5 °C, in the dark)

Expiry date: May 23, 2025

2. Vehicle and/or positive control Vehicle control: Artificial soil mixed with demineralized water
 Positive control: Reference item

Reference item Dimethoate

3. Test organism

Species Predatory mite, *Hypoaspis aculeifer* Canestrini (Acari: Laelapidae)
Source In-house culture
Age Synchronous adult females (approx. 30 d old)
Diet The test organisms were fed cheese mites (*Tyrophagus putrescentiae*) at experimental start and on day 2, 5, 7, 9 and 12.
Test units Glass containers (volume: 100 mL; diameter: 5 cm), tight screw top closure to avoid water evaporation, filled with approximately 20 g ± 1.0 g artificial soil dry weight.

4. Environmental conditions

Soil Artificial soil was prepared with the following constituents (percent-age distribution on dry weight basis):

Sphagnum peat	5%
Kaolin clay	20%
Industrial quartz sand	74.8%
Calcium carbonate CaCO ₃	0.2%

CaCO₃ was added to adjust the pH to 6.0 ± 0.5.

The maximum water holding capacity (WHC) was measured to be 32% of the dry weight. The soil was pre-moistened to half of the final water content 2 days before the application. Water content of the test soil was brought to 50% of the maximum WHC during soil preparation.

Temperature Nominal: 20 ± 2°C
Water holding capacity At test start: 17.5% to 17.8% (54.6% to 55.6% of the maximum water holding capacity). At test end: 16.1% to 17.4% (50.4% to 54.3% of the maximum water holding capacity).
pH Nominal: 6.0 ± 0.5 at test start; actual: 5.8 - 6.0 at test start and 5.7 - 5.8 at test end
Photoperiod 16 h light (light intensity: nominal: 400 - 800 Lux) to 8 h dark photoperiod

B. STUDY DESIGN AND METHODS

1. In-life dates 20 Sept 2023 to 06 Oct 2023 (experimental period)

2. Experimental conditions

Test design

Adult female mites were exposed to soil treated with Azoxystrobin 160 + Prothioconazole 200 g/L SC at eight concentrations for a period of 14 d. Demineralized water was used as a control treatment and the reference item dimethoate was tested in parallel. At the end of the exposure period, the surviving individuals

were extracted from the test units. The number of juveniles per test unit and additionally the number of surviving females were determined.

Number of animals per treatment

Ten female mites/replicate; four replicates/test item treatment; eight replicates for the control and reference item group; in addition, one replicate per treatment group without test organisms was prepared for water content and pH measurements.

Test concentrations

Azoxystrobin 160 + Prothioconazole 200 g/L SC was tested at nominal concentrations of 16.3, 29.4, 52.9, 95.3, 171, 309, 556 and 1000 mg product/kg dry soil. Test rates were chosen based on a non-GLP range finding test. A control (demineralized water) was tested in parallel and the reference item dimethoate was tested at a single test rate of 2.23 a.s./kg dry soil.

Treatment/Application

For the test item treatments, a stock solution (= application solution for the highest test item group) was prepared by dissolving 1100 mg of test item in deionised water to obtain a final net weight of 97.1 g. Application solutions for the remaining test item groups were prepared by diluting appropriate volumes of the stock solution with water. The soil for each treatment group was mixed with a laboratory mixer to ensure a homogeneous distribution. Each group was treated in one batch and then split into the replicates. After adding the application solutions, the soil moisture was at 50% of WHC_{max}. Subsequently, approx. 20 g of corresponding dry substrate were transferred into each vessel.

The control was prepared by mixing demineralized water into the artificial soil.

3. Sampling and measurements

After 14 days of exposure, the soil was filled into Millipore pots with attached plastic containers for collecting the escaping mites. These extraction units were placed in a heat extractor. The soil including the mites was exposed to a temperature of approximately 25°C to 30°C for approximately 2 days. Escaping mites were collected in a fixing liquid, cooled at a temperature of approximately 16°C. The fixing liquid contained glycol and a detergent. Adults not found were considered dead.

The extraction efficiency of the extractor was determined to be 93.4% in a method validation in June 2023 (required: > 90%). The validation was done in duplicates using vessels containing a known number of juveniles and adult mites.

Prior to the start of the test, the water holding capacity (WHC) was determined in five samples of the artificial soil. At the start of the test, the soil moisture was determined with the soil for each treatment group. At test end, measurements were made with extra vessels prepared for each treatment group. In addition, water content in the test vessels was analysed after 7 d based on the weight of the test vessels. Loss of water was not compensated as it did not deviate by more than 2% from the initial water content.

The pH was determined at the start and end of the test, and temperature was measured continuously.

4. Calculation of toxicity

Mortality (number of dead adults) in percent for each treatment group was calculated. The reproductive output was calculated based on the number of juveniles for each treatment group.

5. Statistics

Mortality data were analyzed using Fisher's Exact Binomial Test (multiple comparison, with Bonferroni Correction, $\alpha = 0.05$, one-sided greater). The LC_{50} at day 14 was not determined by statistical analysis as no mortality above 50% was observed.

Reproduction data were tested for normal distribution and homogeneity of variance using Kolmogorov-Smirnov test and Cochran's test ($\alpha = 0.01$). Since the reproduction data were normally distributed and homogeneous but did not follow a monotonicity trend (contrast trend) the Dunnett's t-test was used to compare treatment and control values (multiple comparison, $\alpha = 0.05$, one-sided smaller). The determination of the NOEC and LOEC values was based on the results of the statistical evaluation. The EC values could not be determined by statistical analysis since there was no adequate concentration response.

The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ToxRat® Solutions GmbH.

Results and Discussion

A. BIOLOGICAL RESULTS

In the control group, mean mortality of adult test organisms after 14 d was 9%. Mean mortality in the test item groups ranged between 5 to 13% and was not statistically significantly different from the control for any test item group (see following table).

In the control group, the mean number of juveniles per replicate was 239 to 274. The mean reproduction in the test item treatments up to and including 1000 mg product/kg dry soil was between 220 and 300 juveniles being not statistically significantly different from the control. The reference item reduced reproduction by 100% at a nominal concentration of 17.4 mg product/kg dry soil.

No differences in morphology of the mites between the test item treated groups and the control were observed.

