

REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: A22773A

Product name: **ORONDIS EVO**

Chemical active substances:

Azoxystrobin, 250 g/L

Oxathiapiprolin, 12 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(New authorisation)

Applicant: Syngenta

Submission date: November 2021

Evaluation date: October 2022

MS Finalisation date: March 2023

Version history

When	What
19 July 22	<p>9.2 Effects on birds A quantitative chronic combination mixture risk assessment for A22773A has been added according to a request by RMS Poland. The theoretical chronic endpoints have been added to Table 9.2-3. The MAF has been amended from 1.5 to 1.4 in the chronic risk assessment (12-day interval). All table numbers have been updated.</p> <p>9.3 Effects on terrestrial vertebrates other than birds A quantitative chronic combination mixture risk assessment for A22773A has been added according to a request by RMS Poland. The theoretical chronic endpoints have been added to Table 9.3-3. The MAF has been amended from 1.5 to 1.4 in the chronic risk assessment (12-day interval). All table numbers have been updated. Some text on the interception for leafy vegetables and on Rinke, 1991, has been added to the higher-tier risk assessment. A higher tier risk assessment using the theoretical chronic endpoints presented in Table 9.3-3 has been added.</p> <p>9.5 Effects on aquatic organisms The aquatic risk assessment was split into Tier 1 in and Tier 2, following the split in the environmental fate section B8 8.9.2.1. The risk assessment using a plant uptake factor of 0 was included (Tier 1). A table using VFSmod for fruiting vegetables was added to Tier 2 (Table 9.5-24). Updated table numbers where appropriate. Updated overall conclusions.</p> <p>9.6 Effect on bees Bumblebee endpoints have been added to Table 9.6-3. A justification for not conducting a bumblebee risk assessment has been added to 9.6.3. A bumblebee study has been added to Appendix 1 and Appendix 2. Table numbers have been updated in Appendix 2.</p> <p>Appendix 2 Correction of formulation number in Table A-17.</p>
October 2022	zRMS finalized dRR evaluation
March 2023	Table 9.8-3 corrected according to the comment.

Table of Contents

9	Ecotoxicology (KCP 10).....	6
9.1	Critical GAP and overall conclusions.....	7
9.1.1	Overall conclusions.....	9
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).....	9
9.1.1.2	Effects on aquatic organisms (KCP 10.2).....	9
9.1.1.3	Effects on bees (KCP 10.3.1).....	11
9.1.1.4	Effects on arthropods other than bees (KCP 10.3.2)	11
9.1.1.5	Effects on non-target soil meso- and macrofauna (KCP 10.4), Effects on soil microbial activity (KCP 10.5)	11
9.1.1.6	Effects on non-target terrestrial plants (KCP 10.6)	12
9.1.1.7	Effects on other terrestrial organisms (flora and fauna) (KCP 10.7)	12
9.1.2	Grouping of intended uses for risk assessment.....	12
9.1.3	Consideration of metabolites	14
9.2	Effects on birds (KCP 10.1.1).....	16
9.2.1	Toxicity data	16
9.2.1.1	Justification for new endpoints	17
9.2.2	Risk assessment for spray applications.....	20
9.2.2.1	First-tier assessment (screening/generic focal species)	20
9.2.2.2	Higher-tier risk assessment	24
9.2.2.3	Drinking water exposure.....	24
9.2.2.4	Effects of secondary poisoning.....	26
9.2.2.5	Biomagnification in terrestrial food chains.....	29
9.2.3	Risk assessment for baits, pellets, granules, prills or treated seed.....	29
9.2.4	Overall conclusions.....	29
9.3	Effects on terrestrial vertebrates other than birds (KCP 10.1.2).....	29
9.3.1	Toxicity data	29
9.3.1.1	Justification for new endpoints	31
9.3.2	Risk assessment for spray applications.....	33
9.3.2.1	First-tier assessment (screening/generic focal species)	33
9.3.2.2	Higher-tier risk assessment	42
9.3.2.3	Drinking water exposure.....	51
9.3.2.4	Effects of secondary poisoning.....	52
9.3.2.5	Biomagnification in terrestrial food chains.....	55
9.3.3	Risk assessment for baits, pellets, granules, prills or treated seed.....	55
9.3.4	Overall conclusions.....	55
9.4	Effects on other terrestrial vertebrate wildlife (reptiles and amphibians) (KCP 10.1.3)	56
9.5	Effects on aquatic organisms (KCP 10.2).....	57
9.5.1	Toxicity data	57
9.5.1.1	Justification for new endpoints	64
9.5.2	Risk assessment	67
9.5.3	Overall conclusions.....	114
9.6	Effects on bees (KCP 10.3.1).....	116
9.6.1	Toxicity data	116
9.6.1.1	Justification for new endpoints	119

9.6.2	Risk assessment	119
9.6.2.1	Hazard quotients for bees.....	121
9.6.2.2	Higher-tier risk assessment for bees (tunnel test, field studies).....	133
9.6.3	Effects on bumble bees	133
9.6.4	Effects on solitary bees	134
9.6.5	Overall conclusions.....	134
9.7	Effects on arthropods other than bees (KCP 10.3.2)	136
9.7.1	Toxicity data	136
9.7.1.1	Justification for new endpoints	137
9.7.2	Risk assessment	137
9.7.2.1	Risk assessment for in-field exposure.....	137
9.7.2.2	Risk assessment for off-field exposure	138
9.7.2.3	Additional higher-tier risk assessment.....	138
9.7.2.4	Risk mitigation measures	139
9.7.3	Overall conclusions.....	139
9.8	Effects on non-target soil meso- and macrofauna (KCP 10.4)	139
9.8.1	Toxicity data	139
9.8.1.1	Justification for new endpoints	143
9.8.2	Risk assessment	143
9.8.2.1	First-tier risk assessment.....	144
9.8.2.2	Higher-tier risk assessment.....	145
9.8.3	Overall conclusions.....	145
9.9	Effects on soil microbial activity (KCP 10.5).....	146
9.9.1	Toxicity data	146
9.9.1.1	Justification for new endpoints	147
9.9.2	Risk assessment	147
9.9.3	Overall conclusions.....	148
9.10	Effects on non-target terrestrial plants (KCP 10.6)	148
9.10.1	Toxicity data	148
9.10.1.1	Justification for new endpoints	150
9.10.2	Risk assessment	150
9.10.2.1	Tier-1 risk assessment (based screening data)	150
9.10.2.2	Tier-2 risk assessment (based on dose-response data).....	150
9.10.2.3	Higher-tier risk assessment.....	151
9.10.2.4	Risk mitigation measures	151
9.10.3	Overall conclusions.....	151
9.11	Effects on other terrestrial organisms (flora and fauna) (KCP 10.7)	152
9.12	Monitoring data (KCP 10.8)	152
9.13	Classification and Labelling	152
Appendix 1	Lists of data considered in support of the evaluation	154
Appendix 2	Detailed evaluation of the new studies	167
A 2.1	KCP 10.1 Effects on birds and other terrestrial vertebrates.....	167
A 2.1.1	KCP 10.1.1 Effects on birds	167
A 2.1.2	KCP 10.1.2 Effects on terrestrial vertebrates other than birds	169
A 2.1.3	KCP 10.1.3 Effects on other terrestrial vertebrate wildlife (reptiles and amphibians).....	189
A 2.2	KCP 10.2 Effects on aquatic organisms	189

A 2.2.1	KCP 10.2.1 Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes	189
A 2.2.2	KCP 10.2.2 Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms.....	200
A 2.2.3	KCP 10.2.3 Further testing on aquatic organisms	200
A 2.3	KCP 10.3 Effects on arthropods	200
A 2.3.1	KCP 10.3.1 Effects on bees	200
A 2.3.2	KCP 10.3.2 Effects on non-target arthropods other than bees.....	227
A 2.4	KCP 10.4 Effects on non-target soil meso- and macrofauna.....	232
A 2.4.1	KCP 10.4.1 Earthworms	232
A 2.4.2	KCP 10.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)	241
A 2.5	KCP 10.5 Effects on soil nitrogen transformation.....	255
A 2.6	KCP 10.6 Effects on terrestrial non-target higher plants.....	258
A 2.6.1	KCP 10.6.1 Summary of screening data	258
A 2.6.2	KCP 10.6.2 Testing on non-target plants.....	258
A 2.6.3	KCP 10.6.3 Extended laboratory studies on non-target plants	264
A 2.6.4	KCP 10.6.4 Semi-field and field tests on non-target plants.....	264
A 2.7	KCP 10.7 Effects on other terrestrial organisms (flora and fauna).....	264
A 2.8	KCP 10.8 Monitoring data.....	264

9 Ecotoxicology (KCP 10)

Oxathiapiprolin is owned by Corteva Agriscience International Sàrl (formally DuPont International Operations Sàrl; change effective January 4, 2021) (hereafter called “Corteva”), Syngenta has a Letter of Access in place to access data owned by Corteva relevant for this evaluation. Study summaries for new studies on oxathiapiprolin can be found in Appendix 2. For actual reports, please refer to data owner.

Review Comments:

This document describes the acceptable use conditions required for registration of A22773A, a suspension concentrate containing 250 g/L azoxystrobin and 12 g/L oxathiapiprolin for use as a fungicide in hop, bulbs and onion like crops, fruiting and leafy vegetables.

This Part B document only reviews data and additional information that has not previously been considered within the EU review process.

Since this document is based on the information provided by the applicant, all review comments, additions and corrections have been made using commenting boxes or highlighted in grey.

9.1 Critical GAP and overall conclusions

Table 9.1-1: Table of critical GAPs

1	2	3	4	5	6	7	8	9	10	11		12	13	14	15	16	17	18	19	20	21
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application					Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion						
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applicatio ns (days)	L product/ha a) max. rate per appl. b) max. total rate per crop/season	g OXTP/ha a) max. rate per appl. b) max. total rate per crop/season	g AZT/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha Min / max			Birds	Mammals	Aquatic organisms	Bees	Non-target	Soil organisms	Non-target plants
Zonal uses (field or outdoor uses, certain types of protected crops)																					
PL-17	Poland	Hop, HUMLU	F	<i>Pseudoperonospora humuli</i>	foliar	BBCH 21-89	a) 2 b) 2	12-16	a) 1 b) 2	a) 12 b) 24	a) 250 b) 500	700-3300	28								
PL-20	Poland	Lettuce, LACSA (covers salad plants, spinach and similar leaves, sweet basil)	F	<i>Bremia lactucae</i>	foliar	BBCH 11 - 49	a) 2 b) 2	7	a) 1 b) 2	a) 12 b) 24	a) 250 b) 500	200-800	14	maximum 2 application per year on the same field							
PL-28	Poland	Tomato, LYPES (covers bell pepper, eggplant, cucumber, melon, pumpkin, squash, watermelon, zucchini)	F	<i>Alternaria sp.</i>	foliar	BBCH 44 40 - 89	a) 2 b) 2	7	a) 1 b) 2	a) 12 b) 24	a) 250 b) 500	200-1200	3								
PL-30	Poland	Leek, ALLPO (covers spring onion)	F	<i>Alternaria porri</i>	foliar	BBCH 11 - 49	a) 2 b) 2	12-14	a) 1 b) 2	a) 12 b) 24	a) 250 b) 500	200-800	7								

* Use number(s) in accordance with the list of all intended GAPs in Part B, Section 0 should be given in column 1. The critical use patterns are reported only for zRMS Poland as they represent all

the other intended uses as listed in the GAP table in Part B, Section 0.

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

Explanation for column 15 – 21 “Conclusion”

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

Remarks table:

- (1) Numeration necessary to allow references
- (2) Use official codes/nomenclatures of EU
- (3) For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (*e.g.* fumigation of a structure)
- (4) F: professional field use, Fn: non-professional field use, Fpn: professional and non-professional field use, G: professional greenhouse use, Gn: non-professional greenhouse use, Gpn: professional and non-professional greenhouse use, I: indoor application
- (5) Scientific names and EPPO-Codes of target pests/diseases/ weeds or when relevant the common names of the pest groups (*e.g.* biting and sucking insects, soil born insects, foliar fungi, weeds) and the developmental stages of the pests and pest groups at the moment of application must be named
- (6) Method, *e.g.* high volume spraying, low volume spraying, spreading, dusting, drench
Kind, *e.g.* overall, broadcast, aerial spraying, row, individual plant, between the plants - type of equipment used must be indicated
- (7) Growth stage at first and last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
- (8) The maximum number of application possible under practical conditions of use must be provided
- (9) Minimum interval (in days) between applications of the same product.
- (10) For specific uses other specifications might be possible, *e.g.*: g/m³ in case of fumigation of empty rooms. See also EPPO-Guideline PP 1/239 Dose expression for plant protection products
- (11) The dimension (g, kg) must be clearly specified. (Maximum) dose of a.s. per treatment (usually g, kg or L product / ha).
- (12) If water volume range depends on application equipments (*e.g.* ULVA or LVA) it should be mentioned under “application: method/kind”.
- (13) PHI - minimum pre-harvest interval
- (14) Remarks may include: Extent of use/economic importance/restrictions

9.1.1 Overall conclusions

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

Birds

The acute and long-term risks of A22773A to birds were assessed from toxicity exposure ratios between toxicity endpoints, estimated from studies with A22773A, azoxystrobin and oxathiapiprolin, and maximum residues occurring on food items following applications according to the proposed use pattern.

Risk of secondary poisoning has also been assessed, as oxathiapiprolin and some of its metabolites have log P_{ow} values of > 3.0. The risk to birds from exposure via drinking water has also been assessed.

The TER values, calculated for recommended scenarios, all exceed the trigger values of 10 for acute risk and 5 for long-term risk (including secondary poisoning), indicating that the risk to birds is acceptable following use of A22773A according to the proposed use pattern.

Mammals

The acute and long-term risks of A22773A to mammals were assessed from toxicity exposure ratios between toxicity endpoints, estimated from studies with A22773A, azoxystrobin and oxathiapiprolin, and maximum residues occurring on food items following applications according to the proposed use pattern.

Risk of secondary poisoning has also been assessed, as oxathiapiprolin and some of its metabolites have log P_{ow} values of > 3.0. The risk to mammals from exposure via drinking water has also been assessed.

The TER values, calculated for recommended scenarios, all exceed the trigger value of 10 for acute risk, and nearly all exceed the trigger value of 5 for long-term risk (including drinking water and secondary poisoning), indicating that the risk to mammals is acceptable following use of A22773A according to the proposed use pattern. For acute risk the small herbivorous mammals (voles) were below the trigger of 10 for the formulation A22773A in fruiting and leafy vegetables. The TER_a value of > 9.6 for the small herbivorous mammal “vole” scenarios was based on the unbound (“greater than”) endpoint for A22773A (LD_{50} > 2 000 mg A22773A/kg bw). The results of the acute oral toxicity study (xxxxxxx 2021; VV-892044) suggest that the actual LD_{50} for A22773A would be significantly higher than the tested dose of 2 000 mg/kg bw, resulting in a TER_a value exceeding the trigger of 10. Syngenta therefore believe that the acute risk assessment for the small herbivorous mammal “vole” indicate acceptable risk.

The exception was for small herbivorous mammals (voles) for which chronic Tier 1 TER values for azoxystrobin were below the trigger of 5 for the use of A22773A in fruiting and leafy vegetables and bulbs/onion-like crops. Higher-tier risk assessment based on newer crop interception values and vole diet, resulted in all scenarios showing acceptable risk except for uses in fruiting vegetables at BBCH 11-39.

9.1.1.2 Effects on aquatic organisms (KCP 10.2)

The PEC/RAC ratios, using worst-case Step 1 PEC_{sw} values for oxathiapiprolin, are less than the trigger value of 1, for all aquatic organisms.

The PEC/RAC ratios, using worst-case PEC_{sw} values for A22773A, are less than the trigger value of 1, for all aquatic organisms, with the exception of fish (acute) and invertebrates (acute and chronic) when based on FOCUS Step 3 calculations and exposed to azoxystrobin. A refined risk assessment is conducted for aquatic invertebrates (acute and chronic) only, since the higher tier RAC for invertebrates is lower than that for fish, and thus covers the risk for all organisms taking into account appropriate mitigation measures.

The potential risk to aquatic invertebrates has been refined by using FOCUS Step 4 PEC_{sw} considering reduced exposure of surface water bodies after consideration of different non-sprayed buffer zones and vegetated filter strips (with regard to run-off mitigation). In addition, the PEC/RAC ratios were calculated based on FOCUS Step 4 PEC_{sw} with VFSmod for Poland only.

The PEC/RAC ratios are below the trigger value of 1 for azoxystrobin when based on FOCUS Step 4 PEC_{sw} considering reduced exposure of surface water bodies as an additional refinement option.

Considering a plant uptake factor of 0 (Tier 1), the PEC/RAC ratios are below the trigger value of 1 for A22773A when consideration is given to a 20 m vegetated filter strip following application of 2 x 1 L A22773A/ha in fruiting vegetables, leafy vegetables or to a 10 m vegetated filter strip following application of 2 x 1 L A22773A/ha in bulb and onion like crops. Following application of 2 x 1 L A22773A/ha to hops, PEC/RAC ratios are below the trigger value of 1 for A22773A considering a 10m no-spray buffer with 75% nozzle reduction or a 15 m no-spray buffer.

Considering a plant uptake factor of 0.5 (Tier 2), the PEC/RAC ratios are below the trigger value of 1 for A22773A when consideration is given to a 20 m vegetated filter strip following application of 2 x 1 L A22773A/ha in fruiting vegetables, leafy vegetables, and to a 20 m no spray buffer following application of 2 x 1 L A22773A/ha to hops or to a 10 m vegetated filter strip following application of 2 x 1 L A22773A/ha in bulb and onion like crops, respectively.

In case VFS_{mod} is taken into consideration, the PEC/RAC ratios are below the trigger value of 1 following use of 2 x 1 L of A22773A/ha on hops with no need of mitigations and when consideration is given to a 5 m vegetated filter strip on leafy, fruiting and bulb vegetables.

The toxic unit analysis indicates that azoxystrobin is driving the toxicity to all organism groups when considered alongside oxathiapiprolin.

There was acceptable risk to aquatic organisms following use of A22773A when the following mitigation steps are considered: 20 m vegetated filter strip following the application of 2 x 1 L A22773A/ha in fruiting vegetables, leafy vegetables and 20 m no spray buffer following application of 2 x 1 L A22773A/ha to hops and a 10 m vegetated filter strip following the application of 2 x 1 L A22773A/ha in bulb and onion like crops. Following application of 2 x 1 L A22773A/ha to hops and using Tier 1, there was acceptable risk to aquatic organisms when considering a 10m no spray buffer with 75% nozzle reduction or a 15 m no spray buffer, but a 20 m no spray buffer was required using Tier 2.

In case VFS_{mod} is taken into consideration, there was acceptable risk to aquatic organisms following use of 2 x 1 L of A22773A/ha on hops with no need of mitigations and when consideration is given to a 5 m vegetated filter strip on leafy fruiting and bulb vegetables.

Tier 1 (plant uptake factor = 0)

Fruiting vegetables (2 x 1 L A22773A/ha)

20m vegetated filter strip
or 5m vegetated filter strip with VFSmod

Leafy vegetables (2 x 1 L A22773A/ha)

20m vegetated filter strip
or 5m vegetated filter strip with VFSmod

Bulb and onion like crop (2 x 1 L A22773A/ha)

10m vegetated filter strip
or 5m vegetated filter strip with VFSmod

Hops (2 x 1 L A22773A/ha)

10m no-spray buffer with 75% nozzle reduction
or 15m no-spray buffer

Tier 2 (plant uptake factor = 0.5)

Fruiting vegetables (2 x 1 L A22773A/ha)

20m vegetated filter strip
or 5m vegetated filter strip with VFSmod

Leafy vegetables (2 x 1 L A22773A/ha)

20m vegetated filter strip
or 5m vegetated filter strip with VFSmod

Bulb and onion like crop (2 x 1 L A22773A/ha)

10m vegetated filter strip

Hops (2 x 1 L A22773A/ha)

or 5m vegetated filter strip with VFSmod

20m no spray buffer

9.1.1.3 Effects on bees (KCP 10.3.1)

The risk to honeybees was assessed following SANCO/10329/2002 rev.2 and EPPO, 2010 as proposed in the list of guidance documents relevant to the implementation of Regulation 1107/2009, published in the official EU Journal 2013/C 95/01 and 95/02.

The risk of A22773A to honeybees was assessed from hazard quotients, estimated from acute oral and contact studies with azoxystrobin, oxathiapiprolin and A22773A. The acute oral and contact hazard quotients were less than the relevant trigger of 50, indicating that the risk to honeybees is acceptable following use of A22773A according to the proposed use pattern.

In addition, the acute risk to honeybees was assessed from hazard quotients (HQ) and Exposure Toxicity Ratios (ETRs) following EFSA Bee Guidance Document, 2013, using endpoints from acute oral and contact studies with azoxystrobin and oxathiapiprolin. Acute contact HQ and oral ETRs were less than the relevant triggers at the screening step, indicating acceptable acute risk to adult honeybees.

The chronic adult and larval risk of A22773A to honeybees was assessed from ETRs following EFSA Bee Guidance Document, 2013, using endpoints from chronic adult and larval studies with azoxystrobin and oxathiapiprolin. The Tier 1 ETR values for azoxystrobin and oxathiapiprolin for the treated crop were less than the relevant triggers, indicating acceptable chronic risk to adult honeybees and bee larvae, with the following exception: The Tier 1 risk assessment indicated a potential chronic risk to adult honeybees for azoxystrobin from downward spray in fruiting vegetables, leafy vegetables and bulb and other alliaceous crops. A refined risk assessment was therefore conducted.

Since azoxystrobin is clearly driving the toxicity in the mixture, the calculations for the chronic Tier 1 refinement were conducted with the azoxystrobin endpoints.

In the refined risk assessment for adult honeybees for azoxystrobin, the dose resulting in 0% mortality under continuous feeding conditions in the laboratory (LDD₀), is above the predicted worst-case exposure in-field of adult bees indicating an acceptable chronic oral risk to adult honeybees (Approach 1 of the refinement). In addition, the in-field ETR values for azoxystrobin are below the EFSA trigger of 0.03 indicating an acceptable chronic oral risk to adult honeybees (Approach 2 of the refinement) for azoxystrobin. Therefore, the chronic risk to adult honeybees is acceptable following use of A22773A according to the proposed use pattern.

9.1.1.4 Effects on arthropods other than bees (KCP 10.3.2)

At Tier I, the in-field and off-field HQ values based on the LR₅₀ were below the trigger value for the worst-case intended use scenarios indicating that the risk to non-target arthropods is acceptable following the use of A22773A according to the proposed use pattern.

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

Soil meso- and macrofauna

The risk of A22773A to earthworms was assessed from acute and long-term toxicity exposure ratios (TERs) between the selected toxicity endpoints for A22773A, azoxystrobin, oxathiapiprolin and their relevant metabolites, and the maximum PEC_{soil}. The acute and long-term TER values derived are greater than the

Regulation (EU) 546/2011 triggers of 10 and 5, respectively, indicating that the risk to earthworms is acceptable following use of A22773A according to the proposed use pattern.

The risk of A22773A to other non-target soil macro-organisms, as represented by *Folsomia* and *Hypoaspis* was assessed from long-term toxicity exposure ratios (TERs) between the selected no-effect concentrations, derived from laboratory tests on A22773A, azoxystrobin, oxathiapiprolin and their relevant metabolites, and the maximum PEC_{soil}. The long-term TER values are all greater than the recommended trigger value of 5, indicating that the risk to soil macro-organisms is acceptable following use of A22773A according to the proposed use pattern.

Soil micro-organisms

The risk of A22773A, azoxystrobin, oxathiapiprolin and their relevant metabolites to soil micro-organisms was evaluated by comparison of the maximum concentrations with effects < 25 % derived from laboratory tests, with the maximum PEC_{soil}.

All the effect levels exceeded the relevant PEC_{soil} values, indicating that the risk to soil micro-organisms is acceptable following the use of A22773A according to the proposed use pattern.

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

Less than 50% effect on seedling emergence on all six species was observed at the maximum single use rate of 1 000 mL A22773A/ha. This indicates that the risk to non-target terrestrial plants for seedling emergence in off-crop areas is acceptable following use of A22773A according to the proposed use pattern. However, the vegetative vigour screening test showed effects below the highest field application rate therefore a Tier 2 risk assessment is conducted.

The risk of A22773A to non-target terrestrial plants was assessed from toxicity exposure ratios (TERs) using the formulation toxicity data from Tier II vegetative vigour study, and the maximum off-field predicted environmental residue (PER) indicating an acceptable risk.

The risk to non-target terrestrial plants in off-crop areas is therefore acceptable following use of A22773A according to the proposed use pattern.

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

Tests on other non-target species are not required.

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).

Table 9.1-2: Critical use pattern of A22773A grouped according to crops

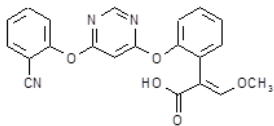
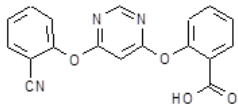
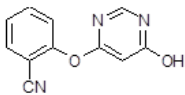
Grouping according to criterion			
Use no.	Intended uses	relevant use parameters for grouping	relevant parameter or value for sorting
PL-1, PL-2, PL-3, PL-4, PL-5, PL-6, PL-7, PL-8, PL-9, PL-10, PL-11, PL-12, PL-13, PL-14, PL-15, PL-16, PL-23, PL-24, PL-25, PL-26, PL-27, PL-28, PL-29 CZ-1, CZ-2, CZ-3, CZ-10, CZ-11, CZ-12, CZ-13, CZ-14, CZ-15, CZ-16, CZ-17, CZ-29, HU-1, HU-2, HU-3, HU-4, HU-5, HU-6, HU-8, HU-9, HU-10, HU-11, HU-12, HU-13, HU-14, HU-15, HU-16, HU-17, HU-18, RO-1, RO-2, RO-3, RO-4, RO-5, RO-6, RO-8, RO-9, RO-10, RO-11, RO-12, RO-13, RO-14, RO-15, RO-16, RO-17, RO-18, SK-1, SK-2, SK-3, SK-4, SK-5, SK-11, SK-12, SK-13, SK-14, SK-15, SK-16, SK-17, SK-18, SK-35, SI-1, SI-2, SI-3, SI-4, SI-5, SI-6, SI-13, SI-14, SI-15, SI-16, SI-17, SI-18, SI-19, SI-20, SI-21, SI-22, SI-23	Fruiting vegetables (cucurbits with edible and non edible peel - cucumber, melon, pumpkin, squash, watermelon, zucchini; tomato, bell pepper, eggplant)	Crop group: Growth stage: BBCH 11 - 89 Application rate: 1 L A22773A/ha Max. number of applications: 2 Min. application interval: 7 d	<u>Relevant scenario for:</u> Birds and mammal risk assessment Aquatic organism risk assessment Bees risk assessment
PL-17 AT-1, BE-1, CZ-4, DE-1, DE-15, HU-7, IE-1, NL-1, RO-7, SI-7	Hop	Crop group: hop Growth stage: BBCH 21 - 89 Application rate: 1 L A22773A/ha Max. number of applications: 2 Min. application interval: 12-16 d	<u>Relevant scenario for:</u> Birds and mammal risk assessment Aquatic organism risk assessment Bees risk assessment Non-target arthropods risk assessment NTTP risk assessment
PL-18, PL-19, PL-20, PL-21, PL-38, PL-39, PL-40 AT-6, BE-2, CZ-9, CZ-31, DE-16, DE-6, IE-6, NL-6, SK-10, SK-37, SK-39, SI-12	Leafy vegetables (lettuce, salad plants, spinach and similar leaves, sweet basil)	Crop group: leafy vegetables Growth stage: BBCH 11 - 49 Application rate: 1 L A22773A/ha Max. number of applications: 2 Min. application interval: 7 d	<u>Relevant scenario for:</u> Birds and mammal risk assessment Aquatic organism risk assessment Bees risk assessment

Grouping according to criterion			
Use no.	Intended uses	relevant use parameters for grouping	relevant parameter or value for sorting
PL-30, PL-31, PL-32, PL-33, PL-71 AT-2, AT-3, AT-4, AT-5, BE-3, BE-4, BE-5, BE-6, BE-7, CZ-5, CZ-6, CZ-7, CZ-8, CZ-30, DE-2, DE-3, DE-4, DE-5, IE-2, IE-3, IE-4, IE-5, NL-2, NL-3, NL-4, NL-5, NL-8, SK-6, SK-7, SK-8, SK-9, SK-36, SI-8, SI-9, SI-10, SI-11	Bulb vegetables (leek, spring onion)	Crop group: bulb and onion like crops Growth stage: BBCH 11 - 49 Application rate: 1 L A22773A/ha Max. number of applications: 2 Min. application interval: 12 - 14 d	Relevant scenario for: Birds and mammal risk assessment Aquatic organism risk assessment Bees risk assessment

9.1.3 Consideration of metabolites

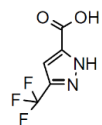
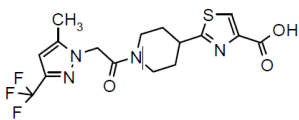
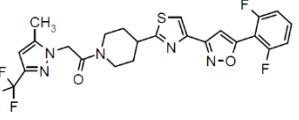
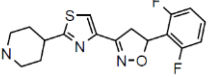
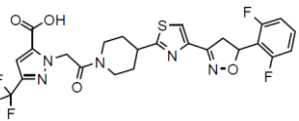
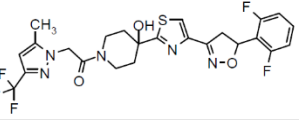
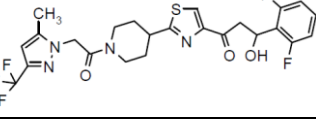
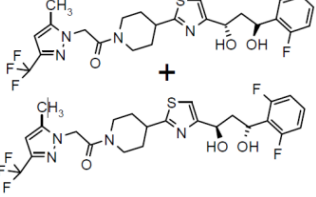
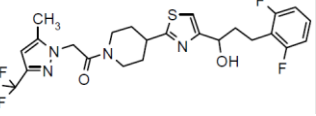
A list of metabolites found in environmental compartments is provided below. The need for conducting a metabolite-specific risk assessment in the context of the evaluation of A22773A is indicated in the tables below.

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

Metabolite	Molar mass	Chemical structure	Maximum occurrence in compartments	Risk assessment required?
R234886	389.4		> 10 % of a.s. in soil > 10 % of a.s. in water > 10 % of a.s. in sediment	Soil = Yes Surface water = Yes Sediment: No*
R402173	333.3		> 10 % of a.s. in soil < 5 % of a.s. in water / sediment	Soil = Yes Surface water = Yes Sediment = No
R401553	213.2		> 10 % of a.s. in soil > 5 % of a.s. in 2 sequential measurements in water / sediment	Soil = Yes Surface water = Yes Sediment = No*

* The trigger of 10% is met, however no risk assessment is required for any of these metabolites, because they are not relevant.

Table 9.1-4: Metabolites of oxathiapiprolin potentially relevant for exposure assessment

Metabolite	Chemical structure	Molar mass	Maximum observed occurrence in compartments (% AR)	Risk assessment required?
IN-E8S72		180.09	Soil: 10.3 ^c Surface Water: not observed Sediment: not observed	Soil = Yes Surface water = Yes Sediment = No
IN-P3X26		402.40	Soil: not observed Surface Water: 14.0 Sediment: not observed	Soil = No Surface water = Yes Sediment = No
IN-Q7D41		537.51	Soil: 2.5 Surface Water: 1.5 Sediment: 10.5	Soil = No Surface water = Yes Sediment = Yes
IN-QPS10		349.41	Soil: 8.7 ^a Surface Water: not observed Sediment: not observed	Soil = Yes Surface water = Yes Sediment = No
IN-RAB06		569.51	Soil: 13.5 Surface Water: 4.2 Sediment: 5.2	Soil = Yes Surface water = Yes Sediment = No
IN-RDT31		555.53	Soil: 9.4 ^a Surface Water: not observed Sediment: not observed	Soil = Yes Surface water = Yes Sediment = No
IN-RSE01		510.47 ^{\$}	Soil: not observed Surface Water: 3.8 Sediment: 8.6 ^a	Soil = No Surface water = Yes Sediment = Yes
IN-RYJ52		544.54	Soil: not observed Surface Water: 7.9 Sediment: 14.7	Soil = No Surface water = Yes Sediment = Yes
IN-S2K66		528.54	Soil: not observed Surface Water: not observed Sediment: 8.7 ^b	Soil = No Surface water = Yes Sediment = Yes

AR: Applied radioactivity

^a > 5 % at two consecutive sampling points.

^b > 5 % and rising at the end of study.

^c Maximum from the field study exceeds maximum amount observed in laboratory of 6.72 % reported in DAR, 2016¹, volume 1, List of endpoints.

^{\$} Incorrect input value was used in the modelling. Using the correct molar mass (542.53), higher PEC values by a factor of 1.06 (differences between correct and incorrect molar weight correction factor) would be obtained. This is foreseen to have no impact on the aquatic risk assessment for metabolite IN-RSE01 because of the high safety factor.

¹ DAR, 2016: Draft Assessment Report for Oxathiapiprolin (February 2016).

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

Avian toxicity studies have been carried out with azoxystrobin and oxathiapiprolin. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on birds of A22773A were not evaluated as part of the EU assessment of azoxystrobin and oxathiapiprolin. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

The selection of endpoints for the risk assessment deviates from the results of the EU review process. Justifications are provided below where necessary.

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

Species	Substance	Exposure System	Results	Reference
Bobwhite quail (<i>Colinus virginianus</i>)	Azoxystrobin	Oral 1 d Acute	LD ₅₀ > 2 000 mg a.s./kg bw Corrected LD ₅₀ = 3 776 mg a.s./kg bw ^a	EFSA, 2010, Hakin <i>et al.</i> , 1992, ICI5504/0852
Bobwhite quail (<i>Colinus virginianus</i>)	Azoxystrobin	Dietary 5 d Short-term	LDD ₅₀ > 1 179 ^b mg a.s./kg bw/d	EFSA, 2010, Hakin <i>et al.</i> , 1992, ICI5504/0854
Bobwhite quail (<i>Colinus virginianus</i>)	Azoxystrobin	Dietary Reproductive toxicity	NOEL = 117 ^b mg a.s./kg bw/d	EFSA 2010, Johnson, 1994, ICI5504/0855

Endpoints used in risk assessment are shown in **bold**.
EFSA Journal 2010; 8(4):1542.

^a The endpoint was extrapolated according to EFSA/2009/1438 as explained under 9.2.1.1 Justification for new endpoints.

^b re-calculated endpoints in terms of daily dietary dose; it is the belief of Syngenta that the units are erroneously displayed in the EFSA Conclusion (2010); 8(4):1542.

Table 9.2-2: Endpoints and effect values relevant for the risk assessment for birds – oxathiapiprolin

Species	Substance	Exposure System	Results	Reference
Bobwhite quail (<i>Colinus virginianus</i>)	Oxathiapiprolin	Oral 1 d Acute	LD ₅₀ > 2 250 mg a.s./kg bw Corrected LD ₅₀ = 4 248 mg a.s./kg bw	EFSA, 2016, DuPont-31753
Zebra finch (<i>Poephila guttata</i>)	Oxathiapiprolin	Oral 1 d Acute	LD ₅₀ > 2 250 mg a.s./kg bw Corrected LD ₅₀ = 4 248 mg a.s./kg bw	EFSA, 2016, DuPont-31764
Bobwhite quail (<i>Colinus virginianus</i>)	Oxathiapiprolin	Dietary 8 d Short-term	LC ₅₀ > 5 620 mg a.s./kg feed (equivalent to > 1 280 mg a.s./kg bw/day)	EFSA, 2016, DuPont-31754
Mallard duck (<i>Anas platyrhynchos</i>)	Oxathiapiprolin	Dietary 8 d	LC ₅₀ > 5 620 mg a.s./ kg feed	EFSA, 2016, DuPont-31765

Species	Substance	Exposure System	Results	Reference
		Short-term	(equivalent to > 2 728 mg a.s./kg bw/day)	
Bobwhite quail (<i>Colinus virginianus</i>)	Oxathiapiprolin	Dietary Reproductive toxicity	NOEL = 1 200 mg a.s./kg feed (equivalent to 106.7 mg a.s./kg bw/day)	EFSA, 2016, DuPont-31755
Mallard duck (<i>Anas platyrhynchos</i>)	Oxathiapiprolin	Dietary Reproductive toxicity	NOEL = 1 200 mg a.s./kg feed (equivalent to 156.3 mg a.s./kg bw/day)	EFSA, 2016, DuPont-31763

Endpoints used in risk assessment are shown in **bold**.
EFSA Journal 2016;14(7):4504.

Metabolites

Studies performed with mammals indicated that plant metabolites of oxathiapiprolin are less toxic than the parent. Moreover, metabolites of oxathiapiprolin are not included in the definition of residues in plants and exposure via these food sources is not expected. For this reason, metabolites were not considered in the dietary risk assessment.

Table 9.2-3: Endpoints and effect values relevant for the risk assessment for birds – A22773A

Species	Substance	Exposure System	Results	Reference
Bobwhite quail (<i>Colinus virginianus</i>)	A22773A	Oral 1 d Acute	LD ₅₀ > 2 000 mg/kg bw Corrected LD ₅₀ = 3 228 mg/kg bw^a (760 mg a.s./kg bw) ^b	xxxxxxx 2020, VV-870400 Study not evaluated by zRMS
-	Theoretical Mixture azoxystrobin/oxathiapiprolin	Oral 1 d Acute	Calculated LD ₅₀ = 3 795 mg a.s./kg bw	Refer to 9.2.1.1
	Theoretical Mixture azoxystrobin/oxathiapiprolin	Dietary Reproductive toxicity	Calculated NOAEL = 116.5 (mg a.s./kg bw/d)	Refer to 9.2.1.1

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

^a The endpoint was extrapolated according to EFSA/2009/1438 as explained under 9.2.1.1 Justification for new endpoints.

^b Formulation endpoint expressed as total a.s. (based on the total concentration of azoxystrobin and oxathiapiprolin in the formulation (actual 258.2 g a.s./L) and considering formulation density of 1.096 g/cm³).

9.2.1.1 Justification for new endpoints

Consideration of acute endpoint for azoxystrobin used in the risk assessment

In the acute oral toxicity study conducted with the bobwhite quail (*Hakin et al., 1992; ICI5504/0852*) no mortalities were observed and therefore the LD₅₀ was reported as > 2 000 mg/kg bw. Under Point 2.1.2 of 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/2009/1438²) a method has been proposed to extrapolate

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

upwards the LD₅₀ value. The extrapolation is carried out assuming a 50% binomial probability bound that mortality could have occurred but had simply been missed by chance in the test. The extrapolation factors are presented in Table 1 of the guidance document and are dependent upon the number of animals tested and whether no, or a single mortality, was observed in the study. The acute toxicity value for the bobwhite quail has been extrapolated and is presented in the table below.

Table 9.2-4: Extrapolation of the acute oral toxicity values for azoxystrobin

Study	Test species	Experimental LD ₅₀ (mg a.s./kg bw)	Number of animals tested	Number of mortalities	Extrapolation factor ^a	Corrected LD ₅₀ (mg a.s./kg bw)
Hakin <i>et al.</i> , 1992, ICI5504/0852	Bobwhite quail	> 2 000	10	0	1.888	3 776

^a The extrapolation factor is presented in Table 1 of the guidance document (Point 2.1.2)

The extrapolated LD₅₀ value of 3776 mg/kg bw will therefore be used in the subsequent risk assessment.

Consideration of acute toxicity endpoint for A22773A used in the risk assessment

In the acute oral toxicity study conducted with the bobwhite quail (xxxxxxx, 2020; VV-870400) no mortalities were observed and therefore the LD₅₀ was reported as > 2 000 mg/kg bw. Under Point 2.1.2 of EFSA/2009/1438² a method has been proposed to extrapolate upwards the LD₅₀ value. The extrapolation is carried out assuming a 50% binomial probability bound that mortality could have occurred but had simply been missed by chance in the test. The extrapolation factors are presented in Table 1 of the guidance document and are dependent upon the number of animals tested and whether no, or a single mortality, was observed in the study. The acute toxicity value for the bobwhite quail has been extrapolated and is presented in the table below.

Table 9.2-5: Extrapolation of the acute oral toxicity values for A22773A

Study	Test species	Experimental LD ₅₀ (mg/kg bw)	Number of animals tested	Number of mortalities	Extrapolation factor ^a	Corrected LD ₅₀ (mg/kg bw)
xxxxxxx 2020, VV-870400	Bobwhite quail	> 2 000	5	0	1.614	3 228

^a The extrapolation factor is presented in Table 1 of the guidance document (Point 2.1.2)

The extrapolated LD₅₀ value of 3 228 mg/kg bw will therefore be used in the subsequent risk assessment.

Consideration of acute mixture toxicity

According to EFSA/2009/1438³ combined action of several toxicants must be specifically considered in the risk assessment when it is obvious that such exposure situations will occur for animals.

For the assessment of acute effects (mortality), a surrogate LD₅₀ can be calculated. The EFSA Guidance Document indicates that the following equation should be used for deriving a surrogate LD₅₀ for a mixture of active substances with known toxicity assuming dose additivity:

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

where:

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

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

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

Table 9.2-6: Acute LD_{50} for the mixture of azoxystrobin and oxathiapiprolin

Test substance	Concentration of active substance in formulation A22773A (g a.s./L)	Fraction of active substance in the formulation mixture ^A	Acute toxicity endpoint (mg a.s./kg bw)	Fraction of active substance/ LD_{50} for the active substance	LD_{50} mix (mg a.s./kg bw)
Azoxystrobin	250	0.954	3 776	0.000253	3 795
Oxathiapiprolin	12	0.046	4 248	0.000011	
Total	262	1	-	0.000263	

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

Note: Calculations undertaken using unrounded values consequently they may not be reproducible when using the figures given in the table.

According to the EFSA Guidance Document (2009; Appendix B, Step 2a), the surrogate LD_{50} of 3795 mg a.s./kg bw for mixture toxicity should be compared to the acute oral toxicity of the formulation, using the following equation:

$$\sum_i \frac{X(a.s.i)}{LD_{50}(a.s.i)} = \frac{1}{LD_{50}(prod.)}$$

where:

$X(a.s.i)$ = fraction of active substance (i) in the formulation mixture
 $LD_{50}(a.s.i)$ = acute toxicity for the active substance (i)
 $LD_{50}(prod.)$ = measured acute toxicity value for the formulated mixture

A comparison of measured and predicted toxicity of A22773A is provided in the table below.

Table 9.2-7: Comparison of the measured formulation with the predicted mixture toxicity assuming dose additivity

Test substance	Azoxystrobin	Oxathiapiprolin	Sum	$1/LD_{50}$ (prod.) (PPP as a.s.)
Fraction of a.s. in formulation	0.954	0.046	1	1
LD_{50} (a.s.)	3 776	4 248	-	760 ^a
Fraction of a.s. in formulation/ LD_{50} for the individual active substance	0.000253	0.000011	0.000263	0.0013

^a Formulation endpoint expressed as total a.s. (based on the total concentration of azoxystrobin and oxathiapiprolin in the formulation (258.2 g a.s./L) and considering formulation density of 1.096 g/cm³).

A greater value on the right side of the equation indicates that the formulation is more toxic than predicted from the toxicity of the individual components (active substances and co-formulants of known toxicity). This may be due to, e.g. further toxic co-formulants, toxicokinetic interaction or synergism/potential of effect. It may also reflect the inherent variability of toxicity testing. In all these cases, the use of the LD_{50} for the formulation is recommended for the first-tier assessment, because it cannot be excluded that such effects would also occur after exposure of animals to residues in the environment.

If, in contrast, the measured toxicity of a formulation is lower than predicted, the predicted mixture toxicity according to Step 1 should be used in the first-tier risk assessment.

In the case of A22773A, this results in a value of 0.00026 on the left and 0.0013 on the right, indicating that the formulation is more toxic than predicted. However, this is not conclusive because the LD₅₀ for A22773A is above the highest dose tested. Nevertheless, the formulation endpoint will be used in the risk assessment.

Consideration of chronic mixture toxicity

According to EFSA/2009/1438⁴ combined action of several toxicants must be specifically considered in the risk assessment when it is obvious that such exposure situations will occur for animals.

For the assessment of chronic effects, a mixture tox risk assessment was conducted following the concentration addition (CA) method. The EFSA Guidance Document indicates that the following equation should be used for deriving a surrogate LD₅₀ for a mixture of active substances with known toxicity assuming dose additivity. A similar approach should be adopted for the chronic risk assessment.

$$NOAEL (mix) = \left(\sum \frac{X(a.s._i)}{NOAEL (a.s._i)} \right)$$

where:

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

$NOAEL(a.s._i)$ = chronic toxicity for the active substance (i)

The avian NOAEL of the mix is summarised in the table below.

Table 9.2-8: Theoretical NOAEL for the mixture of azoxystrobin and oxathiapiprolin

Test substance	Concentration of active substance in formulation A22773A (g a.s./L)	Fraction of active substance in the formulation mixture ^A	Chronic toxicity endpoint (mg a.s./kg bw/d)	Fraction of active substance/NOAEL for the active substance	NOAELmix (mg a.s./kg bw/d)
Azoxystrobin	250	0.954	117	0.008158	116.5
Oxathiapiprolin	12	0.046	106.7	0.000431	
Total	262	1		0.008589	

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

Note: Calculations undertaken using unrounded values consequently they may not be reproducible when using the figures given in the table.

9.2.2 Risk assessment for spray applications

The risk assessment is based on the methods presented in EFSA/2009/1438².

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessments for the uses in fruiting vegetables, leafy vegetables, bulb and onion like crops and hop cover the risk for birds from all intended uses (see 9.1.2).

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

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

⁴ European Food Safety Authority; Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA. EFSA journal 2009; 7(12):1438. [139 pp.]

Table 9.2-9: Screening assessment of the acute and long-term/reproductive risk for birds due to the use of A22773A – Azoxystrobin

Active substance		Azoxystrobin				
Acute toxicity (mg/kg bw)		3 776				
TER criterion		10				
Crop scenario	Application rate (g a.s./ha)	Indicator species	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a
Fruiting vegetables, BBCH 11 - 89	2 x 250 (7-d interval)	Small omnivorous bird	158.8	1.4	55.6	68
Leafy vegetables, BBCH 11 - 49	2 x 250 (7-d interval)	Small omnivorous bird	158.8	1.4	55.6	68
Bulbs and onion like crops, BBCH 11 - 49	2 x 250 (12-d interval)	Small omnivorous bird	158.8	1.3	51.6	73
Hop, BBCH 21 - 89	2 x 250 (12-d interval)	Small granivorous bird	24.7 25.3	1.3	8.03 8.22	470 459
Reprod. Toxicity (mg/kg bw/d)		117				
TER criterion		5				
Crop scenario	Application rate (g a.s./ha)	Indicator species	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{it}
Fruiting vegetables, BBCH 11 - 89	2 x 250 (7-d interval)	Small omnivorous bird	64.8	1.6 × 0.53	13.7	8.5
Leafy vegetables, BBCH 11 - 49	2 x 250 (7-d interval)	Small omnivorous bird	64.8	1.6 × 0.53	13.7	8.5
Bulbs and onion like crops, BBCH 11 - 49	2 x 250 (12-d interval)	Small omnivorous bird	64.8	1.4 × 0.53	12.0	9.7
Hop, BBCH 21 - 89	2 x 250 (12-d interval)	Small granivorous bird	11.4	1.4 x 0.53	2.1	55

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 9.2-10: Screening assessment of the acute and long-term/reproductive risk for birds due to the use of A22773A – Oxathiapiprolin

Active substance		Oxathiapiprolin				
Acute toxicity (mg/kg bw)		4 248				
TER criterion		10				
Crop scenario	Application rate (g a.s./ha)	Indicator species	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a
Fruiting vegetables, BBCH 11 - 89	2 x 12 (7-d interval)	Small omnivorous bird	158.8	1.4	2.67	1 600
Leafy vegetables, BBCH 11 - 49	2 x 12 (7-d interval)	Small omnivorous bird	158.8	1.4	2.67	1 600

Bulbs and onion like crops, BBCH 11 - 49	2 x 12 (12-d interval)	Small omnivorous bird	158.8	1.3	2.5	1 715
Hop, BBCH 21 - 89	2 x 12 (12-d interval)	Small granivorous bird	24.7 25.3	1.3	0.385 0.395	11 000 10 754
Reprod. Toxicity (mg/kg bw/d)		106.7				
TER criterion		5				
Crop scenario Growth stage	Application rate (g a.s./ha)	Indicator species	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_t
Fruiting vegetables, BBCH 11 - 89	2 x 12 (7-d interval)	Small omnivorous bird	64.8	1.6 × 0.53	0.659	160
Leafy vegetables, BBCH 11 - 49	2 x 12 (7-d interval)	Small omnivorous bird	64.8	1.6 × 0.53	0.659	160
Bulbs and onion like crops, BBCH 11 - 49	2 x 12 (12-d interval)	Small omnivorous bird	64.8	1.4 × 0.53	0.58	185
Hop, BBCH 21 - 89	2 x 12 (12-d interval)	Small granivorous bird	11.4	1.4 x 0.53	0.102	1051

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

The acute and chronic screening assessment for azoxystrobin and oxathiapiprolin, for all indicator species concludes TER values greater than the trigger of 10 for acute risk and 5 for chronic risk, indicating that risk to birds is acceptable following use of A22773A according to the proposed use pattern.

Azoxystrobin/Oxathiapiprolin mixture assessment

Acute risk

The experimentally determined LD₅₀ of the formulation A22773A is used as the endpoint for the acute combination mixture risk assessment (see 9.2.1.1).

Table 9.2-11: Screening assessment of the acute risk for birds due to the use of A22773A

Product		A22773A				
Acute toxicity (mg prod./kg bw)		3 228				
TER criterion		10				
Crop scenario Growth stage	Application rate (g prod./ha)	Indicator species	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a
Fruiting vegetables, BBCH 11 - 89	2 x 1 097 ^a (7-d interval)	Small omnivorous bird	158.8	1.4	240	13
Leafy vegetables, BBCH 11 - 49	2 x 1 097 ^a (7-d interval)	Small omnivorous bird	158.8	1.4	240	13
Bulbs and onion like crops, BBCH 11 - 49	2 x 1 097 ^a (12-d interval)	Small omnivorous bird	158.8	1.3	226	14
Hop, BBCH 21 - 89	2 x 1 097 ^a (12-d interval)	Small granivorous bird	24.7	1.3	35.2	92

SV: shortcut value; MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values

shown in bold fall below the relevant trigger.

^a Based on an application rate of 1 L/ha and a nominal formulation density of 1.097 g/cm³

The TER_a values for the mixture toxicity are greater than the relevant trigger value indicating that acute risk to birds is acceptable following use of A22773A according to the proposed use patterns.

Chronic risk

For assessment of chronic effects, according to EFSA/2009/1438², 'if a given formulation contains several active substances all known to cause similar effects via a similar biochemical mechanism (e.g. aromatase inhibition) and if this type of effect is actually driving the risk assessment, it is thus recommended to perform an assessment for combined effects on a case by case basis'.

For A22773A the active ingredients, azoxystrobin (a methoxy-acrylate with activity by disrupting the mitochondrial respiration of fungi) has a different mode of action in fungi than the active ingredient oxathiapiprolin (a piperidiny l thiazole isoxazoline with activity by inhibition of oxysterol binding protein (OSBP) homologue). Their toxicity profiles are different as demonstrated in mammalian studies (refer to 9.3.2.1). Consequently, an assessment for combined effects is considered to not be required.

Moreover, in the reproductive toxicity studies with oxathiapiprolin no effects on survival and reproduction were determined in Bobwhite quail and Mallard duck up to the highest dose level tested (1 200 mg a.s./kg feed). An assessment for combined effects based on the NOEL derived from these studies is therefore not considered justified.

However, RMS Poland requested Syngenta to include a quantitative chronic combination mixture risk assessment for A22773A.

Chronic risk

The calculated NOAEL of the mixture of azoxystrobin/oxathiapiprolin is used as the endpoint for the chronic combination mixture risk assessment (see 9.2.1.1).

Table 9.2-12: Screening assessment of the long-term/reproductive risk for birds due to the use of A22773A

Active substance		azoxystrobin/oxathiapiprolin				
Chronic toxicity (mg a.s./kg bw/d)		116.5				
TER criterion		5				
Crop scenario Growth stage	Application rate (g active substance/ha)	Indicator species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{it}
Fruiting vegetables, BBCH 11 - 89	2 x 262 ^a (7-d interval)	Small omnivorous bird	64.8	1.6 × 0.53	14.4	8.1
Leafy vegetables, BBCH 11 - 49	2 x 262 ^a (7-d interval)	Small omnivorous bird	64.8	1.6 × 0.53	14.4	8.1
Bulbs and onion like crops, BBCH 11 - 49	2 x 262 ^a (12-d interval)	Small omnivorous bird	64.8	1.4 × 0.53	12.6	9.2
Hop, BBCH 21 - 89	2 x 262 ^a (12-d interval)	Small granivorous bird	11.4	1.4 x 0.53	2.2	52.6

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

^a Application rate of azoxystrobin/oxathiapiprolin mixture is the sum of both active substances i.e. sum of 250 g

azoxystrobin/ha + 12 g oxathiapiprolin /ha = 262 g/ha

The TER values for the mixture toxicity are greater than the relevant trigger value for all crops indicating that chronic risk to birds is acceptable following use of A22773A according to the proposed use patterns.

Combined reproductive toxicity

As requested in the Working document on Risk Assessment of Plant Protection Products in the Central Zone – Ecotoxicology (May 2021), a calculation of long-term combitox risk according to the concentration addition (CA) model should be presented for Tier 1.

This virtual compound chronic TER was calculated according to the concentration addition approach and thus gives the same value as if calculated using equation $TER_{LT\text{combi}} = \text{trigger}/((\text{trigger}/TER_{\text{substance 1}}) + (\text{trigger}/TER_{\text{substance 2}}))$.

The $TER_{LT\text{combi}}$ value in the screening step is above the trigger value of 5. Thus, it can be concluded that the reproductive risk for birds for the combined exposure to the two active substances in the application of A22773A according to good agricultural practice is low and acceptable.

9.2.2.2 Higher-tier risk assessment

Not required.

9.2.2.3 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 A22773A is 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 must be considered. Here, the maximum single use rate for leafy vegetables of 1 L A22773A/ha equivalent to 250 g azoxystrobin/ha and 12 g oxathiapiprolin/ha is used in combination with the minimum water volume of 200 L/ha to cover the risk to birds from all intended uses (see 9.1.2).

Table 9.2-134: Assessment of the acute risk for birds due to exposure to azoxystrobin via contaminated drinking water in leaf whorls

Active substance		Azoxystrobin				
Maximum application rate (g a.s./ha)		2 x 250				
Acute toxicity (mg/kg bw)		3 776				
TER criterion		10				
(Single) application rate (g/ha)	Water application rate (L/ha)	C _{spray-sol.} (mg/L)	PEC _{leaf-whorl} = C _{spray-sol.} /5 (mg/L)	DW uptake (L/kg bw/d)	Daily dose (mg/kg bw/d)	TER _a
250	200	1 250	250	0.46	115.0	33

C_{spray-sol.}: concentration in spray solution; PEC_{leaf-whorl}: concentration in pools in leaf whorls; DW: drinking water; TER: toxicity to

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

Table 9.2-145: Assessment of the acute risk for birds due to exposure to oxathiapiprolin via contaminated drinking water in leaf whorls

Active substance		Oxathiapiprolin				
Maximum application rate (g a.s./ha)		2 x 12				
Acute toxicity (mg/kg bw)		4 248				
TER criterion		10				
(Single) application rate (g/ha)	Water application rate (L/ha)	C_{spray-sol.} (mg/L)	PEC_{leaf-whorl} = C_{spray-sol.}/5 (mg/L)	DW uptake (L/kg bw/d)	Daily dose (mg/kg bw/d)	TER_a
12	200	60	12	0.46	5.52	770

C_{spray-sol.}: concentration in spray solution; PEC_{leaf-whorl}: concentration in pools in leaf whorls; DW: drinking water; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

The TER_a values for azoxystrobin and oxathiapiprolin are greater than the trigger value of 10 indicating that acute risk to birds due to exposure via contaminated drinking water in leaf whorls is acceptable following use of A22773A on leafy vegetables according to the proposed use patterns.

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 3 000 in the case of more sorptive substances (K_{oc} ≥ 500 L/kg).

With a K(f)_{oc} of 423 mL/g, azoxystrobin belongs to the group of less sorptive substances and with a K(f)_{oc} of 6 243 mL/g, oxathiapiprolin belongs to the group of more sorptive substances.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the maximum use rate of 2 x 250 g azoxystrobin/ha (7 d interval) and 2 x 12 g oxathiapiprolin/ha (7 d interval) is used to cover the risk to birds from all intended uses (see 9.1.2).

Azoxystrobin			
Effective application rate (g/ha)* =	500		
Acute toxicity (mg/kg bw) =	3 776	quotient =	0.1
Reprod. Toxicity (mg/kg bw/d) =	117	quotient =	4.3

* Effective application rate = Maximum application rate x MAF of 2.0

MAF is based on 2 applications, 7-day application interval and soil DT₅₀ value of 262.0 days

Oxathiapiprolin			
Effective application rate (g/ha)* =	24		
Acute toxicity (mg/kg bw) =	4 248	quotient =	0.01
Reprod. Toxicity (mg/kg bw/d) =	106.7	quotient =	0.22

* Effective application rate = Maximum application rate x MAF of 2.0

MAF is based on 2 applications, 7-day application interval and soil DT₅₀ value of 121.2 days

The resulting ratios fall below the trigger of 50 for azoxystrobin (less sorptive) and 3000 for oxathiapiprolin

(more sorptive), indicating that further assessment of the acute and long-term risk to birds from drinking water from puddles is not required.

9.2.2.4 Effects of secondary poisoning

According to EFSA/2009/1438², substances with a log P_{ow} of > 3 have a potential for bioaccumulation and as such consideration of the potential effects of secondary poisoning to birds and mammals are required.

The log P_{ow} of azoxystrobin amounts to 2.5 and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

The log P_{ow} of oxathiapiprolin is 3.67 (at pH 7) and thus exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is required. The log P_{ow} values of the oxathiapiprolin metabolites IN-Q7D41, IN-S2K66 and IN-RDT31 were reported to be 4.3, 3.4, and 4.1, respectively, and exceed the trigger value of 3. Therefore, a risk assessment for effects due to secondary poisoning is required. IN-S2K66 and IN-Q7D41 were not found in soil and the risk assessment was therefore performed for fish-eating birds only.

Risk assessment for earthworm-eating birds via secondary poisoning

According to EFSA/2009/1438², the risk for vermivorous birds is assessed for a bird of 100 g body weight with a daily food consumption of 104.6 g. Bioaccumulation in earthworms is estimated based on predicted concentrations in soil.

Oxathiapiprolin

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the worst-case 21-day time-weighted average soil PEC following 2 x 12 g a.s./ha applications to onions (see Section 8 - Environmental Fate, Chapter 8.7.2).

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

Parameter	Oxathiapiprolin	Comments
PEC _{soil} (twa = 21 d) (mg/kg soil)	0.027	Onions, 2 x 12 g a.s./ha, crop interception 10%, soil layer depth 20 cm with tillage after year 10, DT ₅₀ = 205.3 d, twa interval = 21 d
log P_{ow} / K_{ow}	3.67 / 4 677	EFSA, 2016
K_{oc}	6 243	Arithmetic mean (n = 5)
f_{oc}	0.02	Default
BCF _{worm}	1*	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + 0.012 \times K_{ow}) / f_{oc} \times K_{oc}$
PEC _{worm}	0.03	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.03	DDD = $PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	106.7	Bobwhite quail (<i>Colinus virginianus</i>)
TER _{lt}	3 764	≥ 5 ; acceptable risk

TER values shown in bold fall below the relevant trigger.

* calculated BCF = 0.46. A BCF of 1 was used for TER_{lt} estimation as a worst-case considering soil in gut of earthworms if relevant.

Table 9.2-16: Assessment of the risk for earthworm-eating birds due to exposure to IN-RDT31 via bioaccumulation in earthworms (secondary poisoning)

Parameter	IN-RDT31	Comments
PEC _{soil} (initial) (mg/kg soil)	0.003	Initial PEC _{soil} for application on onions (refer to Section 8, Chapter 8.7.2)
log P _{ow} / K _{ow}	4.1 / 12 589	EFSA, 2016
K _{oc}	1 168	Arithmetic mean (n = 5)
f _{oc}	0.02	Default
BCF _{worm}	6.50	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + 0.012 \times K_{ow}) / f_{oc} \times K_{oc}$
PEC _{worm}	0.02	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.02	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	10.67	10x parent toxicity assumed as a worst case ^a
TER _{lt}	521	≥ 5; acceptable risk

TER values shown in bold fall below the relevant trigger.

^a In accordance with the DAR, 2016, volume 3, Annex B.9 PPP

Risk assessment for fish-eating birds via secondary poisoning

According to EFSA/2009/1438², 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.

Oxathiapiprolin

To achieve a concise risk assessment, the risk envelope approach is applied. The worst-case 21-day time-weighted average surface water PEC (FOCUS Step 1) following 2 x 12 g a.s./ha applications to hops was used for oxathiapiprolin. For the metabolites the maximum FOCUS Step 1 PEC_{sw} values were used. See Section 8 (Environmental Fate), Chapter 8.9.2.

Table 9.2-17: Assessment of the risk for fish-eating birds due to exposure to oxathiapiprolin via bioaccumulation in fish (secondary poisoning)

Parameter	Oxathiapiprolin	Comments
PEC _{sw} (twa = 21 d) (mg/L)	0.000958	Max. FOCUS Step 1 21-d TWA PEC _{sw} for application to hops
BCF _{fish}	87	EFSA, 2016
BMF	-	Biomagnification factor (relevant for BCF ≥ 2000)
PEC _{fish}	0.083	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.01	$DDD = PEC_{fish} \times 0.159$
NOEL (mg/kg bw/d)	106.7	Bobwhite quail (<i>Colinus virginianus</i>)
TER _{lt}	8 052	≥ 5; acceptable risk

TER values shown in bold fall below the relevant trigger.

Table 9.2-18: Assessment of the risk for fish-eating birds due to exposure to IN-Q7D41 via bioaccumulation in fish (secondary poisoning)

Parameter	IN-Q7D41	Comments
PEC _{sw} (maximum) (mg/L)	0.00111	Max. FOCUS Step 1 PEC _{sw} for application to hops
BCF _{fish}	533	EFSA, 2016
BMF	-	Biomagnification factor (relevant for BCF ≥ 2000)
PEC _{fish}	0.592	PEC _{fish} = PEC _{water} × BCF _{fish}
Daily dietary dose (mg/kg bw/d)	0.09	DDD = PEC _{fish} × 0.159
NOEL (mg/kg bw/d)	10.67	10x parent toxicity assumed as a worst-case ^a
TER _{lt}	113	≥ 5; acceptable risk

TER values shown in bold fall below the relevant trigger.