Table 1: Effects of Azoxystrobin 160 + Prothioconazole 200 g/L SC on survival and reproduction of *Hypoaspis aculeifer*

Nominal test concentrations	Mortality after 14 d ¹	Reproductive output after 14 d ²	
[mg product/kg dry soil]	[%]	Mean no. Juveniles ± SD	Reproduction in [%] of Control (day 14)
Control: Demineralized water			
Control	9	255	-
Test item: Azoxystrobin 160 + Prothioconazole 200 g/L SC			
16.3	10	273	107
29.4	5	281	110
52.9	10	268	105
95.3	8	300	118
171	5	274	108
309	13	220	86
556	10	251	98
1000	5	259	102
Reference item: Dimethoate			
17.4	100	0	0*
14 d endpoints			
		[mg product/kg dry soil]	
NOEC (reproduction)		≥1000	
LOEC (reproduction)		>1000	
EC₁₀ (95% CI)		n.d. due to lack of dose-response	
EC₂₀ (95% CI)		>1000 ³	
EC₅₀ (95% CI)		>1000 ³	
NOEC (mortality)		≥1000	
LOEC (mortality)		>1000	
LC₅₀ (95% CI)		>1000	

n.d.= not determined; - = not applicable

* Statistically significantly different compared to control (Williams t-test, $\alpha = 0.05$, one-sided smaller)

¹ Fisher's Exact Test, $\alpha = 0.05$, one-sided greater

² Dunnett's t-test, $\alpha = 0.05$, one-sided smaller

³ Estimated value

B. VALIDITY CRITERIA

The mortality of female adults in the control was 9% (required $\leq 20\%$) at the end of the test, the mean reproduction rate was 239 to 274 juveniles in the control (required ≥ 50) and the coefficient of variation of reproduction in the control was 5.9% (required $\leq 30\%$). Therefore, the validity criteria of OECD 226 (2016) were fulfilled.

Conclusion

In this study on the chronic toxicity of Azoxystrobin 160 + Prothioconazole 200 g/L SC to *Hypoaspis aculeifer*, the overall NOEC was determined to be ≥ 1000 mg product/kg dry soil based on reproduction. The LC₅₀ was estimated to be >1000 mg test item/kg soil dry weight. The EC₁₀ could not be determined by statistical analysis since there was no adequate concentration response. However, the EC₂₀ and EC₅₀ were estimated to be >1000 mg test item/kg soil dry weight. The validity criteria of OECD 226 (2016) were fulfilled.

A 2.4.2.1.2 Study 15

Comment from zRMS:	The study was conducted in line with OECD 232 with no deviations. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: NOEC = 171 mg prod./kg soil dw EC₁₀ = 211.9 mg prod./kg soil dw (196.6 - 229.8)
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Reference:	KCP 10.4.2.1/02
Report	Azoxystrobin 160 + Prothioconazole 200 g/L SC: Effects on Reproduction of <i>Folsomia candida</i> in Artificial Soil, Hübner, S. (2024), Study no. 179211016
Guideline(s):	OECD 232 (2016)
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Executive summary

Juvenile springtails were exposed to soil treated with Azoxystrobin 160 + Prothioconazole 200 g/L SC at eight concentrations for a period of four weeks. A water control and a reference item were tested in parallel. After four weeks of exposure, the number of adults was counted and mortality was determined. The reproduction output was determined by counting the number of offspring (F1) that emerged from the eggs laid by the test animals.

In this study on the toxicity of Azoxystrobin 160 + Prothioconazole 200 g/L SC to *Folsomia candida*, the NOEC based on mortality was determined to be ≥ 1000 mg product/kg dry soil (equivalent to ≥ 144 mg Azoxystrobin + ≥ 179 mg Prothioconazole/kg soil dry weight). The NOEC based on reproduction was determined to be 171 mg product/kg dry soil (equivalent to 24.8 mg Azoxystrobin + 30.6 mg Prothioconazole/kg soil dry weight). The EC₁₀ was calculated to be 211.9 mg product/kg dry soil (equivalent to 30.6 mg Azoxystrobin + 37.9 mg Prothioconazole/kg soil dry weight) based on reproduction. The validity criteria of OECD 232 (2016) were met in the study.

Materials and Methods

A. MATERIALS

1. Test material	Azoxystrobin 160 + Prothioconazole 200 g/L SC
Description	Liquid, light brown, SC (suspension concentrate)
Lot/Batch #	ARD/AP/360/SC/36
Content of aa.ss.	Azoxystrobin: 162.20 ± 0.48 g/L ($14.45 \pm 0.04\%$ w/w) Prothioconazole: 200.54 ± 0.65 g/L ($17.86 \pm 0.06\%$ w/w) Density: 1.1228 ± 0.0012 g/mL
Stability of test material	Stable under storage conditions (20 ± 5 °C, in the dark) Expiry date: May 23, 2025
2. Vehicle and/or positive control	Vehicle control: Artificial soil mixed with demineralized water Positive control: Reference item

Reference item Boric Acid

3. Test organism

Species Springtail, *Folsomia candida* Willem (Collembola: Isotomidae)
Source In-house culture
Age Synchronous female juvenile springtails (9 - 12 d old)
Diet At the beginning of the test and after a period of 14 d, approx. 2 mg of granulated dry yeast was added to each test vessel.
Test units Glass vessels (volume: 100 mL; diameter: 5 cm) containing ~ 30 g of dry soil; closed tightly to avoid water evaporation

4. Environmental conditions

Soil Artificial soil was prepared with the following constituents (percentage distribution on dry weight basis):

Sphagnum peat	5%
Kaolin clay	20%
Industrial quartz sand	74.8%
Calcium carbonate CaCO ₃	0.2%

CaCO₃ was added to adjust the pH to 6.0 ± 0.5 .

The maximum water holding capacity (WHC) was measured to be 32% of the dry weight. The soil was pre-moistened to half of the final water content 2 days before the application. Water content of the test soil was brought to 50% of the maximum WHC during soil preparation.

Temperature Nominal: 20 ± 2 °C
Water holding capacity At test start: 17.5% to 17.8% (54.6% to 55.6% of the maximum water holding capacity). At test end: 15.3% to 16.4% (47.9% to 51.4% of the maximum water holding capacity).
pH Nominal: 6.0 ± 0.5 at test start; actual: 5.8 - 6.0 at test start and 5.6 - 5.8 at test end
Photoperiod 16 h light (light intensity: nominal: 400 - 800 Lux) to 8 h dark photoperiod

B. STUDY DESIGN AND METHODS

1. In-life dates 20 Sept 2023 to 19 Oct 2023 (experimental period)

2. Experimental conditions

Test design

Juvenile springtails were exposed to soil treated with Azoxystrobin 160 + Prothioconazole 200 g/L SC at eight concentrations for a period of four weeks. A water control and a reference item were tested in parallel. After four weeks of exposure, the number of adults was counted and mortality was determined. The reproduction output was determined by counting the number of offspring (F1) that emerged from the eggs laid by the test animals.

Number of animals per treatment

Four replicates per test item/reference item group and eight replicates for the control group were used with ten springtails per replicate.