^a In accordance with the DAR, 2016, volume 3, Annex B.9 PPP

Table 9.2-19: Assessment of the risk for fish-eating birds due to exposure to IN-S2K66 via bioaccumulation in fish (secondary poisoning)

Parameter	IN-S2K66	Comments
PEC _{sw} (maximum) (mg/L)	0.000805	Max. FOCUS Step 1 PEC _{sw} for application to hops
BCF _{fish}	115	EFSA, 2016
BMF	-	Biomagnification factor (relevant for BCF ≥ 2000)
PEC _{fish}	0.093	PEC _{fish} = PEC _{water} × BCF _{fish}
Daily dietary dose (mg/kg bw/d)	0.01	DDD = PEC _{fish} × 0.159
NOEL (mg/kg bw/d)	10.67	10x parent toxicity assumed as a worst case ^a
TER _{lt}	725	≥ 5; acceptable risk

TER values shown in bold fall below the relevant trigger.

^a In accordance with the DAR, 2016, volume 3, Annex B.9 PPP

Table 9.2-20: Assessment of the risk for fish-eating birds due to exposure to IN-RDT31 via bioaccumulation in fish (secondary poisoning)

Parameter	IN-RDT31	Comments
PEC _{sw} (maximum) (mg/L)	0.000303	Max. FOCUS Step 1 PEC _{sw} for application to leafy vegetables, fruiting vegetables, bulb vegetables and hops
BCF _{fish}	78	EFSA, 2016
BMF	-	Biomagnification factor (relevant for BCF ≥ 2000)
PEC _{fish}	0.024	PEC _{fish} = PEC _{water} × BCF _{fish}
Daily dietary dose (mg/kg bw/d)	0.00376	DDD = PEC _{fish} × 0.159
NOEL (mg/kg bw/d)	10.67	10x parent toxicity assumed as a worst case ^a
TER _{lt}	2 839	≥ 5; acceptable risk

TER values shown in bold fall below the relevant trigger.

^a In accordance with the DAR, 2016, volume 3, Annex B.9 PPP

Risk of secondary poisoning was assessed for oxathiapiprolin (log P_{ow} = 3.67 at pH 7) and some of its metabolites (IN-Q7D41, IN-S2K66 and IN-RDT31 with log P_{ow} values of 4.3, 3.4, and 4.1, respectively). All calculated TER_{lt} values clearly exceed the trigger value of 5 indicating that the risk of secondary

poisoning to birds is acceptable following use of A22773A according to the proposed use pattern.

9.2.2.5 Biomagnification in terrestrial food chains

Not relevant.

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

Not relevant.

9.2.4 Overall conclusions

The acute and long-term risks of A22773A to birds were assessed from toxicity exposure ratios between toxicity endpoints, estimated from studies with A22773A, azoxystrobin and oxathiapiprolin, and maximum residues occurring on food items following applications according to the proposed use pattern.

Risk of secondary poisoning has also been assessed, as oxathiapiprolin and some of its metabolites have log P_{ow} values of > 3.0 . The risk to birds from exposure via drinking water has also been assessed.

The TER values, calculated for recommended scenarios, all exceed the trigger values of 10 for acute risk and 5 for long-term risk (including secondary poisoning), indicating that the risk to birds is acceptable following use of A22773A according to the proposed use pattern.

Review Comments:

The acute and chronic risks of A22773A to birds were assessed from toxicity exposure ratios between toxicity endpoints, estimated from study with active ingredients and maximum residues occurring on food items.

All TER values exceed the relevant triggers indicating that A22773A does not pose an unacceptable risk to birds following applications according to recommended use pattern.

Evaluation of exposing to birds through the drinking water demonstrated the acceptable risk. The potential risk of secondary poisoning is low.

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

9.3.1 Toxicity data

Mammalian toxicity studies have been carried out with azoxystrobin and oxathiapiprolin. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on mammals of A22773A were not evaluated as part of the EU assessment of azoxystrobin and oxathiapiprolin. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2 (see also section B6 (Toxicology) for study summary).

The selection of endpoints for the risk assessment deviates from the results of the EU review process. Justifications are provided below where necessary.

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

Species	Substance	Exposure System	Results	Reference
Rat	Azoxystrobin	Oral 1 d Acute	LD₅₀ > 5 000 mg a.s./kg bw	EFSA, 2010, Robinson, 1991, ICI5504/0081
Rat	Azoxystrobin	Dietary Reproductive toxicity Two-generation study	NOAEL = 32 mg a.s./kg bw/d (offspring effects on pup weight)	EFSA, 2010, Moxon, 1994, ICI5504/0117

Endpoints used in risk assessment are shown in **bold**.
EFSA Journal 2010; 8(4):1542.

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

Species	Substance	Exposure System	Results	Reference
Rat	Oxathiapiprolin	Oral 1 d Acute	LD₅₀ > 5 000 mg a.s./kg bw	EFSA, 2016, DuPont-29441
Rat	Oxathiapiprolin	Dietary Reproductive toxicity Two-generation study	NOAEL = 86.37 mg a.s./kg bw/day	EFSA, 2016, DuPont-30258

Endpoints used in risk assessment are shown in **bold**.
EFSA Journal 2016;14(7):4504.

Metabolites

Studies performed with mammals indicated that plant metabolites of oxathiapiprolin are less toxic than the parent. Moreover, metabolites of oxathiapiprolin are not included in the definition of residues in plants and exposure via these food sources is not expected. For this reason, metabolites were not considered in the dietary risk assessment.

Table 9.3-3: Endpoints and effect values relevant for the risk assessment for mammals – A22773A

Species	Substance	Exposure System	Results	Reference
Rat	A22773A	Oral 1 d Acute	LD₅₀ > 2 000 mg/kg bw (> 471 mg a.s./kg bw) ^b	xxxxxx 2021, VV-892044
-	Theoretical Mixture azoxystrobin/oxathiapiprolin	Oral 1 d Acute	Calculated LD ₅₀ > 5 000 mg a.s./kg bw	Refer to 9.3.1.1
-	Theoretical Mixture azoxystrobin/oxathiapiprolin	Dietary Reproductive toxicity Two-generation study	Calculated NOAEL = 33 mg a.s./kg bw	Refer to 9.3.1.1

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

^b Formulation endpoint expressed as total a.s. (based on the total concentration of azoxystrobin and oxathiapiprolin in the formulation (actual 258.2 g a.s./L) and considering formulation density of 1.096 g/cm³).

9.3.1.1 Justification for new endpoints

Consideration of acute mixture toxicity

According to EFSA/2009/1438² combined action of several toxicants must be specifically considered in the risk assessment when it is obvious that such exposure situations will occur for animals.

For the assessment of acute effects (mortality), a surrogate LD₅₀ can be calculated. The EFSA Guidance Document indicates that the following equation should be used for deriving a surrogate LD₅₀ for a mixture of active substances with known toxicity assuming dose additivity:

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

where:

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

$LD_{50}(a.s._i)$ = acute toxicity for the active substance (i)

The LD₅₀ of the mix is summarised in the table below.

Table 9.3-4: Acute LD₅₀ for the mixture of azoxystrobin and oxathiapiprolin

Test substance	Concentration of active substance in formulation A22773A (g a.s./L)	Fraction of active substance in the formulation mixture ^A	Acute toxicity endpoint (mg a.s./kg bw)	Fraction of active substance/LD ₅₀ for the active substance	LD ₅₀ mix (mg a.s./kg bw)
Azoxystrobin	250	0.954	> 5 000	< 0.000009	> 5 000
Oxathiapiprolin	12	0.046	> 5 000	< 0.00019	
Total	262	1	-	< 0.00020	

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

Note: Calculations undertaken using unrounded values consequently they may not be reproducible when using the figures given in the table.

According to the EFSA Guidance Document (2009; Appendix B, Step 2a), the surrogate LD₅₀ of > 5 000 mg a.s./kg bw for mixture toxicity should be compared to the acute oral toxicity of the formulation, using the following equation:

$$\sum_i \frac{X(a.s._i)}{LD_{50}(a.s._i)} = \frac{1}{LD_{50}(prod.)}$$

where:

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

$LD_{50}(a.s._i)$ = acute toxicity for the active substance (i)

$LD_{50}(prod.)$ = measured acute toxicity value for the formulated mixture

A comparison of measured and predicted toxicity of A22773A is provided in the table below.

Table 9.3-5: Comparison of the measured formulation with the predicted mixture toxicity assuming dose additivity

Test substance	Azoxystrobin	Oxathiapiprolin	Sum	1/LD ₅₀ (prod.) (PPP as a.s.)
Fraction of a.s. in formulation	0.954	0.046	1	1
LD ₅₀ (a.s.)	> 5 000	> 5 000	-	> 471 ^a
Fraction of a.s. in formulation/ LD ₅₀ for the individual active substance	< 0.0000092	< 0.00019	< 0.00020	< 0.0021

^a Formulation endpoint expressed as total a.s. (based on the total concentration of azoxystrobin and oxathiapiprolin in the formulation (actual 258.2 g a.s./L) and considering formulation density of 1.096 g/cm³).

A greater value on the right side of the equation indicates that the formulation is more toxic than predicted from the toxicity of the individual components (active substances and co-formulants of known toxicity). This may be due to, e.g. further toxic co-formulants, toxicokinetic interaction or synergism/potential of effect. It may also reflect the inherent variability of toxicity testing. In all these cases, the use of the LD₅₀ for the formulation is recommended for the first-tier assessment, because it cannot be excluded that such effects would also occur after exposure of animals to residues in the environment.

If, in contrast, the measured toxicity of a formulation is lower than predicted, the predicted mixture toxicity according to Step 1 should be used in the first-tier risk assessment.

In the case of A22773A, this results in a value of < 0.00020 on the left and < 0.0021 on the right, indicating that the formulation is more toxic than predicted. However, this is not conclusive because the LD₅₀ for A22773A is above the highest dose tested. Nevertheless, the formulation endpoint will be used in the risk assessment.

Consideration of chronic mixture toxicity

According to EFSA/2009/1438⁵ combined action of several toxicants must be specifically considered in the risk assessment when it is obvious that such exposure situations will occur for animals.

For the assessment of chronic effects, a mixture tox risk assessment was conducted following the concentration addition (CA) method. The EFSA Guidance Document indicates that the following equation should be used for deriving a surrogate LD₅₀ for a mixture of active substances with known toxicity assuming dose additivity. A similar approach should be adopted for the chronic risk assessment.

$$NOAEL (mix) = \left(\sum_i \frac{X(a.s._i)}{NOAEL(a.s._i)} \right)$$

where:

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

$NOAEL(a.s._i)$ = chronic toxicity for the active substance (i)

The mammalian NOAEL of the mix is summarised in the table below.

⁵ European Food Safety Authority; Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA. EFSA journal 2009; 7(12):1438. [139 pp.]

Table 9.3-6: Chronic NOAEL for the mixture of azoxystrobin and oxathiapiprolin

Test substance	Concentration of active substance in formulation A22773A (g a.s./L)	Fraction of active substance in the formulation mixture ^A	Chronic toxicity endpoint (mg a.s./kg bw/d)	Fraction of active substance/NOAEL for the active substance	NOAEL mix (mg a.s./kg bw/d)
Azoxystrobin	250	0.954	32	0.0298125	33
Oxathiapiprolin	12	0.046	86.37	0.0005326	
Total	262	1	-	0.0303451	

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

Note: Calculations undertaken using unrounded values consequently they may not be reproducible when using the figures given in the table.

9.3.2 Risk assessment for spray applications

The risk assessment is based on the methods presented in EFSA/2009/1438².

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessments for the uses in fruiting vegetables, leafy, bulb and onion like crops and hop cover the risk for mammals from all intended uses (see 9.1.2).

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

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

Table 9.3-7: Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of A22773A – Azoxystrobin

Active substance		Azoxystrobin				
Acute toxicity (mg/kg bw)		> 5 000				
TER criterion		10				
Crop scenario Growth stage	Application rate (g a.s./ha)	Indicator species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a
Fruiting vegetables, BBCH 11 - 89	2 x 250 (7-d interval)	Small herbivorous mammal	136.4	1.4	47.7	> 105
Leafy vegetables, BBCH 11 - 49	2 x 250 (7-d interval)	Small herbivorous mammal	136.4	1.4	47.7	> 105
Bulbs and onion like crops, BBCH 11 - 49	2 x 250 (12-d interval)	Small herbivorous mammal	118.4	1.3	38.5	> 130
Tier 1 – worst case SV ₉₀ value						
Hop BBCH ≥40	2 x 250 (12-d interval)	Small herbivorous mammal (vole)	40.9	1.3	13.29	>376
Hop, BBCH 21 – 89	2 x 250 (12-d interval)	Small herbivorous mammal	118.4	1.3	38.5	>130

Reprod. Toxicity (mg/kg bw/d)		32				
TER criterion		5				
Crop scenario	Application rate (g a.s./ha)	Indicator species	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{It}
Fruiting vegetables, BBCH 11 - 89	2 x 250 (7-d interval)	Small herbivorous mammal	72.3	1.6 × 0.53	15.3	2.1
Leafy vegetables, BBCH 11 - 49	2 x 250 (7-d interval)	Small herbivorous mammal	72.3	1.6 × 0.53	15.3	2.1
Bulbs and onion like crops, BBCH 11 - 49	2 x 250 (12-d interval)	Small herbivorous mammal	48.3	1.4 × 0.53	8.96	3.6
Hop, BBCH 21 - 89	2 x 250 (12-d interval)	Small herbivorous mammal	48.3	1.4 × 0.53	8.96	3.6

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.

Review Comments:

For screening assessment, the shortcut value for mammals is not given for hop scenario. For Tier 1 reproductive risk assessment please refer to Tables 9.3-9 and 9.3-10.

Table 9.3-8: Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of A22773A – Oxathiapiprolin

Active substance		Oxathiapiprolin				
Acute toxicity (mg/kg bw)		> 5 000				
TER criterion		10				
Crop scenario	Application rate (g a.s./ha)	Indicator species	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a
Fruiting vegetables, BBCH 11 - 89	2 x 12 (7-d interval)	Small herbivorous mammal	136.4	1.4	2.29	> 2 200
Leafy vegetables, BBCH 11 - 49	2 x 12 (7-d interval)	Small herbivorous mammal	136.4	1.4	2.29	> 2 200
Bulbs and onion like crops, BBCH 11 - 49	2 x 12 (12-d interval)	Small herbivorous mammal	118.4	1.3	1.9	> 2 707
Tier 1 – worst case SV ₉₀ value						
Hop BBCH ≥40	2 x 12 (12-d interval)	Small herbivorous mammal (vole)	40.9	1.3	0.64	>7812
Hop, BBCH 21 - 89	2 x 12 (12-d interval)	Small herbivorous mammal	118.4	1.3	1.85	> 2 700

Reprod. Toxicity (mg/kg bw/d)		86.37				
TER criterion		5				
Crop scenario Growth stage	Application rate (g a.s./ha)	Indicator species	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{lt}
Fruiting vegetables, BBCH 11 - 89	2 x 12 (7-d interval)	Small herbivorous mammal	72.3	1.6 × 0.53	0.736	120
Leafy vegetables, BBCH 11 - 49	2 x 12 (7-d interval)	Small herbivorous mammal	72.3	1.6 × 0.53	0.736	120
Bulbs and onion like crops, BBCH 11 - 49	2 x 12 (12-d interval)	Small herbivorous mammal	48.3	1.4 × 0.53	0.430	201
Tier 1 – worst case SV _{mean} value						
Hop BBCH ≥40	2 x 12 (12-d interval)	Small herbivorous mammal (vole)	21.7	1.4 x 0.53	0.19	455
Hop, BBCH 21 – 89	2 x 12 (12-d interval)	Small herbivorous mammal	48.3	1.4 × 0.53	0.430	201

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.

Review Comments:

For screening assessment, the shortcut value for mammals is not given for hop scenario.

The acute screening assessment for azoxystrobin and oxathiapiprolin, for all indicator species concludes TER_a values greater than the trigger of 10, indicating that acute risk to mammals is acceptable following use of A22773A according to the proposed use pattern. The TER_{lt} values for oxathiapiprolin are greater than the trigger of 5, indicating that the chronic risk to mammals is acceptable. However, the TER_{lt} values for azoxystrobin for the proposed uses are below the trigger value of 5, indicating that a Tier 1 assessment is required to evaluate the chronic risk to mammals.

Table 9.3-9: Tier 1 assessment of the long-term/reproductive risk for mammals due to the use of A22773A – Azoxystrobin

Active substance/product		Azoxystrobin				
Reprod. Toxicity (mg/kg bw/d)		32				
TER criterion		5				
Crop scenario Growth stage	Application rate (g a.s./ha)	Generic focal species	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{lt}
Fruiting vegetables, BBCH 11 - 89	2 x 250 (7-d interval)	Frugivorous mammal “rat” Fruit stage BBCH 71 - 89	25.2	1.6 × 0.53	5.34	6.0
		Small insectivorous mammal “shrew” BBCH 10 - 19	4.2		0.890	36
		Small insectivorous mammal “shrew” BBCH ≥ 20	1.9		0.403	79

Active substance/product		Azoxystrobin				
Reprod. Toxicity (mg/kg bw/d)		32				
TER criterion		5				
Crop scenario Growth stage	Application rate (g a.s./ha)	Generic focal species	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{It}
		Small herbivorous mammal “vole” BBCH 10 - 49	72.3		15.3	2.1
		Small herbivorous mammal “vole” BBCH ≥ 50	21.7		4.60	7.0
		Small omnivorous mammal “mouse” BBCH 10 - 49	7.8		1.65	19
		Small omnivorous mammal “mouse” BBCH ≥ 50	2.3		0.488	66
Leafy vegetables, BBCH 11 - 49	2 x 250 (7-d interval)	Small insectivorous mammal “shrew” BBCH 10 - 19	4.2	1.6 × 0.53	0.890	36
		Small insectivorous mammal “shrew” BBCH ≥ 20	1.9		0.403	79
		Small herbivorous mammal “vole” BBCH 40 - 49	72.3		15.3	2.1
		Large herbivorous mammal “lagomorph” All season	14.3		3.03	11
		Small omnivorous mammal “mouse” BBCH 10 - 49	7.8		1.65	19
Bulbs and onion like crops, BBCH 11 - 49	2 x 250 (12-d interval)	Small insectivorous mammal “shrew” BBCH 10 - 19	4.2	1.4 × 0.53	0.78	41
		Small insectivorous mammal “shrew” BBCH ≥ 20	1.9		0.35	91
		Small herbivorous mammal “vole” BBCH ≥ 40	43.4		8.05	4.0
		Small omnivorous mammal “mouse” BBCH 10 - 39	7.8		1.45	22
		Small omnivorous mammal “mouse” BBCH ≥ 40	4.7		0.87	37

Active substance/product		Azoxystrobin				
Reprod. Toxicity (mg/kg bw/d)		32				
TER criterion		5				
Crop scenario Growth stage	Application rate (g a.s./ha)	Generic focal species	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{It}
Hop, BBCH 21 - 89	2 x 250 (12-d interval)	Small insectivorous mammal “shrew” BBCH ≥ 20	1.9	1.4 × 0.53	0.352	91
		Small herbivorous mammal “vole” BBCH ≥ 40	21.7		4.03	7.9
		Small omnivorous mammal “mouse” BBCH 20 - 39	3.9		0.723	44
		Small omnivorous mammal “mouse” BBCH ≥ 40	2.3		0.427	75

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

For azoxystrobin for the following scenarios, the chronic TER value was below the trigger of 5:

- Fruiting vegetables, small herbivorous mammal “vole”, BBCH 10-49
- Leafy vegetables, small herbivorous mammal “vole”, BBCH 40-49
- Bulbs and onion like crops, small herbivorous mammal “vole”, BBCH ≥ 40

Azoxystrobin/Oxathiapiprolin mixture assessment

Acute risk

The experimentally determined LD₅₀ of the formulation A22773A is used as the endpoint for the acute combination mixture risk assessment (see 9.3.1.1).

Table 9.3-10: Screening assessment of the acute risk for mammals due to the use of A22773A

Product		A22773A				
Acute toxicity (mg prod./kg bw)		> 2 000				
TER criterion		10				
Crop scenario Growth stage	Application rate (g prod./ha)	Indicator species	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a
Fruiting vegetables, BBCH 11 - 89	2 x 1 097 ^a (7-d interval)	Small herbivorous mammal	136.4	1.4	209	> 9.5
Leafy vegetables, BBCH 11 - 49	2 x 1 097 ^a (7-d interval)	Small herbivorous mammal	136.4	1.4	209	> 9.5
Bulbs and onion like crops, BBCH 11 - 49	2 x 1 097 ^a (12-d interval)	Small herbivorous mammal	118.4	1.3	169	> 12

Tier 1 – worst case SV ₉₀ value						
Hop BBCH ≥40	2 x 1 097 ^a (12-d interval)	Small herbivorous mammal (vole)	40.9	1.3	58.33	>34.3
Hop; BBCH 21 – 89	2 x 1 097 ^a (12-d interval)	Small herbivorous mammal	118.4	1.3	169	>12

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

^a Based on an application rate of 1 L/ha and a nominal formulation density of 1.097 g/cm³

Review Comments:

For screening assessment, the shortcut value for mammals is not given for hop scenario. For Tier 1 reproductive risk assessment please refer to Table 9.3-13.

The TER_a values for the formulation A22773A for the proposed uses in fruiting vegetables and leafy vegetables are below the trigger value of 10, indicating that a Tier 1 assessment is required to evaluate the acute risk to mammals. However, the highest dose tested in the study was 2000 mg/kg bw, which did not cause any mortality and thus the real LD₅₀ would be significantly higher.

Table 9.3-11: Tier 1 assessment of the acute risk for mammals due to the use of A22773A

Product		A22773A				
Acute toxicity (mg prod./kg bw)		> 2 000				
TER criterion		10				
Crop scenario Growth stage	Application rate (g prod./ha)	Generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a
Fruiting vegetables, BBCH 11 - 89	2 x 1 097 ^a (7-d interval)	Frugivorous mammal “rat” fruit stage BBCH 71 - 89	45.2	1.4	69.42	> 29
		Small insectivorous mammal “shrew” BBCH 10 - 19	7.6		11.67	> 170
		Small insectivorous mammal “shrew” BBCH ≥ 20	5.4		8.293	> 240
		Small herbivorous mammal “vole” BBCH 10 - 49	136		208.87	> 9.6
		Small herbivorous mammal “vole” BBCH ≥ 50	40.9		62.81	> 32
		Small omnivorous mammal “mouse” BBCH 10 - 49	17.2		26.42	> 76
		Small omnivorous mammal “mouse” BBCH ≥ 50	5.2		7.986	> 250
Leafy vegetables, BBCH 11 - 49	2 x 1 097 ^a (7-d interval)	Small insectivorous mammal “shrew” BBCH 10 - 19	7.6	1.4	11.67	> 170

Product		A22773A				
Acute toxicity (mg prod./kg bw)		> 2 000				
TER criterion		10				
Crop scenario Growth stage	Application rate (g prod./ha)	Generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a
		Small insectivorous mammal “shrew” BBCH ≥ 20	5.4		8.293	> 240
		Small herbivorous mammal “vole” BBCH 40 - 49	136		208.87	> 9.6
		Large herbivorous mammal “lagomorph” all season	35.1		53.91	> 37
		Small omnivorous mammal “mouse” BBCH 10 - 49	17.2		26.42	> 76

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

^a Based on an application rate of 1 L/ha and a nominal formulation density of 1.097 g/cm³

The acute Tier 1 risk assessment for the formulation A22773A for the proposed uses in fruiting vegetables and leafy vegetables, concludes TER_a values greater than the trigger of 10 for all indicator species with the exception of the small herbivorous mammal “vole” scenarios at crop stages up to BBCH 49 for which the TER_a was calculated to be > 9.6.

The TER_a value of > 9.6 for the small herbivorous mammal “vole” scenarios was based on the unbound (“greater than”) endpoint for A22773A (LD₅₀ > 2 000 mg A22773A/kg bw). In the acute oral toxicity study with rat (xxxxxx; VV-892044), no mortality or effects on body weight were determined at the limit dose of 2 000 mg A22773A/kg bw. Further, no clinical signs were observed from the day after treatment. The results of the acute oral toxicity study suggest that the actual LD₅₀ for A22773A would be significantly higher than the tested dose of 2 000 mg/kg bw, resulting in a TER_a value exceeding the trigger of 10. Syngenta therefore believe that the acute risk assessment for the small herbivorous mammal “vole” indicate acceptable risk.

Moreover, the active substances azoxystrobin and oxathiapiprolin are both of low acute toxicity to mammals (LD₅₀ > 5 000 mg/kg bw for both active substances). Based on the calculated LD₅₀ (mix) (see 9.3.1.1), a TER_a value of > 100 is obtained for the “vole” scenario.

Chronic risk

For assessment of chronic effects, according to EFSA/2009/1438², ‘if a given formulation contains several active substances all known to cause similar effects via a similar biochemical mechanism (e.g. aromatase inhibition) and if this type of effect is actually driving the risk assessment, it is thus recommended to perform an assessment for combined effects on a case by case basis’.

The toxicity profiles of azoxystrobin and oxathiapiprolin do not have the same toxic effects in tissues, organs or physiological systems (below). Based on the differences in the toxicity profile for each active ingredient, no common effect on the mammalian system, as well as the difference in pesticidal target sites of action, the potential for cumulative toxicity effects is considered to be negligible.

Fungicide	Azoxystrobin	Oxathiapiprolin
Pesticidal MOA	Quinone outside inhibitor (QoI) that disrupts the mitochondrial respiration of fungi by binding to the Quinol outer binding site of the cytochrome bc1 complex.	Inhibition of oxysterol binding protein (OSBP) homologue.
FRAC Code	FRAC Code 11; C3: complex III: cytochrome bc1 (ubiquinol oxidase) at Qo site (cyt b gene)	FRAC Code 49; piperidiny-thiazole-isoxazoline fungicide, targets lipid homeostasis and transfer/storage.

The toxicology database for azoxystrobin is considered to be adequate. The liver was the target organ in repeated dose studies, with effects such as increase weight, altered clinical chemistry profile and, at high doses, histopathological changes in rat and dose studies. There were no effects on developmental and reproductive toxicity, neurotoxicity, mutagenicity or carcinogenicity after exposure to azoxystrobin.

The toxicology database for oxathiapiprolin is considered to be complete. In the toxicity studies for oxathiapiprolin, no treatment related effects were seen in any species at doses up to the limit dose (1000 mg/kg/day). Effects were observed in offspring animals in rat reproduction studies at doses above the limit dose. However, the offspring NOAEL was set at 86.37 mg/kg bw/day based on an effect of delayed preputial separation observed in the two-generation rat study which was considered ecologically relevant (refer to the DAR, 2016, volume 3, Annex B.9 PPP).

For A22773A, the active ingredients azoxystrobin and oxathiapiprolin showed no common target organs in the wider database and a combined risk assessment is considered not necessary.

However, RMS Poland requested Syngenta to include a quantitative chronic combination mixture risk assessment for A22773A.

Chronic risk

The calculated NOAEL of the mixture of azoxystrobin/oxathiapiprolin is used as the endpoint for the chronic combination mixture risk assessment (see 9.2.1.1).

Table 9.3-12: Screening assessment of the long-term/reproductive risk for mammals due to the use of A22773A

Active substance		azoxystrobin/oxathiapiprolin				
Chronic toxicity (mg a.s./kg bw/d)		33				
TER criterion		5				
Crop scenario	Application rate (g prod./ha)	Indicator species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{it}
Fruiting vegetables, BBCH 11 - 89	2 x 262 ^a (7-d interval)	Small herbivorous mammal	72.3	1.6 × 0.53	16.1	2.1
Leafy vegetables, BBCH 11 - 49	2 x 262 ^a (7-d interval)	Small herbivorous mammal	72.3	1.6 × 0.53	16.1	2.1
Bulbs and onion like crops, BBCH 11 - 49	2 x 262 ^a (12-d interval)	Small herbivorous mammal	48.3	1.4 × 0.53	9.4	3.5
Hop, BBCH 21 - 89	2 x 262 ^a (12-d interval)	Small herbivorous mammal	48.3	1.4 × 0.53	9.4	3.5

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

^a Application rate of azoxystrobin/oxathiapiprolin mixture is the sum of both active substances i.e. sum of 250 g azoxystrobin/ha + 12 g oxathiapiprolin /ha = 262 g/ha.

Table 9.3-13: Tier 1 assessment of the long-term/reproductive risk for mammals due to the use of A22773A

Active substance/product		azoxystrobin/oxathiapiprolin				
Reprod. Toxicity (mg/kg bw/d)		33				
TER criterion		5				
Crop scenario Growth stage	Application rate (g a.s./ha)	Generic focal species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{It}
Fruiting vegetables, BBCH 11 - 89	2 x 262 ^a (7-d interval)	Frugivorous mammal "rat" Fruit stage BBCH 71 - 89	25.2	1.6 × 0.53	5.7	5.9
		Small insectivorous mammal "shrew" BBCH 10 - 19	4.2		0.9	35.4
		Small insectivorous mammal "shrew" BBCH ≥ 20	1.9		0.4	78.2
		Small herbivorous mammal "vole" BBCH 10 - 49	72.3		16.1	2.1
		Small herbivorous mammal "vole" BBCH ≥ 50	21.7		4.8	6.8
		Small omnivorous mammal "mouse" BBCH 10 - 49	7.8		1.7	19.0
		Small omnivorous mammal "mouse" BBCH ≥ 50	2.3		0.5	64.6
Leafy vegetables, BBCH 11 - 49	2 x 262 ^a (7-d interval)	Small insectivorous mammal "shrew" BBCH 10 - 19	4.2	1.6 × 0.53	0.9	35.4
		Small insectivorous mammal "shrew" BBCH ≥ 20	1.9		0.4	78.2
		Small herbivorous mammal "vole" BBCH 40 - 49	72.3		16.1	2.1
		Large herbivorous mammal "lagomorph" All season	14.3		3.2	10.4
		Small omnivorous mammal "mouse" BBCH 10 - 49	7.8		1.7	19.0
Bulbs and onion like crops, BBCH 11 - 49	2 x 262 ^a (12-d interval)	Small insectivorous mammal "shrew" BBCH 10 - 19	4.2	1.4 × 0.53	0.8	40.4

Active substance/product		azoxystrobin/oxathiapiprolin				
Reprod. Toxicity (mg/kg bw/d)		33				
TER criterion		5				
Crop scenario Growth stage	Application rate (g a.s./ha)	Generic focal species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{it}
		Small insectivorous mammal “shrew” BBCH ≥ 20	1.9		0.4	89.3
		Small herbivorous mammal “vole” BBCH ≥ 40	43.4		8.4	3.9
		Small omnivorous mammal “mouse” BBCH 10 - 39	7.8		1.5	21.8
		Small omnivorous mammal “mouse” BBCH ≥ 40	4.7		0.9	36.1
Hop, BBCH 21 - 89	2 x 262 ^a (12-d interval)	Small insectivorous mammal “shrew” BBCH ≥ 20	1.9	1.4 × 0.53	0.4	89.3
		Small herbivorous mammal “vole” BBCH ≥ 40	21.7		4.2	7.8
		Small omnivorous mammal “mouse” BBCH 20 - 39	3.9		0.8	43.5
		Small omnivorous mammal “mouse” BBCH ≥ 40	2.3		0.4	73.8

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.

^a Application rate of azoxystrobin/oxathiapiprolin mixture is the sum of both active substances i.e. sum of 250 g azoxystrobin/ha + 12 g oxathiapiprolin /ha = 262 g/ha.

For A22773A for the following scenarios, the chronic TER value was below the trigger of 5:

- Fruiting vegetables
 - small herbivorous mammal “vole”, BBCH 10-49
- Leafy vegetables
 - small herbivorous mammal “vole”, BBCH 40-49
- Bulbs and onion like crops
 - small herbivorous mammal “vole”, BBCH ≥ 40

9.3.2.2 Higher-tier risk assessment

The Tier 1 risk assessment showed that the small herbivore (vole) is potentially at risk from azoxystrobin residues from the proposed uses of A22773A, requiring further consideration which is given below.

Refinement of fTWA and MAF

Data is available for azoxystrobin, measuring the foliar decline of residues to determine a substance specific DT₅₀. The decline studies and kinetic evaluation reports are summarised below (Ertus C, 2018, VV-469438; Ford S, 2018, VV-631889).

For azoxystrobin the rate of foliage decline was studied in five sites in Germany, Northern France and the United Kingdom and kinetic models were fitted to the total residue to calculate the DT₅₀. The summary of the results is shown below.

Table 9.3-14: Foliar decline summary for azoxystrobin in grass

Site	Selected kinetic model	χ ² -error (%)	DT ₅₀ (days)
BW1	SFO	5.11	3.22
BW2	SFO	7.65	3.61
MA1	SFO	11.9	4.32
ND1	SFO	9.05	2.60
UK1	SFO	9.03	5.44
Geometric mean			3.72

The geometric mean DT₅₀ of 3.72 days will be used in the risk assessment for the food type grass.

Table 9.3-15: Calculation of MAF x TWA (time-moving window) for use in fruiting and leafy vegetables and bulbs/onions

Diet	Geometric mean DT ₅₀	MAF x TWA (time-moving window)
Fruiting and Leafy Vegetables (2 applications, 7 d interval)		
Grass	3.72	0.487
Bulbs and onions (2 applications, 12 d interval)		
Grass	3.72	0.458

Crop interception

Fruiting vegetables

A refined deposition factor for the risk assessment for small herbivorous mammals is proposed following the guidance for FOCUS ground water assessments (EFSA, 2014⁶). Crop interception for tomatoes at different BBCH stages are mentioned: BBCH 10 - 19 deposition factor = 0.5, BBCH 20 - 39 deposition factor = 0.3, BBCH 40 - 89 deposition factor = 0.2. In fruiting vegetable applications are intended to take place between BBCH 11 and BBCH 89. Therefore, the worst-case deposition factor of 0.5 will be used in the refined risk assessment for the proposed uses on tomato and eggplant.

Leafy vegetables

A field study was conducted to assess crop coverage (Münderle *et al.*, 2020; VV-867392, see Appendix 2). The aim of this generic study was to provide reliable data on crop coverage values for leafy vegetables (cabbage, broccoli, and cauliflower) during BBCH growth stages 41 - 49 (Meier and Bleiholder 2016⁷).

⁶ EFSA, 2014: European Food Safety Authority, 2014. EFSA Guidance Document for evaluating laboratory and field dissipation studies to obtain DegT50 values of active substances of plant protection products and transformation products of these active substances in soil. EFSA Journal 2014;12(5):3662, 37 pp., doi:10.2903/j.efsa.2014.3662

⁷ Meier, U. & Bleiholder, H. 2016. BBCH-Skala. Phänologische Entwicklungsstadien wichtiger gartenbaulicher Kulturen, einschließlich Unkräuter. Band 2, Erling Verlag GmbH & Co. KG, 82 pp.

Digital photographs were taken at two different time points (early and late growth stages) in Northern Germany (Lower Saxony and Schleswig-Holstein) and in Southern Germany (Bavaria and Rhineland-Palatinate) using a camera-carrying drone DJI Mavic Pro II. A total of 1230 JPEG files were taken between 19 June 2019 and 21 September 2019, and subsequently analysed with RifPic v 1.0 to determine the crop coverage by the proportion of green parts per investigated area. Since a digital photograph represents a two-dimensional area, it is assumed that ground cover by growing crop plants represents conservative estimation for soil coverage by crops using this method.

Drone files were analysed separately for early (BBCH 41 - 45) and late growth stages (BBCH 46 - 49). Each file covered cropped area only.

Table 9.3-16: Crop coverage for leafy vegetables during different BBCH crop growth stages

Crop growth stages	Number of study fields	Number of drone files	Crop coverage (%)	
			AM \pm SD	Median
BBCH 41 - 45 (early)	63	640	73 \pm 20	78
BBCH 46 - 49 (late)	58	590	91 \pm 6	92
Total (BBCH 41 - 49)	121	1230	81 \pm 18	88

AM: Arithmetic mean, SD: Standard deviation

This study shows that the mean crop coverage value for all leafy vegetables is > 80 % and close to 100 % within BBCH 46 - 49. The study data supports the interception of 70 % for BBCH > 40 upwards in the FOCUS Ground Water Guidance Document (EFSA, 2014) as a conservative estimate and provides clear evidence that assuming no crop interception for leafy vegetables at BBCH 40 - 49 as per EFSA Birds & Mammals Guidance Document is overly conservative. It should be noted that the new draft guidance document for Birds and mammals Risk Assessment from EFSA (2021) proposes a deposition value of 30% (interception of 70%) for weed foliage in leafy vegetable crops at BBCH stages > 40.

Based on the above it can be concluded that the minimum crop coverage of leafy vegetable at BBCH 41 - 49 is 78 %. Therefore, a deposition factor of 0.25 seems to be appropriate for foliar applications in leafy vegetable fields at BBCH 40 - 49. This value will be used in the higher tier risk assessment.

Translation: Meier, U. & Bleiholder, H. 2016. BBCH scale. Phenological development stages of important horticultural crops, including weeds. Volume 2, Erling Verlag GmbH & Co. KG (publisher), 82 pp.

Review Comments:

According to “Working document on Risk Assessment of Plant Protection Products in the Central Zone – Ecotoxicology” (May 2021), point 3.2.15, the interception values following EFSA Guidance Document to obtain DegT50 values (EFSA Journal 2014;12(5):3662), can be used in the Tier 2 risk assessment. It should be noted that this rule applies only to the later stages of crop growth.

In Appendix E of EFSA B&M guidance the following recommendation is given:

“It was concluded that estimation of residues on undergrowth vegetation using FOCUS interception factors would become increasingly uncertain with decreasing soil cover of the crop and increasing height of weeds in relation to the crop. Thus reliable predictions are only deemed possible where the largest part of the soil surface is actually covered by the crop from a bird’s eye view and undergrowth vegetation is clearly smaller than the crop plants. Weeds or grasses overgrowing the crop at those stages are deemed unlikely to occur in intensive agriculture, but would anyway not form a part of the diet of small to medium herbivores.”

Therefore, the refined DF values are considered acceptable for those crops type and BBCH stages:

1. Fruiting vegetables

In Table 1 Appendix E for solanaceous fruit and cucurbits BBCH ≥ 51 growth stages are recognized satisfactorily high soil coverage by crop plants.

In Table 1.5 of EFSA Guidance Document to obtain DegT50 values for BBCH 40-89 growth stages of tomatoes the interception is 80%. It is the highest percentage of ground cover for fruiting vegetables.

There is no justification for the different approach to IF for BBCH stages ≥ 40 and ≥ 51 . Therefore, in zRMS opinion, for BBCH growth stages of tomatoes ≥ 40 , the DF of 0.2 can be used in the risk assessment.

Taken into consideration acceptable risk to voles based on Tier 1 TER calculation in Table 9.3-9 for BBCH ≥ 51 , further evaluation for tomatoes BBCH ≥ 40 is not required.

2. Leafy vegetables

In Table 1 Appendix E for leafy vegetables BBCH ≥ 51 growth stages are recognized satisfactorily high soil coverage by crop plants.

In Table 1.5 of EFSA Guidance Document to obtain DegT50 values for BBCH 40-89 growth stages of cabbage the interception is 70%.

There is no justification for the different approach to IF for BBCH stages ≥ 40 and ≥ 51 . Therefore, in zRMS opinion, for BBCH growth stages of leafy vegetables ≥ 40 , the DF of 0.3 can be used in the risk assessment.

The study by M nderle, M., et al (Syngenta file No. VV-867392) confirm the correctness of those assumptions. The mean crop cover for leafy vegetables in Germany was 73 ± 20 % during early growth stages (BBCH 41 – 45) and 91 ± 6 % during late growth stages (BBCH 46 – 49).

3. Bulbs and onion like crops

In Table 1 Appendix E for bulb vegetables BBCH ≥ 41 growth stages are recognized satisfactorily high soil coverage by crop plants.

In Table 1.5 of EFSA Guidance Document to obtain DegT50 values for BBCH 40-89 growth stages of onion the interception is 40%.

Therefore, the revised DF of 0.6 is considered acceptable.

Occurrence of voles in tomato fields

A field study on the occurrence of Savi’s pine voles (*Microtus savii*) was conducted in tomato fields in Italy (Sainz-Elise & Hahne 2014, VV-410659, see Appendix 2). Aim of the study was to determine the

attractiveness of commercially managed tomato fields for Savi's pine voles. In order to quantify the use of tomato fields by Savi's pine voles, regular trapping sessions were conducted on tomato fields as well as in the adjacent off-crop habitats. After the wood mouse (*Apodemus sylvaticus*) Savi's pine voles were the most frequently trapped species. The species was trapped in 13 out of 14 study sites. In the adjacent off-crop habitats 7.54 captures per 100 trap nights were obtained. However, not a single individual was trapped with the cropped fields, irrespective of the growth stage of the tomato plants. Based on this study, it is concluded that tomato fields are of low attractiveness to small herbivorous mammals.

Another study was carried out on four tomato fields in Italy (Barfknecht 2003, VV-338885, see Appendix 2). On these fields the occurrence of herbivorous mammals was assessed. In addition, the composition of vegetation and the availability of food for herbivorous mammals were assessed. Small mammals were monitored using life traps and marking animals with radio-collars.

One species of vole was trapped during live-trapping: Savi's pine vole (*Microtus savii*), which were only trapped in the adjacent off-crop habitat. In total four Savi's pine voles were radio-tracked. Radio-tracked pine voles were never recorded in tomato fields and only a single individual made a small and slow movement across the border stripe of field 3.

Review Comments:

For the purposes of this assessment, the field studies performed in SEU are not taken to consideration due to the lack of a focal species required for CEU.

Vole diet

It may also be considered that the FIR/bw value used in EFSA/2009/1438² of 1.33 is highly conservative and would mean that a vole ate 1.33 times its bodyweight each day, whereas a considerably lower value is likely (e.g. FIR/bw of 0.5; see Jacob *et al*, 2014⁸). Reports in the literature show that Common voles prefer to consume dicotyledons rather than monocotyledons like grasses; e.g. Rinke, 1990⁹ found that for voles on grassland the dicotyledons, *Taraxacum officinale* and *Trifolium pratense*, were preferred. Rinke, 1991¹⁰ reported that dicotyledons comprised a mean volume percentage of 63.5 % of stomach contents of common vole. Therefore, the risk assessment can be refined by considering a Common vole consuming a diet comprising 50 % dicotyledons (non-grass herbs) and 50 % grasses. This is also proposed by Ctgb in their Evaluation Manual for the Authorisation of plant protection products according to Regulation (EC) No 1107/2009, version 2.2, April 2017).

The bodyweight of the Common vole is given in the EFSA/2009/1438² as 25 g but real measurements across large samples of voles across Slovakia (Baláz, 2010¹¹) have demonstrated mean bodyweights of 26.6 g for males and 26.1 g for females. The more conservative, lower value of 26.1 g for females will be used in refined risk assessment below.

Calculations based on EFSA/2009/1438 indicate a DEE of 67.1 kJ/day for a vole of 26.1 g bodyweight and using energy content of food items specified in EFSA/2009/1438², the daily consumption of the diet components was calculated as shown below.

⁸ Jacob, J. 2014. Common vole (*Microtus arvalis*) ecology and management: implications for risk assessment of plant protection products. Pest Manag Sci 2014; 70: 869–878.

⁹ Rinke, T. 1990. Zur Nahrungsökologie von *Microtus arvalis* (Pallas, 1778) auf Dauergrünland. I. Allgemeine Nahrungspräferenzen. Zeitschrift für Säugetierkunde 55: 106-114. Syngenta File No. VV-138198.

¹⁰ Rinke, T. 1991. Percentage of volume versus number of species: availability and intake of grasses and forbs in *Microtus arvalis*. Folia Zoologica 40 (2): 143 – 151. Syngenta File No. VV-138196.

¹¹ Baláz, I. 2010. The influence of the altitude on somatic characteristics size of common vole (*Microtus arvalis*) in Slovakia. Ekologia, 29(2): 174–181. Syngenta File No. VV-733196.

Table 9.3-17: Calculation of daily consumption of different diet components for the vole

Food type	Energetic content of food ^a	Assimilation efficiency ^b	Energetic content of food, weighted by assimilation efficiency	Portion of different food items in diet mix	Energy uptake per gram of each diet item ^c	DEE	FIR Daily food consumption of different food items ^d	Body weight of vole	FIR/bw for specific food type
	(kJ/g wet wt)	(%)	(kJ/g wet wt)	(% of diet wet weight)	(kJ/g wet wt)	(kJ/day)	(g wet wt/day)	(g)	(g fresh wt/g bw/day)
Short grass	4.15	47	1.95	50	0.975	-	18.8	26.1	0.73
Non-grass herbs	2.12	76	1.61	50	0.806	-	18.8		0.73
Total	-	-	-	100	1.781	67.1			

^a Calculated based on energy content (kJ/g dry wt) and moisture content of the food items provided in Appendix G, Table 3 of EFSA/2009/1438

^b Taken from Appendix G, Table 4 of EFSA/2009/1438

^c Energy uptake for food item (kJ/g wet wt) = Energy content of food weighted by assimilation efficiency (kJ/g) x [proportion of crop in diet / 100]

^d Daily consumption of food item (g wet wt/d) = [DEE (kJ/d) / Energy uptake per g of total diet (kJ/g)] x [proportion of food item in diet / 100]

The FIR/bw values given above are used below, with the relevant residue levels, to calculate the DDD and TER_{lt} value for crop scenarios that are still in need of refinement.

Review Comments:

For the purposes of this assessment, the ecological data on PD and FIR/bw for common vole are not taken to consideration, due to the lack of a harmonised approach to Rinke, 1990.

Therefore, new TER calculations without changed of this factor were performed.

Table 9.3-18: Higher-tier assessment of the long-term/reproductive risk for mammals due to the use of A22773A on fruiting vegetables – refined parameters (*) are further described and justified in the text

Intended use		Fruiting vegetables, BBCH 11 – 39 89						
Active substance		Azoxystrobin						
Application rate (g/ha)		2 × 250 (7-d interval)						
Reprod. toxicity (mg/kg bw/d)		32						
TER criterion		5						
Generic focal species, growth stage	Food category, % in diet	FIR/bw	RUD_m × DF (mg/kg food)	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{lt}		
Small herbivorous mammal "vole" BBCH 10-49	Short grass, 50 %	0.73 (*)	54.2 × 0.5 (*)	0.487 (*)	2.41			
	Non grass herbs, 50 %	0.73 (*)	28.7 × 0.5 (*)	1.6 × 0.53	2.22			
	Total				4.63			
Food type	FIR/bw	PD_i, fresh	PT	RUD_{mean} [mg a.s./kg]	Maximum 21-d twa factor	DF	Use rate [kg a.s./ha]	DDD [mg a.s./kg bw/d]
Grasses/ Cereal shoots	1.33	1.0	1.0	54.2	0.487 ¹⁾	1.0	0.25	8.78
Toxicity endpoint [mg a.s./kg bw/d]								32
TER_{LT}								3.6

FIR/bw: Food intake rate per body weight; RUD: residue unit dose; DF: deposition factor (considering possible interception by the crop); MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

¹⁾ Refined parameter

Table 9.3-19: Higher-tier assessment of the long-term/reproductive risk for mammals due to the use of A22773A on leafy vegetables – refined parameters (*) are further described and justified in the text

Intended use		Leafy vegetables, BBCH 11 - 49						
Active substance		Azoxystrobin						
Application rate (g/ha)		2 × 250 (7-d interval)						
Reprod. toxicity (mg/kg bw/d)		32						
TER criterion		5						
Generic focal species, growth stage	Food category, % in diet	FIR/bw	RUD_m × DF (mg/kg food)	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{lt}		
Small herbivorous mammal "vole" BBCH 40-49	Short grass, 50 %	0.73 (*)	54.2 × 0.25 (*)	0.487 (*)	1.20			
	Non grass herbs, 50 %	0.73 (*)	28.7 × 0.25 (*)	1.6 × 0.53	1.11			
	Total				2.31			
Food type	FIR/bw	PD_i, fresh	PT	RUD_{mean} [mg a.s./kg]	Maximum 21-d twa factor	DF	Use rate [kg a.s./ha]	DDD [mg a.s./kg bw/d]
Grasses/ Cereal shoots	1.33	1.0	1.0	54.2	0.487 ¹⁾	0.3 ¹⁾	0.25	2.633
Toxicity endpoint [mg a.s./kg bw/d]								32
TER_{LT}								12.2

FIR/bw: Food intake rate per body weight; RUD: residue unit dose; DF: deposition factor (considering possible interception by the crop); MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

²⁾ Refined parameter

Table 9.3-20: Higher-tier assessment of the long-term/reproductive risk for mammals due to the use of A22773A on bulbs and onion like crops – refined parameters (*) are further described and justified in the text

Intended use				Bulbs and onion like crops, BBCH 11 - 49				
Active substance				Azoxystrobin				
Application rate (g/ha)				2 x 250 (12-d interval)				
Reprod. toxicity (mg/kg bw/d)				32				
TER criterion				5				
Generic focal species, growth stage		Food category, % in diet		FIR/bw	RUD _m × DF (mg/kg food)	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{lt}
Small herbivorous mammal “vole” BBCH ≥ 40		Short grass, 50 %		0.73 (*)	54.2 × 0.6	0.458 (*)	2.72	
		Non grass herbs, 50 %		0.73 (*)	28.7 × 0.6	1.5 × 0.53	2.50	
		Total					5.22	
Food type	FIR/bw	PD _i , fresh	PT	RUD _{mean} [mg a.s./kg]	Maximum 21-d twa factor	DF	Use rate [kg a.s./ha]	DDD [mg a.s./kg bw/d]
Grasses/ Cereal shoots	1.33	1.0	1.0	54.2	0.458 ¹⁾	0.6 ¹⁾	0.25	4.95
Toxicity endpoint [mg a.s./kg bw/d]								32
TER _{LT}								6.5

FIR/bw: Food intake rate per body weight; RUD: residue unit dose; DF: deposition factor (considering possible interception by the crop); MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

¹⁾ Refined parameter

Table 9.3-21: Higher-tier assessment of the long-term/reproductive risk for mammals due to the use of A22773A on fruiting vegetables – refined parameters (*) are further described and justified in the text

Intended use		Fruiting vegetables, BBCH 11 – 89					
Active substance		azoxystrobin/oxathiapiprolin					
Application rate (g/ha)		2 × 262 (7 d interval)					
Reprod. toxicity (mg/kg bw/d)		32					
TER criterion		5					
Generic focal species, growth stage	Food category, % in diet	FIR/bw	Use rate (g a.s./ha)	RUD_m × DF (mg/kg food)	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{lt}
Small herbivorous mammal "vole" BBCH 10-49	Short grass, 50 %	0.73 (*)	250 [†]	54.2 × 0.5 (*)	0.487 (*)	2.41	
	Short grass, 50 %	0.73 (*)	12 [†]	54.2 × 0.5 (*)	1.6 × 0.53	0.10	
	Non grass herbs, 50 %	0.73 (*)	262	28.7 × 0.5 (*)	1.6 × 0.53	2.33	
	Total				4.84	6.8	

FIR/bw: Food intake rate per body weight; RUD: residue unit dose; DF: deposition factor (considering possible interception by the crop); MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

[†]— In order to account for refined foliar dissipation data for azoxystrobin on monocots, the refined MAF x TWA is only applied for 250 g a.s./ha, which is the use rate of azoxystrobin

Table 9.3 22: Higher tier assessment of the long-term/reproductive risk for mammals due to the use of A22773A on leafy vegetables—refined parameters (*) are further described and justified in the text

Intended use		Leafy vegetables, BBCH 11—49						
Active substance		azoxystrobin/oxathiapiprolin						
Application rate (g/ha)		2 x 262 (7 d interval)						
Reprod. toxicity (mg/kg bw/d)		33						
TER criterion		5						
Generic focal species, growth stage	Food category, % in diet	FIR/bw	Use rate (g a.s./ha)	RUD_m × DF (mg/kg food)	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{ult}	
Small herbivorous mammal "vole" BBCH 40-49	Short grass, 50 %	0.73 (*)	250 [†]	54.2 × 0.25 (*)	0.487 (*)	1.20		
	Short grass, 50 %	0.73 (*)	12 [†]	54.2 × 0.25 (*)	1.6 × 0.53	0.10		
	Non grass herbs, 50 %	0.73 (*)	262	28.7 × 0.25 (*)	1.6 × 0.53	1.16		
				Total		2.46	13.4	

FIR/bw: Food intake rate per body weight; RUD: residue unit dose; DF: deposition factor (considering possible interception by the crop); MAF: multiple application factor; TWA: time weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

[†]— In order to account for refined foliar dissipation data for azoxystrobin on monocots, the refined MAF x TWA is only applied for 250 g a.s./ha, which is the use rate of azoxystrobin

Table 9.3 23: Higher tier assessment of the long-term/reproductive risk for mammals due to the use of A22773A on bulbs and onion like crops—refined parameters (*) are further described and justified in the text

Intended use		Bulbs and onion like crops, BBCH 11—49						
Active substance		azoxystrobin/oxathiapiprolin						
Application rate (g/ha)		2 x 262 (12 d interval)						
Reprod. toxicity (mg/kg bw/d)		33						
TER criterion		5						
Generic focal species, growth stage	Food category, % in diet	FIR/bw	Use rate (g a.s./ha)	RUD_m × DF (mg/kg food)	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{ult}	
Small herbivorous mammal "vole" BBCH ≥ 40	Short grass, 50 %	0.73 (*)	250 [†]	54.2 × 0.6	0.458 (*)	2.72		
	Short grass, 50 %	0.73 (*)	12 [†]	54.2 × 0.6	1.4 × 0.53	0.21		
	Non grass herbs, 50 %	0.73 (*)	262	28.7 × 0.6	1.4 × 0.53	2.44		
				Total		5.37	6.1	

FIR/bw: Food intake rate per body weight; RUD: residue unit dose; DF: deposition factor (considering possible interception by the crop); MAF: multiple application factor; TWA: time weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

Combined reproductive toxicity

As requested in the Working document on Risk Assessment of Plant Protection Products in the Central Zone – Ecotoxicology (May 2021), a calculation of long-term combitox risk according to the concentration addition (CA) model should be presented for Tier 1.

This virtual compound chronic TER was calculated according to the concentration addition approach and thus gives the same value as if calculated using equation $TER_{LT\text{combi}} = \text{trigger} / ((\text{trigger} / TER_{\text{substance 1}}) + (\text{trigger} / TER_{\text{substance 2}}))$.

The combined TER_{LT} value is calculated according to the following formula:

$$TER_{LT\text{combi}} = \text{trigger} / ((\text{trigger} / TER_{LT\text{ substance 1}}) + (\text{trigger} / TER_{LT\text{ substance 2}}))$$

An acceptable risk is expected when $TER_{LT\text{combi}} > \text{trigger}$.

The $TER_{LT\text{combi}}$ values are calculated based on screening step TER values for the oxathiapiprolin and lowest one for azoxystrobin. The calculations of the cumulative ecotoxicological effects are summarized in table below.

Table 9.3-13: Combined reproductive toxicity risk assessment for birds due to the use of A22773A for the crop group “fruiting / leafy / bulb vegetables”

Crop scenario and/or indicator species		Higher TER_{LT} ¹⁾ azoxystrobin	TER_{LT} ¹⁾ oxathiapiprolin	$TER_{LT\text{combi}}$	Trigger
Reproductive					
Fruiting vegetables BBCH 10-49	Small herbivorous mammal „vole“	3.6	120	3.5	5
Fruiting vegetables BBCH ≥50	Small herbivorous mammal „vole“	7.0 (Tier 1)	120	6.6	
Fruiting vegetables	Fruiting mammal „rat“	6.0 (Tier 1)	120	5.7	
Leafy vegetables	Small herbivorous mammal „vole“	12.2	120	11.1	
Bulb vegetables	Small herbivorous mammal „vole“	6.5	201	6.3	

¹⁾ lowest reproductive TER values

The refined TER_{lt} values for the proposed uses of A22773A on fruiting vegetables BBCH ≥50 (cover BBCH ≥40 based on higher tier assessment), leafy vegetables and bulb and onion like crops are above the trigger value of 5, indicating acceptable risk.

Finally, it must be considered that the Common vole is a pest species and subject to control plans in some areas; and thus, attitudes towards protection of the Common vole vary between EU member states.

9.3.2.3 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 K(f)oc of 423 mL/g, azoxystrobin belongs to the group of less sorptive substances and with a K(f)oc of 6 243 mL/g, oxathiapiprolin belongs to the group of more sorptive substances.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the maximum use rate of 2 x 250 g azoxystrobin/ha (7-d interval) and 2 x 12 g oxathiapiprolin/ha (7 d interval) is used to cover the risk to birds from all intended uses (see 9.1.2).

Azoxystrobin			
Effective application rate (g/ha)* =	500		
Acute toxicity (mg/kg bw) =	> 5 000	quotient =	< 0.10
Reprod. Toxicity (mg/kg bw/d) =	32	quotient =	16

* Effective application rate = Maximum application rate x MAF of 2.0
MAF is based on 2 applications, 7-day application interval and soil DT₅₀ value of 262.0 days

Oxathiapiprolin			
Effective application rate (g/ha)* =	24		
Acute toxicity (mg/kg bw) =	> 5 000	quotient =	< 0.0048
Reprod. Toxicity (mg/kg bw/d) =	86.37	quotient =	0.28

* Effective application rate = Maximum application rate x MAF of 2.0
MAF is based on 2 applications, 7-day application interval and soil DT₅₀ value of 121.2 days

The resulting ratios fall below the trigger of 50 for azoxystrobin (less sorptive) and 3 000 for oxathiapiprolin (more sorptive), indicating that further assessment of the acute and long-term risk to mammals from drinking water from puddles is not required.

9.3.2.4 Effects of secondary poisoning

According to EFSA/2009/1438², substances with a log P_{ow} of > 3 have a potential for bioaccumulation and as such consideration of the potential effects of secondary poisoning to birds and mammals are required.

The log P_{ow} of azoxystrobin amounts to 2.5 and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

The log P_{ow} of oxathiapiprolin is 3.67 (at pH 7) and thus exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is required. The log P_{ow} values of the oxathiapiprolin metabolites IN-Q7D41, IN-S2K66 and IN-RDT31 were reported to be 4.3, 3.4, and 4.1, respectively, and exceed the trigger value of 3. Therefore, a risk assessment for effects due to secondary poisoning is required. IN-S2K66 and IN-Q7D41 were not found in soil and the risk assessment was therefore performed for fish-eating mammals only.

Risk assessment for earthworm-eating mammals via secondary poisoning

According to EFSA/2009/1438², the risk for vermivorous mammals is assessed for a small mammal of 10 g body weight with a daily food consumption of 12.8 g. Bioaccumulation in earthworms is estimated based on predicted concentrations in soil.

Oxathiapiprolin

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the worst-case 21-day time-weighted average soil PEC following 2 x 12 g a.s./ha applications to onions was used (see Section 8 - Environmental Fate, Chapter 8.7.2).

Table 9.3-24: Assessment of the risk for earthworm-eating mammals due to exposure to oxathiapiprolin via bioaccumulation in earthworms (secondary poisoning)

Parameter	Oxathiapiprolin	Comments
PEC _{soil} (twa = 21 d) (mg/kg soil)	0.027	Onions, 2 x 12 g a.s./ha, crop interception 10%, soil layer depth 20 cm with tillage after year 10, DT ₅₀ = 205.3 d, twa interval = 21 d
log P _{ow} / K _{ow}	3.67 / 4 677	EFSA, 2016
K _{oc}	6 243	Arithmetic mean (n = 5)
f _{oc}	0.02	Default
BCF _{worm}	1*	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + 0.012 \times K_{ow}) / f_{oc} \times K_{oc}$
PEC _{worm}	0.03	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.03	DDD = PEC _{worm} × 1.28
NOEL (mg/kg bw/d)	86.37	Rat
TER _{lt}	2 499	≥ 5; acceptable risk

TER values shown in bold fall below the relevant trigger.

* calculated BCF = 0.46. A BCF of 1 was used for TER_{lt} estimation as a worst-case considering soil in gut of earthworms if relevant.

Table 9.3-25: Assessment of the risk for earthworm-eating mammals due to exposure to IN-RDT31 via bioaccumulation in earthworms (secondary poisoning)

Parameter	IN-RDT31	Comments
PEC _{soil} (initial) (mg/kg soil)	0.003	Initial PEC _{soil} for application on onions (refer to Section 8, Chapter 8.7.2)
log P _{ow} / K _{ow}	4.1 / 12 589	EFSA, 2016
K _{oc}	1 168	Arithmetic mean (n = 5)
f _{oc}	0.02	Default
BCF _{worm}	6.50	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + 0.012 \times K_{ow}) / f_{oc} \times K_{oc}$
PEC _{worm}	0.02	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.02	DDD = PEC _{worm} × 1.28
NOEL (mg/kg bw/d)	8.64	10x parent toxicity assumed as a worst case ^a
TER _{lt}	346	≥ 5; acceptable risk

TER values shown in bold fall below the relevant trigger.

^a In accordance with the DAR, 2016, volume 3, Annex B.9 PPP

Risk assessment for fish-eating mammals via secondary poisoning

According to EFSA/2009/1438², the risk for piscivorous mammals is assessed for a mammal of 3 000 g body weight with a daily food consumption of 425 g. Bioaccumulation in fish is estimated based on predicted concentrations in surface water.

Oxathiapiprolin

To achieve a concise risk assessment, the risk envelope approach is applied. The worst-case 21-day time-weighted average surface water PEC (FOCUS Step 1) following 2 x 12 g a.s./ha applications to hops was used for oxathiapiprolin. For the metabolites the maximum FOCUS Step 1 PEC_{sw} values were used. See Section 8 (Environmental Fate), Chapter 8.9.2.

Table 9.3-26: Assessment of the risk for fish-eating mammals due to exposure to oxathiapiprolin via bioaccumulation in fish (secondary poisoning)

Parameter	Oxathiapiprolin	Comments
PEC _{sw} (twa = 21 d) (mg/L)	0.000958	Max. FOCUS Step 1 21-d TWA PEC _{sw} for application to hops
BCF _{fish}	87	EFSA, 2016
BMF	-	Biomagnification factor (relevant for BCF ≥ 2000)
PEC _{fish}	0.083	PEC _{fish} = PEC _{water} × BCF _{fish}
Daily dietary dose (mg/kg bw/d)	0.01	DDD = PEC _{fish} × 0.142
NOEL (mg/kg bw/d)	86.37	Rat
TER _{it}	7 298	≥ 5; acceptable risk

TER values shown in bold fall below the relevant trigger.

Table 9.3-27: Assessment of the risk for fish-eating mammals due to exposure to IN-Q7D41 via bioaccumulation in fish (secondary poisoning)

Parameter	IN-Q7D41	Comments
PEC _{sw} (maximum) (mg/L)	0.00111	Max. FOCUS Step 1 PEC _{sw} for application to hops
BCF _{fish}	533	EFSA, 2016
BMF	-	Biomagnification factor (relevant for BCF ≥ 2000)
PEC _{fish}	0.592	PEC _{fish} = PEC _{water} × BCF _{fish}
Daily dietary dose (mg/kg bw/d)	0.08	DDD = PEC _{fish} × 0.142
NOEL (mg/kg bw/d)	8.64	10x parent toxicity assumed as a worst case ^a
TER _{it}	103	≥ 5; acceptable risk

TER values shown in bold fall below the relevant trigger.

^a In accordance with the DAR, 2016, volume 3, Annex B.9 PPP

Table 9.3-28: Assessment of the risk for fish-eating mammals due to exposure to IN-S2K66 via bioaccumulation in fish (secondary poisoning)

Parameter	IN-S2K66	Comments
PEC _{sw} (maximum) (mg/L)	0.000805	Max. FOCUS Step 1 PEC _{sw} for application to hops
BCF _{fish}	115	EFSA, 2016
BMF	-	Biomagnification factor (relevant for BCF ≥ 2000)
PEC _{fish}	0.093	PEC _{fish} = PEC _{water} × BCF _{fish}
Daily dietary dose (mg/kg bw/d)	0.01	DDD = PEC _{fish} × 0.142
NOEL (mg/kg bw/d)	8.64	10x parent toxicity assumed as a worst case ^a
TER _{lt}	657	≥ 5; acceptable risk

TER values shown in bold fall below the relevant trigger.

^a In accordance with the DAR, 2016, volume 3, Annex B.9 PPP

Table 9.3-29: Assessment of the risk for fish-eating mammals due to exposure to IN-RDT31 via bioaccumulation in fish (secondary poisoning)

Parameter	IN-RDT31	Comments
PEC _{sw} (maximum) (mg/L)	0.000303	Max. FOCUS Step 1 PEC _{sw} for application to leafy vegetables, fruiting vegetables, bulb vegetables and hops
BCF _{fish}	78	EFSA, 2016
BMF	-	Biomagnification factor (relevant for BCF ≥ 2000)
PEC _{fish}	0.024	PEC _{fish} = PEC _{water} × BCF _{fish}
Daily dietary dose (mg/kg bw/d)	0.00336	DDD = PEC _{fish} × 0.142
NOEL (mg/kg bw/d)	8.64	10x parent toxicity assumed as a worst case ^a
TER _{lt}	2 574	≥ 5; acceptable risk

TER values shown in bold fall below the relevant trigger.

^a In accordance with the DAR, 2016, volume 3, Annex B.9 PPP

Risk of secondary poisoning was assessed for oxathiapiprolin (log P_{ow} = 3.67 at pH 7) and some of its metabolites (IN-Q7D41, IN-S2K66 and IN-RDT31 with log P_{ow} values of 4.3, 3.4, and 4.1, respectively). All calculated TER_{lt} values clearly exceed the trigger value of 5 indicating that the risk of secondary poisoning to mammals is acceptable following use of A22773A according to the proposed use pattern.

9.3.2.5 Biomagnification in terrestrial food chains

Not relevant.

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

Not relevant.

9.3.4 Overall conclusions

The acute and long-term risks of A22773A to mammals were assessed from toxicity exposure ratios between toxicity endpoints, estimated from studies with A22773A, azoxystrobin and oxathiapiprolin, and

maximum residues occurring on food items following applications according to the proposed use pattern.

Risk of secondary poisoning has also been assessed, as oxathiapiprolin and some of its metabolites have log P_{ow} values of > 3.0. The risk to mammals from exposure via drinking water has also been assessed.

The TER values, calculated for recommended scenarios, all exceed the trigger value of 10 for acute risk, and nearly all exceed the trigger value of 5 for long-term risk (including drinking water and secondary poisoning), indicating that the risk to mammals is acceptable following use of A22773A according to the proposed use pattern. For acute risk the small herbivorous mammals (voles) were slightly below the trigger of 10 for the formulation A22773A in fruiting and leafy vegetables. The TER_a value of > 9.6 for the small herbivorous mammal “vole” scenarios was based on the unbound (“greater than”) endpoint for A22773A ($LD_{50} > 2\,000$ mg A22773A/kg bw). The results of the acute oral toxicity study (xxxxxx, 2021; VV-892044) suggest that the actual LD_{50} for A22773A would be significantly higher than the tested dose of 2 000 mg/kg bw. In addition, the very low toxicity of the active substances results in a significantly higher calculated LD_{50} value, resulting in a TER_a value exceeding the trigger of 10. Syngenta therefore believe that the acute risk assessment for the small herbivorous mammal “vole” indicate acceptable risk.

The exception was for small herbivorous mammals (voles) for which chronic Tier 1 TER values for azoxystrobin were below the trigger of 5 for the use of A22773A in fruiting and leafy vegetables and bulbs/onion-like crops. Higher-tier risk assessment based on decline data, newer crop interception values and vole diet, resulted in all scenarios showing acceptable risk.

Review Comments:

The acute and chronic risks of A22773A to mammals were assessed from toxicity exposure ratios between toxicity endpoints, estimated from study with active ingredients and maximum residues occurring on food items. An acute oral toxicity study with A22773A in rats was taken to consideration in the evaluation.

All TER values exceed the relevant triggers in the screening step or Tier 1 risk assessment for oxathiapiprolin and for combined active substances (virtual compound approach). Based on the higher tier risk assessment for azoxystrobin, where the deposition factor and DT_{50} in plants were modified, for azoxystrobin and combined active substances risk assessment (chronic), the TERs exceed the trigger values set by Commission regulation (EU) 546/2011 for acceptability of effects except for uses in fruiting vegetables at BBCH 11-39.

Evaluation of exposing to mammals through the drinking water demonstrated the acceptable risk. The potential risk of secondary poisoning is low.

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

No relevant data on amphibians and reptiles are available for azoxystrobin and oxathiapiprolin, consequently no further assessment of potential effects on reptiles and amphibians will be presented in this document.

Studies with terrestrial amphibian and reptile species are not data requirements under Regulation (EU) No 283/2013 and 284/2013. In addition, there is currently no guidance addressing terrestrial life stages of amphibians and reptiles in PPP risk assessments. Therefore, the risk assessment provided above for birds and mammals is considered to be protective of terrestrial amphibian and reptile species.

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, oxathiapiprolin and their mayor metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on aquatic organisms of A22773A were not evaluated as part of the EU assessment of azoxystrobin and oxathiapiprolin. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

The selection of studies and endpoints for the risk assessment deviates from the results of the EU review process. Justifications are provided below where necessary.

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

Species	Substance	Exposure System	Results	Reference
Fish				
<i>Oncorhynchus mykiss</i>	Azoxystrobin	96 h, f	LC ₅₀ = 0.47 mg a.s./L _{mm}	EFSA, 2010, Craig <i>et al.</i> , 1993, ICI5504/0909
<i>Lepomis macrochirus</i>	Azoxystrobin	96 h, f	LC ₅₀ = 1.1 mg a.s./L _{mm}	EFSA, 2010, Sankey <i>et al.</i> , 1993, ICI5504/0910
<i>Oncorhynchus mykiss</i>	250 SC	96 h, f	LC ₅₀ = 0.28 mg a.s./L _{nom}	EFSA, 2010, Kent <i>et al.</i> , 1994, ICI5504/0915
<i>Oncorhynchus mykiss</i>	R234886	96 h, f	LC ₅₀ > 150 mg a.s./L _{mm}	EFSA, 2010, Kent <i>et al.</i> , 1993, ICI5504/0913
<i>Oncorhynchus mykiss</i>	R402173	96 h, s	LC ₅₀ = 62 mg a.s./L _{nom}	EFSA, 2010, Wallace, 2002, SYN511114/0001
<i>Oncorhynchus mykiss</i>	R401553	96 h, s	LC ₅₀ > 120 mg a.s./L _{nom}	EFSA, 2010, Bowles and Wallace, 2002, SYN501657/0002
<i>Pimephales promelas</i>	Azoxystrobin	33 d (ELS), f	NOEC = 0.147 mg a.s./L _{mm}	EFSA, 2010, Rhodes <i>et al.</i> , 1994, ICI5504/0924
Aquatic invertebrates				
<i>Daphnia magna</i>	Azoxystrobin	48 h, s	EC ₅₀ = 0.23 mg a.s./L _{mm}	EFSA, 2010, Farrelly and Hamer, 1994, ICIA5504/0931
<i>Macrocyclus fuscus</i>	Azoxystrobin	48 h, s	EC ₅₀ = 0.13 mg a.s./L _{nom}	EFSA, 2010, Farrelly <i>et al.</i> , 1995, ICIA5504/0940

Species	Substance	Exposure System	Results	Reference
<i>Americamysis bahia</i> (formerly <i>Mysidopsis bahia</i>)	Azoxystrobin	96 h, s	EC ₅₀ = 0.055 mg a.s./L _{nom}	EFSA, 2010, Kent <i>et al.</i> , 1993, ICI5504/0925
<i>Americamysis bahia</i> (formerly <i>Mysidopsis bahia</i>)	Azoxystrobin	48 h, s	EC ₅₀ = 0.068 mg a.s./L _{nom}	EFSA, 2010, Kent <i>et al.</i> , 1993, ICI5504/0925
<i>Crassostrea gigas</i>	Azoxystrobin	48 h, s	EC ₅₀ = 1.3 mg a.s./L _{nom}	EFSA, 2010, Kent <i>et al.</i> , 1994a, ICI5504/0927
<i>Daphnia magna</i>	250 SC	48 h, s	EC ₅₀ = 0.11 mg a.s./L _{nom}	EFSA, 2010, Rapley <i>et al.</i> , 1994, ICI5504/0929
<i>Daphnia magna</i>	R234886	48 h, s	EC ₅₀ > 180 mg a.s./L _{nom}	EFSA, 2010, Kent <i>et al.</i> , 1993b, ICI5504/0926
<i>Daphnia magna</i>	R402173	48 h, s	EC ₅₀ > 100 mg a.s./L _{nom}	EFSA, 2010, Wallace, 2002a, SYN511114/0002
<i>Daphnia magna</i>	R401553	48 h, s	EC ₅₀ > 120 mg a.s./L _{nom}	EFSA, 2010, Bowles and Wallace, 2002a, SYN501657/0003
<i>Daphnia magna</i>	Azoxystrobin	21 d, s	NOEC = 0.044 mg a.s./L _{mm}	EFSA, 2010, Rapley <i>et al.</i> , 1994, ICI5504/0957
<i>Americamysis bahia</i> (formerly <i>Mysidopsis bahia</i>)	Azoxystrobin	28 d, s	NOEC = 0.00954 mg a.s./L _{mm}	EFSA, 2010, Boeri <i>et al.</i> , 1997, ICI5504/0952
Sediment dwelling organisms				
<i>Chironomus riparius</i>	Azoxystrobin	28 d, s	NOEC = 0.8 mg a.s./L _{nom}	EFSA, 2010, Gentle and Rapley, 1997, ICI5504/0956
<i>Chironomus riparius</i>	Azoxystrobin	28 d, s	NOEC = 23 mg a.s./kg _{nom}	DAR, 2010, Gentle, 1997, ICI5504/0954
Algae				
<i>Pseudokirchneriella subcapitata</i>	Azoxystrobin	72 h, s	ErC ₅₀ = 1.47 0.36 mg a.s./L _{mm} EbC ₅₀ = 0.183 mg a.s./L _{mm} ^a	EFSA, 2010, Smyth <i>et al.</i> , 1993, ICI5504/0961
<i>Skeletonema costatum</i>	Azoxystrobin	72 h, s	ErC ₅₀ = 0.3 mg a.s./L _{nom} EbC ₅₀ = 0.098 mg a.s./L _{nom}	EFSA, 2010, Smyth <i>et al.</i> , 1994, ICI5504/0966
<i>Navicula pelliculosa</i>	Azoxystrobin	120 h, s	ErC ₅₀ = 0.146 mg a.s./L _{nom} EbC ₅₀ = 0.014 mg a.s./L _{nom}	EFSA, 2010, Smyth <i>et al.</i> , 1994a, ICI5504/0965
<i>Anabaena flos-aquae</i>	Azoxystrobin	120 h, s	ErC ₅₀ = 13.9 mg a.s./L _{mm} EbC ₅₀ = 9.5 mg a.s./L _{mm}	EFSA, 2010, Smyth <i>et al.</i> , 1994b, ICI5504/0967
<i>Pseudokirchneriella</i>	250 SC	72 h, s	EbC ₅₀ = 0.16 mg a.s./L _{nom}	EFSA, 2010,

Species	Substance	Exposure System	Results	Reference
<i>subcapitata</i>				Smyth <i>et al.</i> , 1994c, ICI5504/0969
<i>Pseudokirchneriella subcapitata</i>	R234886	72 h, s	E_rC₅₀ = 80 mg/L_{mm} E_bC₅₀ = 47 mg/L_{mm}	EFSA, 2010, Smyth <i>et al.</i> , 1993a, ICI5504/0962
<i>Pseudokirchneriella subcapitata</i>	R402173	72 h, s	E_rC₅₀ = 67 mg/L_{nom}	EFSA, 2010, Wallace and Woodyer, 2002, SYN511114/0003
<i>Pseudokirchneriella subcapitata</i>	R401553	72 h, s	E_rC₅₀ > 120 mg/L_{nom}	EFSA, 2010, Bowles and Wallace, 2002b, SYN501657/0004
Aquatic macrophytes				
<i>Lemna gibba</i>	Azoxystrobin	14 d, ss	EC₅₀ = 3.2 mg a.s./L_{nom}	EFSA, 2010, Smyth <i>et al.</i> , 1994d, ICI5504/0963
Higher-tier studies (micro- or mesocosm studies)				
<p>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.</p> <p>Species/groups were present in sufficient numbers to allow appropriate statistical analysis.</p> <p>The Notifier proposed that the no observed ecologically adverse effects concentration (NOEAEC) is 10 µg/L. No uncertainty or assessment factor was proposed.</p> <p>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:</p> <ul style="list-style-type: none"> - <i>Daphnia</i> 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. <p>The following groups increased and were probably the result of indirect effects:</p> <ul style="list-style-type: none"> - 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. <p>It should however be noted that there was only one application and there was only chemical analysis 21 hours after application; due to this it is proposed that the effect concentrations should be based on the initial nominal concentrations.</p>			<p>EFSA, 2010, Cole, J.F.H., Everett, C.J., Gentle, W., Ashwell, J.A., Goggin, U., 2000, ICI5504/0976</p>	

Species	Substance	Exposure System	Results	Reference
Based on all available lines of evidence, the acute and chronic RAC for aquatic invertebrates is 3.3 µg a.s./L				EFSA, 2010

Endpoints used for RAC derivation are shown in **bold**.

EFSA Journal 2010; 8(4):1542.

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

^a The 72 and 96 hour E₅₀ values of azoxystrobin for *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*) are 0.183 mg/L and 0.36 mg/L respectively; it is in the belief of Syngenta that the 72-h EC₅₀ endpoint is erroneously presented as 0.36 mg/L in the EFSA Conclusion (2010); 8(4):1542.

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

Species	Substance	Exposure System	Results	Reference
Fish				
<i>Oncorhynchus mykiss</i>	Oxathiapiprolin	96 h, s	LC ₅₀ > 0.69 mg a.s./L _{mm} ^a	EFSA, 2016, DuPont-32481
<i>Lepomis macrochirus</i>	Oxathiapiprolin	96 h, s	LC ₅₀ > 0.72 mg a.s./L _{mm} ^a	EFSA, 2016, DuPont-32818
<i>Cyprinodon variegatus</i>	Oxathiapiprolin	96 h, s	LC₅₀ > 0.65 mg a.s./L_{mm}^a	EFSA, 2016, DuPont-32819
<i>Cyprinodon variegatus</i>	Oxathiapiprolin	35 d ELS, f	NOEC_{growth and survival} = 0.34 mg a.s./L_{mm}	EFSA, 2016, DuPont-32820
<i>Oncorhynchus mykiss</i>	Oxathiapiprolin	88 d ELS, f	NOEC _{growth} = 0.46 mg a.s./L _{mm}	EFSA, 2016, DuPont-32482
<i>Oncorhynchus mykiss</i>	IN-E8S72	96 h, s	LC ₅₀ > 100 mg/L_{nom}	EFSA, 2016, DuPont-34396
<i>Oncorhynchus mykiss</i>	IN-P3X26	96 h, s	LC ₅₀ > 67.72 mg/L_{mm}^a	EFSA, 2016, DuPont-32662
<i>Oncorhynchus mykiss</i>	IN-Q7D41	96 h, ss	LC ₅₀ > 0.18 mg/L_{mm}^a	EFSA, 2016, DuPont-32660
<i>Oncorhynchus mykiss</i>	IN-QFD61 ^b	96 h, s	LC ₅₀ > 7.38 mg/L _{mm} ^a	EFSA, 2016, DuPont-34403
<i>Oncorhynchus mykiss</i>	IN-QPS10	96 h, s	LC ₅₀ = 6.96 mg/L_{mm}	EFSA, 2016, DuPont-34395
<i>Oncorhynchus mykiss</i>	IN-RAB06	96 h, s	LC ₅₀ > 50.0 mg/L_{mm}	EFSA, 2016, DuPont-34401
<i>Oncorhynchus mykiss</i>	IN-RDT31	96 h, s	LC ₅₀ > 11.56 mg/L_{mm}^a	EFSA, 2016, DuPont- 34397
<i>Oncorhynchus mykiss</i>	IN-RSE01	96 h, ss	LC ₅₀ > 9.84 mg/L_{mm}^a	EFSA, 2016, DuPont-32661
<i>Oncorhynchus mykiss</i>	IN-RYJ52	96 h, s	LC ₅₀ > 13.8 mg/L_{mm}^a	EFSA, 2016, DuPont-32659
<i>Oncorhynchus mykiss</i>	IN-S2K66	96 h, s	LC ₅₀ > 7.48 mg/L_{mm}^a	EFSA, 2016, DuPont-34394

Species	Substance	Exposure System	Results	Reference
<i>Oncorhynchus mykiss</i>	IN-S2K67 ^b	96 h, s	LC ₅₀ > 82.5 mg/L _{mm} ^a	EFSA, 2016, DuPont-34410
Aquatic invertebrates				
<i>Daphnia magna</i>	Oxathiapiprolin	48 h, s	EC ₅₀ = 0.67 mg a.s./L _{mm}	EFSA, 2016, DuPont-32484
<i>Americamysis bahia</i>	Oxathiapiprolin	96 h, s	EC ₅₀ > 0.64 mg a.s./L _{mm} ^a	EFSA, 2016, DuPont-32485
<i>Crassostrea virginica</i>	Oxathiapiprolin	96 h, f	EC ₅₀ > 0.33 mg a.s./L_{mm}^a	EFSA, 2016, DuPont-32453
<i>Daphnia magna</i>	Oxathiapiprolin	21 d, ss	NOEC _{reproduction} = 0.75 mg a.s./L _{mm} ^a	EFSA, 2016, DuPont-32455
<i>Americamysis bahia</i>	Oxathiapiprolin	32 d, f	NOEC _{reproduction} = 0.058 mg a.s./L_{mm}	EFSA, 2016, DuPont-32456
<i>Daphnia magna</i>	IN-E8S72	48 h, s	EC ₅₀ > 100.0 mg/L_{nom}	EFSA, 2016, DuPont-34400
<i>Daphnia magna</i>	IN-P3X26	48 h, s	EC ₅₀ > 67.74 mg/L_{mm}^a	EFSA, 2016, DuPont-32653
<i>Daphnia magna</i>	IN-Q7D41	48 h, ss	EC ₅₀ > 0.15 mg/L_{mm}^a	EFSA, 2016, DuPont-32651
<i>Daphnia magna</i>	IN-QFD61 ^b	48 h, s	EC ₅₀ = 6.29 mg/L _{mm}	EFSA, 2016, DuPont-34404
<i>Daphnia magna</i>	IN-QPS10	48 h, s	EC ₅₀ = 15.87 mg/L_{mm}	EFSA, 2016, DuPont-34399
<i>Daphnia magna</i>	IN-RAB06	48 h, s	EC ₅₀ > 100.0 mg/L_{nom}	EFSA, 2016, DuPont-33941
<i>Daphnia magna</i>	IN-RDT31	48 h, s	EC ₅₀ > 10.49 mg/L_{mm}^a	EFSA, 2016, DuPont-34398
<i>Daphnia magna</i>	IN-RSE01	48 h, s	EC ₅₀ > 10.16 mg/L_{mm}^a	EFSA, 2016, DuPont-32652
<i>Daphnia magna</i>	IN-RYJ52	48 h, s	EC ₅₀ > 16.21 mg/L_{mm}^a	EFSA, 2016, DuPont-32663
<i>Daphnia magna</i>	IN-S2K66	48 h, s	EC ₅₀ = 0.86 mg/L_{mm}	EFSA, 2016, DuPont-34409
<i>Daphnia magna</i>	IN-S2K67 ^b	48 h, s	EC ₅₀ = 66.91 mg/L _{mm}	EFSA, 2016, DuPont-34594
Sediment dwelling organisms				
<i>Chironomus riparius</i>	Oxathiapiprolin	48 h, s	EC ₅₀ > 0.56 mg a.s./L _{mm} ^a	EFSA, 2016, DuPont-32454
<i>Chironomus riparius</i>	Oxathiapiprolin	28 d, s, water spiked	NOEC = 0.11 mg a.s./L_{mm}	EFSA, 2016, DuPont-36043
<i>Chironomus riparius</i>	Oxathiapiprolin	28 d, spiked sediment	NOEC = 2.80 mg/kg sediment_{mm}	EFSA, 2016, DuPont-35835
<i>Chironomus riparius</i>	IN-Q7D41	28 d, spiked sediment	NOEC = 72.0 mg/kg sediment_{mm}	EFSA, 2016, DuPont-35845

Species	Substance	Exposure System	Results	Reference
<i>Chironomus riparius</i>	IN-RYJ52	10x parent toxicity	NOEC = 0.011 mg met./L ^c	EFSA, 2016
<i>Chironomus riparius</i>	IN-RYJ52	10x parent toxicity	NOEC = 0.280 mg met./kg sediment ^c	EFSA, 2016
<i>Chironomus riparius</i>	IN-S2K66	10x parent toxicity	NOEC = 0.011 mg met./L ^c	EFSA, 2016
<i>Chironomus riparius</i>	IN-S2K66	10x parent toxicity	NOEC = 0.280 mg met./kg sediment ^c	EFSA, 2016
<i>Chironomus riparius</i>	IN-RSE01	10x parent toxicity	NOEC = 0.011 mg met./L ^c	EFSA, 2016
<i>Chironomus riparius</i>	IN-RSE01	10x parent toxicity	NOEC = 0.280 mg met./kg sediment ^c	EFSA, 2016
Algae				
<i>Pseudokirchneriella subcapitata</i>	Oxathiapiprolin	96 h, s	E _r C ₅₀ > 0.142 mg a.s./L _{mm} E _y C ₅₀ > 0.142 mg a.s./L _{mm} NOEC ≥ 0.142 mg a.s./L _{mm}	EFSA, 2016, DuPont-29275
<i>Anabaena flos-aquae</i>	Oxathiapiprolin	96 h, s	E _r C ₅₀ > 0.193 mg a.s./L _{mm} E _y C ₅₀ > 0.193 mg a.s./L _{mm} NOEC ≥ 0.193 mg a.s./L _{mm}	EFSA, 2016, DuPont-29320
<i>Skeletonema costatum</i>	Oxathiapiprolin	72 h, s	E _r C ₅₀ > 0.351 mg a.s./L _{mm} E _y C ₅₀ = 0.348 mg a.s./L _{mm} E _b C ₅₀ > 0.351 mg a.s./L _{mm} NOEC = 0.141 mg a.s./L _{mm}	EFSA, 2016, DuPont-35834
<i>Navicula pelliculosa</i>	Oxathiapiprolin	72 h, s	E _r C ₅₀ > 0.163 mg a.s./L _{mm} E _y C ₅₀ > 0.163 mg a.s./L _{mm} E _b C ₅₀ > 0.163 mg a.s./L _{mm} NOEC ≥ 0.163 mg a.s./L _{mm}	EFSA, 2016, DuPont-35843
<i>Pseudokirchneriella subcapitata</i>	IN-E8S72	72 h, s	E _r C ₅₀ > 100.0 mg/L _{nom} E _y C ₅₀ > 100.0 mg/L _{nom} NOEC ≥ 100.0 mg/L _{nom}	EFSA, 2016, DuPont-32817
<i>Pseudokirchneriella subcapitata</i>	IN-P3X26	72 h, s	E _r C ₅₀ > 66.64 mg/L _{mm} E _y C ₅₀ > 66.64 mg/L _{mm} E _b C ₅₀ > 66.64 mg/L _{mm} NOEC ≥ 66.64 mg/L _{mm}	EFSA, 2016, DuPont-32657
<i>Pseudokirchneriella subcapitata</i>	IN-Q7D41	72 h, s	E _r C ₅₀ > 0.21 mg/L _{mm} E _y C ₅₀ > 0.21 mg/L _{mm} E _b C ₅₀ > 0.21 mg/L _{mm} NOEC ≥ 0.21 mg/L _{mm}	EFSA, 2016, DuPont-32654
<i>Pseudokirchneriella subcapitata</i>	IN-QFD61 ^b	72 h, s	E _r C ₅₀ > 7.53 mg/L _{mm} E _y C ₅₀ > 7.53 mg/L _{mm} E _b C ₅₀ > 7.53 mg/L _{mm} NOEC ≥ 1.29 mg/L _{mm}	EFSA, 2016, DuPont-34402
<i>Pseudokirchneriella subcapitata</i>	IN-QPS10	72 h, s	E _r C ₅₀ = 2.32 mg/L _{mm} E _y C ₅₀ = 0.86 mg/L _{mm} E _b C ₅₀ = 0.86 mg/L _{mm} NOE _r C = 0.48 mg/L _{mm} NOE _{by} C = 0.20 mg/L _{mm}	EFSA, 2016, DuPont-32816

Species	Substance	Exposure System	Results	Reference
<i>Pseudokirchneriella subcapitata</i>	IN-RAB06	72 h, s	E_rC₅₀ > 100.0 mg/L_{nom} E _y C ₅₀ > 100.0 mg/L _{nom} NOEC ≥ 100.0 mg/L _{nom}	EFSA, 2016, DuPont-32825
<i>Pseudokirchneriella subcapitata</i>	IN-RDT31	72 h, s	E_rC₅₀ > 11.43 mg/L_{mm} E _y C ₅₀ > 11.43 mg/L _{mm} E _b C ₅₀ > 11.43 mg/L _{mm} NOEC = 1.97 mg/L _{mm}	EFSA, 2016, DuPont-32826
<i>Pseudokirchneriella subcapitata</i>	IN-RSE01	72 h, s	E_rC₅₀ > 10.80 mg/L_{mm} E _y C ₅₀ > 10.80 mg/L _{mm} E _b C ₅₀ > 10.80 mg/L _{mm} NOEC = 0.31 mg/L _{mm}	EFSA, 2016, DuPont-32656
<i>Pseudokirchneriella subcapitata</i>	IN-RYJ52	72 h, s	E_rC₅₀ > 15.34 mg/L_{mm} E _y C ₅₀ > 15.34 mg/L _{mm} E _b C ₅₀ > 15.34 mg/L _{mm} NOEC = 0.14 mg/L _{mm}	EFSA, 2016, DuPont-32655
<i>Pseudokirchneriella subcapitata</i>	IN-S2K66	72 h, s	E_rC₅₀ > 7.56 mg/L_{mm} E _y C ₅₀ > 7.56 mg/L _{mm} E _b C ₅₀ > 7.56 mg/L _{mm} NOEC = 4.71 mg/L _{mm}	EFSA, 2016, DuPont-34507
<i>Pseudokirchneriella subcapitata</i>	IN-S2K67 ^b	72 h, s	E _r C ₅₀ > 83.51 mg/L _{mm} E _y C ₅₀ > 83.51 mg/L _{mm} E _b C ₅₀ > 83.51 mg/L _{mm} NOEC = 0.28 mg/L _{mm}	EFSA, 2016, DuPont-32658
Aquatic macrophytes				
<i>Lemna gibba</i>	Oxathiapiprolin	7 d, ss	EC ₅₀ (frond count) > 0.79 mg a.s./L _{mm} ^a E_rC₅₀ (frond count) > 0.79 mg a.s./L_{mm} E _y C ₅₀ (frond count) > 0.79 mg a.s./L _{mm} EC ₅₀ (biomass) > 0.79 mg a.s./L _{mm} E _r C ₅₀ (biomass) > 0.79 mg a.s./L _{mm} E _y C ₅₀ (biomass) > 0.79 mg a.s./L _{mm} NOEC ≥ 0.79 mg a.s./L _{mm}	EFSA, 2016, DuPont-32480
Higher-tier studies (micro- or mesocosm studies)				
Not performed, not required				

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

Endpoints used for RAC derivation are shown in **bold**.

EFSA Journal 2016;14(7):4504.

^a Endpoints above the highest tested concentration; nominal concentrations of the test item were chosen with consideration of water solubility limit.

^b IN-QFD61 and IN-S2K67 are considered minor aquatic metabolites and their endpoints are given in the table for reasons of completeness. Taking into account their low toxicity to aquatic organisms compared to the parent compound, evaluation was deemed not necessary (refer to DAR, 2016, Vol. 3, Annex B.9 PPP and EFSA, 2016).

^c Since no endpoint is available the metabolite is assumed to be 10 times more toxic than the parent (in accordance with EFSA, 2016).

Table 9.5-3: Endpoints and effect values relevant for the risk assessment for aquatic organisms – A22773A

Species	Substance	Exposure System	Results	Reference
<i>Oncorhynchus mykiss</i>	A22773A	96 h, s	LC₅₀ = 3.20 mg/L_{nom}	xxxxxxx., 2020, VV-884613
<i>Daphnia magna</i>	A22773A	48 h, s	EC₅₀ = 0.659 mg/L_{nom}	Beuter, L.K., 2020, VV-884821
<i>Raphidocelis subcapitata</i> (formerly known as <i>Pseudokirchneriella subcapitata</i>)	A22773A	96 h, s	E_rC₅₀ (72 h) = 3.32 mg/L_{nom} E _y C ₅₀ (72 h) = 0.616 mg/L _{nom} E _b C ₅₀ (72 h) = 0.692 mg/L _{nom} NOEC (72 h) = 0.0954 mg/L _{nom} E _r C ₅₀ (96 h) = 4.46 mg/L _{nom} E _y C ₅₀ (96 h) = 0.807 mg/L _{nom} E _b C ₅₀ (96 h) = 0.739 mg/L _{nom} NOEC (96 h) = 0.0954 mg/L _{nom}	Obert-Rausser, P., 2020, VV-884825
Higher-tier studies (micro- or mesocosm studies)				
Not performed, not required				

Endpoints used for RAC derivation are shown in **bold**.
s: static; nom: based on nominal concentrations

In the studies with A22773A dosing was confirmed with analysis of azoxystrobin. Analysis of oxathiapiprolin is not considered necessary. Assuming concentration addition provides an acceptable assessment of potential combination toxicity (confirmed by MDR analysis, see Table 9.5-4), oxathiapiprolin would be expected to contribute a negligible amount (<< 10%) of the toxicity of the formulation to fish, *Daphnia* and algae.

9.5.1.1 Justification for new endpoints

New studies are available for formulation A22773A which are required to fulfil the data requirements for plant protection products in accordance with Regulation (EC) No 1107/2009. The endpoints are summarised in Table 9.5-3.

Algal endpoint for azoxystrobin and the metabolite R234886

Following the EFSA Aquatic Guidance (2013), the lowest tier 1 E_rC₅₀ will be used for the algal risk assessment for azoxystrobin, *i.e.* the E_rC₅₀ of 146 µg a.s./L for *Navicula pelliculosa*. Further, the E_rC₅₀ of 80 mg/L for *Pseudokirchneriella subcapitata* will be used for the algal risk assessment for the azoxystrobin metabolite R234886.

Acute and chronic aquatic invertebrate endpoint for azoxystrobin

In the case of azoxystrobin, laboratory acute toxicity data are available for 12 freshwater and two marine invertebrate species. The effects of azoxystrobin on phytoplankton, zooplankton and macro-invertebrate populations have also been evaluated in an outdoor mesocosm study. The NOAEC from this mesocosm study was considered to be 10 µg a.s./L.

These studies were evaluated during the Annex 1 Review and were used to derive a regulatory acceptable concentration (RAC) for azoxystrobin. Several approaches were considered in the derivation of a RAC.

- Geometric mean of acute laboratory endpoints of 8.9 µg a.s./L
- Lower limit of the HC₅ based on acute laboratory data of 7.15 µg a.s./L

- Mesocosm study NOAEC of 10 µg a.s./L.

Following consideration of all lines of evidence, the RAC for azoxystrobin was set at 3.3 µg a.s./L.

Consideration of mixture toxicity of Azoxystrobin/Oxathiapiprolin in A22773A

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

Equation 13 of the EFSA Aquatic Guidance (2013, page 148) details the calculated mixture toxicity by concentration addition.

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

where:

n = number of mixture components

i = index from 1... n mixture components

p_i = the i^{th} component as a relative fraction of the mixture composition (note $\sum p_i$ must be 1)

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

The deviation between calculated and measured toxicity is termed Model Deviation Ratio (MDR) and is calculated using equation 15 of the EFSA Aquatic Guidance (page 149).

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

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

The acute toxicity of A22773A to fish, aquatic invertebrates, algae and aquatic macrophytes expected according to the assumption of concentration addition are given in the table below.

Table 9.5-4: Toxicity of A22773A to aquatic organisms, measured and calculated according to assumption of concentration addition, together with and MDR analysis

Species	Test substance	Concentration of active substance in formulation A22773A (g/L)	Fraction of active substance in the formulation mixture	LC/EC ₅₀ for active substance (mg/L)	Fraction of active substance / LC/EC ₅₀ for the active substance	Calculated LC/EC ₅₀ mix-CA (mg a.s./L) ^a	Measured LC/EC ₅₀ ppp (mg form./L)	Measured LC/EC ₅₀ ppp (mg a.s./L) ^b	MDR ^c LC/EC ₅₀ mix-CA / LC/EC ₅₀ ppp
Fish (<i>Oncorhynchus mykiss</i>)	Azoxystrobin	250	0.954	0.47	2.030	0.477 – 0.493*	3.20	0.764	0.62 – 0.65*
	Oxathiapiprolin	12	0.046	> 0.69	< 0.066				
Total	-	262	1.000	-	2.097	-	-	-	-
Aquatic invertebrates (<i>Daphnia magna</i>)	Azoxystrobin	250	0.954	0.23	4.149	0.237	0.659	0.157	1.51
	Oxathiapiprolin	12	0.046	0.67	0.068				
Total	-	262	1.000	-	4.217	-	-	-	-
Algae (<i>R. subcapitata</i>) ^d	Azoxystrobin	250	0.954	1.47 0.36	0.649 2.65	1.03 – 1.54*	3.32	0.793	1.30 – 1.94*
	Oxathiapiprolin	12	0.046	> 0.142	< 0.323	0.336			0.42
Total	-	262	1.000	-	0.972	-	-	-	-
Macrophytes (<i>Lemna gibba</i>)	Azoxystrobin	250	0.954	3.2		2.81	-	-	-
	Oxathiapiprolin	12	0.046	0.79					
Total	-	262	1.000			-	-	-	-

Note: Calculations undertaken using unrounded values consequently they may not be reproducible when using the figures given in the table.

^a Predicted mixture toxicity under assumption of concentration-addition

^b Formulation endpoint calculation based on the nominal total content of azoxystrobin and oxathiapiprolin in the formulation (262 g a.s./L) and a nominal formulation density of 1.097 g/cm³.

^c In accordance with EFSA Aquatic Guidance (2013), mixture toxicity conforms to assumptions of concentration-addition when model deviation ratio (MDR; EC_x mix-CA/EC_x ppp) is between 0.2 and 5.

^d *Raphidocelis subcapitata*, formerly known as *Pseudokirchneriella subcapitata* and *Selenastrum capricornutum*. Endpoints based on 72h E_rC₅₀.

* The lower limit uses an LC/EC₅₀ at the > value i.e. if >0.69, 0.69 is used as the LC/EC₅₀, the upper limit has been calculated based on the assumption that oxathiapiprolin does not contribute at all to the toxicity of the mixture.

The model deviation ratio (MDR) values in Table 9.5-4 above indicate that toxicity of A22773A to fish, aquatic invertebrates and algae is as predicted on the assumption of concentration addition ($0.2 < \text{MDR} < 5$). This means that the measured values could be used for a mixture risk assessment, although it would be equally valid to use the calculated values.

9.5.2 Risk assessment

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

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

From the endpoints and effect values relevant for the risk assessment for aquatic organisms the following Regulatory Acceptable Concentrations (RAC) are derived for use in the Tier 1 risk assessment.

Table 9.5-5: Derivation of RAC values used in the risk assessment – Azoxystrobin and relevant metabolites

Species	Substance	Exposure system	Results ($\mu\text{g/L}$)	Assessment Safety factor	RAC ($\mu\text{g/L}$)
<i>Oncorhynchus mykiss</i>	Azoxystrobin	96 h, f	$\text{EC}_{50} = 470$	100	4.7
<i>Oncorhynchus mykiss</i>	R234886	96 h, f	$\text{EC}_{50} > 150\,000$	100	$> 1\,500$
<i>Oncorhynchus mykiss</i>	R402173	96 h, s	$\text{EC}_{50} = 62\,000$	100	620
<i>Oncorhynchus mykiss</i>	R401553	96 h, s	$\text{EC}_{50} > 120\,000$	100	$> 1\,200$
<i>Pimephales promelas</i>	Azoxystrobin	33 d (ELS), f	$\text{NOEC} = 147$	10	14.7
<i>Americamysis bahia</i> (formerly <i>Mysidopsis bahia</i>)	Azoxystrobin	96 h, s	$\text{EC}_{50} = 55$	100	0.55
<i>Americamysis bahia</i>	Azoxystrobin	21 d, s	$\text{NOEC} = 9.54$	10	0.954
Aquatic invertebrates, acute and chronic	Azoxystrobin	All lines of evidence	3.3	1	3.3
<i>Daphnia magna</i>	R234886	48 h, s	$\text{EC}_{50} > 180\,000$	100	$> 1\,800$
<i>Daphnia magna</i>	R402173	48 h, s	$\text{EC}_{50} > 100\,000$	100	$> 1\,000$
<i>Daphnia magna</i>	R401553	48 h, s	$\text{EC}_{50} > 120\,000$	100	$> 1\,200$
<i>Chironomus riparius</i>	Azoxystrobin	28 d, spiked water	$\text{NOEC} = 800$	10	80
<i>Chironomus riparius</i>	Azoxystrobin	28 d, s	$\text{NOEC} = 23\,000\, \mu\text{g/kg}$	10	$2\,300\, \mu\text{g/kg}$
<i>Navicula pelliculosa</i>	Azoxystrobin	120 h, s	$\text{E}_r\text{C}_{50} = 146$	10	14.6
<i>Pseudokirchneriella subcapitata</i>	R234886	72 h, s	$\text{E}_r\text{C}_{50} = 80\,000$	10	8 000
<i>Pseudokirchneriella subcapitata</i>	R402173	72 h, s	$\text{E}_r\text{C}_{50} = 67\,000$	10	6 700

Species	Substance	Exposure system	Results (µg/L)	Assessment Safety factor	RAC (µg/L)
<i>Pseudokirchneriella subcapitata</i>	R401553	72 h, s	E _r C ₅₀ > 120 000	10	> 12 000
<i>Lemna gibba</i>	Azoxystrobin	14 d, s	EC ₅₀ = 3 200	10	320

Table 9.5-6: Derivation of RAC values used in the risk assessment – Oxathiapiprolin and relevant metabolites

Species	Substance	Exposure System	Results (µg/L)	Assessment Safety factor	RAC (µg/L)
<i>Cyprinodon variegatus</i>	Oxathiapiprolin	96 h, s	LC ₅₀ >650	100	>6.5
<i>Cyprinodon variegatus</i>	Oxathiapiprolin	35-day, f, ELS	NOEC = 340	10	34
<i>Oncorhynchus mykiss</i>	IN-E8S72	96 h, s	LC ₅₀ >100 000	100	>1 000
<i>Oncorhynchus mykiss</i>	IN-P3X26	96 h, s	LC ₅₀ >67 720	100	>677.2
<i>Oncorhynchus mykiss</i>	IN-Q7D41	96 h, ss	LC ₅₀ >180	100	>1.8
<i>Oncorhynchus mykiss</i>	IN-QPS10	96 h, s	LC ₅₀ = 6 960	100	69.6
<i>Oncorhynchus mykiss</i>	IN-RAB06	96 h, s	LC ₅₀ >50 000	100	>500
<i>Oncorhynchus mykiss</i>	IN-RDT31	96 h, s	LC ₅₀ >11 560	100	>115.6
<i>Oncorhynchus mykiss</i>	IN-RSE01	96 h, ss	LC ₅₀ >9 840	100	>98.4
<i>Oncorhynchus mykiss</i>	IN-RYJ52	96 h, s	LC ₅₀ >13 800	100	>138
<i>Oncorhynchus mykiss</i>	IN-S2K66	96 h, s	LC ₅₀ >7 480	100	>74.8
<i>Crassostrea virginica</i>	Oxathiapiprolin	96 h, f	EC ₅₀ >330	100	>3.3
<i>Americamysis bahia</i>	Oxathiapiprolin	32 d, f	NOEC = 58	10	5.8
<i>Daphnia magna</i>	IN-E8S72	48 h, s	EC ₅₀ >100 000	100	>1 000
<i>Daphnia magna</i>	IN-P3X26	48 h, s	EC ₅₀ >67 740	100	>677.4
<i>Daphnia magna</i>	IN-Q7D41	48 h, ss	EC ₅₀ >150	100	>1.5
<i>Daphnia magna</i>	IN-QPS10	48 h, s	EC ₅₀ = 15 870	100	158.7
<i>Daphnia magna</i>	IN-RAB06	48 h, s	EC ₅₀ >100 000	100	>1 000
<i>Daphnia magna</i>	IN-RDT31	48 h, s	EC ₅₀ >10 490	100	>104.9
<i>Daphnia magna</i>	IN-RSE01	48 h, s	EC ₅₀ >10 160	100	>101.6
<i>Daphnia magna</i>	IN-RYJ52	48 h, s	EC ₅₀ >16 210	100	>162.1
<i>Daphnia magna</i>	IN-S2K66	48 h, s	EC ₅₀ = 860	100	8.6
<i>Chironomus riparius</i>	Oxathiapiprolin	28 d, s, water spiked	NOEC = 110	10	11
<i>Chironomus riparius</i>	Oxathiapiprolin	28 d, spiked sediment	NOEC = 2 800 µg/kg sediment	10	280 µg/kg
<i>Chironomus riparius</i>	IN-Q7D41	28 d, spiked sediment	NOEC = 72 000	10	7 200 µg/kg
<i>Chironomus riparius</i>	IN-RYJ52	10x parent toxicity	NOEC = 11	10	1.1

Species	Substance	Exposure System	Results (µg/L)	Assessment Safety factor	RAC (µg/L)
<i>Chironomus riparius</i>	IN-RYJ52	10x parent toxicity	NOEC = 280 µg/kg sediment	10	28 µg/kg
<i>Chironomus riparius</i>	IN-S2K66	10x parent toxicity	NOEC = 11	10	1.1
<i>Chironomus riparius</i>	IN-S2K66	10x parent toxicity	NOEC = 280 µg/kg sediment	10	28 µg/kg
<i>Chironomus riparius</i>	IN-RSE01	10x parent toxicity	NOEC = 11	10	1.1
<i>Chironomus riparius</i>	IN-RSE01	10x parent toxicity	NOEC = 280 µg/kg sediment	10	28 µg/kg
<i>Pseudokirchneriella subcapitata</i>	Oxathiapiprolin	96 h, s	ErC ₅₀ > 142	10	>14.2
<i>Pseudokirchneriella subcapitata</i>	IN-E8S72	72 h, s	ErC ₅₀ > 100 000	10	>10 000
<i>Pseudokirchneriella subcapitata</i>	IN-P3X26	72 h, s	ErC ₅₀ >66 640	10	>6 664
<i>Pseudokirchneriella subcapitata</i>	IN-Q7D41	72 h, s	ErC ₅₀ >210	10	>21
<i>Pseudokirchneriella subcapitata</i>	IN-QPS10	72 h, s	ErC ₅₀ = 2 320	10	232
<i>Pseudokirchneriella subcapitata</i>	IN-RAB06	72 h, s	ErC ₅₀ >100 000	10	>10 000
<i>Pseudokirchneriella subcapitata</i>	IN-RDT31	72 h, s	ErC ₅₀ >11 430	10	>1 143
<i>Pseudokirchneriella subcapitata</i>	IN-RSE01	72 h, s	ErC ₅₀ >10 800	10	>1 080
<i>Pseudokirchneriella subcapitata</i>	IN-RYJ52	72 h, s	ErC ₅₀ >15 340	10	>1 534
<i>Pseudokirchneriella subcapitata</i>	IN-S2K66	72 h, s	ErC ₅₀ >7 560	10	>756
<i>Lemna gibba</i>	Oxathiapiprolin	7 d, ss	ErC ₅₀ (frond count) >790	10	>79

Table 9.5-7: Derivation of RAC values used in the risk assessment – A22773A

Species	Substance	Exposure System	Results (µg/L)	Assessment Safety factor	RAC (µg/L)
<i>Oncorhynchus mykiss</i>	A22773A	96 h, s	LC ₅₀ = 3200	100	32
<i>Daphnia magna</i>	A22773A	48 h, s	EC ₅₀ = 659	100	6.59
<i>Raphidocelis subcapitata</i> (formerly known as <i>Pseudokirchneriella subcapitata</i>)	A22773A	96 h, s	ErC ₅₀ (72 h) = 3320	10	332

In the following tables, the ratios between predicted environmental concentrations in surface water bodies (PEC_{SW} , PEC_{SED}) and regulatory acceptable concentrations (RAC) for aquatic organisms are given per intended use for each FOCUS scenario and each organism group. Please note that for the risk assessment for fruiting vegetables, additional scenarios from leafy vegetables should be considered to fulfil the requirements of the central zone.

Azoxystrobin

For azoxystrobin, the maximum FOCUS Steps 1, 2 and 3 PEC_{sw} and PEC_{SED} for risk assessments for all field uses and the resulting PEC/RAC ratios are presented in the tables below. Upon request of RMS Poland, the risk assessment was conducted using PEC_{sw} values calculated with two differing plant uptake factors. Results are shown below under Tier 1 and Tier 2.

Review Comments:

In zRMS opinion, PEC_{sw} values calculated with $PUF=0$ should be used in the RA for aquatic organisms. Thus, Tier 2 was not considered further.

Tier 1

Tier 1 risk assessment uses $PEC_{sw, max}$ values derived from PEC_{sw} calculations using a plant uptake factor of 0 (see environmental fate section B8 8.9.2.1).

Table 9.5-8: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for azoxystrobin for each organism group based on the maximum FOCUS Steps 1, 2 and 3 calculations for the use of A22773A in fruiting vegetables (BBCH 11-89, 2 x 250 g a.s./ha, application interval of 7 days)

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Inverteb. acute & prolonged ¹	Algae	Aquatic macrophytes	Sed. dwell. prolonged		Sed. dwell. prolonged (spiked sediment)
RAC (µg/L)		4.70	14.7	0.55	0.954	3.3	14.6	320	80	RAC (µg/kg)	2 300
FOCUS Scenario	PEC _{sw, max} (µg/L)	PEC/RAC ratios								PEC _{sed, max} (µg/kg)	PEC/RAC ratios
Step 1											
I	111	24	7.6	202	117	34	7.6	0.35	1.4	462	0.20
Step 2											
N-Europe	17.8	3.8	1.2	32	19	5.4	1.2	*	0.22	73.8	*
S-Europe	32.8	7.0	2.2	60	34	9.9	2.2	*	0.41	137	*
Step 3											
R2 stream	1.90	0.40	0.13	3.5	2.0	0.58	0.13	*	*	5.52	*
R3 stream	5.55	1.2	0.38	10	5.8	1.7	0.38	*	*	5.72	*
R4 stream	8.52	1.8	0.58	15	8.9	2.6	0.58	*	*	4.66	*

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

* No further assessment is required as an acceptable risk has been demonstrated in a more conservative FOCUS scenario

¹ endpoint derived from all lines of evidence and used for higher tier risk assessment (for details see Table 9.5-1 and chapter 9.5.1.1)

Table 9.5-9: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for azoxystrobin for each organism group based on the maximum FOCUS Steps 1, 2 and 3 calculations for the use of A22773A in leafy vegetables (BBCH 11-49, 2 x 250 g a.s./ha, application interval of 7 days)

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Inverteb. acute & prolonged ¹	Algae	Aquatic macrophytes	Sed. dwell. prolonged		Sed. dwell. Prolonged (sediment)
RAC (µg/L)		4.70	14.7	0.55	0.954	3.3	14.6	320	80	RAC (µg/kg)	2 300
FOCUS Scenario	PEC _{sw, max} (µg/L)	PEC/RAC ratios								PEC _{sed, max} (µg/kg)	PEC/RAC ratios
Step 1											
I	111	24	7.6	202	117	34	7.6	0.35	1.4	462	0.20
Step 2											
N-Europe	40.3	8.6	2.7	73	42	12	2.8	*	0.50	168	*
S-Europe	32.8	7.0	2.2	60	34	9.9	2.2	*	0.41	137	*
Step 3											
D3 ditch	1.59	0.34	0.11	2.9	1.7	0.48	0.11	*	*	0.935	*
D3 ditch 2nd	1.59	0.34	0.11	2.9	1.7	0.48	0.11	*	*	0.962	*
D4 pond	1.40	0.30	0.095	2.5	1.5	0.42	0.10	*	*	8.50	*
D4 stream	1.68	0.36	0.11	3.1	1.8	0.51	0.12	*	*	3.33	*
R1 pond	0.897	0.19	0.061	1.6	0.94	0.27	0.061	*	*	5.50	*
R1 pond 2nd	0.721	0.15	0.049	1.3	0.76	0.22	0.049	*	*	4.23	*
R1 stream	4.91	1.0	0.33	8.9	5.1	1.5	0.34	*	*	4.93	*
R1 stream 2nd	4.26	0.91	0.29	7.7	4.5	1.3	0.29	*	*	2.19	*
R2 stream	2.51	0.53	0.17	4.6	2.6	0.76	0.17	*	*	5.18	*
R2 stream 2nd	1.41	0.30	0.096	2.6	1.5	0.43	0.097	*	*	4.92	*
R3 stream	4.38	0.93	0.30	8.0	4.6	1.3	0.30	*	*	3.05	*
R3 stream 2nd	5.77	1.2	0.39	10	6.0	1.7	0.40	*	*	7.88	*
R4 stream	7.15	1.5	0.49	13	7.5	2.2	0.49	*	*	3.95	*
R4 stream 2nd	8.52	1.8	0.58	15	8.9	2.6	0.58	*	*	4.82	*

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

* No further assessment is required as an acceptable risk has been demonstrated in a more conservative FOCUS scenario

¹ endpoint derived from all lines of evidence and used for higher tier risk assessment (for details see Table 9.5-1 and chapter 9.5.1.1)

Table 9.5-10: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for azoxystrobin for each organism group based on the maximum FOCUS Steps 1, 2 and 3 calculations for the use of A22773A in bulbs and onion like crops (BBCH 11-49, 2 x 250 g a.s./ha, application interval of 12 days)

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Inverteb. acute & prolonged ¹	Algae	Aquatic macrophytes	Sed. dwell. prolonged		Sed. dwell. prolonged (spiked sediment)
RAC (µg/L)		4.70	14.7	0.55	0.954	3.3	14.6	320	80	RAC (µg/kg)	2 300
FOCUS Scenario	PEC _{sw, max} (µg/L)	PEC/RAC ratios								PEC _{sed, max} (µg/kg)	PEC/RAC ratios
Step 1											
I	111	24	7.6	202	117	34	7.6	0.35	1.4	462	0.20
Step 2											
N-Europe	46.8	10	3.2	85	49	14	3.2	*	0.59	196	*
S-Europe	38	8.1	2.6	69	40	12	2.6	*	0.48	159	*
Step 3											
D3 ditch	1.59	0.34	0.11	2.9	1.7	0.48	0.11	*	*	0.921	*
D4 pond	1.36	0.29	0.093	2.5	1.4	0.41	0.093	*	*	8.37	*
D4 stream	1.61	0.34	0.11	2.9	1.7	0.49	0.11	*	*	3.18	*
R1 pond	0.493	0.10	0.034	0.90	0.52	0.15	0.034	*	*	2.69	*
R1 stream	5.52	1.2	0.38	10	5.8	1.7	0.38	*	*	2.23	*
R2 stream	2.42	0.51	0.16	4.4	2.5	0.73	0.17	*	*	4.06	*
R3 stream	5.07	1.1	0.34	9.2	5.3	1.5	0.35	*	*	2.71	*
R4 stream	6.45	1.4	0.44	12	6.8	2.0	0.44	*	*	3.92	*

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

* No further assessment is required as an acceptable risk has been demonstrated in a more conservative FOCUS scenario

¹ endpoint derived from all lines of evidence and used for higher tier risk assessment (for details see Table 9.5-1 and chapter 9.5.1.1)

Table 9.5-11: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for azoxystrobin for each organism group based on the maximum FOCUS Steps 1, 2 and 3 calculations for the use of A22773A in hops (BBCH 21-89, 2 x 250 g a.s./ha, application interval of 12 days)

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Inverteb. acute & prolonged ¹	Algae	Aquatic macrophytes	Sed. dwell. prolonged		Sed. dwell. prolonged (spiked sediment)
RAC (µg/L)		4.70	14.7	0.55	0.954	3.3	14.6	320	80	RAC (µg/kg)	2 300
FOCUS Scenario	PEC _{sw, max} (µg/L)	PEC/RAC ratios								PEC _{sed, max} (µg/kg)	PEC/RAC ratios
Step 1											
I	139	30	9.5	253	146	42	9.5	0.43	1.7	536	0.23
Step 2											
N-Europe	45.2	9.6	3.1	82	47	14	3.1	*	0.57	180	*
S-Europe	40.3	8.6	2.7	73	42	12	2.8	*	0.50	159	*
Step 3											
R1 pond	0.847	0.18	0.058	1.5	0.88	0.26	0.058	*	*	3.89	*
R1 stream	9.46	2.0	0.64	17	9.9	2.9	0.65	*	*	1.33	*

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

* No further assessment is required as an acceptable risk has been demonstrated in a more conservative FOCUS scenario

¹ endpoint derived from all lines of evidence and used for higher tier risk assessment (for details see Table 9.5-1 and chapter 9.5.1.1)

Refined risk assessment azoxystrobin – FOCUS Step 4

An acceptable risk cannot be demonstrated for fish (acute) and invertebrates (acute and chronic) when based on FOCUS Step 3 calculations. For scenarios that are not acceptable at FOCUS Step 3, further PEC/RAC ratios were calculated based on FOCUS Step 4 PEC_{sw} considering reduced exposure of surface water bodies after consideration of different non-sprayed buffer zones and vegetated filter strips (with regard to run-off mitigation). In addition, the PEC/RAC ratios were calculated based on FOCUS Step 4 PEC_{sw} with VFSmod for Poland only. For VFSmod, fruiting vegetables do not have a R1 scenario. Thus, leafy vegetables act as a surrogate crop for fruiting vegetables and no separate results are presented for fruiting vegetables.

To achieve a concise risk assessment, the risk envelope approach is applied, using the maximum FOCUS Step 4 PEC_{sw} values (arithmetic mean of DT₅₀, for more details please refer to the B8). The RAC for fish acute (4.70 µg/L) is higher than the Tier 3 RAC of 3.3 µg/L for aquatic invertebrates. Here, the risk assessment is based on the invertebrates (acute & chronic) RAC of 3.3 µg a.s./L and covers the risk for all aquatic organisms from all intended uses.

Step 4 - Invertebrates acute and chronic (mesocosm) with a RAC = 3.3 µg a.s./L

Table 9.5-12: Aquatic organisms: PEC calculation (µg/L) and acceptability of risk (PEC/RAC < 1) for Azoxystrobin, based on FOCUS Step 4 calculations and refined RAC for invertebrates acute and chronic with mitigation of spray drift and run-off for the use in vegetables, fruiting BBCH 11-89 (2 × 250 g a.s./ha, with application interval of 7 days)

Intended use		Fruiting vegetables, BBCH 11-89		
Active substance		Azoxystrobin		
Application rate (g/ha)		2 × 250		
Nozzle reduction	No-spray buffer (m)	none	none	none
	Vegetated filter strip (m)	default	10	20
None	R2 stream	1.90	1.40	1.40
None	R3 stream	5.55	2.53	1.48
None	R4 stream	8.52	3.86	2.02
RAC (µg/L)		3.3		
		PEC/RAC ratio		
None	R2 stream	0.58	*	*
None	R3 stream	1.7	0.77	*
None	R4 stream	2.6	1.2	0.61

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

* No further assessment is required as an acceptable risk has been demonstrated in a more conservative step

Table 9.5-13: Aquatic organisms: PEC calculation (µg/L) and acceptability of risk (PEC/RAC < 1) for Azoxystrobin, based on FOCUS Step 4 calculations and refined RAC for invertebrates acute and chronic with mitigation of spray drift and run-off (VFS_{mod}) for the use in vegetables, fruiting BBCH 11-89 (2 × 250 g a.s./ha, with application interval of 7 days) (Poland only)

Intended use		Fruiting vegetables, BBCH 11-89	
Active substance		Azoxystrobin	
Application rate (g/ha)		2 × 250	
Nozzle reduction	No-spray buffer (m)	none	none
	Vegetated filter strip (m) VFS_{mod}	default	5
None	R2 stream	1.90	1.40
None	R3 stream	5.55	1.48
None	R4 stream	8.52	1.05
RAC (µg/L)		3.3	
		PEC/RAC ratio	
None	R2 stream	0.58	*
None	R3 stream	1.7	0.45
None	R4 stream	2.6	0.32

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

* No further assessment is required as an acceptable risk has been demonstrated in a more conservative step

- See dRR B8, chapter 8.9.2.1

Table 9.5-14: Aquatic organisms: PEC calculation (µg/L) and acceptability of risk (PEC/RAC < 1) for Azoxystrobin, based on FOCUS Step 4 calculations and refined RAC for invertebrates acute and chronic with mitigation of spray drift and run-off for the use in vegetables, leafy BBCH 11-49 (2 × 250 g a.s./ha, with application interval of 7 days)

Intended use		Leafy vegetables, BBCH 11-49		
Active substance		Azoxystrobin		
Application rate (g/ha)		2 × 250		
Nozzle reduction	No-spray buffer (m)	none	none	none
	Vegetated filter strip (m)	default	10	20
None	D3 ditch	1.59		
None	D3 ditch, 2nd	1.59		
None	D4 pond	1.40		
None	D4 stream	1.68		
None	R1 pond	0.897	0.407	0.240
None	R1 pond, 2nd	0.721	0.323	0.182
None	R1 stream	4.91	2.22	1.16
None	R1 stream, 2nd	4.26	1.94	1.05
None	R2 stream	2.51	1.40	1.40
None	R2 stream, 2nd	1.41	1.40	1.40
None	R3 stream	4.38	1.99	1.48
None	R3 stream, 2nd	5.77	2.63	1.47
None	R4 stream	7.15	3.24	1.54
None	R4 stream, 2nd	8.52	3.87	2.03
RAC (µg/L)		3.3		
		PEC/RAC ratio		
None	D3 ditch	0.48	*	
None	D3 ditch, 2nd	0.48	*	
None	D4 pond	0.42	*	
None	D4 stream	0.51	*	
None	R1 pond	0.27	*	*
None	R1 pond, 2nd	0.22	*	*
None	R1 stream	1.5	0.67	*
None	R1 stream, 2nd	1.3	0.59	*
None	R2 stream	0.76	*	*
None	R2 stream, 2nd	0.43	*	*

Intended use		Leafy vegetables, BBCH 11-49		
Active substance		Azoxystrobin		
Application rate (g/ha)		2 × 250		
Nozzle reduction	No-spray buffer (m)	none	none	none
	Vegetated filter strip (m)	default	10	20
None	R3 stream	1.3	0.60	*
None	R3 stream, 2nd	1.7	0.80	*
None	R4 stream	2.2	0.98	*
None	R4 stream, 2nd	2.6	1.2	0.62

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

* No further assessment is required as an acceptable risk has been demonstrated in a more conservative step

- See dRR B8, chapter 8.9.2.1

2nd: See dRR B8, chapter 8.9.2.1

Table 9.5-15: Aquatic organisms: PEC calculation (µg/L) and acceptability of risk (PEC/RAC < 1) for Azoxystrobin, based on FOCUS Step 4 calculations and refined RAC for invertebrates acute and chronic with mitigation of spray drift and run-off (VFS_{mod}) for the use in vegetables, leafy BBCH 11-49 (2 × 250 g a.s./ha, with application interval of 7 days) (Poland only)

Intended use		Leafy vegetables, BBCH 11-49	
Active substance		Azoxystrobin	
Application rate (g/ha)		2 × 250	
Nozzle reduction	No-spray buffer (m)	none	none
	Vegetated filter strip (m) VFS_{mod}	default	5
None	D3 ditch	1.59	!
None	D3 ditch, 2nd	1.59	!
None	D4 pond	1.40	!
None	D4 stream	1.68	!
None	R1 pond	0.897	0.234
None	R1 pond, 2nd	0.721	0.171
None	R1 stream	4.91	1.05
None	R1 stream, 2nd	4.26	1.05
None	R2 stream	2.51	1.40
None	R2 stream, 2nd	1.41	1.40
None	R3 stream	4.38	1.61
None	R3 stream, 2nd	5.77	1.47
None	R4 stream	7.15	1.04
None	R4 stream, 2nd	8.52	1.05
RAC (µg/L)		3.3	
		PEC/RAC ratio	
None	D3 ditch	0.48	!
None	D3 ditch, 2nd	0.48	!
None	D4 pond	0.42	!
None	D4 stream	0.51	!
None	R1 pond	0.27	*
None	R1 pond, 2nd	0.22	*
None	R1 stream	1.5	0.32
None	R1 stream, 2nd	1.3	0.32
None	R2 stream	0.76	*

None	R2 stream, 2nd	0.43	*
None	R3 stream	1.3	0.49
None	R3 stream, 2nd	1.7	0.45
None	R4 stream	2.2	0.32
None	R4 stream, 2nd	2.6	0.32

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

* No further assessment is required as an acceptable risk has been demonstrated in a more conservative step

- See dRR B8, chapter 8.9.2.1

2nd: See dRR B8, chapter 8.9.2.1

Table 9.5-16: Aquatic organisms: PEC calculation (µg/L) and acceptability of risk (PEC/RAC < 1) for Azoxystrobin, based on FOCUS Step 4 calculations and refined RAC for invertebrates acute and chronic with mitigation of spray drift and run-off for the use in vegetables, bulb and onion like BBCH 11-49 (2 × 250 g a.s./ha, with application interval of 12 days)

Intended use		Bulb and onion like vegetables, BBCH 11-49		
Active substance		Azoxystrobin		
Application rate (g/ha)		2 × 250		
Nozzle reduction	No-spray buffer (m)	none	none	none
	Vegetated filter strip (m)	default	10	20
None	D3 ditch	1.59	█	█
None	D4 pond	1.36	█	█
None	D4 stream	1.61	█	█
None	R1 pond	0.493	0.236	0.149
None	R1 stream	5.52	2.50	1.31
None	R2 stream	2.42	1.50	1.40
None	R3 stream	5.07	2.29	1.47
None	R4 stream	6.45	2.92	1.52
RAC (µg/L)		3.3		
		PEC/RAC ratio		
None	D3 ditch	0.48	*	*
None	D4 pond	0.41	*	*
None	D4 stream	0.49	*	*
None	R1 pond	0.15	*	*
None	R1 stream	1.7	0.76	*
None	R2 stream	0.73	*	*
None	R3 stream	1.5	0.69	*
None	R4 stream	2.0	0.88	*

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

* No further assessment is required as an acceptable risk has been demonstrated in a more conservative step

- See dRR B8, chapter 8.9.2.1

Table 9.5-17: Aquatic organisms: PEC calculation (µg/L) and acceptability of risk (PEC/RAC < 1) for Azoxystrobin, based on FOCUS Step 4 calculations and refined RAC for invertebrates acute and chronic with mitigation of spray drift and run-off (VFS_{mod}) for the use in vegetables, bulb and onion like BBCH 11-49 (2 × 250 g a.s./ha, with application interval of 12 days) (Poland only)

Intended use		Bulb and onion like vegetables, BBCH 11-49	
Active substance		Azoxystrobin	
Application rate (g/ha)		2 × 250	
Nozzle reduction	No-spray buffer (m)	none	none
	Vegetated filter strip (m) VFS_{mod}	default	5
None	D3 ditch	1.59	!
None	D4 pond	1.36	!
None	D4 stream	1.61	!
None	R1 pond	0.493	0.081
None	R1 stream	5.52	1.04
None	R2 stream	2.42	1.40
None	R3 stream	5.07	1.60
None	R4 stream	6.45	1.03
RAC (µg/L)		3.3	
		PEC/RAC ratio	
None	D3 ditch	0.48	!
None	D4 pond	0.41	!
None	D4 stream	0.49	!
None	R1 pond	0.15	*
None	R1 stream	1.7	0.32
None	R2 stream	0.73	0.42
None	R3 stream	1.5	0.48
None	R4 stream	2.0	0.31

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

* No further assessment is required as an acceptable risk has been demonstrated in a more conservative step

- See dRR B8, chapter 8.9.2.1

Table 9.5-18: Aquatic organisms: PEC calculation (µg/L) and acceptability of risk (PEC/RAC < 1) for Azoxystrobin, based on FOCUS Step 4 calculations and toxicity data for invertebrates acute and chronic with mitigation of spray drift and run-off for the use in hops BBCH 21-89 (2 × 250 g a.s./ha, with application interval of 12 days)

Intended use	Hops, BBCH 21-89							
Active substance	Azoxystrobin							
Application rate (g/ha)	2 × 250							
No-spray buffer (m)	none	10	10	10	15	15	15	20
Vegetated filter strip (m)	default	none	none	none	none	none	none	none
Nozzle reduction (%)	none	none	75	90	none	50	75	none
R1 pond	0.847	0.525	0.143	0.066	0.295	0.155	0.085	0.171
R1 stream	9.46	4.03	1.25	1.25	2.66	1.33	1.25	1.25
RAC (µg/L)	3.3							
	PEC/RAC ratio							
R1 pond	0.26	*	*	*	*	*	*	*
R1 stream	2.9	1.2	0.38	*	*0.81	*	*	*

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

* No further assessment is required as an acceptable risk has been demonstrated in a more conservative step

The PEC/RAC ratios, using worst-case PEC_{sw} values for A22773A, azoxystrobin, oxathiapiprolin and their metabolites are less than the trigger value of 1, indicating that the risk to aquatic organisms is acceptable following use of A22773A according to the proposed use patterns. Therefore, no further assessment is necessary.