Test concentrations

Azoxystrobin 160 + Prothioconazole 200 g/L SC was tested at 16.3, 29.4, 52.9, 95.3, 171, 309, 556 and 1000 mg product/kg dry soil, equivalent to 2.36 + 2.92, 4.25 + 5.25, 7.65 + 9.45, 13.8 + 17.0, 24.8 + 30.6, 44.6 + 55.1, 80.3 + 99.2 and 144 + 179 mg Azoxystrobin + mg Prothioconazole /kg soil dry weight (conversion based on a content of 162.20 g/L Azoxystrobin and 200.54 g/L Prothioconazole and a density of 1.1228 g/mL of the test item). Test item concentrations were chosen based on a non-GLP range finding test. A test water control (demineralized water) was tested in parallel. In addition, boric acid was tested at 30.5, 48.8, 78.1, 125, and 200 mg/kg dry soil as reference item.

Treatment/Application

For the test item treatments, a stock solution (= application solution for the highest test item group) was prepared by dissolving 1100 mg of test item in deionised water to obtain a final net weight of 97.1 g. Application solutions were prepared by diluting appropriate volumes of the stock solution with water. The soil for each treatment group was mixed with a laboratory mixer to ensure a homogeneous distribution. Each group was treated in one batch and then split into the replicates. After adding the application solutions, the soil moisture was at 50% of WHC_{max}. Subsequently, approx. 30 g of corresponding dry substrate were transferred into each vessel.

The control was prepared by mixing demineralized water into the artificial soil.

3. Sampling and measurements

At the end of the study after four weeks of exposure, the content of the test containers was suspended in water, the suspension was tinted with dark ink and stirred with a fine brush. Adult animals were counted once visually, juvenile animals were counted using FolsomiaCounter, a photo based evaluation software (FolsomiaCounter Version 1.30, © 2022 Visionalytics). Missing adult springtails were considered dead. Any symptoms of toxicity and abnormal behavior were recorded.

To determine the average error of counting, a validation of the flotation method was performed prior to this study. To this end, a known number of adults and juveniles were added to the same type of soil as used in this study, and extraction and counting was performed as explained above. Over two replicates, a mean counting efficiency of 98.3% was documented (required: > 95%).

Prior to the start of the test, the water holding capacity (WHC) was determined in five samples of the artificial soil. At the start of the test, the soil moisture was determined with excess soil for each treatment group. At test end, measurements were made in one extra vessel prepared for each treatment group without test organisms. The initial weight of each test vessel and the weight after 14 d were recorded and the water loss did not exceed 2%.

Soil moisture and pH were determined after application of the test item and at test end for the test item and reference groups and for the control.

4. Calculation of toxicity

Mortality (number of dead adults) in percent for each treatment group was calculated. The reproductive output was calculated based on the number of juveniles for each treatment group.

5. Statistics

Mortality data were statistically analyzed using Fisher's Exact Binomial Test (multiple comparison, with Bonferroni Correction, $\alpha = 0.05$, one-sided greater). The LC_{50} at day 28 was not determined by statistical analysis as no mortality above 50% was observed.

Reproduction data were tested for normal distribution and homogeneity of variance using Shapiro-Wilk's test and Levene's test ($\alpha = 0.01$). Since the reproduction data were normally distributed and homogeneous and did follow a monotonicity trend (contrast trend) the Williams t-test (multiple comparison, $\alpha = 0.05$, one-sided smaller) was used to compare treatment and control values. The determination of the NOEC and LOEC values was based on the results of the statistical evaluation. The EC values for reproduction were calculated by 3-param. normal CDF.

The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ToxRat® Solutions GmbH.

Results and Discussion

A. BIOLOGICAL RESULTS

In the control, mean mortality of adult test organisms after four weeks was 6%. Mortality ranged from 3 - 13% in the test item groups and was not statistically significantly different from the control for any test item group (see following table).

In the control, the mean number of juveniles was 860. The mean reproduction in the test item treatments up to and including 171 mg product/kg dry soil was 798 - 882 juveniles which was not statistically significantly different from the control, while in the three higher treatments the number of juveniles was statistically significantly different (see table below).

The reference item showed statistically significant effects on reproduction at concentrations of 30.5 mg/kg soil dry weight and above. The EC_{50} for reproduction was calculated to be 79.3 mg/kg soil dry weight. Reproduction was reduced by 100% compared to the control at a nominal concentration of 200 mg/kg dry soil of boric acid.

No abnormal behavior or other sublethal effects were observed during the test.

Table 1: Effects of Azoxystrobin 160 + Prothioconazole 200 g/L SC on survival and reproduction of *Folsomia candida*

Nominal test concentrations		Mean mortality after 28 d ²	Reproductive output after 28 d ³	
[mg product/kg dry soil]	[mg aa.ss./kg dry soil] ¹	[%]	Mean no. juveniles ± SD	Reproduction in [%] of Control (day 28)
Control: Demineralized water				
Control		6	860	-
Test item: Azoxystrobin 160 + Prothioconazole 200 g/L SC				
16.3	2.36+2.92	0	821	95
29.4	4.25+5.25	0	842	98
52.9	7.65+9.45	3	882	103
95.3	13.8+17.0	3	801	93
171	24.8+30.6	10	798	93
309	44.6+55.1	5	694	81*
556	80.3+99.2	13	465	54*
1000	144+179	10	261	30*
Reference item: Boric acid				
200	-	83*	3	0*
28 d endpoints				
		[mg product/kg dry soil]	[mg aa.ss./kg dry soil]	
NOEC (reproduction)		171	24.8 + 30.6	
LOEC (reproduction)		309	44.6 + 55.1	
EC₁₀ (95% CI)		211.9 (196.6 - 229.8)	30.6 + 37.9 (28.4 - 33.2 + 35.1 - 41.0)	
EC₂₀ (95% CI)		310.0 (292.3 - 329.8)	44.8 + 55.4 (42.2 - 47.6 + 52.2 - 58.9)	
EC₅₀ (95% CI)		641.9 (624.3 - 661.7)	92.7 + 114.6 (90.2 - 95.6 + 111.5 - 118.2)	
NOEC (mortality)		≥1000	≥144 + ≥179	
LOEC (mortality)		>1000	>144 + >179	
LC₅₀		>1000 ⁴	>144 + >179	

n.d.= not determined; - = not applicable

* Statistically significantly different compared to control (Williams t-test, $\alpha = 0.05$, one-sided smaller)

¹ Based on a content of 162.20 g/L Azoxystrobin and 200.54 g/L Prothioconazole and a density of 1.1228 g/mL

² Fisher's Exact Test, $\alpha = 0.05$, one-sided greater

³ Williams t-test, $\alpha = 0.05$, one-sided smaller

⁴ Estimated value

B. VALIDITY CRITERIA

The mean mortality of adults in the control was 6% (required: $\leq 20\%$) at the end of the test, the mean reproduction rate ranged from 724 to 1022 juveniles per control replicate (required: ≥ 100) and the coefficient of variation for reproduction was 12.2% in the control (required: $\leq 30\%$). Therefore, the validity criteria of OECD 232 (2016) were fulfilled.