Tier 2

Tier 2 risk assessment uses $PEC_{sw, max}$ values derived from PEC_{sw} calculations using worst case plant uptake factor of 0.5 (see environmental fate section B8.8.9.2.1).

Table 9.5-19: Aquatic organisms: acceptability of risk ($PEC/RAC < 1$) for azoxystrobin for each organism group based on the maximum FOCUS Steps 1, 2 and 3 calculations for the use of A22773A in fruiting vegetables (BBCH 11-89, 2 x 250 g a.s./ha, application interval of 7 days)

Group		Fish-acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Inverteb. acute & prolonged ¹	Algae	Aquatic macrophytes	Sed. dwell. prolonged		Sed. dwell. prolonged (spiked sediment)
RAC (µg/L)		4.70	14.7	0.55	0.954	3.3	14.6	320	80	RAC (µg/kg)	2-300
FOCUS Scenario	PEC _{sw,max} (µg/L)	PEC/RAC ratios								PEC _{sed,max} (µg/kg)	PEC/RAC ratios
Step 1											
-	111	24	7.6	202	117	34	7.6	0.35	1.4	462	0.20
Step 2											
N-Europe	17.8	3.8	1.2	32	19	5.4	1.2	*	0.22	73.8	*
S-Europe	32.8	7.0	2.2	60	34	9.9	2.2	*	0.41	137	*
Step 3											
R2-stream	1.89	0.40	0.13	3.4	2.0	0.57	0.13	*	*	5.51	*
R3-stream	5.55	1.2	0.38	10	5.8	1.7	0.38	*	*	5.70	*
R4-stream	8.46	1.8	0.58	15	8.9	2.6	0.58	*	*	4.63	*

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

* No further assessment is required as an acceptable risk has been demonstrated in a more conservative FOCUS scenario

[†] endpoint derived from all lines of evidence and used for higher tier risk assessment (for details see Table 9.5-1 and chapter 9.5.1.1)

Table 9.5-20: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for azoxystrobin for each organism group based on the maximum FOCUS Steps 1, 2 and 3 calculations for the use of A22773A in leafy vegetables (BBCH 11-49, 2 x 250 g a.s./ha, application interval of 7 days)

Group		Fish-acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Inverteb. acute & prolonged [†]	Algae	Aquatic macrophytes	Sed. dwell. prolonged		Sed. dwell. Prolonged (sediment)
RAC (µg/L)		4.70	14.7	0.55	0.954	3.3	14.6	320	80	RAC (µg/kg)	2-300
FOCUS Scenario	PEC _{swr,max} (µg/L)	PEC/RAC-ratios								PEC _{sed,max} (µg/kg)	PEC/RAC ratios
Step 1											
-	111	24	7.6	202	117	34	7.6	0.35	1.4	462	0.20
Step 2											
N-Europe	40.3	8.6	2.7	73	42	12	2.8	*	0.50	168	*
S-Europe	32.8	7.0	2.2	60	34	9.9	2.2	*	0.41	137	*
Step 3											
D3-ditch	1.59	0.34	0.11	2.9	1.7	0.48	0.11	*	*	0.935	*
D3-ditch 2nd	1.59	0.34	0.11	2.9	1.7	0.48	0.11	*	*	0.962	*
D4-pond	1.40	0.30	0.095	2.5	1.5	0.42	0.10	*	*	8.37	*
D4-stream	1.68	0.36	0.11	3.1	1.8	0.51	0.12	*	*	3.28	*
R1-pond	0.892	0.19	0.061	1.6	0.93	0.27	0.061	*	*	5.33	*
R1-pond 2nd	0.705	0.15	0.048	1.3	0.74	0.22	0.048	*	*	4.15	*
R1-stream	4.83	1.0	0.33	8.8	5.1	1.5	0.33	*	*	4.91	*
R1-stream 2nd	4.18	0.89	0.28	7.6	4.4	1.3	0.29	*	*	2.15	*
R2-stream	2.50	0.53	0.17	4.5	2.6	0.76	0.17	*	*	5.18	*
R2-stream 2nd	1.41	0.30	0.096	2.6	1.5	0.43	0.097	*	*	4.92	*
R3-stream	4.33	0.92	0.29	7.9	4.5	1.3	0.30	*	*	2.99	*
R3-stream 2nd	5.69	1.2	0.39	10	6.0	1.7	0.39	*	*	7.86	*
R4-stream	7.10	1.5	0.48	13	7.4	2.2	0.49	*	*	3.93	*
R4-stream 2nd	8.41	1.8	0.57	15	8.8	2.5	0.58	*	*	4.77	*

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

* No further assessment is required as an acceptable risk has been demonstrated in a more conservative FOCUS scenario

[†] endpoint derived from all lines of evidence and used for higher tier risk assessment (for details see Table 9.5-1 and chapter 9.5.1.1)

Table 9.5-21: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for azoxystrobin for each organism group based on the maximum FOCUS Steps 1, 2 and 3 calculations for the use of A22773A in bulbs and onion like crops (BBCH 11-49, 2 x 250 g a.s./ha, application interval of 12 days)

Group		Fish-acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Inverteb. acute & prolonged [†]	Algae	Aquatic macrophytes	Sed. dwell. prolonged		Sed. dwell. prolonged (spiked sediment)
RAC (µg/L)		4.70	14.7	0.55	0.954	3.3	14.6	320	80	RAC (µg/kg)	2-300
FOCUS Scenario	PEC _{sw, max} (µg/L)	PEC/RAC ratios								PEC _{sed, max} (µg/kg)	PEC/RAC ratios
Step 1											
-	111	24	7.6	202	117	34	7.6	0.35	1.4	462	0.20
Step 2											
N-Europe	46.8	10	3.2	85	49	14	3.2	*	0.59	196	*
S-Europe	38	8.1	2.6	69	40	12	2.6	*	0.48	159	*
Step 3											
D3-stream	1.59	0.34	0.11	2.9	1.7	0.48	0.11	*	*	0.921	*
D4-pond	1.34	0.29	0.091	2.4	1.4	0.41	0.092	*	*	8.28	*
D4-stream	1.59	0.34	0.11	2.9	1.7	0.48	0.11	*	*	3.14	*
R1-pond	0.479	0.10	0.033	0.87	0.50	0.15	0.033	*	*	2.60	*
R1-stream	5.37	1.1	0.37	9.8	5.6	1.6	0.37	*	*	2.18	*
R2-stream	2.41	0.51	0.16	4.4	2.5	0.73	0.17	*	*	4.05	*
R3-stream	4.98	1.1	0.34	9.1	5.2	1.5	0.34	*	*	2.63	*
R4-stream	6.40	1.4	0.44	12	6.7	1.9	0.44	*	*	3.89	*

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

* No further assessment is required as an acceptable risk has been demonstrated in a more conservative FOCUS scenario

[†] endpoint derived from all lines of evidence and used for higher tier risk assessment (for details see Table 9.5-1 and chapter 9.5.1.1)

Table 9.5-22: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for azoxystrobin for each organism group based on the maximum FOCUS Steps 1, 2 and 3 calculations for the use of A22773A in hops (BBCH 21-89, 2 x 250 g a.s./ha, application interval of 12 days)

Group		Fish-acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Inverteb. acute & prolonged [†]	Algae	Aquatic macrophytes	Sed.-dwell. prolonged		Sed.-dwell. prolonged (spiked sediment)
RAC (µg/L)		4.70	14.7	0.55	0.954	3.3	14.6	320	80	RAC (µg/kg)	2-300
FOCUS Scenario	PEC _{sw,max} (µg/L)	PEC/RAC-ratios								PEC _{sed,max} (µg/kg)	PEC/RAC ratios
Step 1											
-	139	30	9.5	253	146	42	9.5	0.43	1.7	536	0.23
Step 2											
N-Europe	45.2	9.6	3.1	82	47	14	3.1	*	0.57	180	*
S-Europe	40.3	8.6	2.7	73	42	12	2.8	*	0.50	159	*
Step 3											
R1-pond	0.847	0.18	0.058	1.5	0.88	0.26	0.058	*	*	3.89	*
R1-stream	9.46	2.0	0.64	17	9.9	2.9	0.65	*	*	1.33	*

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

※ No further assessment is required as an acceptable risk has been demonstrated in a more conservative FOCUS scenario

[†] endpoint derived from all lines of evidence and used for higher tier risk assessment (for details see Table 9.5-1 and chapter 9.5.1.1)

Refined risk assessment azoxystrobin—FOCUS Step 4

An acceptable risk cannot be demonstrated for fish (acute) and invertebrates (acute and chronic) when based on FOCUS Step 3 calculations. For scenarios that are not acceptable at FOCUS Step 3, further PEC/RAC ratios were calculated based on FOCUS Step 4 PEC_{sw} considering reduced exposure of surface water bodies after consideration of different non-sprayed buffer zones and vegetated filter strips (with regard to run-off mitigation). In addition, the PEC/RAC ratios were calculated based on FOCUS Step 4 PEC_{sw} with VFSmod for Poland only. For VFSmod, fruiting vegetables do not have a R1 scenario. Thus, leafy vegetables act as a surrogate crop for fruiting vegetables and no separate results are presented for fruiting vegetables.

To achieve a concise risk assessment, the risk envelope approach is applied, using the maximum FOCUS Step 4 PEC_{sw} values (arithmetic mean of DT₅₀, for more details please refer to the B8). The RAC for fish acute (4.70 µg/L) is higher than the Tier 3 RAC of 3.3 µg/L for aquatic invertebrates. Here, the risk assessment is based on the invertebrates (acute & chronic) RAC of 3.3 µg a.s./L and covers the risk for all aquatic organisms from all intended uses.

Step 4 – Invertebrates acute and chronic (mesocosm) with a RAC = 3.3 µg a.s./L

Table 9.5.23: Aquatic organisms: PEC calculation (µg/L) and acceptability of risk (PEC/RAC < 1) for Azoxystrobin, based on FOCUS Step 4 calculations and refined RAC for invertebrates acute and chronic with mitigation of spray drift and run-off for the use in vegetables, fruiting BBCH 11-89 (2 × 250 g a.s./ha, with application interval of 7 days)

Intended use		Fruiting vegetables, BBCH 11-89			
Active substance		Azoxystrobin			
Application rate (g/ha)		2 × 250			
Nozzle reduction	No-spray buffer (m)	none	10	10	20
	Vegetated filter strip (m)	default	none	10	20
None	R2 stream	1.89	1.89	0.862	0.452
None	R3 stream	5.55	5.55	2.53	1.33
None	R4 stream	8.46	8.46	3.83	2.00
RAC (µg/L)		3.3			
		PEC/RAC ratio			
None	R2 stream	0.57	*	*	*
None	R3 stream	1.7	1.7	0.77	*
None	R4 stream	2.6	2.6	1.2	0.61

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

* No further assessment is required as an acceptable risk has been demonstrated in a more conservative step

Table 9.5 24: Aquatic organisms: PEC calculation (µg/L) and acceptability of risk (PEC/RAC < 1) for Azoxystrobin, based on FOCUS Step 4 calculations and refined RAC for invertebrates acute and chronic with mitigation of spray drift and run-off (VFS_{mod}) for the use in vegetables, fruiting BBCH 11-89 (2 × 250 g a.s./ha, with application interval of 7 days) (Poland only)

Intended use		Fruiting vegetables, BBCH 11-89	
Active substance		Azoxystrobin	
Application rate (g/ha)		2 × 250	
Nozzle reduction	No-spray buffer (m)	none	none
	Vegetated filter strip (m) VFS _{mod}	default	5
None	R2 stream	1.89	1.40
None	R3 stream	5.55	1.48
None	R4 stream	8.46	1.05
RAC (µg/L)		3.3	
		PEC/RAC ratio	
None	R2 stream	0.57	0.38
None	R3 stream	1.7	0.45
None	R4 stream	2.6	0.32

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

* No further assessment is required as an acceptable risk has been demonstrated in a more conservative step

See dRR B8, chapter 8.9.2.1

2nd: See dRR B8, chapter 8.9.2.1

Table 9.5-25 — Aquatic organisms: PEC calculation (µg/L) and acceptability of risk (PEC/RAC < 1) for Azoxystrobin, based on FOCUS Step 4 calculations and refined RAC for invertebrates acute and chronic with mitigation of spray drift and run-off for the use in vegetables, leafy BBCH 11-49 (2 × 250 g a.s./ha, with application interval of 7 days)

Intended use		Leafy vegetables, BBCH 11-49			
Active substance		Azoxystrobin			
Application rate (g/ha)		2 × 250			
Nozzle reduction	No-spray buffer (m)	none	10	10	20
	Vegetated filter strip (m)	default	none	10	20
None	D3-ditch	1.59	0.228	–	–
None	D3-ditch, 2nd	1.59	0.228	–	–
None	D4-pond	1.40	1.39	–	–
None	D4-stream	1.68	1.68	–	–
None	R1-pond	0.892	0.864	0.377	0.196
None	R1-pond, 2nd	0.705	0.692	0.303	0.157
None	R1-stream	4.83	4.83	2.19	1.15
None	R1-stream, 2nd	4.18	4.18	1.90	0.996
None	R2-stream	2.50	2.50	1.13	0.591
None	R2-stream, 2nd	1.41	1.41	0.631	0.329
None	R3-stream	4.33	4.33	1.97	1.03
None	R3-stream, 2nd	5.69	5.69	2.59	1.36
None	R4-stream	7.10	7.10	3.22	1.68
None	R4-stream, 2nd	8.41	8.41	3.82	2.00
RAC (µg/L)		3.3			
		PEC/RAC-ratio			
None	D3-ditch	0.48	※	–	–
None	D3-ditch, 2nd	0.48	※	–	–
None	D4-pond	0.42	※	–	–
None	D4-stream	0.51	※	–	–
None	R1-pond	0.27	※	※	※
None	R1-pond, 2nd	0.21	※	※	※
None	R1-stream	1.5	1.5	0.67	※
None	R1-stream, 2nd	1.3	1.3	0.58	※
None	R2-stream	0.76	※	※	※
None	R2-stream, 2nd	0.43	※	※	※

None	R3 stream	1.3	1.3	0.60	*
None	R3 stream, 2nd	1.7	1.7	0.79	*
None	R4 stream	2.2	2.2	0.97	*
None	R4 stream, 2nd	2.5	2.5	1.2	0.61

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

* No further assessment is required as an acceptable risk has been demonstrated in a more conservative step

– See dRR B8, chapter 8.9.2.1

2nd: See dRR B8, chapter 8.9.2.1

Table 9.5 26: Aquatic organisms: PEC calculation (µg/L) and acceptability of risk (PEC/RAC < 1) for Azoxystrobin, based on FOCUS Step 4 calculations and refined RAC for invertebrates acute and chronic with mitigation of spray drift and run-off (VFS_{mod}) for the use in vegetables, leafy BBCH 11-49 (2 × 250 g a.s./ha, with application interval of 7 days) (Poland only)

Intended use		Leafy vegetables, BBCH 11-49	
Active substance		Azoxystrobin	
Application rate (g/ha)		2 × 250	
Nozzle reduction	No-spray buffer (m)	none	none
	Vegetated filter strip (m) VFS _{mod}	default	5
None	D3-ditch	1.59	-
None	D3-ditch, 2nd	1.59	-
None	D4-pond	1.38	-
None	D4-stream	1.68	-
None	R1-pond	0.892	0.233
None	R1-pond, 2nd	0.705	0.168
None	R1-stream	4.83	1.05
None	R1-stream, 2nd	4.18	1.05
RAC (µg/L)		3.3	
		PEC/RAC ratio	
None	D3-ditch	0.48	-
None	D3-ditch, 2nd	0.48	-
None	D4-pond	0.42	-
None	D4-stream	0.51	-
None	R1-pond	0.27	*
None	R1-pond, 2nd	0.21	*
None	R1-stream	1.5	0.32
None	R1-stream, 2nd	1.3	0.32

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

* No further assessment is required as an acceptable risk has been demonstrated in a more conservative step

- See dRR B8, chapter 8.9.2.1

2nd: See dRR B8, chapter 8.9.2.1

Table 9.5 27 — Aquatic organisms: PEC calculation (µg/L) and acceptability of risk (PEC/RAC < 1) for Azoxystrobin, based on FOCUS Step 4 calculations and refined RAC for invertebrates acute and chronic with mitigation of spray drift and run-off for the use in vegetables, bulb and onion like BBCH 11-49 (2 × 250 g a.s./ha, with application interval of 12 days)

Intended use		Bulb and onion like vegetables, BBCH 11-49			
Active substance		Azoxystrobin			
Application rate (g/ha)		2 × 250			
Nozzle reduction	No-spray buffer (m)	none	10	10	20
	Vegetated filter strip (m)	default	none	10	20
None	D3 ditch	1.59	0.228	–	–
None	D4 pond	1.34	1.33	–	–
None	D4 stream	1.59	1.59	–	–
None	R1 pond	0.479	0.454	0.206	0.109
None	R1 stream	5.37	5.37	2.43	1.27
None	R2 stream	2.41	2.41	1.09	0.568
None	R3 stream	4.98	4.98	2.25	1.18
None	R4 stream	6.40	6.40	2.89	1.51
RAC (µg/L)		3.3			
		PEC/RAC ratio			
None	D3 ditch	0.48	※	–	–
None	D4 pond	0.41	※	–	–
None	D4 stream	0.48	※	–	–
None	R1 pond	0.15	※	※	※
None	R1 stream	1.6	1.6	0.74	※
None	R2 stream	0.73	※	※	※
None	R3 stream	1.5	1.5	0.68	※
None	R4 stream	1.9	1.9	0.88	※

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

* No further assessment is required as an acceptable risk has been demonstrated in a more conservative step

– See dRR B8, chapter 8.9.2.1

Table 9.5 28 — Aquatic organisms: PEC calculation (µg/L) and acceptability of risk (PEC/RAC < 1) for Azoxystrobin, based on FOCUS Step 4 calculations and refined RAC for invertebrates acute and chronic with mitigation of spray drift and run-off (VFS_{mod}) for the use in vegetables, bulb and onion like BBCH 11-49 (2 × 250 g a.s./ha, with application interval of 12 days) (Poland only)

Intended use		Bulb and onion like vegetables, BBCH 11-49	
Active substance		Azoxystrobin	
Application rate (g/ha)		2 × 250	
Nozzle reduction	No-spray buffer (m)	none	none
	Vegetated filter strip (m) VFS _{mod}	default	5
None	D3 ditch	1.59	-
None	D4 pond	1.34	-
None	D4 stream	1.59	-
None	R1 pond	0.479	0.081
None	R1 stream	5.37	1.04
RAC (µg/L)		3.3	
		PEC/RAC ratio	
None	D3 ditch	0.48	-
None	D4 pond	0.41	-
None	D4 stream	0.48	-
None	R1 pond	0.15	*
None	R1 stream	1.6	0.32

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

* No further assessment is required as an acceptable risk has been demonstrated in a more conservative step

— See dRR B8, chapter 8.9.2.1

Table 9.5-29 – Aquatic organisms: PEC calculation (µg/L) and acceptability of risk (PEC/RAC < 1) for Azoxystrobin, based on FOCUS Step 4 calculations and toxicity data for invertebrates acute and chronic with mitigation of spray drift and run-off for the use in hops BBCH 21-89 (2 × 250 g a.s./ha, with application interval of 12 days)

Intended use		Hops, BBCH 21-89		
Active substance		Azoxystrobin		
Application rate (g/ha)		2 × 250		
Nozzle reduction	No-spray buffer (m)	none	40	20
	Vegetated filter strip (m)	default	none	none
None	R1-pond	0.847	0.525	0.170
None	R1-stream	9.46	4.03	1.24
RAC (µg/L)		3.3		
		PEC/RAC ratio		
None	R1-pond	0.26	*	*
None	R1-stream	2.9	1.2	0.38

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

* No further assessment is required as an acceptable risk has been demonstrated in a more conservative step

The PEC/RAC ratios, using worst-case PEC_{SW}-values for A22773A, azoxystrobin, oxathiapiprolin and their metabolites are less than the trigger value of 1, indicating that the risk to aquatic organisms is acceptable following use of A22773A according to the proposed use patterns. Therefore, no further assessment is necessary.

Azoxystrobin metabolites

For relevant metabolites of azoxystrobin, a risk envelop approach is applied using maximum FOCUS Steps 1 and 2 PEC_{sw} based on use of A22773A in hops covering all other proposed field uses.

Table 9.5-30: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for azoxystrobin metabolite R234886 for each organism group based on the maximum FOCUS Steps 1 and 2 calculations for the use of A22773A

Group		Fish acute	Inverteb. acute	Algae
RAC (µg/L)		>1 500	>1 800	8 000
FOCUS Scenario	PEC _{sw, max} (µg/L)	PEC/RAC ratios		
Step 1				
	77.6	0.052	0.043	0.010
Step 2				
N-Europe	20.5	*	*	*
S-Europe	17.4	*	*	*

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

* No further assessment is required as an acceptable risk has been demonstrated in a more conservative FOCUS scenario

Table 9.5-31: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for azoxystrobin metabolite R402173 for each organism group based on the maximum FOCUS Steps 1 and 2 calculations for the use of A22773A

Group		Fish acute	Inverteb. acute	Algae
RAC (µg/L)		620	>1 000	6 700
FOCUS Scenario	PEC _{sw, max} (µg/L)	PEC/RAC ratios		
Step 1				
	26.5	0.043	0.026	0.0040
Step 2				
N-Europe	3.14	*	*	*
S-Europe	2.63	*	*	*

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

* No further assessment is required as an acceptable risk has been demonstrated in a more conservative FOCUS scenario

Table 9.5-32: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for azoxystrobin metabolite R401553 for each organism group based on the maximum FOCUS Steps 1 and 2 calculations for the use of A22773A

Group		Fish acute	Inverteb. acute	Algae
RAC (µg/L)		>1 200	>1 200	>12 000
FOCUS Scenario	PEC _{sw, max} (µg/L)	PEC/RAC ratios		
Step 1				
	19.8	0.017	0.017	0.0017
Step 2				
N-Europe	2.74	*	*	*
S-Europe	2.43	*	*	*

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

* No further assessment is required as an acceptable risk has been demonstrated in a more conservative FOCUS scenario

Oxathiapiprolin

For oxathiapiprolin, the maximum FOCUS Steps 1, 2 and 3 PEC_{SW} and PEC_{SED} for risk assessments for all field uses and the resulting PEC/RAC ratios are presented in the tables below.

Table 9.5-33: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for oxathiapiprolin for each organism group based on the maximum FOCUS Steps 1, 2 and 3 calculations for the use of A22773A in fruiting vegetables (BBCH 11-89, 2 x 12 g a.s./ha, application interval of 7 days)

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Aquatic macrophytes	Sed. dwell. prolonged		Sed. dwell. prolonged (spiked sediment)
RAC (µg/L)		>6.5	34	>3.3	5.8	>14.2	>79	11	RAC (µg/kg)	280
FOCUS Scenario	PEC _{sw, max} (µg/L)	PEC/RAC ratios							PEC _{sed, max} (µg/kg)	PEC/RAC ratios
Step 1										
	1.08	0.17	0.032	0.33	0.19	0.076	0.014	0.098	60.1	0.21
Step 2										
N-Europe	0.153	*	*	*	*	*	*	*	9.90	*
S-Europe	0.276	*	*	*	*	*	*	*	18.4	*

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

* No further assessment is required as an acceptable risk has been demonstrated in a more conservative FOCUS scenario

Table 9.5-34: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for oxathiapiprolin for each organism group based on the maximum FOCUS Steps 1, 2 ~~and 3~~ calculations for the use of A22773A in leafy vegetables (BBCH 11-49, 2 x 12 g a.s./ha, application interval of 7 days)

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Aquatic macrophytes	Sed. dwell. prolonged		Sed. dwell. prolonged (spiked sediment)
RAC (µg/L)		>6.5	34	>3.3	5.8	>14.2	>79	11	RAC (µg/kg)	280
FOCUS Scenario	PEC _{sw, max} (µg/L)	PEC/RAC ratios							PEC _{sed, max} (µg/kg)	PEC/RAC ratios
Step 1										
	1.08	0.17	0.032	0.33	0.19	0.076	0.014	0.098	60.1	0.21
Step 2										
N-Europe	0.338	*	*	*	*	*	*	*	22.6	*
S-Europe	0.276	*	*	*	*	*	*	*	18.4	*

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

* No further assessment is required as an acceptable risk has been demonstrated in a more conservative FOCUS scenario

Table 9.5-35: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for oxathiapiprolin for each organism group based on the maximum FOCUS Steps 1, 2 and 3 calculations for the use of A22773A in bulb and onion like vegetables (BBCH 11-49, 2 x 12 g a.s./ha, application interval of 12 days)

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Aquatic macrophyte s	Sed. dwell. prolonged		Sed. dwell. prolonged (spiked sediment)
RAC (µg/L)		>6.5	34	>3.3	5.8	>14.2	>79	11	RAC (µg/kg)	280
FOCUS Scenario	PEC _{sw, max} (µg/L)	PEC/RAC ratios							PEC _{sed, max} (µg/kg)	PEC/RAC ratios
Step 1										
	1.08	0.17	0.032	0.33	0.19	0.076	0.014	0.098	60.1	0.21
Step 2										
N-Europe	0.394	*	*	*	*	*	*	*	26.5	*
S-Europe	0.321	*	*	*	*	*	*	*	21.5	*

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

* No further assessment is required as an acceptable risk has been demonstrated in a more conservative FOCUS scenario

Table 9.5-36: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for oxathiapiprolin for each organism group based on the maximum FOCUS Steps 1, 2 ~~and 3~~ calculations for the use of A22773A in hops (BBCH 21-89, 2 x 12 g a.s./ha, application interval of 12 days)

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Aquatic macrophyte s	Sed. dwell. prolonged		Sed. dwell. prolonged (spiked sediment)
RAC (µg/L)		>6.5	34	>3.3	5.8	>14.2	>79	11	RAC (µg/kg)	280
FOCUS Scenario	PEC _{sw, max} (µg/L)	PEC/RAC ratios							PEC _{sed, max} (µg/kg)	PEC/RAC ratios
Step 1										
	2.40	0.37	0.071	0.73	0.41	0.17	0.030	0.22	69.8	0.25
Step 2										
N-Europe	0.813	*	*	*	*	*	*	*	24.3	*
S-Europe	0.813	*	*	*	*	*	*	*	21.5	*

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

* No further assessment is required as an acceptable risk has been demonstrated in a more conservative FOCUS scenario

Oxathiapiprolin metabolites

For relevant metabolites of oxathiapiprolin, two risk envelop approaches are applied using maximum FOCUS Steps 1 and 2 PEC_{SW} and PEC_{SED} based on uses of A22773A in hops for metabolites IN-P3X26, IN-Q7D41, IN-RAB06, IN-RSE01, IN-RYJ52, IN-S2K66 and bulbs for metabolites IN-E8S72, IN-QPS10, IN-RDT31 covering all other proposed field uses.

Table 9.5-37: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for oxathiapiprolin metabolite IN-E8S72 for each organism group based on maximum FOCUS Steps 1 and 2 calculations for the use of A22773A

Group		Fish acute	Inverteb. acute	Algae
RAC (µg/L)		>1 000	>1 000	>10 000
FOCUS Scenario	PEC _{sw, max} (µg/L)	PEC/RAC ratios		
Step 1				
	0.272	<0.001	<0.001	<0.001
Step 2				
N-Europe	0.120	*	*	*
S-Europe	0.096	*	*	*

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration

PEC/RAC ratios above the relevant trigger of 1 are shown in bold

* No further assessment is required as an acceptable risk has been demonstrated in a more conservative FOCUS scenario

Table 9.5-38: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for oxathiapiprolin metabolite IN-P3X26 for each organism group based on maximum FOCUS Steps 1 and 2 calculations for the use of A22773A

Group		Fish acute	Inverteb. acute	Algae
RAC (µg/L)		>677.2	>677.4	>6 664
FOCUS Scenario	PEC _{sw, max} (µg/L)	PEC/RAC ratios		
Step 1				
	0.977	0.0015	0.0015	<0.001
Step 2				
N-Europe	0.344	*	*	*
S-Europe	0.305	*	*	*

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration

PEC/RAC ratios above the relevant trigger of 1 are shown in bold

* No further assessment is required as an acceptable risk has been demonstrated in a more conservative FOCUS scenario

Table 9.5-39: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for oxathiapiprolin metabolite IN-Q7D41 for each organism group based on maximum FOCUS Steps 1 and 2 calculations for the use of A22773A

Group		Fish acute	Inverteb. acute	Algae		Sed. dwell. prolonged
RAC (µg/L)		>1.8	>1.5	>21		7 200
FOCUS Scenario	PEC _{sw, max} (µg/L)	PEC/RAC ratios			PEC _{sed, max} (µg/kg)	PEC/RAC ratios
Step 1						
	1.11	0.62	0.74	0.053	7.82	0.0011
Step 2						
N-Europe	0.383	*	*		0.04	*
S-Europe	0.340	*	*		0.03	*

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration

PEC/RAC ratios above the relevant trigger of 1 are shown in bold

* No further assessment is required as an acceptable risk has been demonstrated in a more conservative FOCUS scenario

Table 9.5-40: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for oxathiapiprolin metabolite IN-QPS10 for each organism group based on maximum FOCUS Steps 1 and 2 calculations for the use of A22773A

Group		Fish acute	Inverteb. acute	Algae
RAC (µg/L)		69.6	158.7	232
FOCUS Scenario	PEC _{sw, max} (µg/L)	PEC/RAC ratios		
Step 1				
	0.060	<0.001	<0.001	<0.001
Step 2				
N-Europe	0.027	*	*	*
S-Europe	0.021	*	*	*

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration

PEC/RAC ratios above the relevant trigger of 1 are shown in bold

* No further assessment is required as an acceptable risk has been demonstrated in a more conservative FOCUS scenario

Table 9.5-41: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for oxathiapiprolin metabolite IN-RAB06 for each organism group based on maximum FOCUS Steps 1 and 2 calculations for the use of A22773A

Group		Fish acute	Inverteb. acute	Algae
RAC (µg/L)		>500	>1 000	>10 000
FOCUS Scenario	PEC _{sw, max} (µg/L)	PEC/RAC ratios		
Step 1				
	1.33	0.0027	0.0013	<0.001
Step 2				
N-Europe	0.366	*	*	*
S-Europe	0.312	*	*	*

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration

PEC/RAC ratios above the relevant trigger of 1 are shown in bold

* No further assessment is required as an acceptable risk has been demonstrated in a more conservative FOCUS scenario

Table 9.5-42: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for oxathiapiprolin metabolite IN-RDT31 for each organism group based on maximum FOCUS Steps 1 and 2 calculations for the use of A22773A

Group		Fish acute	Inverteb. acute	Algae
RAC (µg/L)		>115.6	>104.9	>1 143
FOCUS Scenario	PEC _{sw, max} (µg/L)	PEC/RAC ratios		
Step 1				
	0.303	0.0026	0.0029	<0.001
Step 2				
N-Europe	0.131	*	*	*
S-Europe	0.104	*	*	*

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration

PEC/RAC ratios above the relevant trigger of 1 are shown in bold

* No further assessment is required as an acceptable risk has been demonstrated in a more conservative FOCUS scenario

Table 9.5-43: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for oxathiapiprolin metabolite IN-RSE01 for each organism group based on maximum FOCUS Steps 1 and 2 calculations for the use of A22773A

Group		Fish acute	Inverteb. acute	Algae	Sed. dwell. prolonged		Sed. dwell. prolonged
RAC (µg/L)		>98.4	>101.6	>1 080	1.1	RAC (µg/kg)	28
FOCUS Scenario	PEC _{sw, max} (µg/L)	PEC/RAC ratios				PEC _{sed, max} (µg/kg)	PEC/RAC ratios
Step 1							
	0.929	0.0095	0.0092	<0.001	0.85	6.55	0.23
Step 2							
N-Europe	0.321	*	*	*	*	2.26	*
S-Europe	0.284	*	*	*	*	2.00	*

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration

PEC/RAC ratios above the relevant trigger of 1 are shown in bold

* No further assessment is required as an acceptable risk has been demonstrated in a more conservative FOCUS scenario

Table 9.5-44: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for oxathiapiprolin metabolite IN-RYJ52 for each organism group based on maximum FOCUS Steps 1 and 2 calculations for the use of A22773A

Group		Fish acute	Inverteb. acute	Algae	Sed. dwell. prolonged		Sed. dwell. prolonged
RAC (µg/L)		>138	>162.1	>1 534	1.1	RAC (µg/kg)	28
FOCUS Scenario	PEC _{sw} , max (µg/L)	PEC/RAC ratios				PEC _{sed} , max (µg/kg)	PEC/RAC ratios
Step 1							
	1.53	0.011	0.0094	<0.001	1.4	10.75	0.38
Step 2							
N-Europe	0.527	*	*	*	0.48	3.71	*
S-Europe	0.466	*	*	*	0.42	3.29	*

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration

PEC/RAC ratios above the relevant trigger of 1 are shown in bold

* No further assessment is required as an acceptable risk has been demonstrated in a more conservative FOCUS scenario

Table 9.5-45: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for oxathiapiprolin metabolite IN-S2K66 for each organism group based on maximum FOCUS Steps 1 and 2 calculations for the use of A22773A

Group		Fish acute	Inverteb. acute	Algae	Sed. dwell. prolonged		Sed. dwell. prolonged
RAC (µg/L)		>74.8	8.6	756	1.1	RAC (µg/kg)	28
FOCUS Scenario	PEC _{sw} , max (µg/L)	PEC/RAC ratios				PEC _{sed} , max (µg/kg)	PEC/RAC ratios
Step 1							
	0.805	0.011	0.094	0.0011	0.74	5.67	0.20
Step 2							
N-Europe	0.278	*	*	*	*	1.96	*
S-Europe	0.246	*	*	*	*	1.74	*

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration

PEC/RAC ratios above the relevant trigger of 1 are shown in bold

* No further assessment is required as an acceptable risk has been demonstrated in a more conservative FOCUS scenario

The PEC/RAC ratios, using worst-case PEC_{sw} values for A22773A, azoxystrobin, oxathiapiprolin and their metabolites are less than the trigger value of 1, indicating that the risk to aquatic organisms is acceptable following use of A22773A according to the proposed use patterns. Therefore, no further assessment is necessary.

Combined risk assessment for A22773A based on calculated mixture toxicity

The EFSA Aquatic Guidance (2013) requires assessment of the risk from a combination of active substances where a product contains more than one active substance. As stated above, comparison of measured and calculated mixture toxicity of A22773A indicates no deviation from that expected on the basis of concentration addition (see 9.5.1.1).

The formulated product A22773A contains azoxystrobin and oxathiapiprolin at 250 g/L and 12 g/L, respectively, which corresponds to a fraction of azoxystrobin of 95% in the formulation. Furthermore, azoxystrobin is more toxic to aquatic organisms than oxathiapiprolin. It is therefore likely that the toxicity of the mixture will be driven by azoxystrobin.

Methodology to establish whether a single substance is driving the toxicity of a mixture

The EFSA Aquatic Guidance (2013), states that where concentration addition gives a reliable estimate of the toxicity of a mixture, when one active substance represents >90% of the risk, it drives the overall mixture risk and no further assessment is required. The MDR calculation (see Table 9.5-4) shows that concentration addition does give a reliable estimate of toxicity, thus this simplified approach is applicable. To evaluate this, toxic units can be calculated at comparable exposures (i.e. FOCUS Steps 1 and 2 which have been modelled for both active substances).

Equation 14 of the EFSA aquatic guidance highlights how to calculate the sum of the toxic units for each component of a mixture:

Equation 14:
$$\sum_{i=1}^n TU_i = \sum_{i=1}^n \frac{c_i}{ECx_i}$$

Table 9.5-46: Aquatic organisms: Toxic Units (TU) approach to identify risk driver – fish acute

Active substance		Azoxystrobin		Oxathiapiprolin		SUM Toxic Units	Toxic Unit as % of total	
Endpoint		<i>Oncorhynchus mykiss</i> <i>LC</i> ₅₀ = 470 µg/L		<i>Cyprinodon variegatus</i> <i>LC</i> ₅₀ > 650 µg/L			Azoxystrobin	Oxathiapiprolin
FOCUS Scenario	Use pattern	PECsw	Toxic Unit	PECsw	Toxic Unit			
Step 1	Fruiting vegetables	111	0.236	1.08	0.002	0.238	99.3	0.7
Step 2 - NEU		17.8	0.038	0.153	0.0002	0.038	99.4	0.6
Step 2 - SEU		32.8	0.070	0.276	0.0004	0.070	99.4	0.6
Step 1	Leafy vegetables	111	0.236	1.08	0.002	0.238	99.3	0.7
Step 2 - NEU		40.3	0.086	0.338	0.001	0.086	99.4	0.6
Step 2 - SEU		32.8	0.070	0.276	0.0004	0.070	99.4	0.6
Step 1	Onion and bulb like crops	111	0.236	1.08	0.002	0.238	99.3	0.7
Step 2 - NEU		46.8	0.100	0.394	0.0006	0.100	99.4	0.6
Step 2 - SEU		38	0.081	0.321	0.0005	0.081	99.4	0.6
Step 1	Hops	139	0.296	2.40	0.004	0.299	98.8	1.2
Step 2 - NEU		42.5	0.090	0.813	0.001	0.092	98.6	1.4
Step 2 - SEU		40.3	0.086	0.813	0.001	0.087	98.6	1.4

TU: Toxic Units

^a If the TU value is > 90%, this indicates the respective component is driving the risk to the taxonomic group and is highlighted in bold.

Table 9.5-47: Aquatic organisms: Toxic Units (TU) approach to identify risk driver – fish chronic

Active substance		Azoxystrobin		Oxathiapiprolin		SUM Toxic Units	Toxic Unit as % of total	
Endpoint		<i>Pimephales promelas</i> NOEC = 147 µg/L		<i>Cyprinodon variegatus</i> NOEC = 340 µg/L			Azoxystrobin	Oxathiapiprolin
FOCUS Scenario	Use pattern	PECsw	Toxic Unit	PECsw	Toxic Unit			
Step 1	Fruiting vegetables	111	0.755	1.08	0.003	0.758	99.6	0.4
Step 2 - NEU		17.8	0.121	0.153	0.0005	0.122	99.6	0.4
Step 2 - SEU		32.8	0.223	0.276	0.0008	0.224	99.6	0.4
Step 1	Leafy vegetables	111	0.755	1.08	0.003	0.758	99.6	0.4
Step 2 - NEU		40.3	0.274	0.338	0.001	0.275	99.6	0.4
Step 2 - SEU		32.8	0.223	0.276	0.0008	0.224	99.6	0.4
Step 1	Onion and bulb like crops	111	0.755	1.08	0.003	0.758	99.6	0.4
Step 2 - NEU		46.8	0.320	0.394	0.001	0.321	99.6	0.4
Step 2 - SEU		38	0.259	0.321	0.001	0.259	99.6	0.4
Step 1	Hops	139	0.946	2.40	0.007	0.953	99.3	0.7
Step 2 - NEU		42.5	0.289	0.813	0.002	0.292	99.2	0.8
Step 2 - SEU		40.3	0.274	0.813	0.002	0.277	99.1	0.9

TU: Toxic Units

^a If the TU value is > 90%, this indicates the respective component is driving the risk to the taxonomic group and is highlighted in bold.

Table 9.5-48: Aquatic organisms: Toxic Units (TU) approach to identify risk driver – invertebrate acute

Active substance		Azoxystrobin		Oxathiapiprolin		SUM Toxic Units	Toxic Unit as % of total	
Endpoint		<i>Americamysis bahia</i> <i>EC</i> ₅₀ = 55 µg/L		<i>Crassostrea virginica</i> <i>EC</i> ₅₀ > 330 µg/L			azoxystrobin	oxathiapiprolin
FOCUS Scenario	Use pattern	PEC _{sw}	Toxic Unit	PEC _{sw}	Toxic Unit			
Step 1	Fruiting vegetables	111	2.018	1.08	0.003	2.021	99.8	0.2
Step 2 - NEU		17.8	0.324	0.153	0.0005	0.324	99.9	0.1
Step 2 - SEU		32.8	0.596	0.276	0.0008	0.597	99.9	0.1
Step 1	Leafy vegetables	111	2.018	1.08	0.003	2.021	99.8	0.2
Step 2 - NEU		40.3	0.733	0.338	0.001	0.734	99.9	0.1
Step 2 - SEU		32.8	0.596	0.276	0.0008	0.597	99.9	0.1
Step 1	Onion and bulb like crops	111	2.018	1.08	0.003	2.021	99.8	0.2
Step 2 - NEU		46.8	0.851	0.394	0.001	0.856	99.9	0.1
Step 2 - SEU		38	0.691	0.321	0.001	0.692	99.9	0.1
Step 1	Hops	139	2.527	2.40	0.007	2.535	99.7	0.3
Step 2 - NEU		42.5	0.773	0.813	0.002	0.775	99.7	0.3
Step 2 - SEU		40.3	0.733	0.813	0.002	0.735	99.7	0.3

TU: Toxic Units

^a If the TU value is > 90%, this indicates the respective component is driving the risk to the taxonomic group and is highlighted in bold.

Table 9.5-49: Aquatic organisms: Toxic Units (TU) approach to identify risk driver – invertebrate prolonged

Active substance		azoxystrobin		oxathiapiprolin		SUM Toxic Units	Toxic Unit as % of total	
Endpoint		<i>Americamysis bahia</i> NOEC = 9.54 µg/L		<i>Americamysis bahia</i> NOEC = 58 µg/L			azoxystrobin	oxathiapiprolin
FOCUS Scenario	Use pattern	PEC _{sw}	Toxic Unit	PEC _{sw}	Toxic Unit			
Step 1	Fruiting vegetables	111	11.64	1.08	0.019	11.65	99.8	0.2
Step 2 - NEU		17.8	1.866	0.153	0.003	1.868	99.9	0.1
Step 2 - SEU		32.8	3.438	0.276	0.005	3.443	99.9	0.1
Step 1	Leafy vegetables	111	11.64	1.08	0.019	11.65	99.8	0.2
Step 2 - NEU		40.3	4.224	0.338	0.006	4.230	99.9	0.1
Step 2 - SEU		32.8	3.438	0.276	0.005	3.443	99.9	0.1
Step 1	Onion and bulb like crops	111	11.64	1.08	0.019	11.65	99.8	0.2
Step 2 - NEU		46.8	4.906	0.394	0.007	4.933	99.9	0.1
Step 2 - SEU		38	3.983	0.321	0.006	3.989	99.9	0.1
Step 1	Hops	139	14.57	2.40	0.041	14.61	99.7	0.3
Step 2 - NEU		42.5	4.455	0.813	0.014	4.469	99.7	0.3
Step 2 - SEU		40.3	4.224	0.813	0.014	4.238	99.7	0.3

TU: Toxic Units

^a If the TU value is > 90%, this indicates the respective component is driving the risk to the taxonomic group and is highlighted in bold.

As the endpoints used in the algal risk assessment for the individual active substances are a mixture of Tier 1 (oxathiapiprolin = E_rC₅₀) and Tier 2a (azoxystrobin = geomean E_bC₅₀) endpoints, the azoxystrobin lowest Tier 1 endpoint used to construct the geometric mean is considered for the toxic unit calculation. This results in comparison of growth rate and biomass endpoints, along with comparison of a diatom to green algae. Therefore, the various combinations of endpoints are considered below.

Table 9.5-50: Aquatic organisms: Toxic Units (TU) approach to identify risk driver – algae

Active substance		Azoxystrobin		Oxathiapiprolin		SUM Toxic Units	Toxic Unit as % of total	
Endpoint		<i>Navicula pelliculosa</i> <i>E_rC₅₀</i> = 146 µg/L		<i>Pseudokirchneriella subcapitata</i> <i>E_rC₅₀</i> >142 µg/L			azoxystrobin	oxathiapiprolin
FOCUS Scenario	Use pattern	PEC _{sw}	Toxic Unit	PEC _{sw}	Toxic Unit			
Step 1	Fruiting vegetables	111	0.760	1.08	0.008	0.768	99.0	1.0
Step 2 - NEU		17.8	0.122	0.153	0.001	0.123	99.2	0.8
Step 2 - SEU		32.8	0.225	0.276	0.002	0.227	99.1	0.9
Step 1	Leafy vegetables	111	0.760	1.08	0.008	0.768	99.0	1.0
Step 2 - NEU		40.3	0.276	0.338	0.002	0.278	99.3	0.7
Step 2 - SEU		32.8	0.225	0.276	0.002	0.227	99.1	0.9
Step 1	Onion and bulb like crops	111	0.760	1.08	0.008	0.768	99.0	1.0
Step 2 - NEU		46.8	0.321	0.394	0.003	0.325	99.1	0.9
Step 2 - SEU		38.0	0.260	0.321	0.002	0.263	99.1	0.9
Step 1	Hops	139	0.952	2.40	0.017	0.969	98.2	1.8
Step 2 - NEU		42.5	0.291	0.813	0.006	0.297	98.0	2.0
Step 2 - SEU		40.3	0.276	0.813	0.006	0.282	97.9	2.1

TU: Toxic Units

^a If the TU value is > 90%, this indicates the respective component is driving the risk to the taxonomic group and is highlighted in bold.

Table 9.5-51: Aquatic organisms: Toxic Units (TU) approach to identify risk driver – macrophytes

Active substance		Azoxystrobin		Oxathiapiprolin		SUM Toxic Units	Toxic Unit as % of total	
Endpoint		<i>Lemna gibba</i> <i>EC50</i> = 3200 µg/L		<i>Lemna gibba</i> <i>NOEC</i> = >790 µg/L			azoxystrobin	Oxathiapiprolin
FOCUS Scenario	Use pattern	PECsw	Toxic Unit	PECsw	Toxic Unit			
Step 1	Fruiting vegetables	111	0.035	1.08	0.0014	0.036	96.2	3.8
Step 2 - NEU		17.8	0.006	0.153	0.0002	0.006	96.6	3.4
Step 2 - SEU		32.8	0.010	0.276	0.0003	0.011	96.7	3.3
Step 1	Leafy vegetables	111	0.035	1.08	0.0014	0.036	96.2	3.8
Step 2 - NEU		40.3	0.013	0.338	0.0004	0.013	96.7	3.3
Step 2 - SEU		32.8	0.010	0.276	0.0003	0.011	96.7	3.3
Step 1	Onion and bulb like crops	111	0.035	1.08	0.0014	0.036	96.2	3.8
Step 2 - NEU		46.8	0.015	0.394	0.0005	0.015	96.7	3.3
Step 2 - SEU		38	0.012	0.321	0.0004	0.012	96.7	3.3
Step 1	Hops	139	0.043	2.40	0.0030	0.046	93.5	6.5
Step 2 - NEU		42.5	0.013	0.813	0.0010	0.014	92.8	7.2
Step 2 - SEU		40.3	0.013	0.813	0.0010	0.014	92.4	7.6

TU: Toxic Units

^a If the TU value is > 90%, this indicates the respective component is driving the risk to the taxonomic group and is highlighted in bold.

The analysis presented in the tables above indicates that azoxystrobin is clearly driving the toxicity and risk to all organism groups when considered alongside oxathiapiprolin. No further assessment of the combination risk of azoxystrobin and oxathiapiprolin is required.

9.5.3 Overall conclusions

The PEC/RAC ratios, using worst-case Step 1 PEC_{SW} values for oxathiapiprolin, are less than the trigger value of 1, for all aquatic organisms.

The PEC/RAC ratios, using worst-case PEC_{SW} values for A22773A, are less than the trigger value of 1, for all aquatic organisms, with the exception of fish (acute) and invertebrates (acute and chronic) when based on FOCUS Step 3 calculations and exposed to azoxystrobin. A refined risk assessment is conducted for aquatic invertebrates (acute and chronic) only, since the higher tier RAC for invertebrates is lower than that for fish, and thus covers the risk for all organisms taking into account appropriate mitigation measures.

The potential risk to aquatic invertebrates has been refined by using FOCUS Step 4 PEC_{SW} considering reduced exposure of surface water bodies after consideration of different non-sprayed buffer zones and vegetated filter strips (with regard to run-off mitigation). In addition, the PEC/RAC ratios were calculated based on FOCUS Step 4 PEC_{SW} with VFSmod for Poland only.

The PEC/RAC ratios are below the trigger value of 1 for azoxystrobin when based on FOCUS Step 4 PEC_{SW} considering reduced exposure of surface water bodies as an additional refinement option.

Considering a plant uptake factor of 0 (Tier 1), the PEC/RAC ratios are below the trigger value of 1 for A22773A when consideration is given to a 20 m vegetated filter strip following application of 2 x 1 L A22773A/ha in fruiting vegetables, leafy vegetables or to a 10 m vegetated filter strip following application of 2 x 1 L A22773A/ha in bulb and onion like crops. Following application of 2 x 1 L A22773A/ha to hops, PEC/RAC ratios are below the trigger value of 1 for A22773A considering a 10m no-spray buffer with 75% nozzle reduction or a 15 m no-spray buffer.

Considering a plant uptake factor of 0.5 (Tier 2), the PEC/RAC ratios are below the trigger value of 1 for A22773A when consideration is given to a 20 m vegetated filter strip following application of 2 x 1 L A22773A/ha in fruiting vegetables, leafy vegetables, and to a 20 m no spray buffer following application of 2 x 1 L A22773A/ha to hops or to a 10 m vegetated filter strip following application of 2 x 1 L A22773A/ha in bulb and onion like crops, respectively.

In case VFS_{mod} is taken into consideration, the PEC/RAC ratios are below the trigger value of 1 following use of 2 x 1 L of A22773A/ha on hops with no need of mitigations and when consideration is given to a 5 m vegetated filter strip on leafy, fruiting and bulb vegetables.

The toxic unit analysis indicates that azoxystrobin is driving the toxicity to all organism groups when considered alongside oxathiapiprolin.

There was acceptable risk to aquatic organisms following use of A22773A when the following mitigation steps are considered: 20 m vegetated filter strip following the application of 2 x 1 L A22773A/ha in fruiting vegetables, leafy vegetables and 20 m no spray buffer following application of 2 x 1 L A22773A/ha to hops and a 10 m vegetated filter strip following the application of 2 x 1 L A22773A/ha in bulb and onion like crops. Following application of 2 x 1 L A22773A/ha to hops and using Tier 1, there was acceptable risk to aquatic organisms when considering a 10m no spray buffer with 75% nozzle reduction or a 15 m no spray buffer, but a 20 m no spray buffer was required using Tier 2.

In case VFS_{mod} is taken into consideration, there was acceptable risk to aquatic organisms following use of 2 x 1 L of A22773A/ha on hops with no need of mitigations and when consideration is given to a 5 m vegetated filter strip on leafy fruiting and bulb vegetables.

Tier 1 (plant uptake factor = 0)

Fruiting vegetables (2 x 1 L A22773A/ha)

20m vegetated filter strip
or 5m vegetated filter strip with VFSmod

Leafy vegetables (2 x 1 L A22773A/ha)

20m vegetated filter strip
or 5m vegetated filter strip with VFSmod

Bulb and onion like crop (2 x 1 L A22773A/ha)

10m vegetated filter strip
or 5m vegetated filter strip with VFSmod

Hops (2 x 1 L A22773A/ha)

10m no-spray buffer with 75% nozzle reduction
or 15m no-spray buffer

Tier 2 (plant uptake factor = 0.5)

Fruiting vegetables (2 x 1 L A22773A/ha)

20m vegetated filter strip
or 5m vegetated filter strip with VFSmod

Leafy vegetables (2 x 1 L A22773A/ha)

20m vegetated filter strip
or 5m vegetated filter strip with VFSmod

Bulb and onion like crop (2 x 1 L A22773A/ha)

10m vegetated filter strip
or 5m vegetated filter strip with VFSmod

Hops (2 x 1 L A22773A/ha)

20m no-spray buffer

Review Comments:

The relevant predicted environmental concentrations in water (PEC_{sw}) for risk assessments covering the proposed use pattern are taken from Part B Section 8 (Environmental Fate). The initial risk assessment was based on the worst case PEC_{sw} values and the results of laboratory toxicity testing.

For active substances and relevant metabolites PEC_{sw} calculations were performed with FOCUS STEPS 1-2 (active substances and metabolites), FOCUS STEP 3-4 (azoxystrobin).

For azoxystrobin the mesocosms study was taken to consideration in the refined risk assessment.

The toxicity of the mixture is driven by azoxystrobin.

The calculated PEC/RAC ratios indicate an acceptable risk for all groups of aquatic organisms with appropriate mitigation measures.

For azoxystrobin the Step 4 calculations were carried out considering $PUF=0$ and the following mitigation methods:

- spray drift reduction by non-sprayed buffer strips of 10 m, 15 m and 20 m for hops only
- spray drift reduction by 50%, 75% and 90% drift reduction nozzles for hops only
- runoff reduction using vegetated buffer strips of 10 m and 20 m using runoff and erosion reduction values as given by the FOCUS Working Group on Landscape and Mitigation Factors (2007) of 60/85% for 10 m and 80/95% for 20 m, for vegetables (leafy, fruiting, bulb).
- Runoff reduction considering 5 m VFSmod for vegetables (leafy, fruiting, bulb).

Based on the risk assessment, it can be concluded that following mitigation measures are required:

1. Fruiting vegetables:

- R3 – 10 m vegetated filter strip
- R4 – 20 m vegetated filter strip
- R3 and R4 (VFSmod) – 5 m vegetated filter strip

2. Leafy vegetables:

- R1 - 10 m vegetated filter strip

- R3 - 10 m vegetated filter strip
- R4 – 20 m vegetated filter strip
- R1, R3 and R4 (VFSmod) – 5 m vegetated filter strip

3. Bulbs and onion like crops:

- R1 - 10 m vegetated filter strip
- R3 - 10 m vegetated filter strip
- R4 – 10 m vegetated filter strip
- R1, R3 and R4 (VFSmod) – 5 m vegetated filter strip

4. Hop:

- R1 - non-sprayed buffer strips of 15 m or 10 m with 75% nozzle reduction.

9.6 Effects on bees (KCP 10.3.1)

9.6.1 Toxicity data

Studies on the toxicity to bees have been carried out with azoxystrobin and oxathiapiprolin. Full details of these studies are provided in the respective EU DAR and related documents as well as in Appendix 2 of this document (for new studies).

Effects on bees of A22773A were not evaluated as part of the EU assessment of azoxystrobin and oxathiapiprolin. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

The selection of studies and endpoints for the acute oral and contact adult bee risk assessments is in line with the results of the EU review process, whilst new studies have been conducted to address the potential chronic exposure of adult honeybees and honeybee larvae.

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

Species	Substance	Exposure System	Results	Reference
<i>Apis mellifera</i>	Azoxystrobin	Acute oral	LD₅₀ > 25 µg/bee	EFSA, 2010, Gough <i>et al.</i> , 1993, ICI5504/0859
<i>Apis mellifera</i>	Azoxystrobin	Acute contact	LD₅₀ > 200 µg/bee	
<i>Apis mellifera</i>	A12705H ^b (250 SC)	Acute oral	LD ₅₀ > 200 µg a.s./bee	EFSA, 2010, Gough and Jackson, 1994, ICI5504/0861
<i>Apis mellifera</i>	A12705H ^b (250 SC)	Acute contact	LD ₅₀ > 200 µg a.s./bee	
<i>Apis mellifera</i>	Azoxystrobin (tested as A12705B) ^a	Chronic oral Adult, 10d	LC ₅₀ = 4.29 mg A12705B/kg diet LDD ₅₀ = 76.7 µg A12705B/bee/day (LDD₅₀ = 17.41 µg a.s./bee/day) NOEC = 2.35 µg A12705B/kg diet NOEDD = 44.1 µg A12705B/bee/day (NOEDD = 10.01 µg a.s./bee/day)	Tänzler, V., 2015, VV-414159 Study not evaluated by zRMS
<i>Apis mellifera</i>	Azoxystrobin (tested as A12705B) ^a	Larval development, repeated exposure, 8d	LD ₅₀ = 56.2 µg product/larva (LD ₅₀ = 12.76 µg a.s./larva) NOED = 39.2 µg product/larva (NOED = 8.90 µg a.s./larva)	Ehmke, A., 2015, VV-414544 Study not evaluated by zRMS
Higher-tier studies (tunnel test, field studies)				
Not performed				

Endpoints used for risk assessment are shown in **bold**.

EFSA Journal 2010; 8(4):1542.

^a Used for conversion of µg A12705B/larva or /bee to µg a.s./larva or /bee. Content of a.s. nominal: 250 g/L. Content of a.s. analysed: 22.7 % w/w corresponding to 248 g/L (density: 1.093 g/cm³)

^b A12705B and A12705H contain the same active ingredient content and a nearly identical co-formulant profile except a marginal decrease for one co-formulants and marginal increases in two further co-formulants within A12705B compared to A12705H.

Table 9.6-2: Endpoints and effect values relevant for the risk assessment for bees – Oxathiapiprolin

Species	Substance	Exposure System	Results	Reference
<i>Apis mellifera</i>	Oxathiapiprolin	Acute oral	LD₅₀ > 40.26 µg a.s./bee	EFSA, 2016, DuPont-32476
<i>Apis mellifera</i>	Oxathiapiprolin	Acute contact	LD₅₀ > 100.0 µg a.s./bee	
<i>Apis mellifera</i>	IN-E8S72	Acute oral	LD ₅₀ > 109.0 µg p.m./bee	EFSA, 2016, DuPont-37896
<i>Apis mellifera</i>	IN-E8S72	Acute contact	LD ₅₀ > 100.0 µg p.m./bee	
<i>Apis mellifera</i>	IN-WR791	Acute oral	LD ₅₀ > 56.2 µg p.m./bee	EFSA, 2016, DuPont-37897
<i>Apis mellifera</i>	IN-WR791	Acute contact	LD ₅₀ > 100.0 µg p.m./bee	
<i>Apis mellifera</i>	Oxathiapiprolin 100 g/L OD	Acute oral	LD ₅₀ > 137.4 µg a.s./bee	EFSA, 2016, DuPont-31006
<i>Apis mellifera</i>	Oxathiapiprolin 100 g/L OD	Acute contact	LD ₅₀ > 100 µg a.s./bee	
<i>Apis mellifera</i>	Oxathiapiprolin 100 g/L OD	Chronic oral Adult, 10d	LDD₅₀ = 34.7 µg a.s./bee/day NOEDD = 24.1 µg a.s./bee/day	Tänzler, V., 2015, DuPont-41989 Study not evaluated by zRMS
<i>Apis mellifera</i>	Oxathiapiprolin	Larval development, repeated exposure, 22d	NOED = 7.02 µg a.s./larva NOEC = 45.6 mg a.s./kg diet	Oberrauch S., 2017, DuPont-48606 Study not evaluated by zRMS
Higher-tier studies (tunnel test, field studies)				
<i>Apis mellifera</i>	Oxathiapiprolin 100 g/L OD	Bee brood, semi-field	NOEC = 180.0 g a.s./ha	EFSA, 2016, DuPont-34268

Endpoints used for risk assessment are shown in **bold**.
EFSA Journal 2016;14(7):4504.