Conclusion

In this study on the toxicity of Azoxystrobin 160 + Prothioconazole 200 g/L SC to *Folsomia candida*, the NOEC based on mortality was determined to be ≥ 1000 mg product/kg dry soil (equivalent to ≥ 144 mg Azoxystrobin + ≥ 179 mg Prothioconazole/kg soil dry weight). The NOEC based on reproduction was determined to be 171 mg product/kg dry soil (equivalent to 24.8 mg Azoxystrobin + 30.6 mg Prothioconazole/kg soil dry weight). The EC₁₀ was calculated to be 211.9 mg product/kg dry soil (equivalent to 30.6 mg Azoxystrobin + 37.9 mg Prothioconazole/kg soil dry weight) based on reproduction. The validity criteria of OECD 232 (2016) were met in the study.

A 2.4.2.2 KCP 10.4.2.2 Higher tier testing

Not required based on the outcome off the risk assessment.

A 2.5 KCP 10.5 Effects on soil nitrogen transformation

A 2.5.1 Study 16

Comment from zRMS:	The study was conducted in line with OECD 216 with no deviations. All the validity criteria were met. Azoxystrobin 160 + Prothioconazole 200 g/L SC had no long-term adverse effects on nitrogen transformation activity of soil microflora after 70 d of incubation when applied at 1.50 and 15.0 mg product/kg dry soil. The validity criterion of OECD 216 (2000) was fulfilled.
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Reference:	KCP 10.5/01
Report	Azoxystrobin 160 + Prothioconazole 200 g/L SC: Effects on the Activity of the Soil Microflora in the Laboratory (Nitrogen Transformation), Hammesfahr, U. (2024), Study no. 179211080
Guideline(s):	OECD 216 (2000)
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Executive Summary

Soil samples were treated once with the test item at two concentrations (low and high concentration) mixed into the soil or remained untreated (control receiving ultrapure water only). The incubation of the soil samples was performed as bulk sample for each test item group and the control. Effects on nitrogen transformation activity of the soil microflora were assessed by determination of the nitrate contents at day 0, 7, 14, and 28. Based on the analysed nitrate contents, nitrate transformation rates were calculated.

Azoxystrobin 160 + Prothioconazole 200 g/L SC had no long-term adverse effects on nitrogen transformation activity of soil microflora after 28 d of incubation when applied at 0.42 and 2.1 mg product/kg dry soil. The validity criterion of OECD 216 (2000) was fulfilled.

Materials and Methods

A. MATERIALS

1. Test material	Azoxystrobin 160 + Prothioconazole 200 g/L SC
Description	Liquid, light brown, SC (suspension concentrate)
Lot/Batch #	ARD/AP/360/SC/36
Content of aa.ss.	Azoxystrobin: 162.20 ± 0.48 g/L ($14.45 \pm 0.04\%$ w/w) Prothioconazole: 200.54 ± 0.65 g/L ($17.86 \pm 0.06\%$ w/w) Density: 1.1228 ± 0.0012 g/mL
Stability of test material	Stable under storage conditions (20 ± 5 °C, in the dark)

Expiry date: May 23, 2025

2. Vehicle and/or positive control	Vehicle control: Ultrapure water Positive control: Reference item																				
Reference item	Sodium chloride																				
3. Test soil	<table> <tr> <td>Soil type:</td><td>Lufa soil F5M; loamy sand</td></tr> <tr> <td>Batch:</td><td>F 5M 4023</td></tr> <tr> <td>Organic C:</td><td>0.97%</td></tr> <tr> <td>pH:</td><td>7.5</td></tr> <tr> <td>Max. water holding cap. (WHC):</td><td>41.6%</td></tr> <tr> <td>Particle size analyses (USDA):</td><td></td></tr> <tr> <td> clay:</td><td>12.4%</td></tr> <tr> <td> silt:</td><td>36.5%</td></tr> <tr> <td> sand:</td><td>51.2%</td></tr> <tr> <td>Microbial biomass:</td><td>121.61 mg C/kg dry soil (1.25% of organic C)</td></tr> </table>	Soil type:	Lufa soil F5M; loamy sand	Batch:	F 5M 4023	Organic C:	0.97%	pH:	7.5	Max. water holding cap. (WHC):	41.6%	Particle size analyses (USDA):		clay:	12.4%	silt:	36.5%	sand:	51.2%	Microbial biomass:	121.61 mg C/kg dry soil (1.25% of organic C)
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clay:	12.4%																				
silt:	36.5%																				
sand:	51.2%																				
Microbial biomass:	121.61 mg C/kg dry soil (1.25% of organic C)																				
Source	Fallow grassland: District authority: Rhineland Palatinate Municipality: Mechtersheim, Germany																				
Soil history	Location: “In der Speyerer Hohl “, No. 977 The soil was sampled from an uncultivated field that had not been subjected to any pesticide or organic fertilizer treatment for at least four years prior to sampling.																				
Soil sampling	6 Oct 2023 The soil was collected from a depth of 0-20 cm, air dried and sieved (mesh 2 mm) at room temperature. The soil was stored at 20°C ± 2°C with appropriate ventilation and periodical moisture adjustment for 15 days. The soil dry weight prior to test start was determined to be 90.0%.																				
Soil preparation	Pre-incubation of the soil under test conditions was initiated 15 d prior to the test.																				
Test units	Disposable plastic boxes 300 g soil (dry weight), box size approximately 0.5 L (dimensions: 0.10 m width x 0.10 m depth x 0.065 m height).																				
4. Environmental conditions																					
Temperature	Nominal: 20 ± 2 °C																				
Soil moisture	Nominal: 40 - 60% of WHC _{max} actual: 45 - 53% of WHC _{max}																				
pH	7.4 - 7.6																				
Photoperiod	Constant darkness																				

B. STUDY DESIGN AND METHODS

1. In-life dates 15 Nov 2023 to 26 Jan 2024

2. Experimental conditions

Test design

Soil samples were treated once with the test item at two concentrations (low and high concentration) mixed into the soil or remained untreated (control receiving ultrapure water only). The incubation of the soil sam-

ples was performed as bulk sample for each test item group and the control. Effects on nitrogen transformation activity of the soil microflora were assessed by determination of the nitrate contents at day 0, 7, 14, 28, 42, 56 and 70. Based on the analysed nitrate contents, nitrate transformation rates were calculated.

Number of replicates per treatment

Three replicates per test item/control group taken from the bulk samples at every sampling time.

Test concentrations

Azoxystrobin 160 + Prothioconazole 200 g/L SC was tested at 1.5 mg product/kg dry soil (low application rate) and 15.0 mg product/kg dry soil (high application rate). In addition, an untreated control (receiving ultrapure water only) was tested in parallel.

Treatment/Application

A stock solution was obtained by dissolving 150 mg of Azoxystrobin 160 + Prothioconazole 200 g/L SC in 100 mL of ultrapure water. Afterwards, appropriate amounts of the stock solution and additionally 0.5% lucerne meal (related to soil dry weight) were mixed to the soil using a laboratory mixer. Throughout the application the soil was ventilated, and the soil water content was adjusted to 48% of WHC. For the control, the soil was treated with ultrapure water only.

3. Sampling and measurements

Soil samples of approximately 25 g dry soil per replicate were taken at 6 h after application and on day 7, 14, 28, 42, 56 and 70 for determination of the nitrate (NO_3) content. The soil samples were extracted with 0.1 M KCl and nitrate concentrations in the extracts were determined at a wavelength of 550 nm by using an AA3 Continuous Flow Analyzer (Software AACE Vers 6.04GLP). The nitrate formation rate was calculated using a cumulative approach i.e. the difference between soil nitrate contents from a sampling date and day 0.

Temperature was recorded continuously. Short-term deviations (≤ 2 hours) from the recommended temperature range are not expected to result in major disturbances of the test performance. Dry weight and water content were determined at test start and on each sampling date gravimetrically. The soil water content was checked once a week until experimental end by re-weighing each test container and adding ultrapure water as needed to compensate water losses. The pH was determined at test start and on days 28, 42, 56 and 70 in one replicate of each treatment group.

4. Calculation of toxicity

For the determination of nitrogen content, soil samples were taken within 6 hours after application and afterwards on each sampling date (7, 14, 28, 42, 56 and 70 days). The nitrogen content was determined in each sample of treated and control soils.

For extraction, data were given as individual values for the three replicates and mean values. Furthermore, the nitrogen transformation rate/time interval/day for exposure intervals related to test start and for incremental exposure intervals were calculated for each treatment group (i.e. day 0-7, 0-14, and 0-28 as well as 0-7, 7-14, and 14-28, etc.). The percentage deviations in the quantities of nitrate formed between the control and the test item treatment groups were calculated for nitrogen transformation rate/time interval/day for the overall exposure periods and incremental exposure periods. Adverse effects were evaluated based on the trigger value of 25% deviation of the nitrogen transformation rate/time interval/day of the treated soils compared to the control.

5. Statistics

Data for soil nitrogen contents were tested for normality and homogeneity of variance using the R/S-Test ($\alpha = 0.01$) and Levene's test ($\alpha = 0.01$), respectively. The Student's t-test (pair wise comparison, two-sided, $\alpha = 0.05$) was used for comparison of treated and control values.

The software used to conduct the statistical analysis was ToxRat Professional, Version 3.3.0. ®.

Results and Discussion

A. BIOLOGICAL RESULTS

Adverse effects of the test item on nitrate content in soil were observed on day 28. On day 28 differences to the control were 18.05% and 42.56% in the low- and high-test item group, respectively. For the overall exposure period no adverse effects of the test item on nitrate content in soil were observed at test end on day 70. On day 70 differences to the control were 4.03% and 11.90% in the low- and high-test item group, respectively. Results are presented in the table below.

Table 1: Effects of Azoxystrobin 160 + Prothioconazole 200 g/L SC on nitrogen transformation in soil

NO ₃ - Contents [mg nitrate/kg dry soil]						
	Control		1.50 mg product/kg dry soil		15.0 mg product/kg dry soil	
Sampling	Nitrate-N Content	Replicate Variation ^{a)}	Nitrate content	[%] Deviation ^{b)}	Nitrate content	[%] Deviation ^{b)}
Day 0	12.624	0.36	12.756	1.05	12.526	-0.78
Day 7	7.632	5.90	16.021	109.92*	24.220	217.35*
Day 14	16.666	2.76	22.163	32.98*	31.016	86.10*
Day 28	28.750	4.13	33.939	18.05*	40.985	42.56*
Day 42	40.472	5.09	43.597	7.72	48.376	19.53*
Day 56	46.321	4.32	50.349	8.70*	55.681	20.21*
Day 70	56.958	1.09	59.254	4.03	63.738	11.90*
NO ₃ - Formation rate [mg/kg dry soil/day]						
	Control		1.50 mg product/kg dry soil		15.0 mg product/kg dry soil	
Interval	Nitrate formation	Replicate Variation ^{a)}	Nitrate formation	[%] Deviation ^{b)}	Nitrate formation	[%] Deviation ^{b)}
Day 0-7	-0.713	9.68	0.466	165.36*	1.671	334.36*
Day 0-14	0.289	11.07	0.672	132.53*	1.321	354.09*
Day 0-28	0.576	7.12	0.756	31.25*	1.016	76.39*
Day 0-42	0.663	7.24	0.734	10.71	0.854	28.81*
Day 0-56	0.602	5.65	0.671	11.46*	0.771	28.07*
Day 0-70	0.633	1.42	0.664	4.90	0.732	15.64*
NO ₃ - Formation rate [mg/kg dry soil/day]						
	Control		1.50 mg product/kg dry soil		15.0 mg product/kg dry soil	
Interval	Nitrate formation	Replicate Variation ^{a)}	Nitrate formation	[%] Deviation ^{b)}	Nitrate formation	[%] Deviation ^{b)}
Day 0-7	-0.713	9.68	0.466	165.36*	1.671	334.36*
Day 7-14	1.291	9.53	0.877	-32.07*	0.971	-24.79*
Day 14-28	0.863	10.54	0.841	-2.55	0.712	-17.50*
Day 28-42	0.838	7.64	0.690	-17.66	0.528	-36.99*
Day 42-56	0.418	11.24	0.482	15.31	0.522	24.88
Day 56-70	0.760	24.47	0.636	-16.32	0.575	-24.34

^{a)} % variation within control replicates (coefficient of variation)

^{b)} % deviation to control; negative values indicate a higher nitrate content compared to the control

* Significance according to Student's t-test (two sided, $\alpha = 0.05$)

B. VALIDITY CRITERIA

The maximum variation in soil nitrate concentrations was 5.90% in the control replicates throughout the entire test period (required: $< \pm 15\%$) and thus the validity criterion of OECD 216 (2000) was fulfilled.

Conclusion

Azoxystrobin 160 + Prothioconazole 200 g/L SC had no long-term adverse effects on nitrogen transformation activity of soil microflora after 70 d of incubation when applied at 1.50 and 15.0 mg product/kg dry soil. The validity criterion of OECD 216 (2000) was fulfilled.