Table 9.6-3: Endpoints and effect values relevant for the risk assessment for bees – A22773A

Species	Substance	Exposure system	Results	Reference
<i>Apis mellifera</i>	A22773A	Acute oral	LD ₅₀ > 1 000 µg prod./bee corresponding to LD₅₀ > 236 µg total a.s./bee¹	Franke, M., 2020, VV-883076
<i>Apis mellifera</i>	A22773A	Acute contact	LD ₅₀ > 1 000 µg prod./bee corresponding to LD₅₀ > 236 µg total a.s./bee¹	
<i>Apis mellifera</i>	A22773A	Chronic oral Adult, 10d	LDD ₅₀ = 378 µg prod./bee/day NOEDD = 112 µg prod./bee/day corresponding to LDD₅₀ = 89.05 µg total a.s./bee/day¹ NOEDD = 26.39 µg total a.s./bee/day ¹	Dreßler, K., 2020, VV-881467
<i>Apis mellifera</i>	A22773A	Larval development, repeated exposure, 22d	NOED = 105 µg product/larva NOEC = 661 mg product/kg diet corresponding to NOED = 24.74 µg total a.s./larva¹	Schmidt, K., 2021, VV-896655
<i>Bombus terrestris</i>	A22773A	Acute oral	LD ₅₀ > 983.9 µg product/bee Corresponding to LD₅₀ > 232 µg a.s./bee¹	Amsel, K., 2022, VV-936507
<i>Bombus terrestris</i>	A22773A	Acute contact	LD ₅₀ > 1000 µg product/bee Corresponding to LD₅₀ > 236 µg a.s./bee¹	

Endpoints used for risk assessment are shown in **bold**.

¹ Endpoint (µg total a.s.) calculated based on active substance content of 258.2 g a.s./L, and a product density of 1.096 g/cm³.

9.6.1.1 Justification for new endpoints

Since active substance approval, new studies with azoxystrobin, oxathiapiprolin and A22773A have been performed in accordance with Regulation (EC) No 1107/2009 to address the data requirements. New endpoints are used in the risk assessment. The results of the studies are summarised in Table 9.6-1 to 9.6-3. Study summaries are provided in Appendix 2.

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 Services¹² (hereafter referred to as SANCO/10329/2002 rev.2) referring to the Environmental risk assessment scheme for plant protection products, Chapter 10: Honeybees (PP 3/10(3), Bulletin OEPP/EPPO Bulletin 40: 323-331, 2010 (hereafter referred to as EPPO, 2010) as proposed in the list of Guidance Documents relevant to the implementation of Regulation (EC) No 1107/2009, published in the official EU Journal 2013/C 95/01 and 95/02.

The applicant considers that risk assessment to the EFSA guidance¹³ is not appropriate for regulatory decision making at EU level as the guidance is not agreed by all member states and as such has not been

¹² SANCO/10329/2002 rev.2: Anonymous, 2002. Guidance Document on terrestrial ecotoxicology under Council Directive 91/414/EEC. SANCO/10329/2002. 17 October 2002.

¹³ EFSA Bee Guidance Document, 2013: European Food Safety Authority, 2013. EFSA Guidance Document on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees). EFSA Journal 2013;11(7):3295, 268 pp., doi:10.2903/j.efsa.2013.3295 – updated 2014.

noted. However, given recent requests by EFSA and many Members States an assessment has been provided by the applicant below. Areas where a lot of uncertainty in approach still exist (e.g., water exposure, HPG assessment and bumble and solitary bee assessments) have not been addressed.

The EFSA risk assessment guidance¹³ is structured in a stepwise manner beginning with a Screening Step assessment, those scenarios which pass the Screening Step are considered to demonstrate acceptable risk and as such will not be considered at higher tiers of assessment.

All calculations are performed using the EFSA Bee calculator Tool (Bee-Tool v.3; Date accessed 14/09/2020)¹⁴. Where the Screening Step indicates a potential risk for acute or chronic exposure to bees and/or bee brood a Tier 1 risk assessment is performed.

The treated crop is considered by the applicant to represent the worst-case¹⁵ exposure as all other scenarios are considered to have lower exposure e.g. field margins, adjacent crop etc. Therefore, only contact and oral exposure in the treated crop is considered at Tier 1 and where this indicates ETR values below the triggers, acceptable risk to bees is demonstrated.

Table 9.6-4: Crop groupings and critical use patterns relevant to the use of A22773A

Test substance	GAP crop species	Application category	Critical use pattern		
			Appl. rate (g a.s./ha)	No. of appl.	Appl. interval (days)
Azoxystrobin	Fruiting vegetables BBCH 11 - 89	Downward spray	250	2	7
Azoxystrobin	Leafy vegetables, lettuce BBCH 11 – 49	Downward spray	250	2	7
Azoxystrobin	Bulb vegetables and other alliaceous crops, onions BBCH 11 – 49	Downward spray	250	2	12
Azoxystrobin	Hops BBCH 21 – 89	Sideward/upward spray	250	2	12
Oxathiapiprolin	Fruiting vegetables BBCH 11 - 89	Downward spray	12	2	7
Oxathiapiprolin	Leafy vegetables, lettuce BBCH 11 – 49	Downward spray	12	2	7
Oxathiapiprolin	Bulb vegetables and other alliaceous crops, onions BBCH 11 – 49	Downward spray	12	2	12
Oxathiapiprolin	Hops BBCH 21 – 89	Sideward/upward spray	12	2	12
A22773A	Fruiting vegetables BBCH 11 - 89	Downward spray	262 ^a	2	7
A22773A	Leafy vegetables, lettuce BBCH 11 – 49	Downward spray	262 ^a	2	7
A22773A	Bulb vegetables and other alliaceous crops, onions BBCH 11 – 49	Downward spray	262 ^a	2	12

¹⁴ EFSA Bee calculator Tool (Bee-Tool v.3) available at <http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2013.3295/full>

¹⁵ Exposure to treated weeds is not considered a relevant exposure scenario according to the guidance as evidence is available to demonstrate that in arable crops flowering attractive weeds are not present at >10% of the area of use: Last *et al.*, 2019; Regulatory report on the occurrence of flowering weeds in agricultural fields. ERM, Harrogate, North Yorkshire, UK. The full regulatory report is available through ECPA on request.

Test substance	GAP crop species	Application category	Critical use pattern		
			Appl. rate (g a.s./ha)	No. of appl.	Appl. interval (days)
A22773A	Hops BBCH 21 – 89	Sideward/upward spray	262 ^a	2	12

^a Based upon an application rate of 1.0 L/ha.

9.6.2.1 Hazard quotients for bees

Risk assessment according to SANCO/10329/2002 rev.2

Acute honeybee studies have been conducted with the active substances azoxystrobin and oxathiapiprolin and the formulated product A22773A according to the data requirements under 1107/2009. The potential risk to honeybees from use of A22773A is assessed using the maximum single application rate and the LD₅₀ values to calculate hazard quotients in accordance with SANCO/10329/2002 rev.2.

$$\text{Hazard Quotient} = \frac{\text{Maximum application rate (g a.s./ha)}}{\text{Acute LD}_{50} (\mu\text{g/bee})}$$

Compounds with hazard quotients below 50 are considered low risk to bees.

Table 9.6-5: First-tier assessment of the risk for honeybees due to the use of formulation A22773A at maximum single use rate – Azoxystrobin

Active substance	Azoxystrobin		
Application rate (g a.s./ha)	250		
Test design	LD ₅₀ (lab.) (μg a.s./bee)	Single application rate (g a.s./ha)	Q _{HO} , Q _{HC} criterion: Q _H ≤ 50
Oral toxicity	> 25	250	< 10
Contact toxicity	> 200	250	< 1.3

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

Table 9.6-6: First-tier assessment of the risk for bees due to the use of A22773A at maximum single use rate – Oxathiapiprolin

Active substance	Oxathiapiprolin		
Application rate (g a.s./ha)	12		
Test design	LD ₅₀ (lab.) (μg a.s./bee)	Single application rate (g a.s./ha)	Q _{HO} , Q _{HC} criterion: Q _H ≤ 50
Oral toxicity	> 40.26	12	< 0.30
Contact toxicity	> 100	12	< 0.12

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

Table 9.6-7: First-tier assessment of the risk for bees due to the use of A22773A at maximum single use rate – A22773A

Product	A22773A		
Application rate (g total a.s./ha)^a	262		
Test design	LD₅₀ (lab.) (µg total a.s./bee)	Single application rate (g total a.s./ha)	Q_{HO}, Q_{HC} criterion: Q_H ≤ 50
Oral toxicity	> 236	262 ^a	< 1.1
Contact toxicity	> 236	262 ^a	< 1.1

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

^a Based upon an application rate of 1.0 L product/ha.

Overall, the acute oral and contact hazard quotients are less than the relevant trigger of 50, indicating that the risk to honeybees is acceptable following use of A22773A according to the proposed use pattern.

Risk assessment according to EFSA Bee Guidance Document (2013)

Screening step - Acute and Chronic Risk Assessment

Acute, chronic adult and larval honeybee studies have been conducted with azoxystrobin and oxathiapiprolin (a.s. or formulated as 100 g/L OD) according to the data requirements under 1107/2009. The endpoints from these studies have been assessed by using EFSA Bee Guidance Document (2013)¹² and EFSA Bee calculator Tool.

Table 9.6-8: Screening Step assessment of the risk for honeybees due to the use of formulation A22773A on fruiting, leafy and bulb vegetables – Azoxystrobin

Intended use	Downward Spray				
Active substance	Azoxystrobin				
Application rate (g a.s./ha)	2 × 250				
Test design	Endpoint (lab.)	Single application rate	Calculation factor (Ef x SV)	HQ / ETR	Trigger
Acute contact toxicity LD ₅₀	> 200 µg a.s./bee	250 g a.s./ha	1	< 1.3	≤42
Acute oral toxicity LD ₅₀	> 25 µg a.s./bee	0.250 kg a.s./ha	7.6	< 0.08	≤0.2
Chronic adult oral toxicity LDD ₅₀	17.41 µg a.s./bee/day	0.250 kg a.s./ha	7.6	0.109	≤0.03
Larval development NOED	8.90 µg a.s./larva	0.250 kg a.s./ha	4.4	0.12	≤0.2

HQ (hazard quotients) and ETR (exposure toxicity ratio) for oral and contact exposure. HQ/ETR values shown in **bold** breach the relevant trigger.

The HQ / ETR values for azoxystrobin are less than the Screening Step trigger values for downward spray indicating that the acute oral and contact risk to adult honeybees and chronic risk to honeybee larvae is acceptable following use of A22773A according to the proposed use pattern. However, the Screening Step risk assessment has indicated a potential chronic risk for adult honeybees following oral exposure and therefore a Tier 1 assessment for the treated crop is provided.

Table 9.6-9: Screening Step assessment of the risk for honeybees due to the use of formulation A22773A on hops – Azoxystrobin

Intended use	Sideward/upward spray				
Active substance	Azoxystrobin				
Application rate (g a.s./ha)	2 × 250				
Test design	Endpoint (lab.)	Single application rate	Calculation factor (Ef x SV)	HQ / ETR	Trigger
Acute contact toxicity LD ₅₀	> 200 µg a.s./bee	250 g a.s./ha	1	< 1.3	≤85
Acute oral toxicity LD ₅₀	> 25 µg a.s./bee	0.250 kg a.s./ha	10.6	< 0.11	≤0.2
Chronic adult oral toxicity LDD ₅₀	17.41 µg a.s./bee/day	0.250 kg a.s./ha	10.6	0.152	≤0.03
Larval development NOED	8.90 µg a.s./larva	0.250 kg a.s./ha	6.1	0.17	≤0.2

HQ (hazard quotients) and ETR (exposure toxicity ratio) for oral and contact exposure. HQ/ETR values shown in **bold** breach the relevant trigger.

The HQ / ETR values for azoxystrobin are less than the Screening Step trigger values for sideward and upward spray indicating that the acute oral and contact risk to adult honeybees and chronic risk to honeybee larvae is acceptable following use of A22773A in hops according to the proposed use pattern. However, the Screening Step risk assessment has indicated a potential chronic risk to adult honeybees following oral exposure and therefore a Tier 1 assessment for the treated crop is provided.

Table 9.6-10: Screening Step assessment of the risk for honeybees due to the use of formulation A22773A on fruiting, leafy and bulb vegetables – Oxathiapiprolin

Intended use	Downward Spray				
Active substance	Oxathiapiprolin				
Application rate (g a.s./ha)	2 × 12				
Test design	Endpoint (lab.)	Single application rate	Calculation factor (Ef x SV)	HQ / ETR	Trigger
Acute contact toxicity LD ₅₀	> 100 µg a.s./bee	12 g a.s./ha	1	< 0.1	≤42
Acute oral toxicity LD ₅₀	> 40.26 µg a.s./bee	0.012 kg a.s./ha	7.6	< 0.01	≤0.2
Chronic adult oral toxicity LDD ₅₀	34.7 µg a.s./bee/day	0.012 kg a.s./ha	7.6	0.003	≤0.03
Larval development NOED	7.02 µg a.s./larva	0.012 kg a.s./ha	4.4	0.01	≤0.2

HQ (hazard quotients) and ETR (exposure toxicity ratio) for oral and contact exposure. HQ/ETR values shown in **bold** breach the relevant trigger.

The HQ / ETR values for oxathiapiprolin are less than the Screening Step trigger values for downward spray indicating that the acute, chronic, and larval risk to honeybees is acceptable following use of A22773A in fruiting, leafy and bulb vegetables according to the proposed use pattern.

Table 9.6-11: Screening Step assessment of the risk for honeybees due to the use of formulation A22773A on hops – Oxathiapiprolin

Intended use	Sideward/upward spray				
Active substance	Oxathiapiprolin				
Application rate (g a.s./ha)	2 × 12				
Test design	Endpoint (lab.)	Single application rate	Calculation factor (Ef x SV)	HQ / ETR	Trigger
Acute contact toxicity LD ₅₀	> 100 µg a.s./bee	12 g a.s./ha	1	< 0.1	≤85
Acute oral toxicity LD ₅₀	> 40.26 µg a.s./bee	0.012 kg a.s./ha	10.6	< 0.01	≤0.2
Chronic adult oral toxicity LDD ₅₀	34.7 µg a.s./bee/day	0.012 kg a.s./ha	10.6	0.004	≤0.03
Larval development NOED	7.02 µg a.s./larva	0.012 kg a.s./ha	6.1	0.01	≤0.2

HQ (hazard quotients) and ETR (exposure toxicity ratio) for oral and contact exposure. HQ/ETR values shown in **bold** breach the relevant trigger.

The HQ / ETR values for oxathiapiprolin are less than the Screening Step trigger values for sideward and upward spray indicating that the acute, chronic and larval risk to honeybees is acceptable following use of A22773A in hops according to the proposed use pattern.

Combination mixture assessment

Acute and chronic mixture toxicity

According to the EFSA Bee Guidance Document, 2013¹², combined action of mixture toxicity and toxicity of formulated products with two or more active substances should be considered in the risk assessment when it is obvious that such exposure situations will occur for bees.

For the assessment of acute and chronic effects, surrogate endpoints (LD₅₀, LDD₅₀, NOED) can be calculated. The EFSA Bee Guidance Document, 2013¹², indicates that the following equation should be used for deriving a surrogate endpoint (LD₅₀, LDD₅₀, NOED) for a mixture of active substances with known toxicity assuming dose additivity:

$$1/EC_x \text{ (mix) or } 1/NOEC \text{ (mix)} = \left(\sum_i \frac{X(a.s._i)}{EC_x \text{ or } NOEC(a.s._i)} \right)$$

where:

$X(a.s._i)$ = fraction of active substance [i] in the mixture (please note that the sum $\sum X(a.s._i)$ must be 1)

$EC_x \text{ or } NOEC(a.s._i)$ = toxicity value for active substance [i] (for the same endpoint)

Where the toxicity value of a formulated product with more than one active substance is available (acute and chronic studies), this value should be compared with the predicted mixture toxicity assuming dose additivity. A different form of the equation is used.

$$\sum_i \frac{X(a.s._i)}{EC_x \text{ or } NOEC(a.s._i)} = \frac{1}{EC_x \text{ or } NOEC(mix)}$$

$X(a.s._i)$ = fraction of active substance [i] in the mixture

ECx or NOEC(a.s.i) = acute toxicity value for active substance [i]
ECx or NOEC(mix) = measured acute toxicity value for the mixture.

A greater value on the right side of the equation indicates that the formulation is more toxic than predicted from the toxicity of the individual components (active substances and co-formulants of known toxicity). If, in contrast, the measured toxicity of a formulation is lower than predicted, the predicted mixture toxicity should be used in the first-tier risk assessment.

The calculated mixture endpoints are summarised in the following table.

Table 9.6-12: Calculated mixture toxicity endpoints for A22773A and comparison to measured endpoints

Exposure system (endpoint)	Test substance	Concentration of active substance in formulation A22773A (g/L)	Fraction of active substance in the formulation mixture ^a	Toxicity endpoint ^b	Predicted endpoint for mixture (µg total a.s.) ^c	Measured endpoint for mixture (based on total a.s.)	Endpoint used for risk assessment based on comparison
Acute oral	Azoxystrobin	250	0.954	> 25	> 25.4	> 236	use predicted endpoint
	Oxathiapiprolin	12	0.046	> 40.26			
	Total	262	1.000	-			
Acute contact	Azoxystrobin	250	0.954	> 200	> 191.2	> 236	use predicted endpoint
	Oxathiapiprolin	12	0.046	> 100			
	Total	262	1.000	-			
Chronic oral, adult	Azoxystrobin	250	0.954	17.41	17.8	89.05	use predicted endpoint
	Oxathiapiprolin	12	0.046	34.7			
	Total	262	1.000	-			
Larval development	Azoxystrobin	250	0.954	8.90	8.8	24.74	use predicted endpoint
	Oxathiapiprolin	12	0.046	7.02			
	Total	262	1.000	-			

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

^b LD₅₀ in µg a.s./bee, LDD₅₀ in µg a.s./bee/day for adult honeybees, NOED for bee larvae in µg a.s./larva/development period

^c Used for comparison with measured acute toxicity of product. Predicted endpoint for the mixture (µg total a.s.) is calculated based on total nominal content of the active substances (262 g a.s./L), and nominal product density of 1.097 g/cm³.

In accordance with the above presented calculation, toxicity endpoints for azoxystrobin and oxathiapiprolin and the formulation endpoint are used in the acute risk assessment according to SANCO/10329/2002 rev.2. Predicted mixture endpoints are used in the acute and chronic adult and larval risk assessment in accordance with the EFSA Bee Guidance Document, 2013¹².

Acute and Chronic Mixture Assessment – Screening Step

Lowest endpoints of azoxystrobin, oxathiapiprolin and formulation A22773A (based on total a.s.) were used to calculate the mixture toxicity endpoints (Table 9.6-12). A comparison of the available formulation data and the calculated endpoints based on toxicity of the active substances indicated that the measured toxicity is lower than predicted. Therefore, predicted endpoints for the formulation are used as a worst-case in the following risk assessment.

Table 9.6-13: Screening Step assessment of the risk for honeybees due to the use of formulation A22773A on fruiting, leafy and bulb vegetables – Azoxystrobin / oxathiapiprolin mixture

Intended use	Downward spray				
Product	A22773A				
Application rate (g total a.s./ha)	2 x 262 ^a				
Test design	Endpoint (predicted)	Single application rate	Calculation factor (Ef x SV)	HQ / ETR	Trigger
Acute contact toxicity LD ₅₀	> 191.2 µg a.s./bee	262 g a.s./ha ^a	1	< 1.4	≤42
Acute oral toxicity LD ₅₀	> 25.4 µg a.s./bee	0.262 kg a.s./ha ^a	7.6	< 0.08	≤0.2
Chronic adult oral toxicity LDD ₅₀	17.8 µg a.s./bee/day	0.262 kg a.s./ha ^a	7.6	0.112	≤0.03
Larval development NOED	8.8 µg a.s./larva	0.262 kg a.s./ha ^a	4.4	0.13	≤0.2

HQ (hazard quotients) and ETR (exposure toxicity ratio) for oral and contact exposure. HQ/ETR values shown in **bold** breach the relevant trigger.

^a Azoxystrobin 250 g/ha + oxathiapiprolin 12 g/ha

The HQ / ETR values for the azoxystrobin/oxathiapiprolin mixture are less than the Screening Step trigger values for downward spray indicating that the acute oral and contact risk to adult bees and chronic risk to honeybee larvae is acceptable following use of A22773A in fruiting, leafy and bulb vegetables according to the proposed use pattern. However, the Screening Step risk assessment has indicated a potential chronic risk for adult honeybees following chronic oral exposure and therefore a Tier 1 assessment for the treated crop is provided.

Table 9.6-14: Screening Step assessment of the risk for honeybees due to the use of formulation A22773A on hops – Azoxystrobin / oxathiapiprolin mixture

Intended use	Sideward/upward spray				
Product	A22773A				
Application rate (g total a.s./ha)	2 x 262 ^a				
Test design	Endpoint (predicted)	Single application rate	Calculation factor (Ef x SV)	HQ / ETR	Trigger
Acute contact toxicity LD ₅₀	> 191.2 µg a.s./bee	262 g a.s./ha ^a	1	< 1.4	≤42
Acute oral toxicity LD ₅₀	> 25.4 µg a.s./bee	0.262 kg a.s./ha ^a	10.6	< 0.11	≤0.2
Chronic adult oral toxicity LDD ₅₀	17.8 µg a.s./bee/day	0.262 kg a.s./ha ^a	10.6	0.156	≤0.03
Larval development NOED	8.8 µg a.s./larva	0.262 kg a.s./ha ^a	6.1	0.18	≤0.2

HQ (hazard quotients) and ETR (exposure toxicity ratio) for oral and contact exposure. HQ/ETR values shown in bold breach the relevant trigger.

^a Azoxystrobin 250 g/ha + oxathiapiprolin 12 g/ha

The HQ / ETR values for the azoxystrobin/oxathiapiprolin mixture are less than the Screening Step trigger values for sideward and upward spray indicating that the acute oral and contact risk and risk to honeybee larvae is acceptable following use of A22773A in hops according to the proposed use pattern. However, the Screening Step risk assessment has indicated a potential chronic risk for adult honeybees following oral exposure and therefore a Tier 1 assessment is provided.

Tier 1 - Chronic Risk Assessment

The Screening Step risk assessment has indicated a potential chronic risk for adult honeybees based on the use of A22773A in fruiting, leafy and bulb vegetables and hops. Therefore, a Tier 1 assessment is provided here.

Methodology to establish whether a single substance is driving the toxicity of a mixture

Table 9.6-15: Toxicity of azoxystrobin and oxathiapiprolin in the mixture

Exposure system (endpoint)	Test substance	Concentration of active substance in formulation A22773A (g a.s./L)	Fraction of active substance in the formulation on mixture ^a	Toxicity endpoint ^b	Fraction of active substance/LD D ₅₀ for the active substance	Toxicity per fraction µg a.s./bee/day	Toxicity per fraction quotient (%)	Single driver of toxicity
Chronic oral, adult	Azoxystrobin	250	0.954	17.41	0.054796	18.25	97.64	yes
	Oxathiapiprolin	12	0.046	34.7	0.001326	754	2.36	no
	Total	262	1	-	-		100	-

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

^b LDD₅₀ in µg a.s./bee/day for adult honeybees

Note: Calculations undertaken using unrounded values consequently they may not be reproducible when using the figures given in the table.

Due to its higher fraction in the formulation azoxystrobin was shown to be 41 times more toxic compared to oxathiapiprolin and is clearly driving the toxicity in the mixture. Therefore, calculations for the chronic Tier 1 were conducted with the azoxystrobin LDD₅₀ endpoint only.

Table 9.6-16: First-tier assessment of the chronic adult risk for honeybees due to the use of formulation A22773A in fruiting vegetables for the treated crop – Azoxystrobin

Intended use		Downward spray (fruiting vegetables, BBCH 11 - 89)						
Active substance		Azoxystrobin						
Application rate (g a.s./ha)		2 × 250						
Test design	Endpoint (lab.)	Single application rate	BBCH	SV (downward spray) ^a	TWA	Ef	ETR	Trigger
Chronic adult oral toxicity LDD ₅₀	17.41 µg a.s./bee/day	0.250 kg a.s./ha	10 - 49	5.8	0.72	1	0.060	≤0.03
			50 - 69	5.8	0.72	1	0.060	
			≥70	0	0.72	1	0	

SV: Shortcut value; TWA: Time-weighted average factor; Ef: Exposure factor; ETR (exposure toxicity ratio) for oral exposure; ETR values shown in **bold** breach the relevant trigger.

^a SV for crop attractive to honey bees for pollen and nectar used as worst-case for all fruiting vegetables including crops potentially visited by honeybees for pollen only (tomato and eggplant).

The Tier 1 ETR values for chronic risk for azoxystrobin are higher than the trigger value for downward spray, indicating that the chronic oral risk to honeybees is not acceptable following use of A22773A in

fruiting vegetables according to the proposed use pattern.

Table 9.6-17: First-tier assessment of the chronic adult risk for bees due to the use of formulation A22773A in leafy vegetables and lettuce for the treated crop – Azoxystrobin

Intended use		Downward spray (leafy vegetables ^a , BBCH 11 - 49)						
Active substance		Azoxystrobin						
Application rate (g a.s./ha)		2 × 250						
Test design	Endpoint (lab.)	Single application rate	BBCH	SV (downward spray)	TWA	Ef	ETR	Trigger
Chronic adult oral toxicity LDD ₅₀	17.41 µg a.s./bee/day	0.250 kg a.s./ha	10 - 49	5.8	0.72	1	0.060	≤0.03

SV: Shortcut value; TWA: Time-weighted average factor; Ef: Exposure factor; ETR (exposure toxicity ratio) for oral exposure; ETR values shown in **bold** breach the relevant trigger.

^a Crop category leafy vegetables used as worst-case covering lettuce

The Tier 1 ETR values for chronic risk for azoxystrobin are above the trigger value for downward spray, indicating potential chronic oral risk to honeybees in the treated crop following use of A22773A in leafy vegetables according to the proposed use pattern.

Table 9.6-18: First-tier assessment of the chronic adult risk for bees due to the use of formulation A22773A in bulb and other alliaceous crops, onions for the treated crop – Azoxystrobin

Intended use		Downward spray (bulb and other alliaceous crops, onions, BBCH 11 - 49)						
Active substance		Azoxystrobin						
Application rate (g a.s./ha)		2 × 250						
Test design	Endpoint (lab.)	Single application rate	BBCH	SV (downward spray)	TWA	Ef	ETR	Trigger
Chronic adult oral toxicity LDD ₅₀	17.41 µg a.s./bee/day	0.250 kg a.s./ha	10 - 39	5.8	0.72	1	0.060	≤0.03
			40 - 69	5.8	0.72	1	0.060	

SV: Shortcut value; TWA: Time-weighted average factor; Ef: Exposure factor; ETR (exposure toxicity ratio) for oral exposure; ETR values shown in **bold** breach the relevant trigger.

The Tier 1 ETR values for chronic risk for azoxystrobin are above the trigger value for downward spray, indicating potential chronic oral risk to honeybees in the treated crop following use of A22773A in bulb vegetables according to the proposed use pattern.

Table 9.6-19: First-tier assessment of the chronic adult risk for bees due to the use of formulation A22773A in hops for the treated crop – Azoxystrobin

Intended use		Sideward/upward spray (hops, BBCH 21 - 89)						
Active substance		Azoxystrobin						
Application rate (g a.s./ha)		2 × 250						
Test design	Endpoint (lab.)	Single application rate	BBCH	SV (downward spray)	TWA	Ef	ETR	Trigger
Chronic adult oral toxicity LDD ₅₀	17.41 µg a.s./bee/day	0.250 kg a.s./ha	20 - 39	0.06	0.72	1	0.001	≤0.03
			40 - 69	0.06	0.72	1	0.001	
			≥70	0	0.72	1	0	

SV: Shortcut value; TWA: Time-weighted average factor; Ef: Exposure factor; ETR (exposure toxicity ratio) for oral exposure; ETR values shown in **bold** breach the relevant trigger.

The Tier 1 ETR values for chronic risk for azoxystrobin are less than the trigger value for sideward and upward spray, indicating that the chronic oral risk to honeybees is acceptable following use of A22773A in hops according to the proposed use pattern.

For uses where the SV is 0 and therefore the ETR is 0 the EFSA risk assessment indicates an acceptable chronic oral risk in-field due to the application timing and/or unattractive crop, therefore for the risk assessment the treated field scenario can no longer be considered worst-case. Therefore, an off-field risk assessment is considered worst-case¹⁶ and is detailed below.

A Tier 1 assessment for chronic oral off-field exposure to azoxystrobin following use of A22773A according to the proposed uses is given in the tables below.

Table 9.6-20: Tier 1 assessment of the chronic oral risk for adult bees due to the use of formulation A22773A on various crops for the field margin – Azoxystrobin

Intended use	Downward Spray (fruiting vegetables) / Sideward/upward spray (hops)						
Active substance	Azoxystrobin						
Application rate (g a.s./ha)	2 x 250						
Crop scenario	LDD₅₀ (µg a.s./bee/day)	Single application rate (g/ha)	Shortcut Value (downward spray)	TWA	fDep/ Ef	ETR	Trigger
Adult chronic oral toxicity							
Fruiting vegetables (BBCH > 70)	17.41	250	2.9	0.72	0.0092	0.00028	≤0.03
Hops (BBCH > 70)	17.41	250	2.9	0.72	0.064	0.0020	

The Tier 1 ETR values for azoxystrobin for the field margin are less than the trigger for downward sprays, according to EFSA 2013, indicating that the acute risk to honeybees is acceptable following use of A22773A according to the proposed use pattern.

¹⁶ Exposure to treated weeds is not considered a relevant exposure scenario according to the guidance as evidence is available to demonstrate that in arable crops flowering attractive weeds are not present at > 10% of the area of use: Last et al., 2019; Regulatory report on the occurrence of flowering weeds in agricultural fields. ERM, Harrogate, North Yorkshire, UK. The full regulatory report is available through ECPA on request.

Table 9.6-21: Tier 1 assessment of the chronic oral risk for adult bees due to the use of formulation A22773A on various crops for the adjacent crop – Azoxystrobin

Intended use	Downward Spray (fruiting vegetables) / Sideward/upward spray (hops)						
Active substance	Azoxystrobin						
Application rate (g a.s./ha)	2 x 250						
Crop scenario	LDD₅₀ (µg a.s./bee/day)	Single application rate (g/ha)	Shortcut Value (downward spray)	TWA	fDep/ Ef	ETR	Trigger
Adult chronic oral toxicity							
Fruiting vegetables (BBCH > 70)	17.41	250	5.8	0.72	0.0033	0.00020	≤0.03
Hops (BBCH > 70)	17.41	250	5.8	0.72	0.041	0.0020	

The Tier 1 ETR values for azoxystrobin are less than the trigger for downward sprays, according to EFSA 2013, indicating that the risk to bees is acceptable for azoxystrobin following use of A22773A according to the proposed use pattern.

Tier 1 Refinement - Chronic Risk Assessment

Adult oral

The EFSA Bee Guidance Document¹² (p. 168) identifies the approach taken for setting the ETR trigger for chronic risk assessment using the honeybee chronic (10 day) LDD₅₀ (referred to as the LC₅₀ in the Guidance Document p. 168).

This assumes:

1. That the maximum increment above the lowest observed background mortality (5.3%) which yields a 7% reduction in colony size derived from the Khoury model is 1.43
2. An assumption that the dose response relationship is linear from the origin to the LC₅₀ (mortality = exposure*50/LC₅₀) and thus the slope varies widely depending on the LC₅₀.
3. The protection goal is met when the exposure = the maximum mortality permitted based on the Khoury model (1.43%) over the 10 days of the study

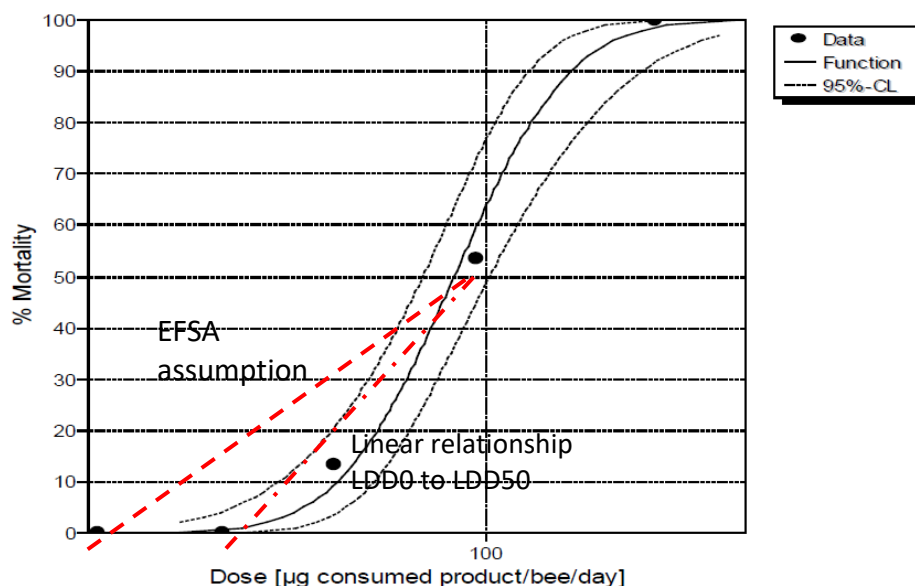
Based on the above, EFSA derived the trigger for the ETR as $1/(LC_{50}/(1.43*(LC_{50}/50)) = 1.43/50 = 0.03$

However, the EFSA assumption of linearity of the dose response through the origin does not take into account either the true shape of the dose-response curve or that for many pesticides the NOEC/NOEDD as well as the LC₅₀/LDD₅₀ is derived and the LDD₀ (0% mortality above control) can usually be directly identified from the data.

Alternative approaches that take into account the data generated in the chronic adult honeybee laboratory studies have been considered and proposed by ECPA¹⁷. These are:

1. To compare predicted daily dose directly with the daily dose resulting in 0% mortality above control (LDD₀) in the 10 day chronic adult laboratory study.
2. To use the true slope between the daily doses resulting in 0% mortality (compared with control) and 50% mortality in the 10 day chronic adult laboratory study.
3. To use the dose-response curve from the 10 day chronic adult laboratory study to identify the dose resulting in 1.43% mortality above control.

Dose-effect curve showing the influence of the test item on mortality of the introduced test organism as observed after 10 d.



Each approach is presented in the following:

Approach 1. Comparison of predicted daily dose directly with the daily dose resulting in 0% mortality (LDD₀) above control in the 10 day chronic adult laboratory study

The first step is to define whether the predicted exposure (daily dose = application rate* shortcut value) is greater than the LDD₀ – if not then clearly the exposure level is below that at which any individual mortality is expected. This is clearly in accordance with the stated EFSA protection goal as it is less than the maximum mortality permitted based on the Khoury model (1.43%) over the 10 days of the study.

From the EFSA approach the exposure of adult honeybees (µg /bee/day) is calculated from application rate (AR) (kg a.s./ha)* short-cut value (SV) * Exposure factor (Ef) * time-weighted average (twa).

For azoxystrobin the LDD₀ (0% mortality above control) was directly recorded in the 10-day chronic bee laboratory study with azoxystrobin, tested as A12705B (Tänzler, 2015 - A12705B_13707) as 6.0 µg a.s./bee/day. In this case the use of the LDD₀ ensures conservatism as no mortality, when compared to the control, was observed at this dose under continuous exposure conditions.

¹⁷ Proposal for a protective and workable regulatory European bee risk assessment scheme based on the EFSA bee guidance and other new data and available approaches. POS/17/LO/28028 (June 2017)
http://www.ecpa.eu/sites/default/files/document_policy/28028_ECPA%20Proposal%20for%20a%20protective%20and%20workable%20EU%20Bee%20Risk%20Assessment%20-%20Version%2009%20June%202017.pdf

Table 9.6-22: Approach 1: Refined Tier 1 assessment of the chronic oral risk for adult bees due to the use of formulation A22773A for the treated crop (worst case) – downward spray

Intended use		Downward spray, treated crop						
Active substance		Azoxystrobin						
Application rate (g a.s./ha)		1 x 250						
Crop scenario	Single application rate (kg/ha)	Shortcut Value (downward spray)^a	Ef	twa	Predicted exposure (µg/bee/day)	LDD₀ (µg/bee/day)	Margin of safety	Trigger
Adult chronic oral toxicity								
Fruiting vegetables (BBCH 10-69)	0.25	5.8	1	0.72	1.044	6	5.7	1
Leafy vegetables, lettuce (BBCH 10-49)	0.25	5.8	1	0.72	1.044	6	5.7	1
Bulb and other alliacious crops, onions (BBCH 10-69)	0.25	5.8	1	0.72	1.044	6	5.7	1

^a SV for crop attractive to honey bees for pollen and nectar used as worst-case for all fruiting vegetables including crops potentially visited by honeybees for pollen only (tomato and eggplant).

The LDD₀, the dose resulting in 0% mortality under continuous feeding conditions in the laboratory, is above the predicted worst-case exposure in-field of adult bees indicating an acceptable chronic oral risk to adult honeybees for azoxystrobin follow the use of A22773A according to the proposed uses. However, for completeness the chronic risk to adult bees will also be assessed using Approach 2 below.

Approach 2. Use of the true slope between the daily doses resulting in 0% mortality (compared with control) and 50% mortality in the 10 day chronic adult laboratory study

The EFSA Bee Guidance document¹² assumes that zero mortality can only occur at a dose of 0 µg/bee/day which is clearly over-conservative. Where the data from the chronic dosing study can be used to generate both a reliable LDD₅₀ and an LDD₀, the existing proposed EFSA chronic trigger of 0.03 (based on the LDD₅₀) can be achieved directly by using LDD₀.

Thus, the LDD₀ from the 10 day dosing study is subtracted from both the predicted daily dose and the LDD₅₀ in the EFSA chronic ETR trigger equation. i.e.

ETR = (predicted daily dose – LDD₀)/(LDD₅₀ – LDD₀) and compared with the EFSA trigger of 0.03 as shown below.

Table 9.6-23: Approach 2: Refined Tier 1 assessment of the chronic oral risk for adult bees due to the use of formulation A22773A for the treated crop (worst case) – downward spray

Intended use		Downward Spray, treated crop							
Active substance		Azoxystrobin							
Application rate (g a.s./ha)		1 x 250							
Crop scenario	Single application rate (kg/ha)	Shortcut Value (downward spray)^a	Ef	twa	Predicted exposure (µg/bee/day)	LDD₅₀ oral (µg a.s./bee/day)	LDD₀ (µg/bee/day)	ETR	Trigger
Adult chronic oral toxicity									
Fruiting vegetables (BBCH 10-69)	0.25	5.8	1	0.72	1.044	17.41	6	-0.43	0.03
Leafy vegetables, lettuce (BBCH 10-49)	0.25	5.8	1	0.72	1.044	17.41	6	-0.43	0.03
Bulb and other alliacious crops, onions (BBCH 10-69)	0.25	5.8	1	0.72	1.044	17.41	6	-0.43	0.03

^a SV for crop attractive to honey bees for pollen and nectar used as worst-case for all fruiting vegetables including crops potentially visited by honeybees for pollen only (tomato and eggplant).

The in-field ETR values for azoxystrobin are below the EFSA trigger of 0.03 indicating an acceptable chronic oral risk to adult honeybees following use of A22773A according to the proposed uses. Therefore, no higher-tier refinement is considered necessary.

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

Not relevant.

9.6.3 Effects on bumble bees

The EFSA Bee Guidance document (2013)¹³ has not yet been noted at the time of the submission of this dossier. In consideration of the recommendations of the “Technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology” (2015)¹⁸ currently no risk assessment for bumble bees is required, given that the EFSA Bee Guidance document (2013)¹³ has not yet been noted. Furthermore, EFSA stated that it cannot be recommended to routinely perform a risk assessment for bumble bees. **Therefore, no data are available for bumble bees.**

Given the recent addition as a data requirement in some Member States, new data for bumblebees are available for A22773A (see section 9.6.1). No specific risk assessment is presented for bumblebees given that no clear methodology is currently agreed. However, endpoints are in line with the acute data for honeybees and no effects were seen in the highest tested concentration for both species indicating the risk assessment for honeybees is protective for bumblebees.

¹⁸ EFSA (European Food Safety Authority), 2015. Technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology. EFSA supporting publication 2015:EN-924. 62 pp.

9.6.4 Effects on solitary bees

In consideration of the recommendations of the “Technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology” (2015)¹⁸ currently no risk assessment for solitary bees is required, given that the EFSA Bee Guidance document (2013)¹³ has not yet been noted and no OECD technical guidance is available for acute tests on solitary bees. Furthermore, EFSA stated that it cannot be recommended to routinely perform a risk assessment for solitary bees, this is also in line with the feedback of different authorities. Therefore, no data or information is currently available for solitary bees.

9.6.5 Overall conclusions

The risk to honeybees was assessed following SANCO/10329/2002 rev.2 and EPPO, 2010 as proposed in the list of guidance documents relevant to the implementation of Regulation 1107/2009, published in the official EU Journal 2013/C 95/01 and 95/02.

The risk of A22773A to honeybees was assessed from hazard quotients, estimated from acute oral and contact studies with azoxystrobin, oxathiapiprolin and A22773A. The acute oral and contact hazard quotients were less than the relevant trigger of 50, indicating that the risk to honeybees is acceptable following use of A22773A according to the proposed use pattern.

In addition, the acute risk to honeybees was assessed from hazard quotients (HQ) and Exposure Toxicity Ratios (ETRs) following EFSA Bee Guidance Document, 2013¹², using endpoints from acute oral and contact studies with azoxystrobin and oxathiapiprolin. Acute contact HQ and oral ETRs were less than the relevant triggers at the screening step, indicating acceptable acute risk to adult honeybees.

The chronic adult and larval risk of A22773A to honeybees was assessed from ETRs following EFSA Bee Guidance Document, 2013¹², using endpoints from chronic adult and larval studies with azoxystrobin and oxathiapiprolin. The Tier 1 ETR values for azoxystrobin and oxathiapiprolin for the treated crop were less than the relevant triggers, indicating acceptable chronic risk to adult honeybees and bee larvae, with the following exception: The Tier 1 risk assessment indicated a potential chronic risk to adult honeybees for azoxystrobin from downward spray in fruiting vegetables, leafy vegetables and bulb and other alliaceous crops. A refined risk assessment was therefore conducted.

Since azoxystrobin is clearly driving the toxicity in the mixture, the calculations for the chronic Tier 1 refinement were conducted with the azoxystrobin endpoints only.

In the refined risk assessment for adult honeybees for azoxystrobin, the dose resulting in 0% mortality under continuous feeding conditions in the laboratory (LDD₀), is above the predicted worst-case exposure in-field of adult bees indicating an acceptable chronic oral risk to adult honeybees (Approach 1 of the refinement). In addition, the in-field ETR values for azoxystrobin are below the EFSA trigger of 0.03 indicating an acceptable chronic oral risk to adult honeybees (Approach 2 of the refinement) for azoxystrobin. Therefore, the chronic risk to adult honeybees is acceptable following use of A22773A according to the proposed use pattern.

Review Comments:

The evaluation of the acute 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). The submitted risk assessment, based on laboratory studies, has been accepted. It can therefore be concluded that there will be negligible acute risk associated with the exposure of *Apis mellifera* to A22773A.

The data requirements in accordance with Commission Regulation (EU) No 284/2013 for the chronic toxicity to adult honeybees and honeybee larvae are fulfilled.

There is not harmonized approach for the chronic risk assessment for bees, therefore, Concerned Member States must decide on the acceptability of EFSA Bee Guidance Document (2013) approach *at* national level. New studies with active substances were not evaluated by zRMS.

9.7 Effects on arthropods other than bees (KCP 10.3.2)

9.7.1 Toxicity data

Studies on the toxicity to non-target arthropods have been carried out with the representative solo formulation of azoxystrobin and oxathiapiprolin. Full details of these studies are provided in the respective EU DAR and related documents, and a summary of the endpoints from laboratory tests is given below.

Effects on non-target arthropods of A22773A were not evaluated as part of the EU assessment of azoxystrobin and oxathiapiprolin. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

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

Species	Substance	Exposure System	Results	Reference
<i>Typhlodromus pyri</i> (protonymphs)	Azoxystrobin (tested as A12705B)	Laboratory test glass plates (2D)	LR ₅₀ > 6 000 ml A12705B/ha LR ₅₀ > 1 500 g a.s./ha	EFSA, 2010, Taruza, S., 2001, ICI5504/0006
<i>Aphidius rhopalosiphi</i> (adults)	Azoxystrobin (tested as A12705B)	Laboratory test glass plates (2D)	LR ₅₀ > 4 000 ml A12705B/ha LR ₅₀ > 1 000 g a.s./ha	EFSA, 2010, Stacey, D. A., 2004, ICI5504/2627
Field or semi-field tests				
Not required				

EFSA Journal 2010; 8(4):1542.

Table 9.7-2: Endpoints and effect values relevant for the risk assessment for non-target arthropods - oxathiapiprolin

Species	Substance	Exposure System	Results	Reference
<i>Typhlodromus pyri</i> (protonymphs)	Oxathiapiprolin 100 g/L OD	Laboratory test glass plates (2D)	LR ₅₀ > 200 g a.s./ha	EFSA, 2016, DuPont-33193
<i>Aphidius rhopalosiphi</i> (adults)	Oxathiapiprolin 100 g/L OD	Laboratory test glass plates (2D)	LR ₅₀ = 116.1 g a.s./ha	EFSA, 2016, DuPont-32696
Field or semi-field tests				
Not required				

EFSA Journal 2016;14(7):4504.

Table 9.7-3: Endpoints and effect values relevant for the risk assessment for non-target arthropods – A22773A

Species	Substance	Exposure System	Results	Reference
<i>Typhlodromus pyri</i> (protonymphs)	A22773A	Laboratory test glass plates (2D)	LR₅₀ > 5 000 ml/ha	Fallowfield, L., 2020, VV-876566
<i>Aphidius rhopalosiphi</i> (adults)	A22773A	Laboratory test glass plates (2D)	LR₅₀ > 5 000 ml/ha	Stevens, J., 2020, VV-875882
Field or semi-field tests				
Not required				

Endpoints used for risk assessment are shown in **bold**.

9.7.1.1 Justification for new endpoints

Studies with non-target arthropods are always conducted with a formulated product and no testing is carried out with unformulated technical material. Therefore, it may not be appropriate to rely on the data from the individual solo formulations, submitted as representative formulations for the EU review, for the risk assessment for non-target arthropods.

The toxicity of A22773A to non-target arthropods has been investigated by carrying out Tier I tests on the representative non-target arthropods *Aphidius rhopalosiphi* and *Typhlodromus pyri* in accordance with ESCORT 2.

9.7.2 Risk assessment

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

9.7.2.1 Risk assessment for in-field exposure

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the application of 2 x 1 000 mL A22773A/ha for hops covers the risk for non-target arthropods from all intended uses of A22773A (see 9.1.2).

The PER_{in-field} values according to ESCORT 2 were calculated as:
Application rate × MAF.

Table 9.7-4: First-tier assessment of the in-field risk for non-target arthropods due to the use of A22773A

Intended use	Hops		
Product	A22773A		
Application rate	2 × 1 000 mL prod./ha		
MAF	1.7 (foliar) / 1.9 (soil)		
Test species Tier I	LR₅₀ (lab.) (mL prod./ha)	PER_{in-field} (mL prod./ha)	HQ_{in-field} criterion: HQ ≤ 2
<i>Typhlodromus pyri</i>	> 5 000	1700 (foliar) ^a 1900 (soil) ^a	< 0.34 (foliar) < 0.38 (soil)
<i>Aphidius rhopalosiphi</i>	> 5 000		< 0.34 (foliar) < 0.38 (soil)

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

^a As a worst-case risk assessment, no crop interception is considered.

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 application of 2 x 1 000 mL A22773A/ha for hops covers the risk for non-target arthropods from all intended uses of A22773A (see 9.1.2).

The PER_{off-field} value according to ESCORT 2 was calculated as:
Application rate × MAF × (drift factor/vegetation distribution factor).

The corrected PER_{off-field} values according to ESCORT 2 was calculated as:
corr. PER_{off-field} = PER_{off-field} × correction factor.

Table 9.7-5: First-tier assessment of the off-field risk for non-target arthropods due to the use of A22773A in hops

Intended use	Hops					
Active substance/product	A22773A					
Application rate	2 x 1 000 mL prod./ha					
MAF	1.7					
Drift rate (%)	17.73 (at a distance of 3 m) ^a					
vdf	10/5 (Tier I, 2D)					
Test species Tier I	LR₅₀ (lab.) (mL prod./ha)	Drift factor	PER_{off-field} (mL prod./ha)	CF	corr. PER_{off-field} (mL prod./ha)	HQ_{off-field} criterion: HQ ≤ 2
<i>Typhlodromus pyri</i>	> 5 000	0.1773	30.1 / 60.3	10	301.4 / 602.8	< 0.060 / 0.121
<i>Aphidius rhopalosiphi</i>	> 5 000					< 0.060 / 0.121

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

^a An overall 90th percentile drift value was used and hence for each application an 82nd percentile drift value was considered.

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

At Tier 1, the in-field and off-field HQ values based on the LR₅₀ were below the trigger value for the worst-case intended use scenarios indicating that the risk to non-target arthropods is acceptable following the use of A22773A according to the proposed use pattern.

Review Comments:

Based on the results of the conducted Tier 1 risk assessment, it can be concluded that low risk for non-target arthropods is expected from the use of A22773A according to the proposed use pattern. No unacceptable effects on non-target arthropods are expected in in-field and off-field habitats.

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

9.8.1 Toxicity data

Studies on the toxicity to earthworms and other non-target soil organisms (meso- and macrofauna) have been carried out with azoxystrobin (and its representative formulations), oxathiapiprolin and their relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents, as well as in Appendix 2 of this document (new studies).

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of A22773A were not evaluated as part of the EU assessment of azoxystrobin or oxathiapiprolin. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

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

Species	Substance	Exposure System	Results	Reference
Acute				
<i>Eisenia fetida</i>	Azoxystrobin	Mixed into substrate 14 d, acute 10 % peat content	LC ₅₀ = 283 mg/kg soil dw LC_{50corr} = 142 mg/kg soil dw*	EFSA, 2010, Flemming <i>et al.</i> , 1993, ICI5504/0904
<i>Eisenia fetida</i>	Azoxystrobin (formulated as 250 SC)	Mixed into substrate 14 d, acute 10 % peat content	LC ₅₀ = 881 mg a.s./kg soil dw	EFSA, 2010, Bembridge <i>et al.</i> , 1994, ICI5504/0905
<i>Eisenia fetida</i>	R234886	Mixed into substrate 14 d, acute 10 % peat content	LC₅₀ > 1 000 mg/kg soil dw	EFSA, 2010, Friedrich, 2002, R234886/0001

Species	Substance	Exposure System	Results	Reference
<i>Eisenia fetida</i>	R402173	Mixed into substrate 14 d, acute 10 % peat content	LC₅₀ > 1 000 mg/kg soil dw	EFSA, 2010, Friedrich, 2008, SYN501114/0001
<i>Eisenia fetida</i>	R401553	Mixed into substrate 14 d, acute 10 % peat content	LC₅₀ > 1 000 mg/kg soil dw	EFSA, 2010, Friedrich, 2008a, SYN501657/0006
Chronic				
<i>Eisenia fetida</i>	Azoxystrobin (formulated as A12705B)	Mixed into substrate 56 d, chronic 10 % peat content	NOEC = 20 mg a.s./kg soil dw NOEC_{corr} = 10 mg a.s./kg soil dw*	EFSA, 2010, Moser and Roembke, 2000, ICI5504/0903
<i>Eisenia fetida</i>	R234886	Mixed into substrate 56 d, chronic 5 % peat content	NOEC = 16 mg/kg soil dw	Friedrich, S., 2010, VV 394786
<i>Folsomia candida</i>	Azoxystrobin (formulated as A12705B)	Mixed into substrate 28 d, chronic 5 % peat content	NOEC = 50 mg a.s./kg soil dw NOEC_{corr} = 25 mg a.s./kg dw soil	EFSA, 2010, Barth, 2001, ICI5504/1319
<i>Folsomia candida</i>	R234886	Mixed into substrate 28 d, chronic 5 % peat content	NOEC = 250 mg/kg soil dw	Friedrich, S., 2019, VV 471930
<i>Hypoaspis aculeifer</i>	Azoxystrobin (formulated as A12705B)	Mixed into substrate 14 d, chronic 5 % peat content	NOEC = 1 000 mg A12705B/kg soil dw (NOEC = 227 mg a.s./kg soil dw^a)	Schulz, L., 2017, VV 467698
<i>Hypoaspis aculeifer</i>	R234886	Mixed into substrate 14 d, chronic 5 % peat content	NOEC = 1 000 mg/kg soil dw	Schulz, L., 2019, VV 471883
Field studies				
Not relevant				
Litter bag test				
Straw degradation in soil	Azoxystrobin (formulated as A12705A)	181 d	max. 5 % deviation from control at 0.5514 mg a.s./kg soil dw	EFSA, 2010, Kollmann, 2004, ICI5504/2319

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

EFSA Journal 2010; 8(4):1542.

* Corrected value derived by dividing the endpoint by a factor of 2 due to lipophilic substance (log Pow > 2) in accordance with the EPPO earthworm scheme 2002.

^a Calculated based on azoxystrobin content for A12705B of 22.7 % w/w.

For azoxystrobin metabolites R402173 and R401553, chronic studies with *Hypoaspis* and *Folsomia* are not considered necessary since metabolites do not show any toxicity in acute tests and the acute toxicity of the parent is significantly higher compared to the metabolites. Further, *Folsomia* and *Hypoaspis* are less sensitive towards azoxystrobin than the earthworm, which is expected to be also the case for azoxystrobin metabolites.

Table 9.8-2: Endpoints and effect values relevant for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) – oxathiapiprolin and relevant metabolites

Species	Substance	Exposure System	Results	Reference
Acute				
<i>Eisenia fetida</i>	Oxathiapiprolin	Mixed into substrate 14 d, acute 5 % peat content	LC ₅₀ > 1 000 mg a.s./kg soil dw LC_{50corr} > 500 mg a.s./kg dw soil	EFSA, 2016, DuPont-45261
Chronic				
<i>Eisenia fetida</i>	Oxathiapiprolin	Mixed into substrate 56 d, chronic 5 % peat content	NOEC = 1 000 mg a.s./kg soil dw NOEC_{corr} = 500 mg a.s./kg dw soil	EFSA, 2016, DuPont-32457
<i>Eisenia fetida</i>	IN-E8S72	Mixed into substrate 56 d, chronic 5 % peat content	NOEC = 100.0 mg a.s./kg soil dw	EFSA, 2016, DuPont-32720
<i>Eisenia fetida</i>	IN-QPS10	Mixed into substrate 56 d, chronic 5 % peat content	NOEC = 100.0 mg a.s./kg soil dw	EFSA, 2016, DuPont-32134
<i>Eisenia fetida</i>	IN-RAB06	Mixed into substrate 56 d, chronic 5 % peat content	NOEC = 100.0 mg a.s./kg soil dw	EFSA, 2016, DuPont-32470
<i>Eisenia fetida</i>	IN-RDT31	Mixed into substrate 56 d, chronic 5 % peat content	NOEC = 100.0 mg a.s./kg soil dw NOEC_{corr} = 50.0 mg a.s./kg dw soil	EFSA, 2016, DuPont-32461
<i>Folsomia candida</i>	Oxathiapiprolin	Mixed into substrate 28 d, chronic 5 % peat content	NOEC = 25 mg a.s./kg soil dw NOEC_{corr} = 12.5 mg a.s./kg dw soil	EFSA, 2016, DuPont-32458
<i>Folsomia candida</i>	IN-E8S72	Mixed into substrate 28 d, chronic 5 % peat content	NOEC = 100.0 mg a.s./kg soil dw	EFSA, 2016, DuPont-32721
<i>Folsomia candida</i>	IN-QPS10	Mixed into substrate 28 d, chronic 5 % peat content	NOEC = 100.0 mg a.s./kg soil dw	EFSA, 2016, DuPont-32135
<i>Folsomia candida</i>	IN-RAB06	Mixed into substrate 28 d, chronic 5 % peat content	NOEC = 100.0 mg a.s./kg soil dw	EFSA, 2016, DuPont-32462
<i>Folsomia candida</i>	IN-RDT31	Mixed into substrate 28 d, chronic 5 % peat content	NOEC = 100 mg a.s./kg soil dw NOEC_{corr} = 50.0 mg a.s./kg dw soil	EFSA, 2016, DuPont-32463
<i>Hypoaspis aculeifer</i>	Oxathiapiprolin	Mixed into substrate 14 d, chronic 5 % peat content	NOEC = 1 000 mg a.s./kg soil dw NOEC_{corr} = 500 mg a.s./kg dw soil	EFSA, 2016, DuPont-33723

Species	Substance	Exposure System	Results	Reference
<i>Hypoaspis aculeifer</i>	IN-E8S72	Mixed into substrate 14 d, chronic 5 % peat content	NOEC = 100.0 mg a.s./kg soil dw	EFSA, 2016, DuPont-32719
<i>Hypoaspis aculeifer</i>	IN-QPS10	Mixed into substrate 14 d, chronic 5 % peat content	NOEC = 50.0 mg a.s./kg soil dw	EFSA, 2016, DuPont-32136
<i>Hypoaspis aculeifer</i>	IN-RAB06	Mixed into substrate 14 d, chronic 5 % peat content	NOEC = 25.0 mg a.s./kg soil dw	EFSA, 2016, DuPont-32464
<i>Hypoaspis aculeifer</i>	IN-RDT31	Mixed into substrate 14 d, chronic 5 % peat content	NOEC = 12.5 mg a.s./kg soil dw NOEC_{corr} = 6.25 mg a.s./kg dw soil	EFSA, 2016, DuPont-32465
Field studies				
Not required				
Litter bag test				
Not required				

Endpoints used in risk assessment are shown in **bold**.
EFSA Journal 2016;14(7):4504.

Table 9.8-3: Endpoints and effect values relevant for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) – A22773A

Species	Substance	Exposure System	Results	Reference
Acute				
<i>Eisenia andrei</i>	A22773A	Mixed into substrate 14 d, acute 10 % peat content	LC₅₀ > 1 000 mg/kg soil dw LC _{50corr} = 500 mg/kg soil dw*	Friedrich, S., 2020, VV-884611
Chronic				
<i>Eisenia andrei</i>	A22773A	Mixed into substrate 56 d, chronic 5 % peat content	NOEC = 95.3 mg/ kg soil dw NOEC_{corr} = 47.65 mg a.s./kg dw soil EC ₅₀ = 271 mg/ kg soil dw EC ₂₀ = 146 mg/ kg soil dw EC ₁₀ = 96.8 mg/ kg soil dw	Friedrich, S., 2020, VV-883029
<i>Folsomia candida</i>	A22773A	Mixed into substrate 28 d, chronic 5 % peat content	NOEC ≥ 1 000 mg/kg soil dw NOEC_{corr} ≥ 500 mg a.s./kg dw soil	Friedrich, S., 2020, VV-882647
<i>Hypoaspis aculeifer</i>	A22773A	Mixed into substrate 14 d, chronic 5 % peat content	NOEC ≥ 1 000 mg/kg soil dw NOEC_{corr} ≥ 500 mg a.s./kg dw soil	Schulz, L., 2020, VV-876276
Field studies				

Species	Substance	Exposure System	Results	Reference
Not required				
Litter bag test				
Not required				

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

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

~~It is the view of Syngenta that when assessing the acute and long-term risk to earthworms from A22773A, the active substances and metabolites, it is not appropriate to divide the LC₅₀ or NOEC by 2 in the case that the endpoint was generated in an artificial soil that contained reduced (5 %) organic matter rather than the 10 % organic matter of standard test soils. Current Guidance (EU Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002 rev 2; EPPO, 2003¹⁹)) states that the correction of endpoints is only necessary when chemicals have a log Pow > 2 and the artificial soil used in the tests contains 10 % peat. When the peat level is reduced to 5 % it is considered more comparable to agricultural soils (EPPO, 2003¹⁸) and thus the correction is not required.~~

~~In addition, the view of Syngenta is that when assessing the long-term risk from A22773A, the active substances and metabolites to *Folsomia candida* and *Hypoaspis* sp. it is not appropriate to divide the NOEC by 2 as the relationship between organic matter and toxicity has only been demonstrated for a small number of substances and only for earthworms (van Gestel, 1992²⁰). Other soil macro-organisms such as *Hypoaspis* sp. and collembolans are not directly involved in organic matter breakdown and thus the correction conservatively applied to earthworms should not be applied here. Furthermore, the endpoints were generated in an artificial soil that contained 5 % organic matter. Current Guidance (EPPO 2003¹⁹) states that the correction of endpoints is only necessary for earthworms when chemicals have a log Pow > 2 and the artificial soil used in the tests contains 10 % peat. When the peat level is reduced to 5 % it is considered more comparable to agricultural soils (EPPO, 2003¹⁹) and thus the correction is not required.~~

Nevertheless, the correction factor in the endpoints of the active substances azoxystrobin, oxathiapiprolin and its metabolite IN-RDT31 was included as a worst-case approach in line with EFSA Journal 2010; 8(4):1542 and EFSA Journal 2016;14(7):4504, respectively.

9.8.1.1 Justification for new endpoints

New studies are available for A22773A which are required to fulfil the data requirements for plant protection products in accordance with Regulation (EC) No 1107/2009. The endpoints are summarised in Table 9.8-3 and study summaries are provided in Appendix 2 to this document.

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).

¹⁹ EPPO, 2003; Anonymous, 2003. Environmental risk assessment scheme for plant protection products Chapter 8: Soil organisms and functions. OEPP/EPPO, Bulletin OEPP/EPPO Bulletin 33, 147–149

²⁰ van Gestel CAM (1992) The influence of soil characteristics on the toxicity of chemicals for earthworms; a review. In: Ecotoxicology of Earthworms (Ed. Becker, H, Edwards, PJ, Greig-Smith, PW & Heimbach, F). Intercept Press, Andover (GB)

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 in soil needs to be considered for oxathiapiprolin and its metabolites IN-RDT31, IN-E8S72, IN-QPS10 and IN-RAB06 and for azoxystrobin and its metabolite R234886.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, for A22773A, azoxystrobin, oxathiapiprolin and their relevant metabolites, the relevant endpoints are compared to the maximum PEC_{soil} ensuring that the risk for earthworms and other non-target soil organisms from all intended uses is covered (see 9.1.2).

Table 9.8-4: First-tier assessment of the acute and chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of A22773A - worst-case

Intended use	All intended uses		
Acute effects on earthworms			
Test substance	LC ₅₀ (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _a (criterion TER ≥ 10)
Azoxystrobin	142*	0.681 ^a	209
R234886	> 1 000	0.171 ^a	5 850
R402173	> 1 000	0.084 ^b	12 000
R401553	> 1 000	0.054 ^b	19 000
Oxathiapiprolin	> 500*	0.031 ^a	16 000
A22773A	1000 > 500*	1.32	758 > 379
Chronic effects on earthworms			
Test substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{lt} (criterion TER ≥ 5)
Azoxystrobin	10*	0.681 ^a	15
R234886	16	0.171 ^a	94
Oxathiapiprolin	500*	0.031 ^a	16 000
IN-E8S72	100	0.001 ^d	100 000
IN-QPS10	100	0.002 ^d	50 000
IN-RAB06	100	0.004 ^c	25 000
IN-RDT31	50*	0.006 ^c	8 333
A22773A	95.3 47.65*	1.32	72 36
Chronic effects on other soil macro- and mesofauna - <i>Folsomia candida</i>			
Test substance	NOEC (mg/kg dw)	PEC _{soil} (mg/kg dw)	TER _{lt} (criterion TER ≥ 5)
Azoxystrobin	50 25*	0.681 ^a	73 37
R234886	250	0.171 ^a	1460
Oxathiapiprolin	12.5*	0.031 ^a	403
IN-E8S72	100	0.001 ^d	100 000
IN-QPS10	100	0.002 ^d	50 000

IN-RAB06	100	0.004 ^c	25 000
IN-RDT31	50*	0.006 ^e	8 333
A22773A	≥ 1000 500*	1.32	758 ≥ 379
Chronic effects on other soil macro- and mesofauna - <i>Hypoaspis aculeifer</i>			
Test substance	NOEC (mg/kg dw)	PEC_{soil} (mg/kg dw)	TER_{lt} (criterion TER ≥ 5)
Azoxystrobin	227	0.681 ^a	333
R234886	1000	0.171 ^a	5-850
Oxathiapiprolin	500*	0.031 ^a	16 000
IN-E8S72	100	0.001 ^d	100 000
IN-QPS10	50	0.002 ^d	25 000
IN-RAB06	25	0.004 ^c	6 250
IN-RDT31	6.25*	0.006 ^e	1 042
A22773A	≥ 1000 500*	1.32	758 ≥ 379

TER values shown in bold fall below the relevant trigger.

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

^a Maximum PEC_{soil} from use on onions (PEC_{soil, accumulation})

^b Maximum PEC_{soil} from use on onions (PEC_{soil, initial})

^c Maximum PEC_{soil} from use on cabbage and onions (PEC_{soil, accumulation})

^d Maximum PEC_{soil} from use on cabbage, onions, and hops (PEC_{soil, accumulation})

^e Maximum PEC_{soil} from use on hops (PEC_{soil, accumulation})

PEC_{soil, accumulation} = PEC_{soil, initial} + PEC_{soil, plateau}

9.8.2.2 Higher-tier risk assessment

Not relevant.

9.8.3 Overall conclusions

The risk of A22773A to earthworms was assessed from acute and long-term toxicity exposure ratios (TERs) between the selected toxicity endpoints for A22773A, azoxystrobin, oxathiapiprolin and their relevant metabolites, and the maximum PEC_{soil}. The acute and long-term TER values derived are greater than the Regulation (EU) 546/2011 triggers of 10 and 5, respectively, indicating that the risk to earthworms is acceptable following use of A22773A according to the proposed use pattern.

The risk of A22773A to other non-target soil macro-organisms, as represented by *Folsomia* and *Hypoaspis* was assessed from long-term toxicity exposure ratios (TERs) between the selected no-effect concentrations, derived from laboratory tests on A22773A, azoxystrobin, oxathiapiprolin and their relevant metabolites, and the maximum PEC_{soil}. The long-term TER values are all greater than the recommended trigger value of 5, indicating that the risk to soil macro-organisms is acceptable following use of A22773A according to the proposed use pattern.

Review Comments:

The long-term risks of A22773A to soil meso- and macro-organisms were assessed from toxicity exposure ratios between toxicity endpoints and maximum PEC_{soil}. The relevant predicted environmental concentration in soil (PEC_{soil}) for risk assessment covering the proposed use pattern was taken from Part B Section 8 (Environmental Fate).

Safe use of A22773A was confirmed based on TER_{LT} calculations for formulation, azoxystrobin, oxathiapiprolin and their relevant metabolites.

9.9 Effects on soil microbial activity (KCP 10.5)

9.9.1 Toxicity data

Studies on effects on soil microorganisms have been carried out with azoxystrobin, oxathiapiprolin and their relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on soil microorganisms of A22773A were not evaluated as part of the EU assessment of azoxystrobin and oxathiapiprolin. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

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

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	Azoxystrobin (formulated as 250 SC)	28 d	< 25 % effect up to 2.5 kg a.s./ha equivalent to 3.3 mg a.s./kg soil dw ^a	EC review report (1998), 7581/VI/97-Final, Mason <i>et al.</i> , 1994, ICI5504/0960
N-mineralisation	R234886	28 d	< 25 % effect up to 10 mg/kg soil dw	EFSA, 2010, Lemnitzer, 2002, R234886/0002
N-mineralisation	R402173	28 d	< 25 % effect up to 4.131 mg/kg soil dw	EFSA, 2010, Schulz, 2008, SYN501114/0002
N-mineralisation	R401553	28 d	< 25 % effect up to 2.643 mg/kg soil dw	EFSA, 2010, Schulz, 2008a, SYN501657/0007

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

EFSA Journal 2010; 8(4):1542.

^a Calculated based on soil density of 1.5 g/mL and soil depth of 5 cm.

Table 9.9-2: Endpoints and effect values relevant for the risk assessment for soil microorganisms – oxathiapiprolin and relevant metabolites

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	Oxathiapiprolin	28 d, aerobic Silty loamy sand	day 28: 2.2% effect at 15.59 mg a.s./kg soil dw	EFSA, 2016, DuPont-32477
N-mineralisation	IN-E8S72	28 d, aerobic Silty loamy sand	day 28: 9.19% effect at 5.38 mg/kg soil dw	EFSA, 2016, DuPont-32824
N-mineralisation	IN-QPS10	28 d, aerobic Loamy sand	day 42: 14.76% effect at 0.68 mg/kg soil dw	EFSA, 2016, DuPont-32823
N-mineralisation	IN-RAB06	28 d, aerobic Silty loamy sand	day 28: 0.67% effect at 5.50 mg/kg soil dw	EFSA, 2016, DuPont-32821
N-mineralisation	IN-RDT31	42/28 d, aerobic Loamy sand	day 42: 23.38% effect at 0.71 mg/kg soil dw	EFSA, 2016, DuPont-32822

Endpoints used in risk assessment are shown in **bold**.
EFSA Journal 2016;14(7):4504.

Table 9.9-3: Endpoints and effect values relevant for the risk assessment for soil microorganisms – A22773A

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	A22773A	28 d, aerobic Loamy sand	< 25 % effect up to 10 L/ha equivalent to 14.61 mg/kg soil dw^a	Schulz, L., 2020, VV-885459

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

^a Calculated based on soil density of 1.5 g/mL and soil depth of 5 cm.

9.9.1.1 Justification for new endpoints

New studies are available for A22773A, which are required to fulfil the data requirements for plant protection products in accordance with Regulation (EC) No 1107/2009. The endpoints are summarised in Table 9.9-3.

9.9.2 Risk assessment

The evaluation of the risk for soil microorganisms was performed in accordance with the recommendations of SANCO/10329/2002 rev 2.

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

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment based on the maximum PEC_{soil} covers the risk for the soil microorganisms from all intended uses (see 9.1.2).

Table 9.9-4: Assessment of the risk for effects on soil micro-organisms due to the use of A22773A – worst case

Intended use	All intended uses		
N-mineralisation			
Product/active substance	Max. conc. with effects ≤ 25 % (mg/kg dw)	PEC _{soil} (mg/kg dw)	Risk acceptable?
Azoxystrobin	3.3	0.681 ^a	Yes
R234886	10	0.171 ^a	Yes
R402173	4.131	0.084 ^b	Yes
R401553	2.643	0.054 ^b	Yes
Oxathiapiprolin	15.59	0.031 ^a	Yes
IN-E8S72	5.38	0.001 ^d	Yes
IN-QPS10	0.68	0.002 ^d	Yes
IN-RAB06	5.50	0.004 ^c	Yes
IN-RDT31	0.71	0.006 ^e	Yes
A22773A	14.61	1.32	Yes

TER values shown in bold fall below the relevant trigger.

^a Maximum PEC_{soil} from use on onions (PEC_{soil, accumulation})

^b Maximum PEC_{soil} from use on onions (PEC_{soil, initial})

^c Maximum PEC_{soil} from use on cabbage and onions (PEC_{soil, accumulation})

^d Maximum PEC_{soil} from use on cabbage, onions, and hops (PEC_{soil, accumulation})

^e Maximum PEC_{soil} from use on hops (PEC_{soil, accumulation})

PEC_{soil, accumulation} = PEC_{soil, initial} + PEC_{soil, plateau}

9.9.3 Overall conclusions

The risk of A22773A, azoxystrobin, oxathiapiprolin and their relevant metabolites to soil micro-organisms was evaluated by comparison of the maximum concentrations with effects < 25 % derived from laboratory tests, with the maximum PEC_{soil}.

All the effect levels exceeded the relevant PEC_{soil} values, indicating that the risk to soil micro-organisms is acceptable following the use of A22773A according to the proposed use pattern.

Review Comments:

The use of A22773A at the proposed rates poses no unacceptable risk to soil micro-organisms.

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 azoxystrobin and oxathiapiprolin formulated as a 100 g/L OD. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on non-target terrestrial plants of A22773A were not evaluated as part of the EU assessment of

azoxystrobin or oxathiapiprolin. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

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

Species	Substance	Exposure System	Results	Reference
<i>Triticum aestivum</i> _m <i>Lactuca sativa</i> _d <i>Raphanus sativus</i> _d	Azoxystrobin	18 d Tier 1 screening data Seedling emergence	ER ₅₀ > 20 mg a.s./kg dw soil	EFSA, 2010, Porch, J.R., Krueger, H.O., 2002, ICI5504/1376

m: monocotyledonous; d: dicotyledonous
EFSA Journal 2010; 8(4):1542.

Table 9.10-2: Endpoints and effect values relevant for the risk assessment for non-target terrestrial plants –oxathiapiprolin

Species	Substance	Exposure System	Results	Reference
<i>Zea mays</i> _m <i>Avena sativa</i> _m <i>Allium cepa</i> _m <i>Lolium perenne</i> _m <i>Cucumis sativus</i> _d <i>Pisum sativum</i> _d <i>Brassica napus</i> _d <i>Glycine max</i> _d <i>Beta vulgaris</i> _d <i>Lycopersicon esculentum</i> _d	Oxathiapiprolin (formulated as 100 g/L OD)	21 d Seedling emergence	ER ₅₀ > 600 g a.s./ha for all species tested	EFSA, 2016, DuPont-32478
<i>Zea mays</i> _m <i>Avena sativa</i> _m <i>Allium cepa</i> _m <i>Lolium perenne</i> _m <i>Cucumis sativus</i> _d <i>Pisum sativum</i> _d <i>Brassica napus</i> _d <i>Glycine max</i> _d <i>Beta vulgaris</i> _d <i>Lycopersicon esculentum</i> _d	Oxathiapiprolin (formulated as 100 g/L OD)	21 d Vegetative vigour	ER ₅₀ > 600 g a.s./ha for all species tested	EFSA, 2016, DuPont-32479

m: monocotyledonous; d: dicotyledonous
EFSA Journal 2016;14(7):4504.

Table 9.10-3: Endpoints and effect values relevant for the risk assessment for non-target terrestrial plants

Species	Substance	Exposure System	Results	Reference
<i>Allium cepa</i> _m ¹⁾ <i>Triticum aestivum</i> _m ¹⁾ <i>Beta vulgaris</i> _d ²⁾ <i>Brassica napus</i> _d ²⁾	A22773A ^a	28 d Tier 1 screening data Seedling emergence	No phytotoxic effects up to and including 1000 mL A22773A/ha ER ₅₀ > 1000 mL A22773A/ha	Jones, K., 2020, VV-880671

Species	Substance	Exposure System	Results	Reference
<i>Cucumis sativus</i> _d ²⁾ <i>Glycine max</i> _d ³⁾		21 d Tier 1 screening data Vegetative vigour	¹⁾ No phytotoxic effects up to and including 1000 mL A22773A/ha ²⁾ No phytotoxic effects up to and including 125 mL A22773A/ha ³⁾ No phytotoxic effects up to and including 250 mL A22773A/ha	
<i>Allium cepa</i> _m <i>Brassica napus</i> _d <i>Brassica oleracea</i> _d <i>Glycine max</i> _d <i>Beta vulgaris</i> _d <i>Lactuca sativa</i> _d <i>Cucumis sativus</i> _d <i>Zea mays</i> _m <i>Lolium perenne</i> _m <i>Avena sativa</i> _m	A22773A	21 d Vegetative vigour (Tier 2)	ER₅₀ > 4103 mL A22773A/ha	Bützler, R., 2021, VV-912999

m: monocotyledonous; d: dicotyledonous

Endpoints used for risk assessment are shown in **bold**.

^a The product A22773A was applied in combination with the adjuvant A12127R.

9.10.1.1 Justification for new endpoints

Studies with non-target terrestrial plants are always conducted with a formulated product and no testing is carried out with unformulated technical material. Therefore, it may not be appropriate to rely on the data from the individual solo formulations, submitted as representative formulations for the EU review, for the risk assessment for non-target terrestrial plants

A screening study and a Tier II vegetative vigour study with formulated product A22773A have been conducted. The data are listed in Appendix 1 and summarised in Appendix 2.

9.10.2 Risk assessment

9.10.2.1 Tier-1 risk assessment (based screening data)

Screening test rates up to and including 1000 mL A22773A /ha were conducted with formulation and effects were below the critical threshold as defined by the “Guidance Document on Terrestrial Ecotoxicology”, (SANCO/10329/2002 rev.2 final, 2002). The test rates equal the highest field application rate and are thus considered an indicator for an acceptable risk based on seedling emergence. However, the vegetative vigour screening test showed effects below the highest field application rate. Therefore, a Tier 2 risk assessment is presented below.

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

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

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the application of 2 x 1 000 mL A22773A/ha for hops covers the risk for non-target plants from all intended uses of A22773A (see 9.1.2), due to the large drift value for this crop group (17.73% at a distance of 3 m) compared to that for vegetables <50 cm and >50 cm (2.77% and 8.02% at a distance of 1 m and 3m, respectively).

The $PER_{\text{off field}}$ was calculated as Application rate \times drift factor.

Table 9.10-4: Assessment of the risk for non-target plants due to the use of A22773A in hops

Intended use		Hops		
Product		A22773A		
Application rate (mL prod./ha)		2 \times 1 000		
Drift rate (%)		17.73% at 3 m		
Test species	ER₅₀ (mL prod./ha)	Drift factor	PER_{off-field} (mL prod./ha)	TER criterion: TER \geq 5
All species	>4 103	0.1773	177.3	23

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

9.10.2.3 Higher-tier risk assessment

Not relevant.

9.10.2.4 Risk mitigation measures

No risk mitigation needed.

9.10.3 Overall conclusions

Less than 50% effect on seedling emergence on all six species was observed at the maximum single use rate of 1 000 mL A22773A/ha. This indicates that the risk to non-target terrestrial plants for seedling emergence in off-crop areas is acceptable following use of A22773A according to the proposed use pattern. However, the vegetative vigour screening test showed effects below the highest field application rate. Therefore, a Tier 2 risk assessment was conducted.

The risk of A22773A to non-target terrestrial plants was assessed from toxicity exposure ratios (TERs) using the formulation toxicity data from a Tier II vegetative vigour study, and the maximum off-field predicted environmental residue (PER) indicating an acceptable risk.

The risk to non-target terrestrial plants in off-crop areas is therefore acceptable following use of A22773A according to the proposed use pattern.

Review Comments:

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

Based on the risk assessment it can be concluded that the proposed use of A22773A poses no unacceptable risk to non-target plants, if applied according to the recommended use pattern. Particular precautions to reduce the environmental concentrations resulting from A22773A applications are not required.

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


Tests on other non-target species are not required.

9.12 Monitoring data (KCP 10.8)

There are no other relevant data for the active substance or product on organisms in the environment generated from monitoring schemes

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:

Hazard class(es), categories	Acute aquatic toxicity, Category 1 Chronic aquatic toxicity, Category 1
Hazard pictograms or Code(s) for hazard pictogram(s)	 GHS09
Signal word	Warning
Hazard statements	H400: Very toxic to aquatic life H410: Very toxic to aquatic life with long lasting effects
Label elements for labelling	Pictogram GHS09 Signal word: Warning H410: Very toxic to aquatic life with long lasting effects P391: Collect spillage P501: Dispose of contents/container to an approved waste disposal plant EUH401: To avoid risks to human health and the environment, comply with the instructions for use.

The classification of the product is derived from data on the ingredients and the summation method is applied. Based on the classification of azoxystrobin and the corresponding M-factors this active substance forms >25% of the composition of the product (ORONDIS EVO/A22773A). Therefore, the product is classified as Aquatic Acute 1 and Aquatic Chronic 1 according to Regulation (EC) No 1272/2008.

Precautionary statements: P391 (Collect spillage) and P501 (Dispose of contents/container to an approved waste disposal plant) are applied. P273 (Avoid release to the environment) is not required as this product is for use in the environment as a fungicide.

Azoxystrobin

Aquatic Acute 1, M-factor 10 and Aquatic Chronic 1, M-factor 10.

C&L driving data:

Acute toxicity to *Daphnia* and other aquatic invertebrates: EC₅₀ *Americamysis bahia* (Mysid shrimp): 0.055 mg/L Exposure time: 96 h

Chronic toxicity to *Daphnia* and other aquatic invertebrates: NOEC *Americamysis bahia* (Mysid shrimp): 0.0095 mg/L Exposure time: 28 d

The substance is not rapidly degradable.

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on (A22773A formulation data)

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 10.1.1.1	Hubbard, P. Temple, D.	28/08/2020	Oxathiapiprolin/Azoxystrobin SC (A22773A) – An Acute Oral Toxicity Study with the Northern Bobwhite using a Sequential Testing Procedure Report No. 528B-602 Document No. VV-870400 Test Facility Eurofins EAG Agrosience LCC GLP Unpublished	Y	SYN
KCP 10.1.2.1	xxxxxx	11/02/2021	Azoxystrobin/Oxathiapiprolin SC (A22773A) - Acute Oral Toxicity Study in Rats (Up and Down Procedure) Report No. 20/130-001P Document No. VV-892044 Test xxxxxxxxxxxxxx GLP Unpublished	Y	SYN
KCP 10.1.2.2	Barfknecht, R.	19/05/2003	Attractiveness of Tomato Fields for Herbivorous Mammals and Birds, Field Monitoring in Lombardia Report No. E307-2304-9/BAR/FS014-IM-232304-01-1 Document No. VV-338885-N/1159 Test Facility Bayer AG, Crop Science Division GLP Unpublished	Y	BCS (Syngenta access)

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 10.1.2.2	Munderle, M. Carlin, B. Nickisch, D. Ludwigs, J.	16/07/2020	GLP-compliant field study to measure crop coverage in leafy vegetable fields via drone image analysis Report No. R1940003 Document No. VV-867392 Test Facility RIFcon GmbH GLP Unpublished	N	SYN
KCP 10.1.2.2	Hahne, J. Sainz-Elipe, S.	01/09/2014	Bayer – Generic Field Study on the Attractiveness of Tomato Fields for Savi's Pine Voles in Italy Report No. B12063-2 Document No. VV-410659 , NA_13506 Test Facility tier3 solutions GmbH GLP Unpublished	Y	BCS (Syngenta access)
KCP 10.1.2.2	Ertus, C.	22/03/2018	Azoxystrobin - Foliar Residue Decline Study on Winter Barley in Northern Europe in 2017 Report No. B7306 Document No. VV-469438 , A12705B_14098 Test Facility Anadiag S.A. GLP Unpublished	N	SYN
KCP 10.1.2.2	Ford, S.	18/05/2018	Azoxystrobin - Total foliage decline kinetics including foliage metabolite R230310 Report No. 0416036-Kin01 Document No. VV-631889 , ICI5504_12231 Test Facility ERM Not GLP Unpublished	N	SYN

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	██████████.	30/11/2020	Oxathiapiprolin/Azoxystrobin SC (A22773A) - Toxicity to the Rainbow Trout <i>Oncorhynchus mykiss</i> under Laboratory Conditions (Acute Toxicity Test –Static) Report No. S20-05053 Document No. VV-884613 Test Facility xxxxxxxx GLP Unpublished	N	SYN
KCP 10.2.1	Beuter, L-K.	30/11/2020	Oxathiapiprolin/Azoxystrobin SC (A22773A) - Toxicity to the Water Flea <i>Daphnia magna</i> Straus under Laboratory Conditions (Acute Immobilisation Test – Static) Report No. S20-05052 Document No. VV-884821 Test Facility Eurofins Agrosience Services EcoTox GmbH GLP Unpublished	N	SYN
KCP 10.2.1	Obert-Rauser, P.	04/12/2020	Oxathiapiprolin/Azoxystrobin SC (A22773A) - Toxicity to the Single Cell Green Alga <i>Raphidocelis subcapitata</i> Korshikov under Laboratory Conditions Report No. S20-05054 Document No. VV-884825 Test Facility Eurofins Agrosience Services EcoTox GmbH GLP Unpublished	N	SYN
KCP 10.3.1.1	Franke, M.	27/11/2020	Oxathiapiprolin/Azoxystrobin SC (A22773A) - Acute Toxicity to the Honeybee <i>Apis mellifera</i> L. under Laboratory Conditions Report No. 20 48 BAA 0129 Document No. VV-883076 Test Facility BioChem agrar GmbH GLP Unpublished	N	SYN

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 10.3.1.1	Amsel, K.	10/01/2022	Oxathiapiprolin/Azoxystrobin SC (A22773A) - Acute toxicity to the bumblebee <i>Bombus terrestris</i> L. under laboratory conditions Report No. 21 48 BBA 0032 Document No. VV-936507 Test Facility BioChem agrar GmbH GLP Unpublished	N	SYN
KCP 10.3.1.2	Dressler, K.	11/11/2020	Oxathiapiprolin/Azoxystrobin SC (A22773A) - Chronic toxicity to the honey bee <i>Apis mellifera</i> L. in a 10-day continuous laboratory feeding study Report No. 20 48 BAC 0043 Document No. VV-881467 Test Facility BioChem agrar GmbH GLP Unpublished	N	SYN
KCP 10.3.1.3	Schmidt, K.	30/03/2021	Oxathiapiprolin/azoxystrobin SC (A22773A) – Repeated Exposure of the Honey Bee Larvae (<i>Apis mellifera</i> L.) under Laboratory Conditions (until Adult Emergence up to Day 22) Report No. 20 48 BLC 0043 Document No. VV-896655 Test Facility BioChem agrar GmbH GLP Unpublished	N	SYN
KCP 10.3.2.1	Fallowfield, L.	20/10/2020	Oxathiapiprolin/azoxystrobin SC (A22773A) – A Rate-Response Laboratory Study to Determine the Effects of Fresh Residues on the Predatory Mite <i>Typhlodromus pyri</i> (Acari: Phytoseiidae) Report No. SYN-20-48 Document No. VV-876566 Test Facility Mambo-Tox, Ltd. GLP Unpublished	N	SYN

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 10.3.2.1	Stevens, J.	22/09/2020	Oxathiapiprolin/azoxystrobin SC (A22773A) - A Rate-Response Laboratory Study to Determine the Effects of Fresh Residues on the Parasitic Wasp Aphidius rhopalosiphi (Hymenoptera, Braconidae) Report No. SYN-20-47 Document No. VV-875882 Test Facility Mambo-Tox, Ltd. GLP Unpublished	N	SYN
KCP 10.4.1	Friedrich, S.	17/11/2020	Oxathiapiprolin/Azoxystrobin SC (A22773A) - Acute Toxicity to the Earthworm Eisenia andrei in Artificial Soil Report No. 20 48 TEA 0018 Document No. VV-884611 Test Facility BioChem agrar GmbH GLP Unpublished	N	SYN
KCP 10.4.1.1	Friedrich, S.	23/11/2020	Oxathiapiprolin/Azoxystrobin SC (A22773A) - Sublethal Effects on the Reproduction of the Earthworm Eisenia andrei in Artificial Soil Report No. 20 48 TEC 0052 Document No. VV-883029 Test Facility BioChem agrar GmbH GLP Unpublished	N	SYN
KCP 10.4.2.1	Friedrich, S.	25/11/2020	Oxathiapiprolin/Azoxystrobin SC (A22773A) - Effects on the Reproduction of the Collembolan Folsomia candida Report No. 20 48 TCC 0049 Document No. VV-882647 Test Facility BioChem agrar GmbH GLP Unpublished	N	SYN

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 10.4.2.1	Schulz, L.	06/10/2020	Oxathiapiprolin/Azoxystrobin SC (A22773A) - Effects on the Reproduction of the Predatory Mite Hypoaspis aculeifer Report No. 20 48 THC 0042 Document No. VV-876276 Test Facility BioChem agrar GmbH GLP Unpublished	N	SYN
KCP 10.5	Schulz, L.	10/12/2020	Oxathiapiprolin/Azoxystrobin SC (A22773A) – Effects on the Activity of Soil Microflora (Nitrogen and Carbon Transformation Tests) Report No. 20 48 SMO 0017 Document No. VV-885459 Test Facility BioChem agrar GmbH GLP Unpublished	N	SYN
KCP 10.6.2	Butzler, R. Kowalczyk, F.	27/07/2021	Oxathiapiprolin/azoxystrobin SC (A22773A) - Effects on Terrestrial (Non-Target) Plants: Vegetative Vigour Test Report No. 159471087 Document No. VV-912999 Test Facility Ibacon GmbH GLP Unpublished	N	SYN
KCP 10.6.2	Jones, K.	06/11/2020	Oxathiapiprolin/azoxystrobin SC (A22773A) plus Adjuvant A12127R - Phytotoxicity to Non-Target Plants Screening Test Report No. ACE-20-101 Document No. VV-880671 Test Facility AgroChemex, Ltd GLP Unpublished	N	SYN

List of data submitted by the applicant and relied on (Azoxystrobin)

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
KCA 8.3.1.2	Tänzler V	03/09/2015	Azoxystrobin SC (A12705B) — Chronic Oral Toxicity Test to the Honey Bee (<i>Apis mellifera</i> L.) in the Laboratory Report No. 100921136 Institut für Biologische Analytik und Consulting IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany GLP Unpublished Syngenta file No VV 414159	N	Syngenta
KCA 8.3.1.3	Ehmke A	19/11/2015	Azoxystrobin SC (A12705B) — Honey Bee (<i>Apis mellifera</i> L.) Larval Toxicity Test, Repeated Exposure Report No. 100921032 Institut für Biologische Analytik und Consulting IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany GLP Unpublished Syngenta file No VV 414544	N	Syngenta
KCA 8.4.1	Friedrich S	29/10/2010	R234886 — Sublethal Toxicity to the Earthworm <i>Eisenia fetida</i> in Artificial Soil with 5 % Peat Report No. 101048078S BioChem agrar, Labor für biologische und chemische, Analytik GmbH, Kupferstr. 6, 04827 Machern OT Gerichshain, Germany GLP Unpublished Syngenta file No VV 394786	N	Syngenta
KCA 8.4.2	Friedrich S	2019	R234886 — Effects on the Reproduction of the Collembolan <i>Folsomia candida</i> Report Number 19-48 TCC 0011. BioChem agrar, Labor für biologische und chemische, Analytik GmbH, Kupferstr. 6, 04827 Machern OT Gerichshain, Germany GLP Unpublished Syngenta File No VV 471930	N	Syngenta

KCA 8.4.2.1	Schulz L	14/06/2017	Azoxystrobin SC (A12705B) – Effects on the Reproduction of the Predatory Mite <i>Hypoaspis aculeifer</i> Report No. 17 48 THC 0019 BioChem agrar, Labor für biologische und chemische, Analytik GmbH, Kupferstr. 6, 04827 Machern OT Gerichshain, Germany GLP Unpublished Syngenta file No VV 467698	N	Syngenta
KCA 8.4.2.1	Schulz L	2019	R234886 – Effects on the Reproduction of the Predatory Mite <i>Hypoaspis aculeifer</i> Report No. 19 48 THC 0004 BioChem agrar, Labor für biologische und chemische, Analytik GmbH, Kupferstr. 6, 04827 Machern OT Gerichshain, Germany GLP Unpublished Syngenta file No VV 471883	N	Syngenta

List of data submitted by the applicant and relied on (Oxathiapiprolin)

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
KCA 8.3.1.2	Tänzler V	2015	Oxathiapiprolin (DPX QGU42) 100 g/L OD: Chronic oral toxicity to the honey bee, <i>Apis mellifera</i> L. (Hymenoptera, Apidae) Report Number 94441136 Institut für Biologische Analytik und Consulting IBACON GmbH, Arheilger Weg 17, 64380 Rüssdorf, Germany GLP Unpublished DuPont Study No. DuPont 41989	N	Corteva (Syngenta access)

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
KCA 8.3.1.3	Oberrauch S	2017	Oxathiapiprolin (DPX QGU42) technical: Honey bee (<i>Apis mellifera</i> L.) 22-day larval toxicity test (repeated exposure) Report Number S17-01639 Eurofins Agroscience Services EcoChem GmbH / Eurofins Agroscience Services Ecotox GmbH, Eutingen Str. 24, 75223 Niefern-Öschelbronn, Germany GLP Unpublished DAS Study No. DuPont-48606	N	Corteva (Syngenta access)

The following tables are to be completed by MS

List of data submitted by the applicant and not relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.1.1.1		28/08/2020	Oxathiapiprolin/Azoxystrobin SC (A22773A) – An Acute Oral Toxicity Study with the Northern Bobwhite using a Sequential Testing Procedure Report No. 528B-602 Document No. VV-870400 Test Facility xxxxxxxxxxxx GLP Unpublished	Y	SYN
KCP 10.1.2.2		19/05/2003	Attractiveness of Tomato Fields for Herbivorous Mammals and Birds, Field Monitoring in Lombardia Report No. E307 2304-9 BAR/FS014 M-232304-01-1 Document No. VV-338885 , N/1159 Test Facility xxxxxxxxxxxx	Y	BCS (Syngenta access)

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			GLP Unpublished		
KCP 10.1.2.2		01/09/2014	Bayer - Generic Field Study on the Attractiveness of Tomato Fields for Savi's Pine Voles in Italy Report No. B12063-2 Document No. VV-410659 , NA_13506 Test Facility xxxxxxxxxxxxxxxx GLP Unpublished	Y	BCS (Syngenta access)
KCA 8.3.1.2	Tänzler V	03/09/2015	Azoxystrobin SC (A12705B) – Chronic Oral Toxicity Test to the Honey Bee (Apis mellifera L.) in the Laboratory Report No. 100921136 Institut für Biologische Analytik und Consulting IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany GLP Unpublished Syngenta file No VV-414159	N	Syngenta
KCA 8.3.1.3	Ehmke A	19/11/2015	Azoxystrobin SC (A12705B) – Honey Bee (Apis mellifera L.) Larval Toxicity Test, Repeated Exposure Report No. 100921032 Institut für Biologische Analytik und Consulting IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany GLP Unpublished Syngenta file No VV-414544	N	Syngenta
KCA 8.4.1	Friedrich S	29/10/2010	R234886 - Sublethal Toxicity to the Earthworm Eisenia fetida in Artificial Soil with 5 % Peat Report No. 101048078S BioChem agrar, Labor für biologische und chemische, Analytik GmbH, Kupferstr. 6, 04827 Machern OT Gerichshain, Germany GLP Unpublished Syngenta file No VV-394786	N	Syngenta

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCA 8.4.2	Friedrich S	2019	R234886 - Effects on the Reproduction of the Collembolan Folsomia candida. Report Number 19 48 TCC 0011. BioChem agrar, Labor für biologische und chemische, Analytik GmbH, Kupferstr. 6, 04827 Machern OT Gerichshain, Germany GLP Unpublished Syngenta File No VV-471930	N	Syngenta
KCA 8.4.2.1	Schulz L	14/06/2017	Azoxystrobin SC (A12705B) - Effects on the Reproduction of the Predatory Mite Hypoaspis aculeifer Report No. 17 48 THC 0019 BioChem agrar, Labor für biologische und chemische, Analytik GmbH, Kupferstr. 6, 04827 Machern OT Gerichshain, Germany GLP Unpublished Syngenta file No VV-467698	N	Syngenta
KCA 8.4.2.1	Schulz L	2019	R234886 - Effects on the Reproduction of the Predatory Mite Hypoaspis aculeifer Report No. 19 48 THC 0004. BioChem agrar, Labor für biologische und chemische, Analytik GmbH, Kupferstr. 6, 04827 Machern OT Gerichshain, Germany GLP Unpublished Syngenta file No VV-471883	N	Syngenta
KCA 8.3.1.2	Tänzler V	2015	Oxathiapiprolin (DPX-QGU42) 100 g/L OD: Chronic oral toxicity to the honey bee, Apis mellifera L. (Hymenoptera, Apidae) Report Number 94441136 Institut für Biologische Analytik und Consulting IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany GLP Unpublished DuPont Study No. DuPont-41989	N	Corteva (Syngenta access)
KCA 8.3.1.3	Oberrauch S	2017	Oxathiapiprolin (DPX-QGU42) technical: Honey bee (Apis mellifera L.) 22 day larval toxicity test (repeated exposure)	N	Corteva (Syngenta)

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			Report Number S17-01639 Eurofins Agrosience Services EcoChem GmbH / Eurofins Agrosience Services Ecotox GmbH, Eutinger Str. 24, 75223 Niefern-Öschelbronn, Germany GLP Unpublished DAS Study No. DuPont-48606		access)

List of data relied on not submitted by the applicant but necessary for evaluation

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

Appendix 2 Detailed evaluation of the new studies

A 2.1 KCP 10.1 Effects on birds and other terrestrial vertebrates

A 2.1.1 KCP 10.1.1 Effects on birds

A 2.1.1.1 KCP 10.1.1.1 Acute oral toxicity

Comments of zRMS:	Study not evaluated.
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Reference:	KCP 10.1.1.1
Report:	(2020), Oxathiapiprolin/Azoxystrobin SC (A22773A) – An Acute Oral Toxicity Study with the Northern Bobwhite using a Sequential Testing Procedure. Report Number 528B-602. xxxxxxxxxxxxxxxxx. (Syngenta file No. VV-870400)
Guideline(s):	OECD Guidelines No. 223 (2016)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication: (if vertebrate study)	No

Executive Summary

The acute oral LD₅₀ value for northern bobwhite exposed to A22773A as a single oral dose was determined to be greater than 2000 mg/kg body weight, the highest dosage tested. The no-mortality and no-observed-effect level was 2000 mg/kg body weight.

Materials

Test material	Azoxystrobin/Oxathiapiprolin SC (250/012); A22773A
Lot/Batch #:	SFI003-174-001; 1127290
Purity:	22.5 % w/w (247 g/L) azoxystrobin & 1.02 % w/w (11.2 g/L) oxathiapiprolin
Description:	Beige liquid
Stability of test compound:	< 30 °C
Reanalysis/expiry date:	End of February 2023
Density:	1096 kg/m ³
Treatments	
Test concentrations:	0 and 2000 mg/kg body weight
Control:	Reverse osmosis deionized water
Test vehicle:	None
Test organisms	
Species:	Northern Bobwhite (<i>Colinus virginianus</i>)
Age at Dosing:	48 weeks
Source:	Trace Pheasantry, Inc. Douglassville, PA
Acclimatisation period:	21 weeks to facility and 6 weeks to test caging
Treatment for disease:	Water soluble antibiotics for 8 days during the first 2 weeks of acclimation
Weight at exposure:	187-228 grams.
Feed:	Basal game bird ration (containing at least 27% protein and 3.7% crude fat,

Water:	and no more than 3.5% crude fibre), <i>ad libitum</i> Municipal tap water, <i>ad libitum</i>
Test design	
Test caging:	Wire mesh pen with galvanized sheet sides, approximate 25 x 51 cm floor, approximate 20 to 26 cm height
Replication:	5 birds per level
Duration:	Single dose with 14 day observation period
Environmental conditions	
Temperature:	21.1 °C (20.4-21.9 °C)
Humidity:	72% (58-80%)
Photoperiod:	8 hour light per day (average of 217 lux)

Study Design and Methods

Experimental dates: 19 June 2020 to 3 July 2020

A limit dose of 2000 mg/kg was used in this study. Five northern bobwhite were randomly assigned to each of the control group and test group pens. Based on the results of the limit test no further testing was needed.

Birds were acclimated to the study facility for 21 weeks and to the caging for 6 weeks prior to test initiation. The birds were fasted for approximately 17 hours prior to dosing. At experimental start, a single dose of the test substance was orally administered by gavage into the crop/proventriculus of each bird. Each bird was individually weighed and dosed on the basis of milligrams of test substance per kilogram of body weight (mg/kg). The control birds received a corresponding dose of reverse osmosis deionized water equivalent to the volume of test substance dosing solution that the birds in the 2000 mg/kg dosage level received.

From test initiation until termination, all birds were observed at least twice daily. A record was maintained of all mortality, signs of toxicity, and abnormal behaviour. Body weights were measured on the day of dosing (Day 0) and on Days 3, 7, and 14 of the test. Feed consumption was determined by pen for approximately 24-hour intervals from Day 0 to 1, Day 1 to 2, and Day 2 to 3. Average daily feed consumption was then determined from Days 3 to 7 and from Days 7 to 14.

Results and Discussion

No mortalities were observed. No regurgitation was observed. When compared to the control group, there were no apparent treatment-related effects on body weight at the 2000 mg/kg bw dosage level. When compared to the control group, there were no apparent effects on feed consumption at the 2000 mg/kg bw dosage level.

Results are summarised in the tables that follow.

Table A 1: Acute oral toxicity of A22773A to Northern Bobwhite

Nominal A22773A dose (mg/kg bw)	Toxicological results ^a	Duration of clinical signs	Time of death
0	0/0/5	N/A	N/A
2000	0/0/5	N/A	N/A

^a Number of animals which died/number of animals with clinical signs/number of animals used
N/A: Not applicable

Table A 2: Summary of endpoints

Test item	A22773A
Test object	Northern bobwhite
LD ₅₀	>2000 mg/kg bw
Lowest observed effect level (LOAEL)	Not determined

Highest tested does without adverse toxic effects (NOAEL)	2000 mg/kg bw
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Validity Criteria

The test was considered valid;

- There was no mortality in the control (must be $\leq 10\%$)

Conclusions

The acute oral LD₅₀ value for northern bobwhite exposed to A22773A as a single oral dose was determined to be greater than 2000 mg/kg body weight, the highest dosage tested. The no-mortality level and no-observed-effect level was 2000 mg/kg body weight.

(Hubbard PM, Temple DT, 2020)

A 2.1.1.2 KCP 10.1.1.2 Higher tier data on birds

A 2.1.2 KCP 10.1.2 Effects on terrestrial vertebrates other than birds

A 2.1.2.1 KCP 10.1.2.1 Acute oral toxicity to mammals

Please refer to Section B6 (Toxicology) for study summary.

Reference:	KCP 10.1.2.1 (KCP 7.1.1)
Report	██████████ (2021), Azoxystrobin/Oxathiapiprolin SC (A22773A) - Acute Oral Toxicity Study in Rats (Up and Down Procedure). Report Number: 20/130-001P. xxxxxxxxxxxx., H-8200 Veszprém, Szabadságpuszta, hrsz. 028/1., Hungary. (Syngenta File No. VV-892044)
Guideline:	OECD 425 (2008), EPA 870.1100 (2002)
Deviations:	The bodyweight variation of the animals at dosing was slightly out of the expected range (mean $\pm 20\%$). This deviation has no effect on the outcome of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

A 2.1.2.2 KCP 10.1.2.2 Higher tier data on mammals

Comments of zRMS:	Study not evaluated because it was conducted in Italy.
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Reference:	KCP 10.1.2.2
Report:	Barfknecht, R., (2003), Attractiveness of Tomato fields for herbivorous mammals and birds; Field monitoring in Lombardia. Report Number BAR/FS014. Bayer CropScience AG, Institute for Ecotoxicology, 40789 Monheim, Germany. (Syngenta file No. VV-338885) (Study owner Bayer CropScience AG, Syngenta have access)

Guideline(s):	Pesticides and Wildlife – Field Testing: Recommendations of an international workshop on terrestrial field testing of pesticides, attached to Pesticide Effects on Terrestrial Wildlife, Somerville & Walker (ed.), Taylor and Francis, London 1990.
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Executive summary

Bird and small mammal species and their abundance in tomato fields compared to adjacent non-crop natural habitats were studied within and around tomato fields in the region of Lombardia, Italy, in June to July 2002. The composition of vegetation and the availability of food for herbivorous birds and mammals were assessed on randomly assigned plots within and in the vicinity of the study fields. The portion of time which selected small mammal species spent actively in tomato fields was monitored using radio-tracking and visual observations. Snap-traps were used to capture wood mice (*Apodemus sylvaticus*) and analysis of their stomach contents was carried out to determine diet composition of these species living in-crop (tomato fields) and in adjacent non-crop habitats. Observations of the bird population were carried at each study field and its vicinity. Observations of medium and large sized mammals were also recorded.

The species caught in these vineyards were predominately wood mouse (*Apodemus sylvaticus*). The analysis of the wood mouse stomach samples showed omnivorous diets. Observations of medium and large sized mammals in the tomato fields included domestic cat, fox, weasel, hedgehog, brown hare and water vole.

The abundance and diversity of birds on the tomato fields were very low (5 %) compared to the surrounding habitats (95%), with the main species being Yellow Wagtail (strictly insectivore) and the Tree- and House Sparrow, both of which feed on the insects within this habitat. Only the Hooded Crow was observed to feed on the inner parts of tomato fruits.

Materials

Test Material	Not applicable for generic field study
Test design	
Test site:	Commercially used tomato fields (3.7 to 7 ha). All growing areas had a stripe of bare soil with a sparse herb and grass layer of 4 to 20 m width along at least two sides. In the majority of cases, this stripe was followed by a grass stripe and a watery or dry ditch.
Location:	Tomato fields in the Province of Lodi in Lombardia, Italy.
Replication:	4. Fields 2 and 4 were near Codogno and fields 1 and 3 were near S Fiorano.
Vegetation coverage:	Field 1: 50 to 90 % Field 2: 8 to 25 % Fields 3 and 4: In between fields 1 and 2
Vegetation stage:	Field 1: tomatoes were ripening and at the end of the study the first red fruits were developed. Partly the branches were pulled under by ripening fruits. Field 2: tomato plants were much younger and the vegetation stage was blooming. Fields 3 and 4: In-between fields 1 and 2.
Bird Monitoring	
Study area:	Whole study field and a stripe of approximately 50 to 100 m around the field
Endpoints:	Species inventory, species abundance (semi quantitatively), bird censuses (number of birds and their position on a map of the area surveyed) and feeding behaviour
Frequency of counts:	Morning or evening (3 counts per field) Whole day counts were performed on two different days per field
Duration:	2 hours for each census

	Hourly and during whole daylight period
Live-trapping test	
Species:	The test organisms were the naturally occurring populations of small mammals at the test site: Wood mouse (<i>Apodemus sylvaticus</i>) Savi's pine vole (<i>Microtus savii</i>)
Type of trap:	Ugglan, Sweden multiple capture live traps, baited with oats
Location of traps:	Only fields 1, 3 and 4 were used for live trapping
Number of traps per plot:	25 (arranged in a H shape)
Duration of trapping session:	Activated in the evening of a trapping session and deactivated the following morning. Active traps were checked around midnight.
Radio-tracked test	
Organisms:	Adult animals recaptured in the vicinity (or inside) the tomato fields if body weight was greater than 20 g. Wood mouse (<i>Apodemus sylvaticus</i>) Savi's pine vole (<i>Microtus savii</i>)
Number of individuals:	During each session, up to four individuals were tracked
Observations:	The position of each individual was recorded every 15 to 30 minutes
Duration:	At least twelve 24-hour observations on different individuals (and species) of small mammals
Radio label:	Radio collars (Biotrack, UK)
Snap-trapping test	
Species:	The test organisms were the naturally occurring populations of small mammals at the test site: Wood mouse (<i>Apodemus sylvaticus</i>)
Number of individuals:	17 wood mice
Type of trap:	Snap-trap
Location:	The four study fields as far away as possible from the live traps
Duration:	Snap-trapping stopped when animals on a field were equipped with radio collars and radio-tracking started.
Environmental test conditions	
Temperature:	Mean daily temperature at noon in Codogno: 26.9 °C
Precipitation:	Calculated rainfall per month: 17.3 mm
Sequence of cultures:	Tomatoes every 4 to 5 years for 2 years (in between maize, soya bean, peas, beets and beans)
Treatment before planting:	Ploughing, fertilisation
Key dates:	Planting: April to 10 th June Flowering: 1 month after planting until harvest Harvesting: 25 th July to beginning of September
Plant height:	Before flowering: 20 cm At time of harvesting: 50 cm Maximum height: 120 cm
Plant spacing:	Plant distance: 20 or 22 cm Row distance: 150 cm Plants per hectare: 30000 or 33000 (depending on plant distance)
Soil coverage:	First month: 25 % Second month: 50 % Third month (before harvest): 95 to 100 %
Irrigation:	30 to 35 mm every 13 days
Treatments:	Nitrification, fungicide, insecticide, hoeing and weeding approximately 12 -15 days after planting. Herbicide treatment 10 and 20 days after planting. Treatments are repeated every 12 to 14 days, if necessary additionally full cover application of copper and sulphur; for insecticide only one or two treatments.
Harvesting technique:	Double-photoelectric cell machine
Fate of plant material after harvest:	Plant material is left on the field and incorporated into the soil.

Study Design and Methods

Field phase experimental dates: 18 June 2002 to 08 July 2002

Aim: To identify those wild small mammal and bird species that use tomato fields in the growing period when pesticide application is principally performed, to determine their habitat use by observation, live trapping and radio-tracking and additionally to estimate the diet composition of these species.

Study area: The study was conducted in four tomato fields in the Province of Lodi in the Lombardi region of Italy, selected to represent a common size and basic structure of the tomato fields in the region. The region is typical of tomato growing areas in Italy, with the agrarian landscape characterised by different fields, hedges, trees and farm building, with a watering system of canals and ditches. The soil of all fields was a middle mixture of clay, silt and sand.

The availability of vegetable food was low. The mean total vegetation coverage in the fields was nearly 50 %, with tomato plants contributing to more than 97 % of the coverage. Other plants identified were mainly *Sorghum halepense*, *Poa* spp. and *Solanum nigrum*.

Tomato plants did not grow outside the growing area. The total coverage of the surrounding vegetation, excluding the stripe of bare soil with a sparse herb and grass layer, was much higher than on the fields (mean of all plots: approximately 66 % of the soil). Approximately 52 % of the plants were vegetative, with 14 % blooming and 0.32 % yielding fruits or seeds.

Method and parameters:

Medium sized and larger mammals

Data were recorded by direct observation around sun rise irregularly made by the mammal team or during bird census or bird observation by the bird team. Additional to observations, the teams searched for tracks, feeding signs or faeces.

Bird monitoring

Bird censuses were performed in the morning or in the evening on the fields and in the surroundings, with a special focus on herbivorous birds. The number of birds as well as their position on a map of the area surveyed was recorded. Species only flying over were recorded qualitatively. The species were summarised according to their feeding behaviour; omnivorous/herbivorous or definitely not herbivorous.

Small Mammal Monitoring

Small mammals were caught, individually marked and released. Twelve animals were equipped with radio collars and tracked for at least 24 hours. Analysis of stomach contents from wood mice caught with snap-traps were used for analysis of diet composition.

Data analysis:

Bird monitoring

The number of individuals and foraging individuals per species were summarised for each day. The datasets were normalised on one observation interval and one ha field area to provide an abundance index value.

Small Mammals Monitoring

Radio-telemetry was used to determine the temporal (PT) and spatial use pattern of small mammals. PT data were analysed by noting the type of habitat of an animal's location in the field, computing a Minimum Convex Polygon of the home range and calculating kernel contours based on estimates of the density indices. Jacobs index was used to assess the habitat preferences within a given home range, where +1 indicates highest preference and -1 complete avoidance.

The speed of each radio-tracked animal was calculated, with all movements within one habitat averaged to an individual mean speed in habitat and movements between two habitat types were regarded as 'changing habitat' speed.

Results and Discussion

Traces or observations of domestic cat (*Felis silvestris f. catus*), fox (*Vulpes vulpes*), weasel (*Mustela nivalis*), hedgehog (*Erinaceus europaeus*), brown hare (*Lepus europaeus*), water vole (*Arvicola terrestris*) and wood mouse (*Apodemus sylvaticus*) were found in the tomato fields and the river rat (*Myocastor coypus*) was observed in the ditches surrounding the fields.

The vegetation constitution of the tomato fields and surrounding habitats are shown in the table below.

Table A 3: Vegetation monitoring

	Mean total coverage of soil (%)	
Tomato fields	Tomatoes	48.4
	Other species	1.5
Surrounding	Tomatoes	0.0
	Other species	66.0

Live-trapping

Only two species, the wood mouse (*Apodemus sylvaticus*) and Savi's pine vole (*Microtus savii*), were found in the traps. The voles were only caught at the borders of the tomato fields, with wood mice found in both the fields and the surrounding habitats.

Radio-tracking

A total of eight wood mice and four Savi's pine voles were radio tracked and the average speed of the movement is shown in the table below.

Table A 4: Number of radio-tracked small mammals and speed of movement in different habitats

Field/ Species	Number of radio tracked small mammals per field				Mean speed of movement in different habitats (m/h)		
	1	2	3	4	Tomato	Surrounding	Changing habitat
Wood mouse	2	-	1	5	29.68	10.38	50.95
Savi's pine vole	-	-	4	-	-	13.19	23.56

The table below shows the percentage of tomato fields of total home range, through MCP and Kernel calculations of habitat content of individual home ranges and preference scores.

Table A 5: Percentage tomato fields of total home range and preference of tomato fields

Species	Home range (%)		Jacobs Index ¹	
	MCP	Kernel	MCP	Kernel
Wood mouse	86.30	72.90	0.04	0.44
	2.3 – 100.0	11.8 – 100.0	-0.84 - +1	-0.89 - +1
Savi's pine vole	0	0	-	-1

¹ -1 = avoidance; +1 = preference

Snap-trapping

Of the 17 trapped wood mice, the stomach content of six included invertebrate animals, nine included seeds and starch and five included green plants and fruits. Stomachs could contain more than one component (see table below).

Table A 6: Stomach content of snap trapped wood mice

Item	Stomach content	
	Number of individuals	Percent
Invertebrates	6	35
Seeds and starch	9	53
Green plants and fruits	5	29

Bird monitoring

During the bird censuses, 10 bird species were observed on the fields, in the surroundings or flying over the fields during all the study activities, with seven of these species being observed only sporadically or singly. Thirty-eight species were observed in the surrounding areas, with three species only observed to be flying over. The lowest number were found around field 3 (24 species) and the maximum number on field 2 (30 species). The main species on the fields were Yellow Wagtail, the Tree and the House Sparrow on all fields and, in much lower abundance, the Hooded Crow and the Stonechat on only one field each. Apart from the two minor species, the general result was the same on all four study fields.

During morning and evening counts, five species were observed on the fields and 30 species occurred in the surroundings. Whole day observations showed a clear time-dependence of occurrence of birds in the fields. A first peak was found in the third hour of daylight and a second peak was observed three hours before sunset.

The relative abundance of birds in tomato fields and surrounding habitats was 5% and 95% respectively, based on the percentage of observations after 12 counts on four fields. The mean absolute number of bird observations per census was 97.08 in the surroundings and 5.58 on the field, although the size of the covered areas of the fields and surroundings were comparable. The absolute abundance of birds in tomato fields was 0.82 individuals per hectare, based on the mean of 128 counts during the whole daylight period on four fields.

Sixteen of the species were omnivore, with potential uptake of greenery, but none of the species were exclusively herbivore. The mean total number of 1.67 (30 %) observations per count on the fields belonged to not herbivorous species, with 3.92 (70 %) to omnivorous ones. Beyond the field borders, omnivorous species were much more dominant (87 % of the mean total number of observations).

Observations of the feeding behaviour of birds on the tomato fields based, on 49 observations are shown in the table below.

Table A 7: Observations of feeding behaviour

Item	Observations	
	Number	Percent
Insects	36	73.5
Unknown items	3	6.1
Tomato fruits	10 ¹	20.4
Tomato plants	0	0.0

¹ Hooded Crow only

Validity criteria

Not applicable as non-standard guideline followed.

Conclusion

Tomato fields are intensively cultivated areas, with approximately 50% vegetation coverage. The majority of the coverage is cultivated tomatoes, with a minor amount of weeds. The abundance and diversity of birds on the tomato fields were very low compared to the surrounding habitats, with the main species being Yellow Wagtail and the Tree- and House Sparrow, both of which feed on the insects within this habitat. Only the Hooded Crow was observed to feed on the inner parts of tomato fruits.

Seven mammal species were observed on the tomato fields, two only occasionally, with two other species

found in the surrounding habitat only. Only the wood mouse had its home range in the tomato fields, and showed a slight preference for this type of habitat when compared to the surroundings. The average speed of wood mice revealed a higher speed inside tomato fields than surrounding habitats, but with changing habitat speeds being even higher. This may indicate that wood mice in tomato fields were searching for rare food items and not feeding on the green parts of tomato plants. Stomach analysis showed that wood mice are omnivorous mammals, feeding on insects and plant material, with a preference for fruits and seeds compared to green material.

(Barfknecht R, 2003)

Comments of zRMS:	<p>The study is considered to be acceptable for regulatory use.</p> <p>The mean crop cover for leafy vegetables in Germany was 73 ± 20 % during early growth stages (BBCH 41 – 45) and 91 ± 6 % during late growth stages (BBCH 46 – 49).</p> <p>The results of the study confirm the correctness of the assumptions used in the higher tier risk assessment for mammals.</p>
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Reference:	KCP 10.1.2.2
Report:	Münderle, M., Carlin, B., Nickisch, D., Ludwigs, J.-D., (2020) - GLP-compliant field study to measure crop coverage in leafy vegetable fields via drone image analysis. Report Number R1940003. RIFCON GmbH, Goldbeckstraße 13, 69493 Hirschberg, Germany. (Syngenta file No. VV-867392)
Guideline(s):	Not applicable
Deviations:	Not applicable
GLP:	Yes
Acceptability:	Yes
Duplication: (if vertebrate study)	No

Executive Summary

The aim of this study was to determine crop coverage values for representative leafy vegetable crops (cabbage, broccoli and cauliflower) during BBCH growth stages 41 – 49 using drone analysis. Digital aerial photographs were taken at two different time points (early and late growth stages) in Northern Germany (Lower Saxony and Schleswig-Holstein) and in Southern Germany (Bavaria and Rhineland-Palatinate) between June and September 2019. Image files were analysed with RifPic v1.0 to determine the crop coverage by the proportion of green parts per investigated area.

The mean crop cover for leafy vegetables in Germany was 73 ± 20 % during early growth stages (BBCH 41 – 45) and 91 ± 6 % during late growth stages (BBCH 46 – 49). Overall, the mean crop cover of all leafy vegetable fields during BBCH growth stages 41 – 49 in Germany was 81 ± 18 %. The data supports the interception of 70 % for BBCH >40 upwards in the EFSA, 2014⁶ guidance as a conservative estimate and provides evidence that assuming no crop interception for leafy vegetables at BBCH 41 – 49 as per the EFSA/2009/1438² guidance is overly conservative.

Materials

Test material	Not applicable
Test organisms	
Species:	Red and white cabbage, cauliflower and broccoli
Test design	
Test site:	Northern Germany (Lower Saxony and Schleswig-Holstein) and Southern Germany (Bavaria and Rhineland-Palatinate)
Number of study sites:	Cabbage: 35 (18 in Northern Germany, 17 in Southern Germany), of which 26 were white cabbage and 9 were red cabbage Cauliflower: 40 (22 in Northern Germany, 18 in Southern Germany) Broccoli: 17 (7 in Northern Germany, 10 in Southern Germany)
Planting distance within the same row:	Cabbage: 40 ± 3 cm (range 30 – 50 cm) in Northern Germany, 46 ± 10 cm (range 30 – 60 cm) in Southern Germany Cauliflower and broccoli: 37 ± 5 cm (range 30 – 40 cm) in Northern Germany, 40 ± 7 cm (range 30 – 50 cm) in Southern Germany

Planting distance between rows:	Cabbage: 48 ± 4 cm (range 40 – 50 cm) in Northern Germany, 49 ± 4 cm (range 40 – 60 cm) in Southern Germany Cauliflower and broccoli: 40 cm in Northern Germany, 49 ± 9 cm (range 40 – 60 cm) in Southern Germany
Observation sessions:	Cabbage: Once in both early and late growth stages Cauliflower and broccoli: Once in either the early or late growth stages
Replication:	10 drone images taken per study sites, per session

Study Design and Methods

Experimental dates: 19 June 2019 to 21 September 2019

The study was conducted at four different study sites in Northern Germany and Southern Germany to cover typical cultivation areas of leafy vegetables. Aerial photographs were taken within BBCH growth stages 41 – 49 at two different time points (early and late). The early time was defined as BBCH growth stages 41 – 45 (i.e. heads begin to form until 45 – 50 % of the expected head size reached) and the late time was defined from BBCH growth stages 46 – 49 (i.e. until typical size, form and firmness of heads reached). Study fields were usually investigated in one photo session in each of the early and late growth stages for cabbage and in either the early or late growth stage for cauliflower and broccoli. The conditions of the crops were good during all surveyed BBCH growth stages.

Digital photographs of the study fields were taken using a camera-carrying drone DJI Mavic Pro II with vision positioning and GPS positioning. The accuracy of the positioning was < 1.5 m. The standard resolution of the photographs was 5472 x 3648 pixels. Ten drone images per study field were taken and were defined as one photo session. The position of each drone image was randomised to avoid human bias by approaching a selected row. The drone was then moved along the row and randomly stopped without analysing the point of interest regarding weed pressure, reduced plant growth, etc. Crop specific agronomic data, flight altitude of the drone, condition of crop, variety of crop and colour variability of crop leaves were recorded.

JPEG files were analysed with RifPic v1.0 to determine the proportion of green parts in the photographs with a pixel colour-based algorithm. Mean coverage values (\pm standard deviation of the mean), ranges (minimum and maximum values) and quantiles (2.5 %, 5.0 %, 95.0 % and 97.5 %) were calculated for each site, crop and growth stage.

Results and Discussion

Summaries of the crop cover of representative leafy vegetables in Germany determined by drone analysis are shown in the tables below.

Table A 8: Percentages of crop cover of cabbage, cauliflower and broccoli fields in Northern and Southern Germany during early and late growth stages

Crop	Site	Growth stage	Number of study fields	Number of drone files ¹	Percentage of crop cover			
					Minimum	Maximum	Average	SD
Cabbage	Northern Germany	Early	15	150	71	100	93	6
		Late	17	180	68	98	92	6
	Southern Germany	Early	16	170	21	90	57	18
		Late	16	160	76	99	92	4
Cauliflower and broccoli	Northern Germany	Early	17	170	59	97	80	10
		Late	12	120	75	97	90	5
	Southern Germany	Early	15	150	21	98	63	20
		Late	13	130	73	100	86	6

SD: Standard deviation

¹ Two photo sessions sometimes conducted in one study field during one growth stage

Table A 9: Quantiles of percentages of crop cover of cabbage, cauliflower and broccoli fields in Northern and Southern Germany during early and late growth stages

Crop	Site	Growth stage	Quantiles			
			2.5%	5.0%	95.0%	97.5%
Cabbage	Northern Germany	Early	78.0	80.0	100.0	100.0
		Late	77.0	78.0	97.0	98.0
	Southern Germany	Early	23.2	27.0	87.6	89.0
		Late	83.0	87.0	99.0	99.0
Cauliflower and broccoli	Northern Germany	Early	61.2	63.0	95.6	97.0
		Late	78.0	79.0	95.0	95.0
	Southern Germany	Early	25.0	26.0	92.0	93.0
		Late	76.0	77.5	96.0	97.8

Table A 10: Crop coverage during different BBCH crop growth stages

BBCH crop growth stage	Number of study fields	Number of drone files	Percentage of crop cover		
			Average	SD	Median
41 – 45 (early)	63	640	73	20	78
46 – 49 (late)	58	590	91	6	92
41 – 49 (overall)	121	1230	81	18	88

The study shows that the mean crop coverage value for all leafy vegetables is > 80 % and close to 100 % within BBCH growth stage 46 – 49. The data supports the interception of 70 % for BBCH growth stage > 40 upwards in the EFSA 2014 guidance as a conservative estimate and provides evidence that assuming no crop interception for leafy vegetables at BBCH growth stage 41 – 49 as per the EFSA 2009 guidance is overly conservative.

The study fields generally had very low weed pressure and visible weeds were present in a few files only (approximately 10 out 1200 image files). The inclusion of weeds in the analysis is not expected to have any notable impact on the crop coverage values. The image analysis also includes crop gaps where no crop was present (e.g. due to malfunction during drilling/ planting seedlings), therefore the coverage values can be considered a worst-case estimate.

Conclusion

The mean crop cover for leafy vegetables in Germany was 73 ± 20 % during early growth stages (BBCH 41 – 45) and 91 ± 6 % during late growth stages (BBCH 46 – 49). Overall, the mean crop cover of all leafy vegetable fields during BBCH growth stages 41 – 49 in Germany was 81 ± 18 %. The data supports the interception of 70 % for BBCH > 40 upwards in the EFSA 2014 guidance as a conservative estimate and provides evidence that assuming no crop interception for leafy vegetables at BBCH 41 – 49 as per the EFSA 2009 guidance is overly conservative.

(Münderle, M. *et al.* 2020)

Comments of zRMS:	Study not evaluated because it was conducted in Italy.
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Reference:	KCP 10.1.2.2
Report:	Sainz-Elise S & Hahne J, (2014), Generic field study on the attractiveness of tomato fields for Savi's pine voles in Italy. Report Number M489745-01-1. Tier3 Solutions GmbH, Leverkusen, Germany. (Syngenta file No. VV-410659) (Study owner Bayer CropScience AG, Syngenta have access)
Guideline(s):	No official test guideline(s) available at present. The study was conducted under consideration of the EFSA Guidance Document on Risk Assessment for Birds & Mammals (EFSA 2009).
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication: (if vertebrate study)	No

Executive summary

The aim of this generic study was to determine the attractiveness of commercially managed tomato fields for Savi's pine voles (*Microtus savii*). The study was conducted in the Province of Lodi/Lombardy as well as in the Province of Piacenza/ Emilia-Romagna, Italy. Live trapping of small mammals was conducted both in-crop and off-field to determine which small mammals use tomato fields. Savi's pine vole was the most frequently trapped small mammal in off-field habitats with captures per trap night more than twice that of the next most frequently captured species, the wood mouse (*Apodemus sylvaticus*). However, Savi's pine voles were never caught in-field and here the wood mouse was most frequently trapped small mammal.