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

No additional data submitted

A 2.6.2 KCP 10.6.2 Testing on non-target plants

A 2.6.2.1 Study 17

Comment from zRMS:	The study was conducted in line with OECD 208 with major deviation in environmental conditions. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: ER₅₀ is > 1.9 L product/ha. The NOER ≥ 1.9 L product/ha, and the LOER was estimated to be > 1.9 L product/ha. No phytotoxic effects were observed for any species tested.
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Reference:	KCP 10.6.2/01
Report	Azoxystrobin 160 + Prothioconazole 200 g/L SC: Effects on Terrestrial (Non-Target) Plants: Seedling Emergence and Seedling Growth Test, Stürtz, S., Horstmann, C. (2024), Study no. 179211086
Guideline(s):	OECD 208 (2006)
Deviations:	None.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Executive summary

The inhibitory effect of Azoxystrobin 160 + Prothioconazole 200 g/L SC on seedling emergence and seedling early growth of four dicotyledons (small radish, cucumber, soybean, and lettuce) and two monocotyledons (garden onion and ryegrass) was investigated in a limit test design over a test period of 21 d following 50% emergence in the control. Azoxystrobin 160 + Prothioconazole 200 g/L SC was sprayed onto the soil substrate where seeds of the test species had been sown shortly before application. The test plants were assessed for seedling emergence, post-emergence mortality and phytotoxicity symptoms on day 14 and 21 of the observation period as well as the growth stage at test termination.

In this study on seedling emergence of plants treated with Azoxystrobin 160 + Prothioconazole 200 g/L SC, no statistically significant effects on seedling emergence and post-emergence mortality were observed. Thus, the ER₅₀ is supposed to be set as >1.9 L/ha. The LOER was estimated to be ≤1.90 L product/ha for small radish and soybean. The validity criteria of OECD 208 (2006) were fulfilled.

Materials and Methods

A. MATERIALS

1. Test material	Azoxystrobin 160 + Prothioconazole 200 g/L SC
Description	Liquid, light brown, SC (suspension concentrate)
Lot/Batch #	ARD/AP/360/SC/36
Content of aa.ss.	Azoxystrobin: 162.20 ± 0.48 g/L ($14.45 \pm 0.04\%$ w/w) Prothioconazole: 200.54 ± 0.65 g/L ($17.86 \pm 0.06\%$ w/w) Density: 1.1228 ± 0.0012 g/mL
Stability of test material	Stable under storage conditions (20 ± 5 °C, in the dark) Expiry date: May 23, 2025
2. Vehicle and/or positive control	Vehicle control: Deionised water
3. Test plants	Four dicotyledonous species: Small radish <i>Raphanus sativus</i> (Brassicaceae) Cucumber <i>Cucumis sativus</i> (Cucurbitaceae) Soybean <i>Glycine max</i> (Fabaceae) Lettuce <i>Lactuca sativa</i> (Asteraceae) Two monocotyledonous species: Garden onion <i>Allium cepa</i> (Amaryllidaceae) Perennial ryegrass <i>Lolium perenne</i> (Poaceae)
Test containers	Commercial plastic flowerpots, diameter of 15 cm
4. Environmental conditions	Test plants were grown under controlled climatic conditions in a growth chamber.
Temperature	Nominal: 22 ± 10 °C; actual mean: 19.8 °C ($16.4 - 22.6$ °C, deviation < 2 h)
Relative humidity	Nominal: $70 \pm 25\%$; actual mean: 63% ($61 - 83\%$, deviation < 2 h)
Photoperiod	16 h light and 8 h dark; mean light intensity: $322 \mu\text{E}/\text{m}^2/\text{s}$ ($300 - 400 \mu\text{E}/\text{m}^2/\text{s}$).
Watering	The water supply was regularly controlled and replenished via the plant saucers.
Fertilization	After development of the first true leaves, Ferty® 9 “Hydro” (Planta-Düngemittel GmbH) at 3 g/L and Terraflor®-AZ (Terraflor GmbH) at 0.4 g/L were added to the water up to two times a week, depending on the development of the plants via the plant saucers.

B. STUDY DESIGN AND METHODS

1. In-life dates 25 Oct 2023 to 21 Nov 2023 (experimental period)

2. Experimental conditions

Test design

The inhibitory effect of Azoxystrobin 160 + Prothioconazole 200 g/L SC on seedling emergence and seedling early growth of four dicotyledons (small radish, cucumber, soybean, and lettuce) and two monocotyledons (onion and ryegrass) was investigated over a test period of 21 d following 50% emergence in the control. Azoxystrobin 160 + Prothioconazole 200 g/L SC was sprayed at 1.90 L/ha onto the soil substrate where seeds of the test species had been sown one day before application. The test plants were assessed for

seedling emergence, post-emergence mortality and phytotoxicity symptoms on day 14 and 21 of the observation period. Furthermore, growth stage and shoot dry weight were determined at test termination.

Number of replicates per treatment

Each treatment group consisted of at least 20 plants, divided into seven to ten pots containing two to three plants each for all dicotyledons, and into five pots containing four seeds each for all monocotyledons.

Test concentrations

The test plants were treated with Azoxystrobin 160 + Prothioconazole 200 g/L SC at a single application rate of 1.90 L product/ha (corresponding to 21.4 g prod./L). For each test species, a control receiving deionised water was tested in addition.

Treatment/Application

Prior to experimental start, the seeds of the test plants were sown in pots containing the soil substrate (soil type: LUFA 2.3, sandy loam), with a pH of 6.0 ± 0.4 and a total organic matter content of $0.66 \pm 0.05\%$. The pots were kept under the same climatic conditions as in the test.

The test substance was applied in a water volume of 100 L/ha. The controls received deionised water at 100 L/ha. Application was performed with a laboratory track-sprayer (Company Schachtner, Ludwigsburg, Germany) with Teejet 80015 EVS nozzles and a spraying pressure of 2.3 bar. Prior to application, the apparatus was calibrated to provide the required application volume.

The spray mixture for the highest rate was prepared by diluting 10.7 g Azoxystrobin 160 + Prothioconazole 200 g/L SC to 500 mL with deionised water. The control received deionised water only.

Analytics

The stock solution and control were checked analytically by HPLC-UV. The analytical method was validated according to SANTE/2020/12830 (Rev.2.).

3. Sampling and measurements

Plant pots for all species were observed 14 and 21 d after 50% of the control seeds had emerged to determine the number of emerged seeds, the cumulative number of dead plants and to visually assess any phytotoxic symptoms. The condition of test plants was assessed during the in-life phase utilizing a rating system based upon EPPO Standard PP 1/135 (2014), considering necrosis, chlorosis and other characteristics that might be treatment related.

Growth stage of the plants was assessed for all treatment groups on the last assessment day.