Study Design and Methods

Experimental dates: 5 June 2013 to 14 July 2013.

The study was conducted in the Province of Lodi/Lombardy and in the Province of Piacenza/ Emilia-Romagna, Italy on tomato fields. Altogether 14 study fields were selected to be representative for commercially managed tomato fields, the potential presence of the focal species and the suitability for the study conduct (e.g. accessibility of fields).

Live trappings of small mammals with individual marking and subsequent recaptures were conducted in tomato fields and in the adjacent off-crop habitats in the period between early June until mid of July, corresponding to growth stages of tomato plants between BBCH 13 and BBCH 77. For regular trappings at each study site live traps were distributed in a regular trapping grid with 53 traps. Two rows of each trapping grid, comprising 15 traps, were set in the adjacent off-crop area, whilst the other 38 traps were distributed within the tomato fields. Additional sets of 20 traps each were installed and frequently moved between fields in order to increase trapping success. Animals which were trapped for the first time were individually marked by means of transponders. For each captured individual the following information was taken: date, location (position in study field and trap identity; i.e. number of trap and row), species, ID of PIT (if applied), first capture or recapture, sex, reproductive state and body weight.

Results

During 8,591 trap nights altogether 395 captures of small mammals were made. Six species were trapped during the study period: the black rat (*Rattus rattus*), the house mouse (*Mus musculus*), the wood mouse (*Apodemus sylvaticus*), the Savi's pine vole (*Microtus savii*), the Eurasian harvest mouse (*Micromys*

minutus) and the bicoloured white-toothed shrew (*Crocidura leucodon*). The wood mouse was the most trapped species (105 individuals) followed by *M. savii* (92 individuals). The highest trapping success, however, was obtained for Savi's pine vole in traps set up in the adjacent off-crop habitats (7.54 captures per 100 trap nights; see table below). This species was trapped in 13 of 14 study sites and not a single *M. savii* was recorded inside the tomato fields in the course of the study i.e. all *M. savii* were trapped in the off-crop habitats adjacent to the tomato fields.

Table A 11: Small mammal trapping in and adjacent to tomato fields

Species	Standardised trapping success [captures/100 trap nights]		
	Tomato field	Adjacent off-crop habitats	TOTAL
<i>Microtus savii</i>	0.00	7.54	1.80
<i>Apodemus sylvaticus</i>	2.16	3.12	2.39
<i>Mus musculus</i>	0.09	0.34	0.15
<i>Rattus rattus</i>	0.00	0.05	0.01
<i>Micromys minutus</i>	0.12	0.10	0.12
<i>Crocidura leucodon</i>	0.00	0.44	0.10
<i>Sorex sp.</i>	0.00	0.10	0.02

Conclusion

In the current study it could be shown that Savi's pine vole was absent on tomato fields. Savi's pine voles were frequently trapped in the off-crop habitats adjacent to tomato fields but not inside the target crop indicating that tomato fields are not a relevant feeding habitat for this species. Not a single *M. savii* was trapped inside of tomato field irrespective of developing growth stages of tomato plants (BBCH stages).

(Sainz-Eliphe S & Haehne J, 2014)

Comments of zRMS:	Five trials on young barley plants under field conditions were conducted in Germany, Northern France and the United Kingdom. The application rate was 0.25 kg a.s./ha. The samplings were carried out at 0, then 1, 2, 4-5, 6, 8-9 and 13-15 days after the application. The sampling schedule gave 7 data points for each trial, which is sufficient to perform the reliable kinetic analysis. There was no rainfall within 24 hours of the application being made except trial B7306 UK1 where 0.5 mm fell on the day after the application. The study is considered to be reliable and suitable for the risk assessment.
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Reference:	KCP 10.1.2.2
Report:	Ertus C, (2018), Azoxystrobin – Foliage Residue Decline Study on Winter Barley in Northern Europe in 2017. Report Number B7306. Anadiag, 16 rue Ampère, 67500 Haguenau, France. (Syngenta file No. VV-469438)
Guideline(s):	Commission of the European Communities, General Recommendations for the Design, Preparation and Realization of Residue Trials; 7029/VI/95 (rev. 5, working document). OECD Guidelines for the Testing of Chemicals – Crop Field Trial, No. 509 (2009) OECD Guidance Document on Crop Field Trials, Series on Pesticides No. 66 and Series on Testing and Assessment No. 164, ENV/JM/MONO(2011)50.
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication: (if vertebrate study)	No

Executive summary

Five residue decline field trials on winter barley were conducted in Germany, Northern France and the United Kingdom during 2017. Each trial consisted of a single plot, except in trial B7306 UK1 where a control plot was used to generate the untreated samples. No control plot was created within each trial however the first sampling was done before application, which served as a control sample (except for trial B7306 UK1).

To plot P1, azoxystrobin was applied to winter barley as A12705B, a flowable concentrate (SC) formulation containing 250 g ai azoxystrobin per litre. One application was made at 250 g ai/ha for azoxystrobin at growth stage BBCH 13-24.

Following the application, treated barley whole plant samples were collected at 0, 1, 2, 4-5, 6, 8-9 and 13-15 days after application (DAA), with untreated barley whole plant specimens being collected from within the trial plot area at 0 day before application (0 DBA).

Samples were analysed for azoxystrobin as the analytes (azoxystrobin and R230310).

The study design as detailed above was successfully carried out leading to the following conclusions:

Residues of azoxystrobin in treated winter barley whole plant samples taken at 0 day after application (DAA) were in the range 8.24 mg/kg to 19.71 mg/kg, at 1 DAA were in the range 6.68 mg/kg to 21.98

mg/kg, at 2 DAA were in the range 4.32 mg/kg to 15.92 mg/kg, at 4-5 DAA were in the range 2.25 mg/kg to 8.97 mg/kg, at 6 DAA were in the range 2.41 mg/kg to 9.62 mg/kg, at 8-9 DAA were in the range 1.01 mg/kg to 6.69 mg/kg and at 13-15 DAA were in the range 0.43 mg/kg to 1.26 mg/kg.

Residues of R230310 in treated winter barley whole plant samples taken at 0 day after application (DAA) were in the range 0.02 mg/kg to 0.05 mg/kg, at 1 DAA were in the range 0.03 mg/kg to 0.07 mg/kg, at 2 DAA were in the range 0.03 mg/kg to 0.18 mg/kg, at 4-5 DAA were in the range 0.02 mg/kg to 0.18 mg/kg, at 6 DAA were in the range 0.03 mg/kg to 0.23 mg/kg, at 8-9 DAA were in the range 0.01 mg/kg to 0.15 mg/kg and at 13-15 DAA were in the range the limit of quantification (0.01 mg/kg) to 0.03 mg/kg.

Residues of azoxystrobin and R230310 in untreated winter barley whole plant samples taken just before application (0 DBA) were below the limit of quantification (0.01 mg/kg).

Materials

Test system:	The following test system is representative of the crop group required for product registration. Winter barley (<i>Hordeum vulgare</i>) EPPO - Code: HORVW	
Test Item(s):	Formulation – Company Code	A12705B
	Formulation Content and Type	250 SC
	Batch No.	GRA5J052A/1
	Valid until:	Nov 2018
	Active ingredient	azoxystrobin
	Nominal Content in Formulation (nominal)	250 g/L
	Actual Content in Formulation (actual)	248 g/L
	Stability	The test item is assumed to be stable for the period of use in the study, pending concurrent batch re-analysis
Treatments:	Test rates:	Trial Number B7306 BW1 (01): Nominal 250 g azoxystrobin/ha (actual*: 247 g azoxystrobin/ha) Trial Number B7306 BW2 (02): Nominal 250 g azoxystrobin/ha (actual*: 253 g azoxystrobin/ha) Trial Number B7306 MA1 (03): Nominal 250 g azoxystrobin/ha (actual*: 269 g azoxystrobin/ha) Trial Number B7306 ND1 (04): Nominal 250 g azoxystrobin/ha (actual*: 247 g azoxystrobin/ha) Trial Number B7306 UK1 (05): Nominal 250 g azoxystrobin/ha (actual*: 239 g azoxystrobin/ha)
	Control:	Untreated plant specimens were collected from the trial plots prior to test item application.
	Application method:	Foliar with boom sprayer
	Pesticides, fertiliser, irrigation:	01: None 02: None 03: None (except one molluscicide against slugs) 04: None 05: None
	Locations:	01: Baden-Württemberg, Germany (Application: 18 Oct 2017; final plant specimen collection: 31 Oct 2017) 02: Baden-Württemberg, Germany (Application: 11 Oct 2017; final plant specimen collection: 25 Oct 2017) 03: Grand Est, France (Application: 11 Oct 2017;

		<p>final plant specimen collection: 25 Oct 2017)</p> <p>04: Hauts-de-France, France (Application: 26 Sep 2017; final plant specimen collection: 11 Oct 2017)</p> <p>05: Oxfordshire, UK (Application: 25 Oct 2017; final plant specimen collection: 08 Nov 2017)</p>
Test organisms	Species:	<p>01: Winter barley <i>Hordeum vulgare</i> var. California</p> <p>02: Winter barley <i>Hordeum vulgare</i> var. Sandra</p> <p>03: Winter barley <i>Hordeum vulgare</i> var. Etincel</p> <p>04: Winter barley <i>Hordeum vulgare</i> var. Etincel</p> <p>05: Winter barley <i>Hordeum vulgare</i> var. Talisman</p>
Test design	Plot size:	<p>01: 25.0 m x 9 m</p> <p>02: 25.0 m x 9 m</p> <p>03: 25.0 m x 6 m</p> <p>04: 40.0 m x 3 m</p> <p>05: 40.0 m x 3 m</p>
	Test soil:	<p>01 : Sandy clay loam</p> <p>02 : Clay loam</p> <p>03 : Clay</p> <p>04 : Silt loam</p> <p>05: Clay loam</p>
	Replication:	One field plot per trial except for trial B7306 UK1, 2 plots (one control plot and one treated plot).
	Sampling interval :	<p>Whole plant samples were collected at 0 (< 2 hrs), 1, 2, 4-5, 6, 8-9 and 13-15 days after application. Untreated control samples were collected at 0 day before application.</p> <p>All samples were analysed for residues of azoxystrobin and its metabolite R230310.</p>
	Duration of test:	13 – 15 days after application
Environmental test conditions (between application and last sample collection)	Temperatures – average minimum to average maximum:	<p>01: Range 5.8 – 16.3 °C</p> <p>02: Range 4.7 – 20.0 °C</p> <p>03: Range 8.5 – 19.2 °C</p> <p>04: Range 9.4 – 18.7 °C</p> <p>05: Range 2.7 – 12.6 °C</p>
	Humidity (average minimum to average maximum):	<p>01: Range 60.0 – 98.0 % RH</p> <p>02: Range 56.0 – 95.4 % RH</p> <p>03: Range 68.0 – 95.7 % RH</p> <p>04: Range 71.7 – 99.9 % RH</p> <p>05: Range 82.1 – 93.1 % RH</p>
	pH of soil:	<p>Trial site 01: 7.2</p> <p>Trial site 02: 7.1</p> <p>Trial site 03: 8.0</p> <p>Trial site 04: 7.0</p> <p>Trial site 05: 6.7</p>
	Total precipitation:	<p>01: 13 mm</p> <p>02: 8 mm</p> <p>03: 9 mm</p> <p>04: 16 mm</p> <p>05: 20.4 mm</p>
	Photoperiod:	<p>Plants were in field plots and exposed to natural sunlight. Total solar energy for each trial were:</p> <p>01: not recorded</p> <p>02: not recorded</p> <p>03: not recorded</p> <p>04: not recorded</p> <p>05: 888.7 W/m²</p>
*For all given test rates, the actual application rate is based on the nominal formulation concentrations		

Study Design and Methods

Five residue field trials on winter barley were conducted in Germany, Northern France and the United Kingdom in 2017.

Experimental dates:

26 Sep 2017 to 08 Nov 2017 (field phase period)

29 Aug 2017 to 11 Jan 2018 (study initiation to experimental completion)

Plants of four varieties of winter barley (*Hordeum vulgare*) each received a single foliar application of azoxystrobin, as A12705B, at a nominal concentration of 250 g azoxystrobin/ha and at growth stage BBCH 13-24. No pesticides or fertilisers were used on the test fields after application of the test item. Prior to application, no pesticides and/or fertilisers were used on trial plots except one product against slugs on the trial site B7306 MA1.

Details of the application of azoxystrobin as formulation A12705B to barley in trials B7306 BW1, BW2, MA1, ND1 and UK1 are summarised in Table 1.

Table A 12: Treatment details for Trials B7306 BW1, B7306 BW2, B7306 MA1, B7306 ND1, B7306 UK1

Trial. B7306	Applications	Application date(s)	Formulation Code	Product rate (L/ha)	Actual spray volume (L/ha)	Growth stage at application (BBCH)	AI application rate (g azoxystrobin/ha)	
							Actual	Target
BW1	1	18 Oct 2017	A12705B	0.988	247	13	247	250
BW2	1	11 Oct 2017	A12705B	1.012	253	13-21	253	250
MA1	1	11 Oct 2017	A12705B	1.076	269	13-21	269	250
ND1	1	26 Sep 2017	A12705B	1.012	247	13-20	247	250
UK1	1	25 Oct 2017	A12705B	0.956	239	20-24	239	250

There was no rainfall within 24 hours of the application being made except maybe for trial B7306 UK1 where 0.5 mm fell on the day after the application but not whilst on site.

Selection of samples to be analysed and shipment:

Following the application, treated barley whole plant samples were collected at 0 (<2 hours), 1, 2, 4-5, 6, 8-9 and 13-15 days after application (DAA), with untreated barley whole plant samples being collected from within the trial plot area at 0 day before application (0 DBA).

Samples were kept deep frozen at or below -18 °C during transport and storage prior to analysis.

Residue analysis

The analytical phase was conducted at the Anadiag facility located in France using method RAM 305/03. The Limit of Quantification required was 0.01 mg/kg for azoxystrobin and its metabolite R230310.

Results

Table A 13: Results of Analysis of Field Trial Samples

Number and Nominal Rate of Application (g ai/ha)	Sampling Interval (hours or days)	Crop Part	Azoxystrobin Residue (mg/kg)				
			Trial B7306 BW1	Trial B7306 BW2	Trial B7306 MA1	Trial B7306 ND1	Trial B7306 UK1
1 x 250	<2 hours after application	Whole plant	12.00	8.80	19.71	8.24	17.39
1 x 250	1 DAA	Whole plant	9.07	8.39	21.98	6.68	9.29
1 x 250	2 DAA	Whole plant	7.85	6.61	15.92	4.32	11.77
1 x 250	4-5 DAA	Whole plant	5.30	3.56	8.97	2.25	8.94
1 x 250	6 DAA	Whole plant	3.57	2.77	9.62	2.41	8.18
1 x 250	8-9 DAA	Whole plant	1.39	2.16	5.47	1.01	6.69
1 x 250	13-15 DAA	Whole plant	0.70	0.74	0.50	0.43	1.26
Control	<2 hours before	Whole plant	<0.01	<0.01	<0.01	<0.01	<0.01

No correction of results for either control residues or recovery values has been performed.

Table A 14: Results of Analysis of Field Trial Samples

Number and Nominal Rate of Application (g ai/ha)	Sampling Interval (hours or days)	Crop Part	R230310 Residue (mg/kg)				
			Trial B7306 BW1	Trial B7306 BW2	Trial B7306 MA1	Trial B7306 ND1	Trial B7306 UK1
1 x 250	<2 hours after application	Whole plant	0.02	0.02	0.05	0.02	0.04
1 x 250	1 DAA	Whole plant	0.06	0.05	0.07	0.03	0.04
1 x 250	2 DAA	Whole plant	0.05	0.07	0.18	0.03	0.10
1 x 250	4-5 DAA	Whole plant	0.04	0.07	0.18	0.02	0.14
1 x 250	6 DAA	Whole plant	0.04	0.07	0.23	0.03	0.13
1 x 250	8-9 DAA	Whole plant	0.04	0.06	0.15	0.01	0.11
1 x 250	13-15 DAA	Whole plant	0.02	0.02	0.01	<0.01	0.03
Control	<2 hours before	Whole plant	<0.01	<0.01	<0.01	<0.01	<0.01

No correction of results for either control residues or recovery values has been performed.

Conclusions

Residues of azoxystrobin in treated winter barley whole plant samples taken at 0 day after application (DAA) were in the range 8.24 mg/kg to 19.71 mg/kg, at 1 DAA were in the range 6.68 mg/kg to 21.98 mg/kg, at 2 DAA were in the range 4.32 mg/kg to 15.92 mg/kg, at 4-5 DAA were in the range 2.25 mg/kg to 8.97 mg/kg, at 6 DAA were in the range 2.41 mg/kg to 9.62 mg/kg, at 8-9 DAA were in the range 1.01 mg/kg to 6.69 mg/kg and at 13-15 DAA were in the range 0.43 mg/kg to 1.26 mg/kg. Residues of R230310 in treated winter barley whole plant samples taken at 0 day after application (DAA) were in the range 0.02

mg/kg to 0.05 mg/kg, at 1 DAA were in the range 0.03 mg/kg to 0.07 mg/kg, at 2 DAA were in the range 0.03 mg/kg to 0.18 mg/kg, at 4-5 DAA were in the range 0.02 mg/kg to 0.18 mg/kg, at 6 DAA were in the range 0.03 mg/kg to 0.23 mg/kg, at 8-9 DAA were in the range 0.01 mg/kg to 0.15 mg/kg and at 13-15 DAA were in the range the limit of quantification (0.01 mg/kg) to 0.03 mg/kg. Residues of azoxystrobin and R230310 in untreated winter barley whole plant samples taken before application were below the limit of quantification (0.01 mg/kg).

(Ertus C, 2018)

Comments of zRMS:	<p>The calculation is considered valid and acceptable for regulatory use.</p> <p>The FOCUS (2006, 2014) degradation kinetics guidance was applied to calculate DT₅₀ endpoints for azoxystrobin modelling from residues measured in five plant residue trials in Europe. The data were described reasonably well by SFO kinetics and acceptable endpoints were derived for all studies.</p> <p>The error value for all trial is below 15%. Therefore, the results were considered to be reliable and suitable for the risk assessment.</p> <p>The calculation is considered valid and acceptable for regulatory use.</p>
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Reference:	KCP 10.1.2.2
Report:	Ford S, (2018), Azoxystrobin – Total foliage decline kinetics including foliage metabolite R230310. Report Number 0416036-Kin01. ERM, the exchange, station parade, Harrogate, Yorkshire, HG1 1TS, United Kingdom. (Syngenta file No. VV-631889)
Guideline(s):	<p>FOCUS (2006). Guidance document on estimating persistence and degradation kinetics from environmental fate studies on pesticides in EU registration. Report of the FOCUS Work Group on Degradation Kinetics, EC Document Reference SANCO/10058/2005, version 2.0,434 pp.</p> <p>FOCUS (2014). Generic guidance for estimating persistence and degradation kinetics from environmental fate studies on pesticides in EU registration. Version 1.1, 440 pp.</p>
Deviations:	No
GLP:	Not applicable
Acceptability:	Yes
Duplication: (if vertebrate study)	No

Executive summary

The report presents the calculation of foliage decline endpoint DT₅₀ values for the active substance azoxystrobin and its metabolite R230310. The rate of azoxystrobin foliage decline has been studied at five field site locations in Northern Europe (Ertus, 2018). The original data from this study was used to calculate the rate of dissipation of total residues of azoxystrobin plus R230310 in foliage considering the guidance in FOCUS Kinetics (2006, 2014).

Kinetic models were fitted to the total residue of azoxystrobin and its metabolite R230310 in each sample. Input data were generated according to the data handling recommendations made in the FOCUS guidance for degradation kinetics (FOCUS, 2006, 2014).

Kinetic modelling following the FOCUS (2006, 2014) kinetics flowchart for modelling endpoints was carried out using CAKE v3.2 (2016).

Results

The table below provides a summary of the DT₅₀ for all sites analysed.

Table A 15: Summary of foliage decline endpoint DT₅₀ values for total azoxystrobin plus R230310 residues

Site	Selected kinetic model	χ ² -error (%)	DT ₅₀ (days)
BW1	SFO	5.11	3.22
BW2	SFO	7.65	3.61

MA1	SFO	11.9	4.32
ND1	SFO	9.05	2.60
UK1	SFO	9.03	5.44
Geometric mean			3.72

Conclusions

Foliage decline endpoints for total azoxystrobin plus R230310 have been calculated from outdoor foliage decline study data from Northern Europe according to the principles of FOCUS (2006, 2014) kinetics guidance. The foliage DT₅₀ values of azoxystrobin ranged from 2.60 days to 5.44 days with a geometric mean of 3.72 days.

(Ford S, 2018)

A 2.1.3 KCP 10.1.3 Effects on other terrestrial vertebrate wildlife (reptiles and amphibians)

A 2.2 KCP 10.2 Effects on aquatic organisms

A 2.2.1 KCP 10.2.1 Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes

Comments of zRMS:	The study was conducted to OECD guideline 203 and according to the principles of GLP. All validity criteria were met. It should be noted that analytical verification of A22773A concentrations in test medium was done by analysing the content of azoxystrobin, only. The study is considered to be reliable and suitable for the risk assessment.
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Reference: KCP 10.2.1

Report: [REDACTED] (2020), Oxathiapiprolin/Azoxystrobin SC (A22773A) - Toxicity to the Rainbow Trout *Oncorhynchus mykiss* under Laboratory Conditions. (Acute Toxicity Test – Static), Report Number S20-05053, xxxxxxxxxxxx, Eutinger Str. 24, 75223 Niefern-Öschelbronn, Germany. Syngenta File No. VV-884613

Guideline(s): OECD Guidelines No. 203 (2019)

Deviations: No

GLP: Yes

Acceptability: Yes

Executive Summary

The acute toxicity of Oxathiapiprolin/Azoxystrobin SC (A22773A) to rainbow trout *Oncorhynchus mykiss* was determined under static conditions. The test was performed as a concentration response test and fish were exposed to nominal concentrations of 10.0, 5.00, 2.50, 1.25 and 0.625 mg A22773A/L including a dilution water control group. Based on nominal concentrations, the 96-hour LC₅₀ was determined to 3.20 mg test item/L (95% confidence limits 2.67 – 3.85 mg/L).

Materials

Test material Oxathiapiprolin/Azoxystrobin SC (A22773A)

Lot/Batch #:	SFI003-174-001
Purity:	Azoxystrobin analysed: 247 g/L Oxathiapiprolin analysed: 11.2 g/L
Description:	Liquid / beige
Stability of test compound:	Stable under standard conditions (ambient, dark, dry 5 - 30 °C)
Reanalysis/expiry date:	28 Feb 2023
Density:	1.096 g/cm ³
Treatments	
Test concentrations:	10.0, 5.00, 2.50, 1.25 and 0.625 mg A22773A/L and a test medium control
Solvent:	None
Analysis of test concentrations:	Analysis of azoxystrobin in all test item concentrations and control at 0 h fresh and 96 hours (aged) by HPLC-MS/MS detection.
Test organisms	
Species:	<i>Oncorhynchus mykiss</i>
Source:	Forellenzucht Peter Störk, D-88348 Bad Saulgau, Germany
Acclimatisation period:	≥ 9 days
Treatment for disease:	None
Weight and length of dilution water control fish at end of exposure period:	49 – 54 mm; 0.93 – 1.25 g
Feeding:	None
Test design	
Test vessels:	18 L glass aquaria, filled with 15 L test solution
Test medium:	Mix of dechlorinated drinking water and deionised water
Replication:	None
No of fish per tank:	7
Exposure regime:	Static
Aeration:	None
Duration:	96 h
Environmental conditions	
Test temperature:	12.7 – 14.0 °C
pH:	7.79 – 8.53
Dissolved oxygen:	≥ 70 % of air saturation
Hardness of dilution water:	10°dH corresponding to approx. 178 mg/L (as CaCO ₃)
Lighting:	16 hours daily / 8 hours darkness daily, with 20 minute transition

Study Design and Methods

Experimental dates: 14 Sep 2020 – 20 Sep 2020

The necessary amount of test item (300 mg) for preparing the stock solution S1 was weighed on a weighing scoop and transferred to a volumetric flask. Test medium was added up to the bench mark and the stock solution was homogenised by shaking and treated with five minutes of ultrasonication. Afterwards, the stock solution S1 was observed turbid. Lower solutions were prepared by dilution of the appropriate solution with test medium. Dilution solutions V1 to V4 were turbid. Defined volumes of the stock solution and dilution solutions were transferred into the respective aquarium filled with test medium in order to achieve the following nominal test concentrations: 0.625, 1.25, 2.50, 5.00 and 10.0 mg/L. The test solutions were homogenized by stirring with a whisk directly in the aquarium.

At the start of the test seven fish were allocated to the test concentrations and to one negative control with untreated test medium. Observations for mortalities and symptoms of toxicity were made at 0 h, 4-6 h, day 1 (24 h), day 2 (48 h, 51-54 h), day 3 (72 h, 75-78 h) and day 4 (96 h) after exposure.

Measurements of temperature, pH-value and oxygen saturation were performed at 24 hour intervals from aged and fresh test solutions, where appropriate. Physico-chemical assessments in a test item concentration were stopped after assessment of 100 % mortality. Water hardness of the untreated control and light intensity was determined at the beginning of the test.

The content of azoxystrobin in the test solution samples were determined by analysing with HPLC-MS/MS.

The LC₅₀-values after 4-6, 24 and 48 hours together with their confidence intervals were estimated following the probit analysis, the LC₅₀-values for 72 and 96 hours together with their confidence intervals were estimated following the Spearman-Kärber distribution. For data evaluation the statistical programme ToxRat Professional 3.3.0 was used. The NOEC (mortality) was established based on the highest test concentration at which no mortality within the allowed control mortality was observed.

Results and Discussion

The initial measured concentrations of azoxystrobin were between 88 % and 110 % of nominal. The measured concentrations after 96 hours were between 81 % and 87 % of nominal. Since all measured concentrations for azoxystrobin at test start were between 80 and 120 % of nominal, the results were calculated using the nominal concentrations of A22773A.

Table A 16: Analytical results – Azoxystrobin

Nominal concentration of A22773A [mg/L]	Nominal concentration of azoxystrobin [mg a.s./L]	Age [hours]	Measured concentration of azoxystrobin [mg a.s./L]	Measured concentration of azoxystrobin [% nominal]
Control	-	0 h	< LOD	-
		96 h aged	< LOD	-
0.625	0.141	0 h	0.132	94
		96 h aged	0.123	87
1.25	0.281	0 h	0.310	110
		96 h aged	0.230	82
2.50	0.563	0 h	0.548	97
		96 h aged	0.484	86
5.00	1.13	0 h	1.00	88
		24 h aged	0.960	85
10.0	2.25	0 h	2.12	94
		24 h aged	1.83	81

LOQ: Limit of quantification: 0.0141 mg/L azoxystrobin

LOD: Limit of detection: 0.00423 mg/L azoxystrobin

- not applicable

The mortality data and calculated LC₅₀ values are shown in the table below:

Table A 17: Effects of A22773A on the survival of *Oncorhynchus mykiss*

Nominal concentration of A22773A [mg/L]	Mortality observed (cumulative number of dead fish) (n = 7)				
	4-6 hours	24 hours	48 hours	72 hours	96 hours
Control	0	0	0	0	0
0.625	0	0	0	0	0
1.25	0	0	0	0	0
2.50	0	0	0	1	1
5.00	7	7	7	7	7
10.0	7	7	7	7	7
LC ₅₀ (mg/L) (Nominal)	3.48 ¹⁾	3.48 ¹⁾	3.48 ¹⁾	3.20 ²⁾	3.20 ²⁾
95% confidence interval	2.86 – 4.28	2.86 – 4.28	2.86 – 4.28	2.67 – 3.85	2.67 – 3.85

NOEC (mortality) (mg /L) (Nominal)	2.50	2.50	2.50	2.50	2.50
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¹⁾ estimates using the probit analysis

²⁾ estimates using Spearman-Kärber

Validity Criteria

According to the OECD 203 (2019) this study can be regarded as valid, since;

- the mortality in the control did not exceed one fish at the end of the test, (actual 0)
- the dissolved oxygen concentration was at least 60 % of the air saturation value throughout the test, (actual ≥ 70 %)
- analytical measurements of test concentrations were conducted

Conclusions

The acute toxicity of Oxathiapiprolin/Azoxystrobin SC (A22773A) to the rainbow trout *Oncorhynchus mykiss* was investigated in a 96-hour static test. Fish were exposed to nominal concentrations of 10.0, 5.00, 2.50, 1.25 and 0.625 mg A22773A/L including a dilution water control group. Based on nominal concentrations, the 96-hour LC₅₀ was determined to 3.20 mg test item/L (95% confidence limits 2.67 – 3.85 mg/L).

(Beuter LK, 2020)

Comments of zRMS:	<p>The study was conducted to OECD guidance 202 and according to the principles of GLP. All validity criteria were met. It should be noted that analytical verification of A22773A concentrations in test medium was done by analysing the content of azoxystrobin, only.</p> <p>The study is considered to be reliable and suitable for the risk assessment.</p>
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Reference:	KCP 10.2.1
Report:	Beuter, L.-K., (2020). Oxathiapiprolin/Azoxystrobin SC (A22773A) - Toxicity to the Water Flea <i>Daphnia magna</i> Straus under Laboratory Conditions (Acute Immobilisation Test –Static), Report Number S20-05052, Eurofins Agrosience Services Ecotox GmbH, Eutinger Str. 24, 75223 Niefern-Öschelbronn, Germany. Syngenta file No. VV-884821
Guideline(s):	OECD Guidelines No. 202 (2004)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Executive Summary

The acute toxicity of Oxathiapiprolin/Azoxystrobin SC (A22773A) to *Daphnia magna* was determined under static conditions. Daphnids were exposed to nominal concentrations of 1.00, 0.500, 0.250, 0.125 and 0.0625 mg A22773A/L alongside a dilution water control. Based on nominal concentrations of the test item A22773A, the 48 hour EC₅₀ was considered to be 0.659 mg A22773A/L.

Materials

Test Material

Name/code:	Oxathiapiprolin/Azoxystrobin SC (A22773A)
Lot/Batch #:	SFI003-174-001
Purity:	Azoxystrobin analysed: 247 g/L Oxathiapiprolin analysed: 11.2 g/L
Description:	Liquid / beige
Stability of test compound:	Stable under standard conditions (ambient, dark, dry 5 - 30 °C)
Reanalysis/Expiry date:	28 Feb 2023
Density:	1.096 g/cm ³
Treatments	
Test concentrations:	1.00, 0.500, 0.250, 0.125 and 0.0625 mg A22773A/L and a test medium control
Solvent:	None
Positive control:	Two concentrations of the reference item potassium dichromate were tested around the same time period as the study.
Analysis of test concentrations:	Analysis of azoxystrobin in all test item concentrations and control at 0 h fresh and 48 hours (aged) by HPLC-MS/MS detection.
Test organisms	
Species:	<i>Daphnia magna</i> STRAUS, Clone V
Source:	Continuously bred in the laboratory, originally obtained from Federal Environment Agency in Berlin/Germany
Feeding:	The animals were fed on single cell green algae (<i>Desmodesmus subspicatus</i> , formerly <i>Scenedesmus subspicatus</i>) at least three times a week. No feeding during testing.

Test design

Test vessels:	100 mL glass beaker, filled with ≥ 50 mL test solution
Test medium:	Elendt M4
Replication:	4 test vessels each with 5 test organisms at all test concentrations and the control (individual organisms were randomly assigned to the treatments and test vessels)

Exposure regime:	Static
Duration:	48 hours
Environmental conditions	
Test temperature:	20.2 – 20.6 °C
pH range:	7.57 – 7.97
Dissolved oxygen:	6.9 – 8.8 mg/L
Total hardness of dilution water:	250 mg/L as CaCO ₃
Lighting:	16 hours photoperiod / 8 hours darkness daily (mean 1283 lux)

Study Design and Methods

Experimental dates: 14 Sep 2020 to 22 Sep 2020

A stock solution of 50.0 mg A22773A/L was prepared by dissolving 50.0 mg of the test item completely in 1000 mL of test medium. Using this stock solution, the nominal test concentrations as stated above were prepared by serial dilution. The control consisted of test medium only.

The immobility of the daphnids was determined by visual observations after 24 and 48 hours of exposure. Organisms unable to swim within 15 seconds after gentle agitation of the test beaker were considered to be immobile.

Temperature, pH and dissolved oxygen were measured at the start and end of the test in each test concentration and the control. The appearance of the test solution was recorded daily.

The test concentrations from all test item concentrations and the control were verified by chemical analysis of azoxystrobin at 0 (fresh) and 48 hours (aged) by HPLC-MS/MS detection.

The median effect concentration (EC₅₀) was defined as the concentration resulting in 50 % immobilisation of the *Daphnia* in the time period specified and was not determined statistically at 24 and 48 hours.

Results and Discussion

At test initiation, the measured concentrations of azoxystrobin were between 93 and 110 % of the nominal test concentrations and between 94 and 109 % of nominal at test end. Since all measured concentrations were between 80 – 120 % of nominal, the results were calculated using the nominal concentrations.

Table A 18: Analytical results

Nominal concentration (mg A22773A/L)	Determined concentration of azoxystrobin at 0 hours (% of nominal)	Determined concentration of azoxystrobin at 48 hours (% of nominal)
Control	-	-
0.0625	110	109
0.125	107	107
0.250	98	102
0.500	99	106
1.00	93	94

-: not applicable

LOQ: 0.00141 mg azoxystrobin/L

LOD: 0.000423 mg azoxystrobin/L

There was no immobility observed in the dilution water control and all test item concentrations. Immobility data and estimated EC₅₀ values are shown in the table below:

Table A 19: Effects of A22773A on *Daphnia magna* following exposure for 48-hours in a static test

Nominal concentration (mg A22773A/L)	Immobilised daphnids after 24 hours		Immobilised daphnids after 48 hours	
	Number	%	Number	%
Control	0	0	0	0
0.0625	0	0	0	0
0.125	0	0	0	0
0.250	1	5	1	5
0.500	4	20	4	20
1.00	16	80	19	95
EC ₅₀ (mg A22773A/L)	0.738		0.659	

Validity criteria

The test was considered valid;

- There was no immobilization in the control (must be $\leq 10\%$)
- Oxygen concentrations at the end of the test were ≥ 6.9 mg/L in the control and treated test vessels (must be ≥ 3 mg/L)

Conclusions

The acute toxicity of Oxathiapiprolin/Azoxystrobin SC (A22773A) to *Daphnia magna* was determined under static conditions. Daphnids were exposed to nominal concentrations of 1.00, 0.500, 0.250, 0.125 and 0.0625 mg A22773A/L alongside a dilution water control. Based on nominal concentrations of the test item A22773A, the 48 hour EC₅₀ was considered to be 0.659 mg A22773A/L.

(Beuter LK, 2020)

Comments of zRMS:	<p>The study was conducted to OECD guidance 201 and according to the principles of GLP. All validity criteria were met. It should be noted that analytical verification of A22773A concentrations in test medium was done by analysing the content of azoxystrobin, only.</p> <p>For the 72-hour E_rC_{50} of 3.32 mg A22773A/L the measured concentrations after 96 hours was above 80%. Thus, the endpoints based on nominal concentrations are accepted.</p> <p>The study is considered to be reliable and suitable for the risk assessment.</p>
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Reference:	KCP 10.2.1
Report:	Obert-Rausser, P. (2020), Oxathiapiprolin/Azoxystrobin SC (A22773A) - Toxicity to the Single Cell Green Alga <i>Raphidocelis subcapitata</i> Korshikov under Laboratory Conditions. Report Number S20-05054. Eurofins Agrosience Services Ecotox GmbH, Eutinger Str. 24, 75223 Niefern-Öschelbronn, Germany. Syngenta File No. VV-884825
Guideline(s):	OECD Guidelines No. 201 (2011)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Executive Summary

The toxicity of A22773A to the green alga *Raphidocelis subcapitata* was investigated in a 96-hour static test. Algae were exposed to nominal concentrations of 0.0954, 0.305, 0.977, 3.31 and 10.0 mg A22773A/L alongside a culture medium control. Based on nominal concentrations, the 72-hour E_rC_{50} was 3.32 mg A22773A/L, the E_yC_{50} was 0.616 mg A22773A/L and the E_bC_{50} was 0.692 mg A22773A/L. The 96-hour E_rC_{50} was 4.46 mg A22773A/L, the E_yC_{50} was 0.807 mg A22773A/L and the E_bC_{50} was 0.739 mg A22773A/L.

Materials

Test Material

Name/Code:	Oxathiapiprolin/Azoxystrobin SC (A22773A)
Lot/Batch #:	SFI003-174-001
Actual content of active ingredient:	Azoxystrobin analysed: 247 g/L Oxathiapiprolin analysed: 11.2 g/L
Description:	Liquid, beige
Stability of test compound:	Sufficient for the test purpose (at least 1 h)
Reanalysis/expiry date:	28 Feb 2023
Density:	1.096 g/cm ³
Treatments	
Test concentrations:	0, 0.0954, 0.305, 0.977, 3.31 and 10.0 mg A22773A/L and test medium control
Solvent:	None
Analysis of test concentrations:	Analysis of azoxystrobin in all concentrations and control at 0 and 96 h
Test organism	
Species:	<i>Raphidocelis subcapitata</i> Korshikov, Strain SAG 61.81
Source:	MBM Sciencebridge GmbH, Hans-Adolf-Krebs-Weg 1, D-37077 Göttingen, Germany

Test design

Test vessels:	100 mL Erlenmeyer flasks with aluminium caps
Test medium:	AAP medium
Replication:	The control was prepared with six replicates and the concentrations were prepared with three replicates at each concentration.
Starting cell density:	5000 cells per mL (nominal)

Exposure regime:	Static
Aeration:	By continuous agitation: Test vessels were placed in an incubator on a pivoted bogie which turns around and induces shaking by regular sudden stops
Duration:	96 h
Environmental conditions	
Test temperature:	22.5 – 23.4 °C
pH:	7.43 – 8.47 within 72 h, up to 8.97 after 96 h
Lighting:	Continuously, 88.6 – 100 $\mu\text{E m}^{-2} \text{s}^{-1}$

Study Design and Methods

Experimental dates: 21 September 2020 – 14 October 2020

One stock solution (S1) containing 25 mg was prepared by direct weighing into 250 mL test medium with algae. The solution was homogenised by shaking and treated with 5 minutes of ultrasonication. The stock solution appeared to be turbid. The further concentrations V1 – V5 were made by diluting the appropriate solutions in test medium with algae to give the required test concentrations. Dilution V1 appeared to be turbid and dilutions V2 – V5 were clear and transparent. The control consisted of culture medium only. An aliquot of approximately 50 mL test solution was placed into each test vessel and the test was started by inoculation of 5,000 algal cells per mL of test medium. The test flasks were continuously agitated in a temperature controlled light incubator.

Small volumes of all test concentrations and controls were taken from all test flasks after 24, 48, 72 and 96 hours of exposure. The algal cell densities in these samples were determined by fluorescence measurements. In addition, after 96 hours exposure, a sample was taken from the control and all test item concentrations and the shape of the algal cells was examined microscopically in these samples. The pH was measured at the start, after 72 hours and at the end of the test. The test temperature was measured continuously in the incubator and recorded daily. The appearance of the test media was also recorded daily.

The test concentrations were verified by chemical analysis of azoxystrobin at 0 and 96 hours, using HPLC-MS/MS.

From the algal cell densities the mean growth rate, yield and integrated biomass were calculated. The statistical evaluation was performed using the 72-hour and 96-hour data for each of the three growth parameters growth rate, yield and integrated biomass using SAS® (2016). A test for normality of the data was performed by using the Shapiro-Wilks test and homogeneity of variance was qualified using the Levene's test. The NOEC and LOEC were determined by using Dunnett's-t-test for growth rate (72 h and 96 h), Jonckheere-Terpstra test for yield and for biomass integral (72 h and 96 h). The calculation of EC₁₀, EC₂₀ and EC₅₀ was performed with probit analysis following normal distribution for growth rate (72 h and 96 h) and logistic distribution for yield and for biomass integral (72 h and 96 h).

Results and Discussion

The analytical verification of A22773A concentrations in test medium was done by analysing the content of azoxystrobin at 0 and 96 hours. The initial measured concentrations were between 82 % and 103 % of nominal. The measured concentrations after 96 hours were between 75 % and 96 % of nominal. Since all initial measured concentrations were between 80 – 120 % of nominal, the results have been calculated using the nominal concentrations.

Table A 20: Analytical results

Nominal concentrations (mg A22773A/L)	Determined concentration of azoxystrobin at 0 hours (% of nominal)	Determined concentration of azoxystrobin at 96 hours (% of nominal)
Control	n.a.	n.a.
0.0954	96	75

0.305	96	78
0.977	82	83
3.31	103	86
10.0	96	96

n.a.: not applicable

LOQ: 0.00215 mg azoxystrobin/L

The morphology of the algae cells was observed microscopically after 96 hours. The cells were considered normal for the control and up to a test item concentration of 10.0 mg/L.

Algal Biomass

The algal density at 0, 24, 48, 72 and 96 hours was measured for each replicate and the calculated means are shown below.

Table A 21: Mean values for the control and test item treatment of A22773A for the density of algal cultures at 24, 48, 72 and 96 hours for *Raphidocelis subcapitata*

Nominal concentrations (mg A22773A/L)	Density of algal cells ^a			
	24 h	48 h	72 h	96 h
Control	2.38	16.08	89.66	271.22
0.0954	2.81	15.49	85.38	265.57
0.305	2.32	13.66	66.34	234.68
0.977	1.50	7.27	31.80	117.45
3.31	0.97	1.86	4.30	10.92
10.0	0.80	1.25	2.34	4.48

^a The density was determined by fluorescence measurements (at least duplicate measurements per replicate) and is given as number of cells (x 10⁴) per milliliter. At the start of the test, the initial cell density was 5100 algal cells/mL

Growth rate, yield and biomass (area under the growth curve)

Table A 22: Mean values for the control and test item treatment of A22773A for the percent inhibition of growth rate, yield and AUC at 72 hours for *Raphidocelis subcapitata*

Nominal concentrations (mg A22773A/L)	0 to 72 h					
	AUC (10 ⁴ *day)	Percentage inhibition of AUC	Growth rate (1/day)	Percentage inhibition of growth rate	Yield (x 10 ⁴)	Percentage inhibition of yield
Control	62.02	0.0	1.72243	0.0	89.15	0.0
0.0954	59.72	3.7	1.70626	0.9	84.87	4.8
0.305	47.88*	22.8	1.62262*	5.8	65.83*	26.2
0.977	23.40*	62.3	1.37749*	20.0	31.29*	64.9
3.31	3.71*	94.0	0.71010*	58.8	3.79*	95.7
10.0	1.95*	96.9	0.50690*	70.6	1.83*	97.9

* mean value significantly lower than in the control (according to Dunnett's t-test / Jonckheere Terpstra test / Jonckheere Terpstra test for growth rate / yield / biomass integral (left-sided, p<0.05))

Table A 23: Mean values for the control and test item treatment of A22773A for the percent inhibition of growth rate, yield and AUC at 96 hours for *Raphidocelis subcapitata*

Nominal concentrations (mg A22773A/L)	0 to 96 h					
	AUC (10 ⁴ *day)	Percentage inhibition of AUC	Growth rate (1/day)	Percentage inhibition of growth rate	Yield (x 10 ⁴)	Percentage inhibition of yield
Control	241.94	0.0	1.56833	0.0	270.71	0.0
0.0954	234.69	3.0	1.56380	0.3	265.06	2.1
0.305	197.88*	18.2	1.53262*	2.3	234.17	13.5
0.977	97.53*	59.7	1.35975*	13.3	116.94*	56.8
3.31	10.82*	95.5	0.76555*	51.2	10.41*	96.2
10.0	4.86*	98.0	0.54306*	65.4	3.97*	98.5

* mean value significantly lower than in the control (according to Dunnett's t-test / Jonckheere Terpstra test / Jonckheere Terpstra test for growth rate / yield / biomass integral (left-sided, p<0.05))

Table A 24: Summary of biological results for toxicity of A22773A to *Raphidocelis subcapitata* after 72 and 96 hours

Parameter	after 72 h (mg A22773A/L)			after 96 h (mg A22773A/L)		
	AUC	Growth rate	Yield	AUC	Growth rate	Yield
EC ₅₀	0.692	3.32	0.616	0.739	4.46	0.807
95% CI	0.580 – 0.824	2.67 – 4.25	0.517 – 0.732	0.626 – 0.871	3.59 – 5.72	0.690 – 0.943
EC ₂₀	0.282	0.884	0.255	0.332	1.31	0.392
95% CI	0.218 – 0.348	0.662 – 1.12	0.197 – 0.313	0.261 – 0.402	0.992 – 1.64	0.314 – 0.469
EC ₁₀	0.167	0.443	0.152	0.207	0.688	0.257
95% CI	0.119 – 0.216	0.300 – 0.598	0.108 – 0.197	0.152 – 0.263	0.474 – 0.914	0.193 – 0.321
NOEC	0.0954	0.0954	0.0954	0.0954	0.0954	0.305
LOEC	0.305	0.305	0.305	0.305	0.305	0.977

Validity criteria

The test was considered valid;

- The algal biomass in the control increased by a factor of 175.8 over 72 hours (must be at least a factor of 16).
- The mean coefficient of variation of the daily growth rates in the control was 11 and 23 % over 72 and 96 hours, respectively (must be ≤ 35%).
- The coefficient of variation of average specific growth rates in replicate control cultures was 1.8 and 1.5 % after 72 and 96 hours, respectively (must be <7%).

Conclusions

The toxicity of A22773A to the green alga *Raphidocelis subcapitata* was investigated in a 96-hour static test. Algae were exposed to nominal concentrations of 0.0954, 0.305, 0.977, 3.31 and 10.0 mg A22773A/L alongside a culture medium control. Based on nominal concentrations, the 72-hour E_rC₅₀ was 3.32 mg A22773A/L, the E_yC₅₀ was 0.616 mg A22773A/L and the E_bC₅₀ was 0.692 mg A22773A/L. The 96-hour E_rC₅₀ was 4.46 mg A22773A/L, the E_yC₅₀ was 0.807 mg A22773A/L and the E_bC₅₀ was 0.739 mg A22773A/L.

(Obert-Rausser P, 2020)

A 2.2.2 KCP 10.2.2 Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms

A 2.2.3 KCP 10.2.3 Further testing on aquatic organisms

A 2.3 KCP 10.3 Effects on arthropods

A 2.3.1 KCP 10.3.1 Effects on bees

A 2.3.1.1 KCP 10.3.1.1 Acute toxicity to bees

A 2.3.1.1.1 KCP 10.3.1.1.1 Acute oral toxicity to bees

Comments of zRMS:	The study was conducted to OECD guidance 213 and 214 and according to the principles of GLP. All validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.
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Reference: KCP 10.3.1.1

Report: Franke, M., (2020), Oxathiapiprolin/Azoxystrobin SC (A22773A) - Acute Toxicity to the Honeybee *Apis mellifera* L. under Laboratory Conditions; Report No. 20 48 BAA 0129, BioChem agrar, Labor für biologische und chemische Analytik GmbH, Kupferstraße 6, 04827 Machern OT Gerichshain, Germany. (Syngenta file No. VV-883076).

Guideline(s): OECD 213 (1998); OECD 214 (1998)

Deviations: No

GLP: Yes

Acceptability: Yes

Executive Summary

In a 48-hr-acute oral toxicity study, honey bees (*Apis mellifera*) were exposed to A22773A via a feeding solution. The 48 h LD₅₀ was > 1000 µg A22773A/bee.

In a 48-hr-acute contact toxicity study, honey bees (*Apis mellifera*) were exposed to A22773A administered topically to adult bees. The 48 h LD₅₀ was > 1000 µg A22773A/bee.

Materials

Test Material

Lot/Batch #: SFI003-174-001

Actual content of active ingredients: Azoxystrobin: 22.5 % w/w corresponding to 247 g/L

Description: Oxathiapiprolin: 1.02 % w/w corresponding to 11.2 g/L

Stability of test compound: SC (suspension concentrate); brown liquid

Reanalysis/expiry date: Stable

Treatments: End of February 2023

Test rates:

Contact test: 1000, 500, 250, 125, 62.5 µg/bee

Oral test (offered): 1000, 500, 250, 125, 62.5 µg/bee

Oral test (consumed): 1000, 500, 250, 125, 62.5 µg/bee

Control: Contact test: water and wetting agent control (Tween solution at 1 % v/v)

Reference item:	Oral test: 50 % w/v sucrose solution Dimethoate EC 400; 429.0 g/L; nominal dose levels of 0.250, 0.188, 0.141 and 0.105 µg a.s./bee in contact test and 0.250, 0.175, 0.123 and 0.086 µg a.s./bee in oral test
Test organisms	
Species:	<i>Apis mellifera</i> L. Buckfast
Source:	BioChem agrar GmbH, Germany
Food:	50 % w/v sucrose solution
Test design	
Test cage description:	Disposable test cages constructed of cardboard, with holes in the bottom for ventilation and a glass plate in front
Replication:	3
No. of bees/rep:	10
Duration of test:	48 hours (contact test); 48 hours (oral test)
Environmental test conditions	
Temperature:	23.9 – 25.4 °C (contact and oral)
Humidity:	49 - 64 % (contact and oral)
Photoperiod:	Darkness (except during observations)

Study Design and Methods

Test facility: BioChem agrar GmbH, Kupferstr. 6, 04827 Machern OT Gerichshain, Germany

Experimental dates: Experimental start: 10 September 2020

Experimental completion: 12 September 2020

Oral test procedures:

Bees were unfed from the time they were collected from the hives for 1-2 hours until treatment. Each group of bees was offered 200 µL (equivalent to 20 µL/bee) of the test material or toxic standard dispersed in aqueous sucrose solution. Treatments were calculated so that the target dose was contained in 200 µL. The doses were measured into the feeding tubes and the feeding tubes were weighed before the doses were made available to the bees. Once all test solutions were consumed, or after six hours (whichever was achieved first), the feeding tubes were replaced with similar tubes containing aqueous sucrose solution. All feeding tubes with test solutions were weighed to calculate actual mean consumption per bee for each treatment.

Contact test procedures:

Bees were treated with 2 µL of droplets of the test solution, control or toxic standard applied to the dorsal surface of the thorax using topical application with a micro applicator. The bees were returned to the test unit, allowed to recover and fed with a continuous supply of 50 % w/v aqueous sucrose solution.

The mortality [%] per treatment was calculated from the number of dead bees and the total number of introduced bees per treatment group. Mortality in the test and reference item treatments was corrected with the corresponding control mortality according to the formula of SCHNEIDER-ORELLI (1947). The LD₅₀ values with 95 % confidence limits of the reference item treatment were calculated by means of a Probit analysis.

Results

Mortality data for the test material and toxic standard are summarised in the table below.

Table A 25: Summary of acute oral toxicity of A22773A to the honeybee

Treatment (target dose) (µg test item/bee)	Treatment (based in consumption) (µg test item/bee)	Mortality (%)		Correct mortality (%) [#]	
		24 h	48 h	24 h	48 h
50 % w/v sucrose solution control		0.0	0.0	-	-
1000	1000	0.0	0.0	-	-
500	500	0.0	0.0	-	-

Treatment (target dose) (µg test item/bee)	Treatment (based in consumption (µg test item/bee)	Mortality (%)		Correct mortality (%) [#]	
		24 h	48 h	24 h	48 h
250	250	0.0	0.0	-	-
125	125	0.0	0.0	-	-
62.5	62.5	0.0	0.0	-	-
48h LD₅₀ (µg test item/bee)		> 1000			
95% C.I.		--			

Mortality results are averages based on 3 replicates consisting of 10 bees each

[#] corrected mortality (according to SCHNEIDER-ORELLI 1947)

No sublethal effects were observed in the oral test.

Table A 26: Summary of acute contact toxicity of A22773A to the honeybee

Treatment (µg test item/bee)	Mortality (%)		Correct mortality (%) [#]	
	24 h	48 h	24 h	48 h
Water control	0.0	0.0	-	-
Solvent control 1 % v/v Tween solution	0.0	0.0	-	-
1000	0.0	0.0	-	-
500	0.0	0.0	-	-
250	0.0	0.0	-	-
125	0.0	0.0	-	-
62.5	0.0	0.0	-	-
48h LD₅₀ (µg test item/bee)		> 1000		
95% C.I.		--		

Mortality results are averages based on 3 replicates consisting of 10 bees each

[#] corrected mortality (according to SCHNEIDER-ORELLI 1947)

No sublethal effects were observed in the contact test.

Validity criteria

- The test was considered valid as the mean mortality in the controls in the oral and contact toxicity test was ≤ 10 %, (actual 0%).
- The 24-h LD₅₀ of the reference item in the oral toxicity test was 0.11 µg a.s./bee (must be within the range of 0.10 to 0.35 µg a.s./bee).
- The 24-h LD₅₀ of the reference item in the contact toxicity test was 0.15 µg a.s./bee (must be within the range of 0.10 to 0.30 µg a.s./bee).

Conclusion

The acute toxicity of A22773A to honeybees was assessed over 48 hours in the contact test and oral test. The contact and oral 48 h LD₅₀ was > 1000 µg A22773A/bee.

(Franke M, 2020)

Comments of zRMS:	The study was conducted to OECD guidance 246 and 247 and according to the principles of GLP. All validity criteria were met. The study is considered to be reliable.
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Reference: KCP 10.3.1.1

Report Amsel, K. (2022), Oxathiapiprolin/Azoxystrobin SC (A22773A) - Acute toxicity to the bumblebee *Bombus terrestris* L. under laboratory conditions. Report No. 21 48 BBA 0032, BioChem agrar, Labor für biologische und chemische Analytik GmbH, Kupferstraße 6, 04827 Machern OT Gerichshain, Germany. (Syngenta file No. VV-936507)

Guideline(s): OECD 246 (2017), OECD 247 (2017)

Deviations: No

GLP: Yes

Acceptability: Yes

Executive Summary

In a 48-hr-acute oral toxicity study, bumble bees (*Bombus terrestris* L.) were exposed orally to A22773A via a feeding solution. The LD₅₀ was > 983.9 µg consumed product/bumble bee and the NOED was 983.9 µg consumed product/bumble bee after 48 hours.

In a 48-hr-acute contact toxicity study, bumble bees (*Bombus terrestris* L.) were exposed to A22773A administered topically to adult bees. the LD₅₀ was > 1000.0 µg product/bumble bee and the NOED was 1000.0 µg product/bumble bee after 48 hours.

Materials

Test Material

Name: Azoxystrobin/Oxathiapiprolin SC (A22773A)
Other product code: ICI5504/SYN546539 SC (250/012)
Product code: A22773A
Lot/Batch #: SFI003-174-001 (other Batch ID: 1127290)
Active substances content: Azoxystrobin: 250 g/L nominal, 247 g/L (22.5% w/w) analysed
Oxathiapiprolin: 12 g/L nominal, 11.2 g/L (1.02% w/w) analysed
Description: Appearance: beige liquid
Density: 1096 kg/m³
Stability of test compound: Stable under recommended handling and storage conditions (< 30 °C)
Reanalysis/expiry date: End of February 2023

Treatments

Test rates: Contact test: 1000.0, 500.0, 250.0, 125.0, 62.5 µg product/bumble bee
Oral test (offered): 1000.0, 500.0, 250.0, 125.0, 62.5 µg product/bumble bee

Controls: Oral test (consumed): 983.9, 495.1, 246.5, 123.4, 61.8 µg product/bumble bee
Contact test: Deionised water, 0.5% TritonX solution
Oral test: Sucrose solution

Reference item: Danadim® Progress (dimethoate EC 400; 401.7 g/L (analysed))
Administration: Contact test: 4 µL/bee
Oral test: 40 µL/bee

Test organisms

Species: *Bombus terrestris* L. (bumble bee), worker bumble bees from queen right hives
Source: Biobest Belgium N.V. Ilse Velden, 18, 2260 Westerlo, Belgium, supplied by: Katz Biotech AG, An der Birkenpfehlheide 10, 15837 Baruth, Germany
Food: 50% (w/v) sucrose solution

Test design

Test cage description:	Nicot cages (part of the Nicot queen bee rearing system) with a length of 7 cm and a diameter of 2 cm.
Number of bees/ test unit (= replicate):	1
No. of bees/rep:	30 (+ 5 additionally replicates/treatment to account non-feeders in the oral test)
Duration of test:	48 hours
Environmental test conditions	
Temperature:	23.0 – 25.2 °C
Humidity:	51 – 80%
Photoperiod:	Constant darkness throughout the test (diffuse artificial light only during handling and assessments)

Study Design and Methods

Test facility: BioChem agrar Labor für biologische und chemische Analytik GmbH
Kupferstr. 6, 04827 Machern OT Gerichshain, Germany
Experimental dates: 14 September – 17 September 2021

Oral test: Plastic syringes (feeders) containing the corresponding application solution were used for application. The application volume was 40 µL/replicate (corresponding to 40 µL/bumble bee). The bumble bees were starved for approx. 4 hours prior to application start. Each unit was provided with the application solution for about 2.5 hours, to ensure a sufficient uptake. The feeders were then removed, and the bumble bees were provided *ad libitum* with an untreated 50 % (w/v) aqueous sucrose solution. Treatments started with the control followed by the test item and finally the reference item. For dose verification the amount of application solution(s) consumed was determined by weighing the feeders before and after feeding using calibrated equipment.

Contact test: The application amount was 4 µL/bumble bee. After having been anaesthetised with CO₂ (the amount of anaesthetic used was minimised), the droplet of the application solution was applied individually to the dorsal side of the thorax of each bumble bee. Treatments started with the control followed by the test item and finally the reference item. For the toxic reference item group and the water treated control group, the water-wetting agent Triton X-100 was mixed into all application solutions. This reduced the surface tension of the applied solution and ensured that the drop of the application solution was spread out immediately after the application. After the application the bees were returned to the test units.

Fisher's Exact Binomial Test with Bonferroni Correction ($\alpha=0.05$; one sided greater) was used for mortality data in the oral test.

Results

Analytical recoveries in dosing solutions are presented below.

Table A 27: Analytical results - oral test

Target dosing solution (µg product/bee)	Measured concentration (mg a.i./kg)	% recovery
Sucrose	< LOD	1
1000.0	198.5	93
500.0	99.09	92
250.0	52.65	98
125.0	26.34	98
62.5	13.11	98

LOD: 0.0026 mg/kg, corresponding to 0.062 µg/L

Mortality data for the test material and toxic standard are summarised in the table below.

Table A 28: Summary of acute oral toxicity of A22773A to the bumble bee

Treatment (target dose)	Treatment (consumed)	Mortality (%)		Corrected mortality (%)	
		24 h	48 h	24 h	48 h
(µg product/bee)					
Sucrose	-	0.0	0.0	-	-
1000.0	983.9	0.0	0.0	-	-
500.0	495.1	0.0	0.0	-	-
250.0	246.5	0.0	0.0	-	-
125.0	123.4	0.0	3.3	-	-
62.5	61.8	0.0	0.0	-	-
Toxic reference	1.46	96.7	100	-	-
LD ₅₀ (µg consumed product/bee)	> 983.9				
95% C.I.	-				
NOED (µg consumed product/bee)	983.9				

Mortality results are averages based on 30 replicates consisting of 1 bumblebee each

No sublethal effects were observed in the oral test.

Table A 29: Analytical results - contact test

Target dosing solution (µg product/bee)	Measured concentration (mg a.i./L)	% recovery
Solvent	< LOD	-
1000.0	2439	96
500.0	1168	92
250.0	571.5	90
125.0	286.4	90
62.5	142.5	89

LOD: 0.0026 mg/L, corresponding to 0.062 µg/L

Table A 30: Summary of acute contact toxicity of A22773A to the bumble bee

Treatment (µg product/bee)	Mortality (%)		Corrected mortality (%)	
	24 h	48 h	24 h	48 h
Water control	0.0	0.0	-	-
0.5% TritonX	0.0	0.0	-	-
1000.0	0.0	0.0	-	-
500.0	0.0	0.0	-	-
250.0	0.0	0.0	-	-
125.0	0.0	0.0	-	-
62.5	0.0	0.0	-	-
Toxic reference	93.3	96.7	-	-
LD ₅₀ (µg product/bee)	> 1000.0			
95% C.I.	-			
NOED (µg product/bee)	≥ 1000.0			

Mortality results are averages based on 30 replicates consisting of 1 bumblebee each

No sublethal effects were observed in the contact test.

Validity criteria

The study is considered valid since the control and reference item validity criteria were met:

- the mean mortality in both control groups of the oral and contact test was $\leq 10\%$ at the end of the test (observed values 0%);
- the mean reference item mortality was $\geq 50\%$ at the end of the test (observed values of 96.7% and 100.0% for the contact and oral tests, respectively)

Conclusion

In a 48-hr-acute oral toxicity study, bumble bees (*Bombus terrestris* L.) were exposed orally to A22773A via a feeding solution. The LD₅₀ was $> 983.9\ \mu\text{g}$ consumed product/bumble bee and the NOED was $983.9\ \mu\text{g}$ consumed product/bumble bee after 48 hours.

In a 48-hr-acute contact toxicity study, bumble bees (*Bombus terrestris* L.) were exposed to A22773A administered topically to adult bees. the LD₅₀ was $> 1000.0\ \mu\text{g}$ product/bumble bee and the NOED was $1000.0\ \mu\text{g}$ product/bumble bee after 48 hours.

(Amsel, K., 2022)

A 2.3.1.1.2 KCP 10.3.1.1.2 Acute contact toxicity to bees

See Section KCP 10.3.1.1.1. above.

A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

Comments of zRMS:	The study was conducted to OECD guidance TG 245 and according to the principles of GLP. All validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.
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Reference:	KCP 10.3.1.2
Report:	Dreßler K., (2020), Oxathiapiprolin/Azoxystrobin SC (A22773A) – Chronic toxicity to the honey bee <i>Apis mellifera</i> L. in a 10-day continuous laboratory feeding study, Report No 20 48 BAC 0043, BioChem agrar, Labor für biologische und chemische Analytik GmbH, Kupferstraße 6, 04827 Machern OT Gerichshain, Germany. (Syngenta file No. VV-881467).
Guideline(s):	OECD TG 245 (2017)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Executive Summary

The toxicity of A22773A to the honeybee *Apis mellifera* was determined in a 10-day continuous oral exposure study.

The 10-day NOEC was determined to be $3.043\ \text{g A22773A/kg sucrose solution}$ and the LC₅₀ is $14.871\ \text{g A22773A/kg sucrose solution}$. Based on actual consumption of the test solutions, the NOEDD was $112\ \mu\text{g consumed A22773A/bee/day}$ and the LDD₅₀ was calculated to be $378\ \mu\text{g consumed A22773A/bee/day}$.