Test temperature and air humidity were recorded continuously throughout the test. Light conditions were measured once a week during the entire study with 5 different measuring points for each species.

4. Calculation of toxicity

Fresh weight, emergence and mortality of the plants treated with the test item were compared with the results of the control plants.

5. Statistics

Fresh weight data were tested for normal distribution and homogeneity of variance using the Shapiro-Wilk's test ($\alpha = 0.01$) and the Levene's test ($\alpha = 0.01$). If the data were normally distributed and homogeneous the Student's t-test (two sample comparison, one-sided smaller, $\alpha = 0.05$) was used, and if the data

were normally distributed but not homogeneous the Welch t-test (two sample comparison, one-sided smaller, $\alpha = 0.05$) was used for comparing the treatment group with the control.

For the mortality and emergence data Fisher's Exact Binomial Test (with Bonferroni Correction, multiple comparison, one-sided greater, $\alpha = 0.05$) was used.

The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ® ToxRat Solutions GmbH.

Results and Discussion

A. VERIFICATION OF APPLICATION RATE

The analytical recovery rate of Azoxystrobin in the stock solution was 103% of the nominal value and the analytical recovery rate of Prothioconazole in the stock solution was 97% of the nominal value. In the control solution no test item ingredients were detected. Therefore, the study endpoints were based on nominal application rates.

B. SEEDLING EMERGENCE

Seedling emergence was not statistically significantly reduced compared to the control for any test species.

C. FRESH WEIGHT

The fresh weight was reduced for *Raphanus sativus* (-9.4%) and *Glycine max* (-11.9%) at 1.90 L test item/ha. Most sensitive species resulted to be *Lolium perenne*, with a reduction in fresh weight of -31.4%.

Table 1. Inhibitory effect of Azoxystrobin 160 + Prothioconazole 200 g/L SC on fresh weight (21 d after 50% emergence in the control)

Plant species	NOER [L product/ha]	LOER [L product/ha]
Small radish (<i>Raphanus sativus</i>)	> 1.90	≤1.90
Cucumber (<i>Cucumis sativus</i>)	1.90	> 1.90
Soybean (<i>Glycine max</i>)	> 1.90	≤1.90
Lettuce (<i>Lactuca sativa</i>)	1.90	> 1.90
Garden onion (<i>Allium cepa</i>)	1.90	> 1.90
Perennial ryegrass (<i>Lolium perenne</i>)	1.90	> 1.90

D. MORTALITY

Mortality was observed for *Allium cepa* at 1.90 L test item/ha (5%). The mortality counts turned out to be not statistically significant.

E. PHYTOTOXICITY

No phytotoxic effects were observed for any species tested.

F. GROWTH STAGE

No differences in the growth stage of the plants were observed between the test item groups and the controls in any of the species tested on the last assessment day.

G. VALIDITY CRITERIA

The seedling emergence in the control was 85 - 100% (required: $\geq 70\%$) at test end. Control plants did not exhibit visible phytotoxic effects and mean plant survival was 100% (required: $\geq 90\%$) at test end. Furthermore, environmental conditions and growing media for a particular species were identical. Therefore, all validity criteria of OECD 208 (2006) were fulfilled.

Conclusion

In this study on seedling emergence of plants treated with Azoxystrobin 160 + Prothioconazole 200 g/L SC, no statistically significant effects on seedling emergence and post-emergence mortality were observed. Thus, the ER₅₀ is supposed to be set as >1.9 L/ha. The LOER was estimated to be ≤ 1.90 L product/ha for small radish and soybean. The validity criteria of OECD 208 (2006) were fulfilled.

A 2.6.2.2 Study 18

Comment from zRMS:	The study was conducted in line with OECD 227 with major deviation in environmental conditions. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: ER₅₀ > 1.9 L product/ha. The NOER ≥ 1.9 L product/ha, and the LOER was estimated to be > 1.9 L product/ha. Phytotoxic effects were detected only in <i>Raphanus sativus</i> (discoloration and necrosis, 3%). No further effects were detected.
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Reference:	KCP 10.6.2/02
Report	Azoxystrobin 160 + Prothioconazole 200 g/L SC: Effects on Terrestrial (Non-Target) Plants: Vegetative Vigour Test, Stürtz, S., Horstmann, C. (2024), Study No. 179211087
Guideline(s):	OECD 227 (2006)
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Executive summary

The inhibitory effect of Azoxystrobin 160 + Prothioconazole 200 g/L SC on vegetative vigour of four dicotyledons (small radish, cucumber, soybean, and lettuce) and two monocotyledons (garden onion and ryegrass) was investigated in a limit test design over a test period of 21 d. Azoxystrobin 160 + Prothioconazole 200 g/L SC was applied when the plants had reached BBCH 12-14. The test plants were assessed for mortality and phytotoxicity symptoms on days 7, 14 and 21, and plant fresh weight and plant growth stage were determined at test termination.

Based on the results of this vegetative vigour test with Azoxystrobin 160 + Prothioconazole 200 g/L SC, no statistically significant effects on mortality and fresh weight were observed. Non-significant phytotoxic effects (i.e., discoloration and necrosis) were observed only in *Raphanus sativus* at 3%. Thus, the ER₅₀ is supposed to be set as >1.9 L/ha. The NOER was determined to be ≥ 1.9 L product/ha, and the LOER was estimated to be > 1.9 L product/ha. All validity criteria of OECD 227 (2006) were fulfilled in this study.

Materials and Methods

A. MATERIALS

1. Test material	Azoxystrobin 160 + Prothioconazole 200 g/L SC
Description	Liquid, light brown, SC (suspension concentrate)
Lot/Batch #	ARD/AP/360/SC/36
Content of aa.ss.	Azoxystrobin: 162.20 ± 0.48 g/L ($14.45 \pm 0.04\%$ w/w) Prothioconazole: 200.54 ± 0.65 g/L ($17.86 \pm 0.06\%$ w/w) Density: 1.1228 ± 0.0012 g/mL
Stability of test material	Stable under storage conditions (20 ± 5 °C, in the dark) Expiry date: May 23, 2025
2. Vehicle and/or positive control	Vehicle control: Deionised water
3. Test plants	Four dicotyledonous species: Small radish <i>Raphanus sativus</i> (Brassicaceae) Cucumber <i>Cucumis sativus</i> (Cucurbitaceae) Soybean <i>Glycine max</i> (Fabaceae) Lettuce <i>Lactuca sativa</i> (Asteraceae) Two monocotyledonous species: Garden onion <i>Allium cepa</i> (Amaryllidaceae) Perennial ryegrass <i>Lolium perenne</i> (Poaceae)
Test containers	Commercial plastic flowerpots, diameter of 15 cm
4. Environmental conditions	Test plants were grown under controlled climatic conditions in a growth chamber.
Temperature	Nominal: 22 ± 10 °C; actual mean: 19.9 °C ($16.4 - 22.6$ °C, deviation < 2 h)
Relative humidity	Nominal: $70 \pm 25\%$; actual mean: 63% ($51 - 82\%$, deviation < 2 h)
Photoperiod	16 h light and 8 h dark; mean light intensity: $341 \mu\text{E}/\text{m}^2/\text{s}$ ($300 - 400 \mu\text{E}/\text{m}^2/\text{s}$).
Watering	The water supply was regularly controlled and replenished via the plant saucers.
Fertilization	After development of the first true leaves, Ferty® 9 “Hydro” (Planta-Düngemittel GmbH) at 3 g/L and Terraflor®-AZ (Terraflor GmbH) at 0.4 g/L were added to the water up to two times a week, depending on the development of the plants via the plant saucers.

B. STUDY DESIGN AND METHODS

1. In-life dates 25 Oct 2023 to 15 Nov 2023 (experimental period)

2. Experimental conditions

Test design

The inhibitory effect of Azoxystrobin 160 + Prothioconazole 200 g/L SC on vegetative vigour of four dicotyledons (small radish, cucumber, soybean, and lettuce) and two monocotyledons (onion and ryegrass) was investigated. Azoxystrobin 160 + Prothioconazole 200 g/L SC was applied when the plants had reached BBCH 12-13. The test plants were assessed for mortality and phytotoxicity symptoms on days 7, 14 and 21, and plant fresh weight and plant growth stage were determined at test termination.

Number of replicates per treatment

Each treatment group consisted of a total of at least 20 plants, divided into seven to ten pots containing two to three plants each for all dicotyledons, and into five pots containing four seeds each for all monocotyledons.

Test concentrations

The test plants were treated with Azoxystrobin 160 + Prothioconazole 200 g/L SC at a single application rate of 1.90 L product/ha (corresponding to 21.4 g prod./L). For each test species, a control receiving deionised water was tested in addition.

Treatment/Application

Prior to experimental start, the seeds of the test plants were sown in pots containing the soil substrate (soil type: LUFA 2.3, sandy loam), with a pH of 6.0 ± 0.4 and a total organic matter content of $0.66 \pm 0.05\%$. The pots were kept under the same climatic conditions as in the test. The test substance was applied when the plants had reached BBCH 12-14 (test start).

The test substance was applied in a water volume of 100 L/ha. The controls received deionised water at 100 L/ha. Application was performed with a laboratory track-sprayer (Company Schachtner, Ludwigsburg, Germany) with Teejet 80015 EVS nozzles and a spraying pressure of 2.3 bar. Prior to application, the apparatus was calibrated to provide the required application volume.

The spray mixture for the highest rate was prepared by diluting 10.7 g Azoxystrobin 160 + Prothioconazole 200 g/L SC to 500 mL with deionised water. The control received deionised water only.

Analytics

The stock solution and control were checked analytically by HPLC-UV. The analytical method was validated according to SANTE/2020/12830 (Rev.2.).

3. Sampling and measurements

The test plants were assessed for mortality and phytotoxicity symptoms on days 7, 14 and 21. Phytotoxicity was assessed during the in-life phase utilizing a rating system based upon EPPO Standard PP 1/135 (2014).

Growth stage of the plants was assessed for all treatment groups on the last assessment day.

Test temperature and air humidity were recorded continuously throughout the test. Light conditions were measured once a week during the entire study with 5 different measuring points for each species.

4. Calculation of toxicity

Fresh weight, emergence and mortality of the plants treated with the test item were compared with the results of the control plants.

5. Statistics

Fresh weight data were tested for normal distribution and homogeneity of variance using the Shapiro-Wilk's test ($\alpha = 0.01$) and the Levene's test ($\alpha = 0.01$). For comparison between control and the treatment groups, the Student's t-test (two sample comparison, one-sided smaller, $\alpha = 0.05$) was used.

The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ® ToxRat Solutions GmbH.

Results and Discussion

A. VERIFICATION OF APPLICATION RATE

The analytical recovery rate of Azoxystrobin in the stock solution was 103% of the nominal value and the analytical recovery rate of Prothioconazole in the stock solution was 97% of the nominal value. In the control solution no test item ingredients were detected. Therefore, the study endpoints were based on nominal application rates.

B. FRESH WEIGHT

The most sensitive species regarding fresh weight resulted to be *Cucumis sativus* with 10.8% of effect recorded, however the effect was not statistically significant. Hence the NOER was determined as ≥ 1.90 L product/ha for all species tested.

Table 1: Inhibitory effect of Azoxystrobin 160 + Prothioconazole 200 g/L SC on fresh weight

Plant species	NOER [L product/ha]	LOER [L product/ha]
Small radish (<i>Raphanus sativus</i>)	≥ 1.90	> 1.90
Cucumber (<i>Cucumis sativus</i>)	≥ 1.90	> 1.90
Soybean (<i>Glycine max</i>)	≥ 1.90	> 1.90
Lettuce (<i>Lactuca sativa</i>)	≥ 1.90	> 1.90
Garden onion (<i>Allium cepa</i>)	≥ 1.90	> 1.90
Perennial ryegrass (<i>Lolium perenne</i>)	≥ 1.90	> 1.90

C. MORTALITY

No mortality occurred in any of the species tested.

D. PHYTOTOXICITY

Phytotoxic effects were detected only in *Raphanus sativus* (discoloration and necrosis, 3%). No further effects were detected.

E. GROWTH STAGE

No differences in the growth stage of the plants were observed between the test item groups and the controls in any of the species tested on the last assessment day.

F. VALIDITY CRITERIA

The control seedling emergence was 71 - 100% (required: $\geq 70\%$). Control plants did not exhibit visible phytotoxic effects and mean plant survival was 100% (required: $\geq 90\%$). Therefore, the validity criteria of OECD 227 (2006) were met.

Conclusion

Based on the results of this vegetative vigour test with Azoxystrobin 160 + Prothioconazole 200 g/L SC, no statistically significant effects on mortality and fresh weight were observed. Non-significant phytotoxic effects (i.e., discoloration and necrosis) were observed only in *Raphanus sativus* at 3%. Thus, the ER₅₀ is supposed to be set as > 1.9 L product/ha. The NOER was determined to be ≥ 1.9 L product/ha, and the LOER was estimated to be > 1.9 L product/ha. All validity criteria of OECD 227 (2006) were fulfilled in this study.

A 2.6.3 KCP 10.6.3 Extended laboratory studies on non-target plants

No additional data submitted.

A 2.7 KCP 10.7 Effects on other terrestrial organisms (flora and fauna)

No additional data submitted.

A 2.8 KCP 10.8 Monitoring data

No additional data submitted.