Materials

Test Material

Name:	Oxathiapiprolin/Azoxystrobin SC (A22773A)
Product code:	A22773A
Lot/Batch #:	SFI003-174-001
Purity:	content of oxathiapiprolin: 12 g/L (nominal); 1.02% w/w corresponding to 11.2 g/L (analysed) content of azoxystrobin: 250 g/L (nominal); 22.5% w/w corresponding to 247 g/L (analysed)
Density:	1096 kg/m ³
Description:	formulation type: SC (suspension concentrate) appearance: beige liquid
Stability of test compound:	Stable under recommended handling and storage conditions (< 30 °C)
Reanalysis/Expiry date:	End of February 2023
Treatments	
Treatment groups:	2 untreated controls, 5 concentrations of A22773A, 1 concentration of the reference item
Test rates:	nominal dose rates of 1255, 697, 387, 215 and 119 µg A22773A/bee/day corresponding to concentrations of 31.950, 17.751, 9.861, 5.479 and 3.043 g A22773A/kg sucrose solution
Controls:	1 untreated control fed with 50% (w/v) sucrose solution and 1 untreated control fed with 50% (w/v) sucrose solution containing 0.1% (w/v) xanthan
Reference item:	Danadim® Progress (dimethoate)
Application method:	daily preparation of feeding solutions and provision <i>ad libitum</i> over a period of 10 days (according to OECD Guideline 245 (2017))
Analysis of test concentrations:	Yes, all applied concentrations of the active ingredient oxathiapiprolin were analytically verified in samples taken on each day of application by RP-HPLC with MS-MS detection. Moreover, the storage stability of the active ingredient oxathiapiprolin in sample matrix (50% (w/v) sucrose solution + 0.1% (w/v) xanthan) over the actual storage period was verified.
Test organisms	
Test organism:	worker honey bee
Species:	<i>Apis mellifera</i> L. subspecies Buckfast
Age:	max. 2 days old
Source:	BioChem agrar GmbH, Kupferstraße 6, 04827 Machern OT Gerichshain, Germany
Food:	50% (w/v) sucrose solution
Test Design	
Test cage description:	aluminium cages; dimensions: 95 mm (width) x 70 mm (height) x 60 mm (depth); with holes in the lateral walls for sufficient air supply and ventilation; two glass plates (one in front and one in the back) for observation of the bees
Replication:	3
No. of bees/replicate:	10
Environmental test conditions	
Temperature:	31.2 – 33.4 °C
Humidity:	55.9 – 64.2 %
Photoperiod:	darkness (diffuse artificial light only during assessments)
Duration of test:	10 days

Study Design and Methods

Test facility: BioChem agrar GmbH, Kupferstraße 6, 04827 Machern OT Gerichshain, Germany
Experimental dates: 30 June to 10 July 2020

A22773A treatments were freshly prepared every day. The target amount of 1.901 g A22773A was weighted into a 50 mL volumetric flask and dissolved in 50% (w/v) sucrose solution containing 0.1% (w/v) xanthan to prepare a 38.02 g/L stock solution (highest concentrated feeding solution). The remaining feeding solutions were prepared by parallel dilution by adding 13.89, 7.716, 4.287 or 2.381 mL of the highest concentrated feeding solution to 50 % (w/v) sucrose solution + 0.1 % (w/v) xanthan (total volume

of each feeding solution: 25 mL).

The control treatments were provided with 50 % (w/v) sucrose solution and 50 % (w/v) sucrose solution containing 0.1 % (w/v) xanthan.

To consider the evaporation from the feeders, three additional test units with untreated 50% (w/v) sucrose solution and untreated 50 % (w/v) sucrose solution + 0.1 % (w/v) xanthan and no bees present were set up. Bees were fed *ad libitum* with treated/untreated sugar solutions presented with syringe feeders, which were renewed every day. Feeders were weighed before and after they were offered, so that the food consumed could be determined by comparison of the weight of the remaining solution with the initial weight. The individual daily consumption was corrected each day by the number of surviving bees at each assessment date as well as by estimated sucrose evaporation.

Direct treatment effects (mortality and other observed biological effects) were assessed at daily intervals during the 10-day exposure period by visual counting of honeybees.

All applied concentrations of the active ingredient oxathiapiprolin were analytically verified in samples taken on each day of application by RP-HPLC with MS-MS detection. No residues of the active ingredient oxathiapiprolin were found in the control samples. Additionally, the storage stability of the active ingredient oxathiapiprolin in sample matrix (50 % (w/v) sucrose solution + 0.1 % (w/v) xanthan) over the actual storage period was verified.

To determine the NOEC and NOEDD, survival data were analysed for statistically significant differences compared to the viscosifier control (with xanthan) using Step-down Cochran-Armitage Test Procedure (one-sided greater, $\alpha = 0.05$). To determine the $LC_{50/20/10}$ and $LDD_{50/20/10}$ values along with their 95% confidence limits, Probit analysis using linear max. likelihood regression was used.

Results

Analytical recoveries in the diets are presented below.

Table A 31: Analytical results

Diet (mg oxathiapipr olin/kg)*	Analytical Recovery in Diet [mg oxathiapiprolin/kg] (% of nominal)									
	D0	D1	D2	D3	D4	D5	D6	D7	D8	D9
Untreated xanthan gum control	< 30% of LOQ	< 30% of LOQ	< 30% of LOQ	< 30% of LOQ	< 30% of LOQ	< 30% of LOQ	< 30% of LOQ	< 30% of LOQ	< 30% of LOQ	< 30% of LOQ
326	270 (83)	284 (87)	287 (88)	281 (86)	284 (87)	289 (89)	281 (86)	293 (90)	296 (91)	296 (91)
181	148 (82)	157 (87)	158 (87)	150 (83)	156 (86)	154 (85)	154 (85)	156 (86)	159 (88)	153 (85)
101	80.1 (80)	85.7 (85)	82.9 (82)	83.0 (83)	87.4 (87)	86.4 (86)	87.9 (87)	88.3 (88)	89.2 (89)	93.0 (92)
55.9	44.4 (80)	46.5 (83)	46.8 (84)	47.3 (85)	55.8 (100)	47.8 (86)	48.3 (86)	47.9 (86)	48.3 (86)	48.9 (87)
31.0	26.0 (84)	26.2 (84)	25.8 (83)	26.2 (84)	29.6 (95)	26.4 (85)	30.7 (99)	25.9 (83)	26.5 (85)	30.4 (98)

LOQ: 0.01 mg oxathiapiprolin/kg, corresponding to 0.25 µg oxathiapiprolin/L in diluted extracts

* based on the analysed content of oxathiapiprolin according to Certificate of Analysis of 06 April 2020 (1.02% w/w)

Mortality data for the test material and reference item are summarised in the table below.

Table A 32: Summary of mortality of bees in the chronic toxicity test after 10 days

Nominal Concentration [g A22773A/kg sucrose solution]	Daily Consumed Dose based on bee consumption [µg A22773A/bee/day]	After 10 days	
		Mean mortality	
		absolute [%]	corrected [%]
Blank control		6.7	--
Xanthan gum control		10.0	--
31.950	699	76.7*	74.1
17.751	498	63.3*	59.3
9.861	227	40.0*	33.3
5.479	157	30.0*	22.2
3.043	112	13.3	3.7
Reference item		96.7	96.4

Results are averages based on 3 replicates, containing 10 bees each; Calculations are performed with non-rounded values. corrected: corrected mortality (according to SCHNEIDER-ORELLI 1947); Mortality of the test item treatment group was corrected for mortality of the untreated xanthan gum control group, whereas mortality of the reference item treatment group was corrected for mortality of the untreated blank control group. Negative values are treated as "0".

* Statistically significant difference in pairwise comparison between treatment and untreated xanthan gum control group (Step-down Cochran-Armitage Test Procedure; $\alpha = 0.05$; one-sided greater)

During the course of the test, behavioural abnormalities were observed in the third highest test item dose (227 µg consumed A22773A/bee/day). One bee out of 26 remaining bees was observed as being moribund on day 5. No other behavioural abnormalities were observed in any test item treatment group on any other assessment day.

Study endpoints are summarised in the table below.

Table A 33: Study endpoints at 10 days

Treatment	Endpoints	After 10 d
Test item doses	LDD ₁₀ [µg consumed A22773A/bee/day]	116 (70.1 – 155)
	LDD ₂₀ [µg consumed A22773A/bee/day]	174 (122 – 219)
	LDD ₅₀ [µg consumed A22773A/bee/day]	378 (307 – 490)
	NOED [µg consumed A22773A/bee/day]	112
Test item concentrations	LC ₁₀ [g A22773A/kg sucrose solution]	3.817 (2.103 – 5.382)
	LC ₂₀ [g A22773A/kg sucrose solution]	6.088 (4.019 – 7.966)
	LC ₅₀ [g A22773A/kg sucrose solution]	14.871 (11.689 – 20.025)
	NOEC [g A22773A/kg sucrose solution]	3.043

Validity Criteria

The test was considered valid;

- Average cumulative mortality was <15% in the sucrose solution control
- Average cumulative mortality was <15% in the xanthan gum control
- Mortality was >50% in the toxic reference

Conclusion

The toxicity of A22773A to the honeybee *Apis mellifera* was determined in a 10-day continuous oral exposure study.

The 10-day NOEC was determined to be 3.043 g A22773A/kg sucrose solution and the LC₅₀ is 14.871 g A22773A/kg sucrose solution. Based on actual consumption of the test solutions, the NOEDD was 112 µg consumed A22773A/bee/day and the LDD₅₀ was calculated to be 378 µg consumed A22773A/bee/day.

(Dressler K, 2020)

Comments of zRMS:	Study not evaluated.
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Reference:	KCA 8.3.1.2
Report:	Tänzler, V. (2015), Azoxystrobin SC (A12705B) - Chronic Oral Toxicity Test on the Honey Bee (<i>Apis mellifera</i> L.) in the Laboratory. Report Number 100921136. ibacon GmbH Arheilger Weg 17 64380 Rossdorf Germany. Syngenta File No. VV-414159
Guideline(s):	OECD Guideline 213 (1998)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Executive Summary

The effects of A12705B were assessed on young adult honey bees, *Apis mellifera*, in a 10 day chronic feeding test under laboratory conditions.

The LC₅₀ was calculated to be 4.29 g product/kg feeding solution and the NOEC was determined to be 2.35 g product/kg feeding solution

The LD₅₀ was calculated to be 76.7 µg product/bee/day and the NOED was determined to be 44.1 µg product/bee/day.

Materials

Test Material	A12705B Azoxystrobin SC
Lot/Batch #:	GRA2L121D
Actual content of active ingredients:	22.7 % w/w corresponding to 248 g/L
Description:	Beige liquid
Stability of test compound:	Stable under test conditions
Reanalysis/Expiry date:	January 2016
Density:	1.093 g/cm ³
Treatments	
Test rates:	14.7, 5.87, 2.35, 0.938 and 0.374 g product/kg feeding solution (corresponding to 3 333, 1 333, 533, 213 and 85 mg a.i./kg feeding solution) Mean dose of 343.3, 97.8, 44.1, 26.4 and 10.6 µg product/bee/day (based on daily actual intake) after 10 days (corresponding to 77.9, 22.2, 10.0, 6.0 and 2.4 µg a.i./bee/day)
Control:	50 % w/v sucrose solution (500 g sucrose/L deionised water)
Toxic standard:	Dimethoate BAS 152 11 I (nominal: 400.0 g/L; measured 400.9 g/L)
Administration:	Oral application in artificial diet
Test organisms	
Species:	<i>Apis mellifera carnica</i> L. (Hymenoptera: Apidae) (young adult worker bees)
Source:	Culture maintained at test facility
Food:	50 % (w/v) sucrose solution continuously ad libitum
Test design	
Test cage description:	Stainless steel chambers (8 x 6 x 4 cm)
Replication:	3 replicates of 10 bees
Duration of test:	10 days
Environmental test conditions	
Temperature:	31 - 33 °C
Humidity:	23 - 62 %; average relative humidity was 59 %
Photoperiod:	Constant darkness

Study Design and Methods

Experimental dates: 27th May to 06th June 2015

Two days prior to test initiation, brood combs containing capped cells which were expected to hatch on the same day were transferred into a climatically controlled chamber from the honey bee colony. Brood combs from two hives were used to guarantee a sufficient number of bees for the test. One day prior to test start the 0 – 3 day old bees were transferred from combs to the test cages and kept under test conditions.

Feeding solutions were placed in syringes, the tips of which had been removed for access, and offered to the bees in each unit *ad libitum*. Bees in one replicate shared the feeding solution and thus received similar doses. Feeding solutions were replaced daily and the amount of feeding solution consumed was determined by weighing the syringe before and after feeding.

The LC₅₀ and LD₅₀ values of the test item were estimated with Probit Analysis (according to Finney 1971). It was not necessary to correct for control mortality, since no control mortality occurred. The NOEC/NOED of the test item was estimated using Fisher's Exact Test (pairwise comparison, 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 2.10.05, © ToxRat Solutions GmbH. A12705B was analysed in each test item and control solution by HPLC-MS/MS.

Results and Discussion

Mortality data for the test material and control are summarised in the table below.

Table A 34: Summary of chronic toxicity of A12705B to honey bees (*Apis mellifera* L.)

Nominal concentration [µg prod./bee/day]	Mean dose [µg prod./bee/day]	Corrected cumulative mortality (%)									
		E1	E2	E3	E4	E5	E6	E7	E8	E9	E10
Control		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
400.0	343.3	0.0	10.0	30.0	63.3	93.3	96.7	96.7	100.0	100.0	100.0
160.0	97.8	0.0	0.0	3.3	6.7	13.3	16.7	23.3	33.3	50.0	66.7
64.0	44.1	3.3	6.7	10.0	10.0	10.0	10.0	10.0	16.7	16.7	16.7
25.6	26.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10.2	10.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Reference Item		0.0	0.0	16.7	43.3	80.0	100.0	100.0	100.0	100.0	100.0
LC ₅₀ [g product/kg feeding solution]		4.29									
NOEC[g product/kg feeding solution]		2.35									
LD ₅₀ [µg product/bee/day]		76.7									
NOED [µg product/bee/day]		44.1									

Table A 35: Accumulated mean uptake of A12705B

Concentration (µg a.i./bee/day)	mean uptaken µg a.i./bee over the course of the study ^a										
	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	Mean
100.0	68.7	41.2	54.1	49.2	72.5	104.2	125.7	107.7	-	-	77.9
40.0	29.7	29.5	19.0	31.9	12.0	16.3	25.2	13.5	19.6	25.2	22.2

Concentration (µg a.i./bee/day)	mean uptaken µg a.i./bee over the course of the study ^a										
	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	Mean
16.0	11.0	17.5	10.8	10.3	5.7	11.5	5.8	10.2	9.8	7.7	10.0
6.4	6.4	6.1	6.6	7.6	7.0	6.2	6.4	5.5	4.2	4.5	6.0
2.55	2.1	2.4	2.6	1.9	3.1	2.6	2.3	2.3	2.5	2.6	2.4
Reference	0.03	0.02	0.02	0.02	0.02	0.03	-	-	-	-	0.02

^a a calculated average per living bees, results are rounded results, calculated from the exact data

Conclusions

The chronic oral toxicity of A12705B was tested on honeybees under laboratory conditions over 10 days.
The 10 day LC₅₀ value was 4.29 g product/kg feeding solution.
The 10 day LD₅₀ value was 76.7 µg product/bee/day.

The 10 day NOEC and NOED values were 2.35 g product/kg feeding solution and 44.1 µg product/bee/day, respectively.

Tänzler V, 2015

Comments of zRMS:	Study not evaluated.
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Reference:	KCA 8.3.1.3
Report:	Ehmke A. (2015), Azoxystrobin SC (A12705B) - Honey Bee (<i>Apis mellifera</i> L.) Larval Toxicity Test, Repeated Exposure, Report Number 100921032. ibacon GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany. Syngenta File No. VV-414544
Guideline(s):	OECD DRAFT Guidance Document for testing chemicals: Honey bee (<i>Apis mellifera</i>) larval toxicity test, repeated exposure (2014) OECD 237 Guidelines: Honey bee (<i>Apis mellifera</i>) larval toxicity test, single exposure (2013)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Executive Summary

The purpose of this study was to determine the semi-chronic toxicity of A12705B to honeybee larvae *Apis mellifera* L. in an *in vitro* test after repeated oral application. The 8 day NOEC was determined to be a food concentration of 0.2544 g A12705B/kg diet. The 8 day NOED was determined to be 39.2 µg product/larva. The 8 day LD₅₀ was determined to be 56.2 µg product/larva.

Materials

Test Material	A12705B
Lot/Batch #:	Azoxystrobin SC GRA2L121D
Actual content of active ingredients:	22.7 % (w/w), corresponding to 248 g/L (analytical)
Description:	Beige liquid
Stability of test compound:	Stable under test conditions
Reanalysis/Expiry date:	January 2016
Density:	1.093 g/cm ³
Treatments	
Test rates:	Total µg product/larva: 352.6, 117.5, 39.2, 13.1, 4.4 and 1.5 Total µg a.i./larva: 80.0, 26.7, 8.9, 3.0, 1.0 and 0.3
Control:	Untreated diet of royal jelly and sucrose solution
Toxic standard:	Dimethoate tech. (BAS 152 I), purity 98.5 %
Application method:	Oral application using a sterile pipette
Test organisms	
Species:	Worker honey bee larvae <i>Apis mellifera</i> L. subspecies <i>carnica</i> P. (Insecta, Hymenoptera, Apoidea)
Age:	First instar (L1) during grafting
Source:	Maintained at test facility
Food:	Artificial diet of royal jelly; sucrose solution, yeast, fructose and glucose
Test Design	
Test cage description:	Sterile 48-well plates(8.5 cm x 2 cm x 12.5 cm) equipped with crystal polystyrene grafting cells Grafting cells were sterilized before introduction of the larvae with a suitable method
Replication:	3
No. of larvae/replicate:	12
Environmental test conditions	
Temperature:	33 – 36 °C
Humidity:	45 – 95 %
Photoperiod:	Constant darkness

Duration of test: 8 day test, endpoints given 120 h after first exposure

Study Design and Methods

Experimental dates: 20th to 25th May 2015

The test/reference item was mixed into sterile filtered aqueous sugar solution. Several dilutions were prepared by adding further sugar solution. The royal jelly was added to each stock solution at a ratio of 1 : 1, based on (w/w), to reach the final test concentrations.

Honeybee larvae *Apis mellifera* L. were exposed to repeated oral application of 80.0, 26.7, 8.9, 3.0, 1.0 and 0.3 µg a.i./larva (equivalent to 352.6, 117.5, 39.2, 13.1, 4.4 and 1.5 µg product/larva) in an *in vitro* test. One control group was included in the test. The larvae of the control treatment were fed with untreated artificial diet, which served as a vehicle for the test item and reference item.

Two days prior to test start, the combs containing the larvae were transported from the hive to an acclimated laboratory room. Larvae were transferred from the combs to the crystal polystyrene grafting cells using a suitable grafting tool. During grafting the larvae were placed on the surface of the artificial diet within the grafting cells. Cells were placed in 48 well plates. Each replicate unit consisted of 12 larvae, and there were 3 replicates per treatment and control. Each larva was fed daily between Day 0 and Day 3 using a sterile pipette.

The number of dead larvae was recorded at 24, 48, 72, 96 and 120 hours. The presence of unconsumed food was recorded qualitatively at test end, 120 h after application. After the last assessment the culture plates with all organisms were placed in a freezer.

The LD₅₀ value of the test item was estimated with Probit Analysis (according to Finney 1971).

The NOED of the test item was estimated using Fisher's Exact Test (pairwise comparison, 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 2.10.05, ® ToxRat Solutions GmbH.

Results and Discussion

Mortality data and other observations for the test material and reference item are summarised in the table below.

Table A 36: Summary of semi-chronic toxicity of test material to honeybee larvae

Item applied	Dosage [µg product/larva]	Day 8	
		Mortality mean %	
		Absolute	Corrected
Control	-	2.8	-
Test item	352.6	100.0*	100.0
	117.5	97.2*	97.1
	39.2	5.6	2.9
	13.1	0.0	-2.9
	4.4	8.3	5.7

Item applied	Dosage [µg product/larva]	Day 8	
		Mortality mean %	
		Absolute	Corrected
	1.5	5.6	2.9
Reference item	6.2	100.0	-
Treatment	Endpoints	Day 8	
Test item doses	NOED [µg product/larva]	39.2	
	LD50 [µg product/ larva] (95 %-CL/lower-upper)	56.2	
Test item concentrations	NOEC [g A12705B/kg diet]	0.2544	

OO: Other observations

* statistically significant according to Fisher`s Exact Test (pairwise comparison, one-sided greater, $\alpha = 0.05$).

Analytical Verification

The actual analysed concentration, was within the required range of 80-120 % of the nominal concentration (actual values 82 %), thus confirming correct preparation of the analysed test item stock solution

Validity Criteria

All of the validity criteria were met:

- Control mortality should be ≤ 15 % for larvae across all control replicates at day 8 (actual value 2.8 %)
- Reference item mortality should be ≥ 50 % for larvae across all reference replicates at day 8 (actual value 100 %)

Conclusions

The chronic larval toxicity of A12705B was tested on honey bee larvae under laboratory conditions.

The 8 day LD₅₀ value was 56.2 µg product/larva. The 8 day NOED value was 39.2 µg product/larva. The 8 day NOEC was determined to be a food concentration of 0.2544 g A12705B/kg diet.

Ehmke A, 2015

Comments of zRMS:	Study not evaluated.
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Reference: KCA 8.3.1.2

Report: Tänzler, V. (2015), Oxathiapiprolin (DPX-QGU42) 100 g/L OD: Chronic oral toxicity to the honey bee, *Apis mellifera* L. (Hymenoptera, Apidae). Report Number 94441136. Institut für Biologische Analytik und Consulting IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany. DuPont Study No. DuPont-41989. (Study owner Corteva, Syngenta have access)

Guideline(s): OECD Guideline No. 213 (1998)
CEB No. 230 (2014)

Deviations: No

GLP: Yes

Acceptability: Yes

Executive Summary

This study was conducted in order to determine the chronic oral toxicity of Oxathiapiprolin 100 g/L OD on the honey bee (*Apis mellifera* L.) under laboratory conditions for a period of ten days. Mortality of the bees was used as the toxic endpoint. Sublethal effects such as changes in behaviour were also monitored and reported.

Under laboratory conditions 30 freshly emerged worker bees (*Apis mellifera* L.) per treatment level were exposed for 10 days to 5 concentrations (1500, 750, 375, 188 and 94 mg a.s./kg food corresponding to 14306, 7153, 3576, 1788 and 894 mg prod./kg food) of the test item-treated sugar solutions *ad libitum*. These concentrations led to actual mean dose levels of 43.6, 24.1, 12.1, 6.5 and 3.7 µg a.s./bee per day (corresponding to 415.8, 229.8, 115.4, 62.0 and 35.3 µg prod./bee per day). An untreated control and a reference item were included in this study.

At test end (10 days after start of exposure) there was 83.3, 13.3, 3.3, 3.3 and 0.0% mortality in the 1500, 750, 375, 188 and 94 ppm treatments, respectively (corresponding to 14306, 7153, 3576, 1788 and 894 ppm prod./kg food). A mortality of 6.7 % was observed in the water control group.

On day 7 two bees and day 9 to 10 three bees from the 1500 ppm treatment level were affected. On day 10 one bee from the 750 ppm treatment level was affected. No further test item-related behavioural effects were observed.

The reference item (dimethoate) at a concentration of 1 ppm (corresponding to a mean dose of 0.021 µg a.s./bee/day) led to 100% mortality on day 10.

Materials

Test material:	Oxathiapiprolin 100 g/L OD
Batch/Lot Number:	QGU42-347
Purity:	100 g a.s./L (nominal); 103.7 g/L (measured)
Description:	OD (oil dispersion)
CAS Registry Number:	None for the formulation 1003318-67-9 for the active substance
Test vehicle:	50 % w/v sucrose solution (500 g sucrose/L deionised water)
Reference item:	Perfekthion (BAS 152 11 I)
Test organism:	Worker honey bees (Insecta, Hymenoptera)
Species:	Adult <i>Apis mellifera carnica</i> L.
Stage and Sex:	Female worker bees
Source:	Honey bee colonies, disease-free and queen-right, bred by IBACON

Collection:	Two days before the start of the test, three brood combs with sealed brood and bees visibly starting to emerge were selected. The comb also contained pollen which served as a first feeding source for the freshly hatched bees. The combs were taken out from the hive and the adult bees were swept away. Then it was enclosed in an excluder box and stored in an incubator in the laboratory. The emerging bees remained in the excluder box for one further day. The following day (test start), the freshly emerged worker bees were taken out from the excluder box with forceps and were transferred to the ready-prepared test units (cages) without the use of smoke and without anaesthetics.
Environmental conditions	
Temperature:	32 – 33 °C
Relative Humidity:	58 – 59 %
Application:	The treated and untreated feeding solutions were offered <i>ad libitum</i> to each cage in syringes. The syringes were weighed daily before introduction into the cages and after the feeding interval (before daily replacement with new syringes containing fresh test solutions).

Study Design and Methods

Experimental dates: 02 September 2014 to 12 September 2014

The study comprised 7 treatment groups (five dose rates of the test item, water control, one dose rate of the reference item) with 3 replicates each containing 10 bees. The final concentration of sugar in the test item feeding solutions offered to the bees was 50 % (w/v). The feeding solutions of the test item were prepared freshly every day.

The reference item was prepared with 50 % (w/v) sucrose solution. The stock solution of the reference item was prepared once at start of the test and stored at 4 °C ± 4 °C over a period of ten days. 50 % (w/v) sucrose solution was used for the untreated control. The feeding solutions of the reference item and untreated control were prepared freshly every 4 days and stored at 4 °C ± 4 °C.

The treated and untreated feeding solutions were offered *ad libitum* to each cage in syringes. The syringes were weighed daily before introduction into the cages and after the feeding interval (before daily replacement with new syringes containing fresh test solutions).

Results and Discussion

All study validity criteria were met.

Under laboratory conditions 30 freshly emerged worker bees (*Apis mellifera* L.) per treatment level were exposed for 10 days to 5 concentrations (1500, 750, 375, 188 and 94 mg a.s./kg food corresponding to 14306, 7153, 3576, 1788 and 894 mg prod./kg food) of the test item-treated sugar solutions *ad libitum*. These concentrations led to actual mean dose levels of 43.6, 24.1, 12.1, 6.5 and 3.7 µg a.s./bee per day (corresponding to 415.8, 229.8, 115.4, 62.0 and 35.3 µg prod./bee per day). An untreated control and a reference item were included in this study.

At test end (10 days after start of exposure) there was 83.3, 13.3, 3.3, 3.3 and 0.0 % mortality in the 1500, 750, 375, 188 and 94 ppm treatments, respectively (corresponding to 14306, 7153, 3576, 1788 and 894 mg prod./kg food). A mortality of 6.7 % was observed in the water control group.

On day 7 two bees and day 9 to 10 three bees from the 1500 ppm treatment level were affected. On day 10 one bee from the 750 ppm treatment level was affected. No further test item-related behavioural effects were observed.

The reference item (dimethoate) at a concentration of 1 ppm (corresponding to a dose of 0.021 µg a.s./bee/day) led to 100% mortality on day 10.

The results are summarised in the table below.

Table A 37: Chronic toxicity of Oxathiapiprolin 100 g/L OD to honeybees

Test Organism			<i>Apis mellifera</i> L.			
Treatment Group	Concentration [mg a.s./kg]	Concentration [mg prod./kg]	Dose Level ^a [µg a.s./bee]	Dose Level ^a [µg prod./bee]	Mortality at day 10 ^b [% mean]	Corrected mortality [%]
Oxathiapiprolin 100 g/L OD	1500	14306	43.6	415.8	83.3 (*)	82.1
	750	7153	24.1	229.8	13.3 (n.s.)	7.1
	375	3576	12.1	115.4	3.3 (n.s.)	0.0
	188	1788	6.5	62.0	3.3 (n.s.)	0.0
	94	894	3.7	35.3	0.0 (n.s.)	0.0
Water Control	0.0		0.0		6.7	-
Reference Item	1.0		0.021		100	100

^a Mean dose per bee per day; dose measured based on consumed feeding solution

^b Mortality at study termination 10 days after start of first feeding

n.s. = no statistical significant difference compared to the control

* = statistically significant difference compared to the control

Table A 38: Chronic toxicity of Oxathiapiprolin 100 g/L OD to honeybees: Endpoints at test termination (day 10)

LC ₅₀	LD ₅₀	NOEC	NOED
1148.1 mg a.s./kg food 10949.6 mg prod./kg food	34.7 µg a.s./bee per day 330.9 µg prod./bee per day	750 mg a.s./kg food 7153 mg prod./kg food	24.1 µg a.s./bee per day 229.8 µg prod./bee per day

Statistical analysis:

LC/LD: according to Probit Analysis (according to Finney 1971).

NOEC/NOED: Fisher's Exact Test, pairwise comparison, one-sided greater, $\alpha = 0.05$

Conclusions

The chronic toxicity of Oxathiapiprolin 100 g/L OD was tested over 10 days:

The LC₅₀ value (10 days) was 1148.1 mg a.s./kg feeding solution (corresponding to 10949.6 mg prod./kg feeding solution). The LD₅₀ value (10 days) was 34.7 µg a.s./bee per day (corresponding to 330.9 µg prod./bee per day). The NOED and NOEC values (10 days) were 24.1 µg a.s./bee/day and 750 mg a.s./kg feeding solution, respectively (corresponding to 229.8 µg prod./bee per day and 7153 mg prod./kg feeding solution, respectively).

In addition, the LC₁₀ value (10 days) and the LC₂₀ value (10 days) were 791.4 mg a.s./kg feeding solution and 899.2 mg a.s./kg feeding solution, respectively (corresponding to 7547.7 mg prod./kg feeding solution and 8575.8 mg prod./kg feeding solution, respectively). Moreover the LD₁₀ value (10 days) and the LD₂₀ value (10 days) were 25.2 µg a.s./bee/day and 28.1 µg a.s./bee/day, respectively (corresponding to 240.3 µg prod./bee/day and 268.0 µg prod./bee/day).

The analytical recovery rate of the active ingredient oxathiapiprolin in the feeding solutions was between 103 and 128% of the nominal value.

(Tänzler V, 2015)

Comments of zRMS:	Study not evaluated.
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Reference: KCA 8.3.1.3

Report: Oberrauch, S., (2017), Oxathiapiprolin (DPX-QGU42) technical: Honey bee (*Apis mellifera* L.) 22 day larval toxicity test (repeated exposure). Report Number S17-01639. Eurofins Agrosience Services EcoChem GmbH / Eurofins Agrosience Services Ecotox GmbH, Eutinger Str. 24, 75223 Niefern-Öschelbronn, Germany. DAS Study No. DuPont-48606. (Study owner Corteva, Syngenta have access)

Guideline(s): OECD Guideline No. 239 (2016)

Deviations: No

GLP: Yes

Acceptability: Yes

Executive Summary

Effects of the test item oxathiapiprolin on the honey bee larvae, *Apis mellifera* L., from repeated feeding exposure in a 22-day *in vitro* test were assessed.

On the first day (D1) of the dose response test synchronised honey bee larvae (*Apis mellifera carnica* Pollmann, first instar, L1) were transferred into 48-well plates where they were fed a standardised amount of artificial diet. On Days 3, 4, 5 and 6 of the test, five different concentrations (7.30, 18.2, 45.6, 114 and 285 mg oxathiapiprolin/kg diet) of the test item and one single concentration of the reference item (48.0 mg dimethoate/kg diet) were applied to the larvae with Diet B and Diet C. A control and solvent control group were included in the test and exposed for the same period of time under identical exposure conditions to the water and acetone treated artificial diet. Assessment of mortality was carried out during the larval phase on Days 4, 5, 6, 7 and 8. The presence of uneaten food was qualitatively recorded on Day 8. Assessment of mortality was carried out during the pupation phase on Days 15 and 22. Assessment of adult emergence was carried out on Day 22.

The No Observed Effect Concentration/Dose (NOEC/NOED) as well as the concentrations and doses causing 10, 20 and 50 % reduction of adult emergence (EC₁₀/ED₁₀, EC₂₀/ED₂₀ and the EC₅₀/ED₅₀) were determined for Day 22, where possible.

The measured concentrations of the test item stock solution and the test item solutions were within 20 % of nominal. The measured concentrations of the test item treated larval diet of the test item groups of 7.30, 18.2 and 45.6 mg oxathiapiprolin/kg diet were between 84 and 130 % of nominal, with mean recoveries across the application days of 104, 104 and 95% of nominal, per test item group. The nominal concentrations of the two highest test item groups of 114 and 285 mg oxathiapiprolin/kg diet were not confirmed. A possible reason for this could be the low water solubility of the test item. Therefore, they were excluded from the statistical evaluation.

On Day 8, the cumulative mortalities in the control and solvent control group were both 0.0 %. The cumulative mortality in the reference item group was 97.9 %. On day 22 the adult emergence rate was 89.6 % in the control group and 85.4 % in the solvent control group.

There were no statistically significant differences in adult emergence compared to the solvent control group in any test item group that was statistically analysed.

On Day 22, the NOEC relating to adult emergence for oxathiapiprolin was determined as ≥ 45.6 mg oxathiapiprolin/kg diet, equivalent to a NOED of ≥ 7.02 µg oxathiapiprolin/larva per developmental period.

On Day 22, the EC₁₀ and the corresponding ED₁₀ relating to adult emergence for oxathiapiprolin could not

be determined due to the lack of a clear concentration response relationship.

On Day 22, the EC₂₀ and EC₅₀ relating to adult emergence for oxathiapiprolin could not be determined since there was no reduction in emergence above 20 % in any of the concentrations that were statistically analysed. However, they can be regarded as > 45.6 mg oxathiapiprolin/kg diet, equivalent to an ED₂₀ and ED₅₀ of > 7.02 µg oxathiapiprolin/larva per developmental period.

During the assessments of mortality and adult emergence no test item related other observations such as deviating sizes, appearances and malformations of the test organisms were made. On Day 8, uneaten food was observed in the solvent control group and in the test item groups T1, T4 and T5 with nominal concentrations of 7.30, 114 and 285 mg oxathiapiprolin/kg diet.

The study was considered valid since all validity criteria were met.

Materials

Test material:	Oxathiapiprolin technical
Batch/Lot Number:	QGU42-174
Purity:	95.8% by analysis
Description:	Solid, chrystalline, off-white
CAS #	1003318-67-9
Stability in solution:	Stable at normal temperatures and storage conditions
Control:	Water-treated Diet B and Diet C containing autoclaved, deionized water as solvent
Solvent control:	Acetone-treated Diet B and Diet C containing acetone as solvent
Test vehicle:	Test item-treated Diet B and Diet C containing aliquots of the test item solution prepared with acetone
Test organism:	Honey Bee larvae
Species:	<i>Apis mellifera carnica</i> Pollmann
Age at grafting:	First instar larvae, L1
Source:	Test facility (Eurofins Agroscience Services EcoChem GmbH)
Place of test:	Eurofins Agroscience Services Ecotox GmbH Neulingen-Göbrichen Field Station, 75245 Neulingen-Göbrichen, Germany All bee hives were located at the field station.
Test chamber:	Crystal polystyrene grafting cells (NICOTPLAST) having a diameter of 9 mm; each cell was placed into a well of a sterile 48-well cellular culture plate. The open plates were placed into a hermetically sealed desiccator, containing a dish filled with a saturated potassium sulphate (K ₂ SO ₄) solution in order to keep a water saturated atmosphere from Day 1 until Day 8. On Day 8, the plates were transferred into a second desiccator containing a saturated sodium chloride (NaCl) solution. The desiccators were placed in an incubator with forced air circulation. On Day 15, each plate was covered by its lid and transferred from the desiccator into an incubator. The incubator contained a dish filled with deionised water in order to keep the adequate relative air humidity.
Environmental conditions	
(biological phase)	
Temperature:	31.4 – 35.2 °C
Relative Humidity:	39.1 – 100 %
Exposure to light:	None, except during grafting, feeding and assessments

Study Design and Methods

Experimental dates: 30 May 2017 to 14 September 2017

Experimental treatments

Two controls, five test item concentrations of 7.30, 18.2, 45.6, 114 and 285 mg oxathiapiprolin/kg diet and one single concentration of the reference item (48.0 mg dimethoate/kg diet) were tested. For each treatment group, 48 larvae from three different hives were tested over 22 days. Each hive equates to one replicate, 16

larvae from each replicate were used. On Days 3, 4, 5 and 6 of the test diet B or diet C containing the test item solutions was applied to the larvae.

Observations

Assessments of mortality were carried out during the larval phase on Days 4, 5, 6, 7 and 8 and during the pupation phase on Days 15 and 22. The presence of uneaten food was qualitatively recorded on Day 8. Assessment of adult emergence was carried out on Day 22. Other observations (appearance, size, malformation of organisms) were recorded to aid in the interpretation of mortality in comparison to the solvent control group.

Statistics

Multiple Fisher's exact test with Bonferroni-Holm adjustment (one-sided greater, $\alpha = 0.05$) was used to evaluate whether there was a statistically significant difference between the mortality data of the test item groups and the solvent control group for larval mortality on Day 8. Multiple Chi²-test with Bonferroni-Holm adjustment (one-sided greater, $\alpha = 0.05$) was used to evaluate whether there was a statistically significant difference between the mortality data of the test item groups and the solvent control group for larval and pupal mortality on Day 15 and pupal mortality from Day 8 through 22 as well as for adult emergence on Day 22. Since the nominal concentrations of the highest two test item groups of 114 and 285 mg oxathiapiprolin/kg diet were not confirmed by the analytical verification, both test item groups were excluded from statistical analysis.

The NOEC was determined for adult emergence on Day 22. The corresponding NOED was calculated by taking into account the density of the larval diet (1.1 g/cm³) and the cumulative feeding volume per larva of 140 µL. The EC₁₀ with 95% confidence limits could not be determined due to the lack of a clear concentration response relationship. The EC₂₀ and EC₅₀ with 95% confidence limits could not be calculated since there was no reduction in emergence above 20 % in any of the concentrations that were statistically analysed, but can be regarded as above the highest concentration tested. The corresponding ED₂₀ and ED₅₀ values were calculated by taking into account the density of the larval diet (1.1 g/cm³) and the cumulative feeding volume per larva of 140 µL.

Results and Discussion

Study results and endpoints are summarised in the table below.

Table A 39: The effects on adult emergence of honey bees until Day 22 after repeated exposure of treated diet in the laboratory

Treatment Group	Concentration		Cumulative Dose		Adult Emergence on Day 22
					(%)
Control	---	---	---	---	89.6
Solvent Control	---	---	---	---	85.4
Test Item (oxathiapiprolin)	7.30	[mg oxathiapiprolin/ kg diet] ^b	1.12	[µg oxathiapiprolin/ larva per developmental period] ^{b c}	87.5
	18.2		2.80		89.6
	45.6		7.02		68.8
	114 ^a		17.6 ^a		83.3
	285 ^a		43.9 ^a		66.7
Endpoints for Adult Emergence on Day 22					
NOEC	EC ₁₀		EC ₂₀		EC ₅₀
[mg oxathiapiprolin/kg diet] ^b					
≥ 45.6	n.d. ^d		> 45.6 ^e		> 45.6 ^e
NOEC	EC ₁₀	EC ₂₀	EC ₅₀	NOEC	EC ₁₀
[µg oxathiapiprolin/larva per developmental period] ^{b,c}					
≥ 7.02	n.d. ^d		≥ 7.02 ^e		≥ 7.02 ^e

^a The test item groups of 114 and 285 mg oxathiapiprolin/kg diet (respectively 17.6 and 43.9 µg oxathiapiprolin/larva per

- developmental period) were excluded from statistical evaluations, since the nominal concentrations were not confirmed by the analytical dose verification
- ^b Based on the analysed purity
 - ^c Based on the total feeding volume of 140 µL and the density of the diet of 1.1 g/cm³
 - ^d The EC₁₀/ED₂₀ values for the adult emergence on Day 22 could not be determined due to the lack of a clear concentration/dose response relationship.
 - ^e The EC₂₀/ED₂₀ and EC₅₀/ED₅₀ for the adult emergence on Day 22 could not be determined, since there was no reduction in emergence above 20 % in any of the concentrations that were statistically analysed. Therefore, the EC₂₀/ED₂₀ and EC₅₀/ED₅₀ values can be regarded as above the highest concentration/dose statistically tested.

Conclusions

The measured concentrations of the test item stock solution and the test item solutions were within ± 20 % of nominal. The measured concentrations of the test item treated larval diet of the test item groups of 7.30, 18.2, and 45.6 mg oxathiapiprolin/kg diet were between 84 and 130 % of nominal, with mean recoveries across the application days of 104, 104 and 95 % of nominal, per test item group.

The nominal concentrations of the two highest test item groups of 114 and 285 mg oxathiapiprolin/kg diet were not confirmed. A possible reason for this could be the low water solubility of the test item. Therefore, they were excluded from the statistical evaluation.

On Day 8 the cumulative mortalities in the control and solvent control group were both 0.0 %. The cumulative mortality in the reference item group was 97.9%. On Day 22, the adult emergence rate was 89.6 % in the control group and 85.4 % in the solvent control group. Thus, the validity criteria were met and the study was deemed valid.

On Day 22, the NOEC relating to adult emergence for oxathiapiprolin was determined as ≥ 45.6 mg oxathiapiprolin/kg diet, equivalent to a NOED of ≥ 7.02 µg oxathiapiprolin/larva per developmental period. On Day 22, the EC₁₀ and the corresponding ED₁₀ relating to adult emergence for oxathiapiprolin could not be determined due to the lack of a clear concentration response relationship.

On Day 22, the EC₂₀ and EC₅₀ relating to adult emergence for oxathiapiprolin could not be determined since there was no reduction in emergence above 20 % in any of the concentrations that were statistically analysed. However, they can be regarded as > 45.6 mg oxathiapiprolin/kg diet, equivalent to an ED₂₀ and ED₅₀ of > 7.02 µg oxathiapiprolin/larva per developmental period.

(Oberrauch S, 2017)

A 2.3.1.3 KCP 10.3.1.3 Effects on honey bee development and other honey bee life stages

Comments of zRMS:	The study was conducted to OECD guidance TG 239 and according to the principles of GLP. All validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.
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Reference:	KCP 10.3.1.3
Report:	Schmidt, K., (2021), Oxathiapiprolin/azoxystrobin SC (A22773A)–Repeated Exposure of the Honey Bee Larvae (<i>Apis mellifera</i> L.) under Laboratory Conditions (until Adult Emergence up to Day 22). Report Number 20 48 BLC 0043, BioChem agrar, Labor für biologische und chemische Analytik GmbH, Kupferstraße 6, 04827 Machern OT Gerichshain, Germany. (Syngenta file No. VV-896655).
Guideline(s):	OECD Guideline No. 239 (2016)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Executive Summary

The toxicity of Oxathiapiprolin/azoxystrobin SC (A22773A) to the larvae of the honeybee *Apis mellifera* was determined in a chronic exposure study over a 22-day period.

The 22-day NOEC was determined to be 661 mg product/kg diet and the EC₅₀ is 1625 mg product/kg diet.

Materials

Test Material	Oxathiapiprolin/azoxystrobin SC (A22773A)
Lot/Batch #:	SFI003-174-001
Purity:	Oxathiapiprolin 1.02% w/w corresponding to 11.2 g/L Azoxystrobin 22.5% w/w corresponding to 247 g/L
Description:	Beige liquid
Stability of test compound:	Stable under the given conditions
Reanalysis/Expiry date:	End of February 2023
Treatments	
Test rates:	µg product/larva: 418, 209, 105, 52, 26 mg product/kg diet: 2643, 1322, 661, 330, 165
Control:	Untreated diet
Toxic standard:	Dimethoate tech., Purity: 98.8 ± 0.5%, 7.6 µg a.i./larva, 48 mg a.i./kg diet
Analysis of test concentrations:	The determination of oxathiapiprolin in final diet was conducted by an in-house developed method using reversed phase - high performance liquid chromatography (RP-HPLC) with mass-spectrometric (MS/MS) detection.
Test organisms	Worker honey bee larvae (Hymenoptera, Apoidea)
Species:	<i>Apis mellifera</i> L., ssp: <i>Buckfast</i>
Age:	The larvae were in first instar stage (L1) at grafting.
Source:	BioChem agrar GmbH
Food:	artificial diets containing glucose, fructose, yeast extract and water mixed with royal jelly at a weight ratio of 1 : 1 (D3-D6)
Test Design	
Test cage description:	Crystal polystyrene grafting cells (CNE Nicotplast, internal diameter 9 mm) were placed in 48 well plates
Replication:	3 replicates of the control, 3 replicates of each test and reference item dosage
No. of larvae/replicate:	12
Environmental test conditions	
Temperature:	34.0 – 35.0 °C

Humidity:	D1-D8: 99.9%
	D8-D15: 79.2 – 91.5%
	D15-D22: 64.1 – 67.6%
Photoperiod:	Illumination: constant darkness within the test chamber (diffuse artificial light only during handling and assessments)
Duration of test:	22 days

Study Design and Methods

Test facility: BioChem agrar GmbH, Kupferstr. 6, 04827 Machern OT Gerichshain, Germany

Experimental dates:	Experimental start date:	17 August 2020
	Experimental completion date:	07 September 2020
	Experimental start date (analytical phase):	02 October 2020
	Experimental completion date (analytical phase):	06 October 2020

To obtain larvae of approximately the same age, the queen was confined for a maximum of 24 hours on a brood comb containing empty cells for egg laying. After removing the queen from the excluder cage, the comb in the colony for approximately 3 days for incubation after which time it was brought into the laboratory for grafting larvae into the individual rearing cells (test units). The diet was deposited at the bottom of each cell, then the young larvae were grafted into the cells using a grafting tool.

During development, larvae were fed with three diet compositions containing the test item on days 3, 4, 5 and 6 of the larval rearing period. The composition of the aqueous part (D-glucose, D-fructose, yeast extract) and the amount of diet per larva varied according to the larval age. The required amount of diet was prepared and stored at $\leq 5^\circ\text{C}$ during the 6 days of feeding.

Mortality was observed and recorded throughout the study period to day 22 by visual assessment. At the end of the study, adult bees which left their cell and/or showed a normal development into an adult honey bee were counted as successfully emerged.

Statistical analysis of larval mortality at day 8, pupal mortality at day 15 and adult bee emergence at day 22 was conducted in order to determine the NOEC. To determine the NOEC and LOEC ($\alpha = 0.05$), survival data was analysed for statistically significant differences compared to the control group using Step-Down Cochran-Armitage Test followed by the Probit analysis using linear maximum likelihood regression and Weibull analysis using linear maximum likelihood regression. Mortality and emergence results were corrected for control mortality using an adaptation of Abbott's formula. Diet consumption and timing of pupal transfer were not evaluated for statistical significance due to the non-quantitative nature of the observations.

Results

Analytical recoveries in the diets are presented below.

Table A 40: Analytical Results

Diet (mg a.i./kg)	Analytical Recovery in Diet	
	Mean measured concentration (mg a.i./kg)	% of nominal
27.0	27.3	101
13.5	13.2	98
6.74	6.65	99
3.37	3.41	101
1.69	1.74	103
0.00	-	-

LOQ: 0.008 mg/kg, corresponding to 0.20 µg/L in diluted extracts

Mortality and emergence data for the test material and reference item are summarised in the table below.

Table A 41: Summary of mortality and emergence over 22 days

Nominal Concentration [mg product/kg diet]	Cumulative dose [µg product/larva]	Larval mortality day 8		Total mortality day 22		
		Actual	Corrected	Actual	Corrected	Adult emergence
Control		0.0	-	19.4	0.0	80.6
2643	418	75.0*	-	80.6	75.9	19.4*
1322	209	22.2*	-	50.0	37.9	50.0*
661	105	0.0	-	30.6	13.8	69.4
330	52	0.0	-	27.8	10.3	72.2
165	26	0.0	-	25.0	6.9	75.0
Reference item		83.3	-	88.9	86.2	11.1

* Statistically significant difference between treatment and control

No sublethal effects were observed.

Study endpoints are summarised in the table below.

Table A 42: Study endpoints

Treatment	Endpoints	After 22 d
Test item doses [µg product/larva per developmental period]	22-day ED ₁₀ [95% CL]	58 (37 - 93)
	22-day ED ₂₀ [95% CL]	105 (77 - 145)
	22-day ED ₅₀ [95% CL]	257 (205 - 322)
	NOED [95% CL]	105
Test item concentrations [mg product/kg diet] ³	22-day LC ₁₀ [95% CL]	369 (231 - 587)
	22-day LC ₂₀ [95% CL]	666 (484 - 916)
	22-day LC ₅₀ [95% CL]	1625 (1299 - 2034)
	NOEC	661

Validity Criteria

The test was considered valid;

- Day 8 larval mortality was $\leq 15\%$ in the control replicates
- Day 22 adult bee emergence was $\geq 70\%$ in the control replicates
- Day 8 mortality in the dimethoate reference item was $\geq 50\%$

Conclusion

The toxicity of Oxathiapiprolin/azoxystrobin SC (A22773A) to the larvae of the honeybee *Apis mellifera* was determined in a chronic exposure study over a 22-day period.

The 22-day NOEC was determined to be 661 mg product/kg diet and the EC₅₀ is 1625 mg product/kg diet.

(Schmidt K, 2021)

A 2.3.1.4 KCP 10.3.1.4 Sub-lethal effects

A 2.3.1.5 KCP 10.3.1.5 Cage and tunnel tests

A 2.3.1.6 KCP 10.3.1.6 Field tests with honeybees

A 2.3.2 KCP 10.3.2 Effects on non-target arthropods other than bees

A 2.3.2.1 KCP 10.3.2.1 Standard laboratory testing for non-target arthropods

Comments of zRMS:	The study follows the guideline specified by Blümel <i>et al.</i> (2000) and according to the principles of GLP. The study is considered to be valid and suitable for the risk assessment.
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Reference: KCP 10.3.2.1

Report: Fallowfield L. (2020). Oxathiapiprolin/azoxystrobin SC (A22773A) – A Rate-Response Laboratory Study to Determine the Effects of Fresh Residues on the Predatory Mite *Typhlodromus pyri* (Acari: Phytoseiidae). Report Number SYN-20-48. Mambo-Tox, A Division of Cawood Scientific Ltd., University Science Park, Southampton SO16 7NP, United Kingdom. (Syngenta file no VV-876566)

Guideline(s): Blümel *et al.* (2000). Laboratory residual contact test with the predatory mite *Typhlodromus pyri* (Acari: Phytoseiidae) for regulatory testing of plant protection products.

Deviations: No

GLP: Yes

Executive Summary

The effects of A22773A on the predatory mite *Typhlodromus pyri* were assessed in a laboratory test. Mites were exposed to rates equivalent to 5000, 2500, 1250, 625 and 312.5 mL test item/ha. The 7-day LR₅₀ value was > 5000 mL test item/ha, the highest rate tested. The NOER with respect to mite survival was 2500 mL test item/ha. In terms of effects on mite reproduction, the ER₅₀ value for reproduction was > 5000 mL test item/ha. The NOER value was 5000 mL test item/ha.

Materials

Test Material	Azoxystrobin/oxathiapiprolin SC (250/012)
Product Code	A22773A
Lot/Batch #:	SFI003-174-001
Actual content of active ingredient:	Azoxystrobin: 22.5% w/w (corresponding to 247 g/L) Oxathiapiprolin: 1.02% w/w (corresponding to 11.2 g/L)
Description:	Beige liquid
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	End February 2023
Density	1096 kg/m ³
Treatments	
Test rates:	5000, 2500, 1250, 625 and 312.5 mL test item/ha
Control:	Purified water
Toxic standard:	Dimethoate (an EC formulation containing 417.0 g a.s./L), applied at a rate of

Spray volume rate:	15 mL product/ha
Application method:	200 L/ha
Test organisms	Calibrated laboratory track-sprayer
Species:	<i>Typhlodromus pyri</i>
Age:	< 24-hr-old protonymphs
Source:	In-house culture, originally obtained in April 1995 from P.K. Nützlingszuchten, Welzheim, Germany and supplemented with further mites from the same source in 1996 and 1997.
Feeding:	1:1 v/v almond (<i>Prunus</i> sp. var. a mix of Aldrich, Nonpareil and Wood Colony) and apple (<i>Malus</i> sp. var. Red Delicious) pollen.
Test design	
Arenas:	Glass plates formed from two microscope slide cover slips (each 2.2 cm x 4.0 cm in area) joined together with two additional cover slips glued to the top and bottom ends of the main cover slips. 12 cm ² arena created on slides by non-drying sticky insect gel barrier.
Replication:	3 per treatment
No. of mites/arena :	20
Duration of test:	14 days
Environmental test conditions	
Temperature:	24.4-26.0 °C
Humidity:	63-79%
Photoperiod:	16 h (450-1120 lux)

Study Design and Methods

Experimental dates: 11 August 2020 to 08 September 2020.

Dilutions of test item were prepared in purified water, shortly before applications were made and the solutions were thoroughly agitated to ensure their homogeneity. Treatments were applied to glass plates which were left to dry and then used to construct the test arenas. Twenty mites were then introduced to the arenas (within 1 h of treatments being applied) and their survival assessed over a 7-day period, by which time the mites in the control were adult. The sex of the adult mites was determined. The control and the highest three treatment rates of the test item resulting in $\leq 60\%$ corrected mortality (5000, 2500 and 1250 mL test item/ha) were then left *in situ* so that their reproduction could be assessed over a further 7 days. The mean number of eggs produced per female between 7 and 14 days after treatment (DAT) was calculated.

The mean percentage mortality after 7 days was calculated for the individual treatments and then corrected for any losses in the control treatment using Abbott's formula. To determine the *no-observed-effect rate* (NOER) in terms of mite survival, the percentage mortality in each test item treatment was compared to that in the control using chi² 2x2 table test with Bonferroni-correction ($\alpha = 0.05$, one-sided, > control). The *median lethal rate* (LR₅₀) with respect to mortality was visually extrapolated from the data. In respect of the NOER for reproduction, the results for the mean number of eggs per female in each replicate were compared statistically.

The data were first checked for normality (Shapiro-Wilk test) and for homogeneity of variance (Levene's test) prior to analysis by multiple sequentially-rejective Welch t-test after Bonferroni-Holm ($\alpha = 0.05$, one-sided, < control). The *median effect rate* (ER₅₀) with respect to reproduction was visually extrapolated from the data.

Results and Discussion

The results for mortality and fecundity assessments are summarised in the table below.

Table A 43: Effects of A22773A on mortality and fecundity of *Typhlodromus pyri*, when exposed under laboratory test conditions

Treatment	% mortality at 7 DAT ^{a)}	Corrected % mortality at 7 DAT ^{b)}	Mean eggs/female from 7 to 14 DAT ^{c)}	% change in reproduction compared to control ^{d)}
Control	6.7	-	4.4	-
5000 mL A22773A/ha	21.7 *	16.1	2.4	45.6
2500 mL A22773A/ha	10.0	3.6	5.0	-11.4
1250 mL A22773A/ha	1.7	-5.4	3.4	23.8
625 mL A22773A/ha	10.0	3.6	~	~
312.5 mL A22773A/ha	0.0	-7.1	~	~
Toxic reference	90.0 *	89.3	~	~

^{a)} Individual test item treatments were compared to the control using chi² 2x2 table test with Bonferroni correction and for the toxic reference treatment Fisher's exact binomial test was used ($\alpha = 0.05$, one-sided, > control). Treatments that differed significantly from the control are indicated with an asterisk (*).

^{b)} Calculated using Abbott's formula. A positive value indicates an increase and a negative value indicates a decrease in mortality, relative to the control.

^{c)} Individual treatments were compared to the control by multiple sequentially-rejective Welch t-test after Bonferroni-Holm ($\alpha = 0.05$, one-sided, < control). No treatment rate differed significantly from the control.

^{d)} A positive value indicates a decrease and a negative value indicates an increase, in egg production.

~ Treatment not assessed.

From the mortality data, the LR₅₀ value was > 5000 mL test item/ha, the highest rate tested. Based on the statistical outcome, the NOER value with respect to survival was 2500 mL test item/ha.

From the fecundity data, based on the statistical outcome, the ER₅₀ value for reproduction was > 5000 mL test item/ha. The NOER value with respect to reproduction was 5000 mL test item/ha.

Validity Criteria

The test was considered valid;

- Mortality in the control treatment over the initial 7 days was 6.7% (should not exceed 20%)
- Corrected mortality in the toxic reference treatment was 89.3% (should exceed 50%)
- The mean cumulative number of eggs produced from 7 to 14 days was 4.4 per female in the control (should be ≥ 4.0 per female).

Conclusions

In a worst case laboratory test to determine the effects of fresh residues of A22773A, on the predatory mite *Typhlodromus pyri*, the 7-day LR₅₀ value was > 5000 mL test item/ha, the highest rate tested. Based on statistical comparison with the control, the NOER value with respect to mite survival was 2500 mL test item/ha.

The ER₅₀ value for reproduction was > 5000 mL test item/ha. Based on statistical comparison with the control, the NOER value with respect to mite reproduction was 5000 mL test item/ha.

(Fallowfield L, 2020)

Comments of zRMS:	The study follows the guideline specified by Mead Briggs <i>et al.</i> and according to the principles of GLP. The study is considered to be valid and suitable for the risk assessment.
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Reference: KCP 10.3.2.1

Report: Stevens, J. (2020). Oxathiapiprolin/azoxystrobin SC (A22773A) – A Rate-Response Laboratory Study to Determine the Effects of Fresh Residues on

the Parasitic Wasp *Aphidius rhopalosiphi* (Hymenoptera, Braconidae). Report Number SYN-20-47. Mambo-Tox, A Division of Cawood Scientific Ltd., 2 Venture Road, University Science Park, Southampton SO16 7NP, United Kingdom. (Syngenta file No. VV-875882)

Guideline(s): Mead-Briggs *et al.* (2000). A laboratory test for evaluating the effects of plant protection products on the parasitic wasp, *Aphidius rhopalosiphi*.

Deviations: No

GLP: Yes

Executive Summary

The effects of A22773A on the parasitic wasp *Aphidius rhopalosiphi* were assessed in a laboratory test. Wasps were exposed to rates equivalent to 5000, 2500, 1250, 625 and 312.5 mL test item/ha. The 48-h LR₅₀ value for A22773A was > 5000 mL/ha. The NOER value with respect to wasp survival was 5000 mL test item/ha, the highest rate tested. In terms of effects on the reproductive performance of surviving wasps, the ER₅₀ value was > 5000 mL test item/ha and the NOER value was 5000 mL test item/ha. The overall NOER value was 5000 mL test item/ha.

Materials

Test Material	azoxystrobin/oxathiapiprolin SC (250/012)
Product Code:	A22773A
Lot/Batch #:	SFI003-174-001 / 1127290
Actual content of active ingredient:	azoxystrobin: 22.5% w/w (247 g/L) oxathiapiprolin: 1.02% w/w (11.2 g/L)
Density:	1096 kg/m ³
Description:	Beige liquid
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	End February 2023
Treatments	
Test rates:	5000, 2500, 1250, 625 and 312.5 mL test item/ha
Control:	Purified water
Toxic standard:	Dimethoate (an EC formulation containing nominally 400 g a.s./L), applied at a rate of 0.05 mL product/ha
Spray volume rate:	200 L/ha
Application method:	Calibrated laboratory track-sprayer
Test organisms	
Species:	<i>Aphidius rhopalosiphi</i> (Hymenoptera, Braconidae)
Age:	< 48 h
Source:	In-house culture, originally established using wasps from a commercial supplier (Katz Biotech AG, Baruth, Germany)
Feeding:	1:3 v/v solution of honey in water on cotton wool
Test design - Mortality phase	
Arenas:	Treated surfaces of glass plates (10 cm x 10 cm) used to form floors and ceilings of shallow test arenas, the walls of which comprised a square frame made from metal casing, with mesh-covered ventilation holes present in the side walls of the casing.
Replication:	4 arenas per treatment
No. of wasps/arena :	10 (> 5 ♀)
Test design - Fecundity phase	
Arenas:	Pots containing approximately 15 barley seedlings (<i>Hordeum vulgare</i> var. Laureate) and previously infested per pot with approximately > 100 adults and nymphs of a mixed cereal aphid culture (<i>Metopolophium dirhodum</i> and <i>Rhopalosiphum padi</i>), were enclosed within clear acrylic cylinders (9 cm in diameter, 20 cm high), the tops of which were covered with nylon mesh netting.
Replication:	15 pots per treatment being assessed
No. of wasps/arena :	1 ♀

Duration of test: 13 days

Environmental test conditions

Temperature: Mortality phase: 20.3-20.8 °C. Fecundity phase: 20.4-20.9 °C

Humidity: Mortality phase: 73-77%.

Photoperiod: Mortality phase: 16 h (1002-1080 lux). Fecundity phase: 16 h (1668-1717 lux for aphid parasitisation phase; 4239-4460 lux for pupal wasp development).

Study Design and Methods

Experimental dates: 08 July 2020 to 24 August 2020.

Dilutions of test item were prepared in purified water shortly before use and the solutions were thoroughly agitated to ensure their homogeneity. Treatments were applied to glass plates which were left to dry and then used to construct the test arenas. The wasps were introduced into these arenas and their mortality was assessed after 2, 24 and 48 h.

To assess any sub-lethal effects, reproduction assessments were then carried out for the control and for the three highest treatment rates of the test item resulting in $\leq 60\%$ corrected mortality. Female wasps were confined individually over untreated aphid-infested barley plants for 24 h, before being removed. The plants were left for a further 10 days before recording the number of aphid ‘mummies’ (pupal wasps) that had developed on plants where wasps had been found alive after the 24-h oviposition period.

The percentage mortality of the wasps in the bioassay over 48 h was calculated. Mortality was defined as the numbers of *moribund* and *dead* wasps combined. The corrected percentage mortality (taking into account any control treatment losses) was derived using Abbott’s formula. Regression analysis of the results proved to be unsuitable. Where there was treatment mortality at 48 h, this was also compared to mortality in the control using multiple sequentially-rejective Fisher test after Bonferroni-Holm (one-sided, $> \text{control}$, $\alpha = 0.05$).

For the reproduction assessments, the data sets from each treatment were checked for normality (Shapiro-Wilk test, $\alpha = 0.05$) and homogeneity of variance (Levene’s test, $\alpha = 0.05$), prior to comparison by Dunnett’s multiple t-test (one-sided, $< \text{control}$, $\alpha = 0.05$).

Results and Discussion

The results of mortality and reproduction are summarised in the table below.

Table A 44: Effects of fresh residues of A22773A on mortality and reproduction of *Aphidius rhopalosiphii*, when exposed under laboratory test conditions.

Treatment	% mortality at 48 h ^a	% corrected mortality at 48 h (M-value) ^b	Number females successfully assessed for reproduction	Mean number mummies per surviving female ^c	% change in reproduction compared to control (R-value) ^d
Control	10.0	-	13	66.2	-
5000 mL A22773A/ha	20.0	11.1	13	67.4	-1.7
2500 mL A22773A/ha	17.5	8.3	14	63.5	4.1
1250 mL A22773A/ha	12.5	2.8	11	66.2	0.1
625 mL A22773A/ha	5.0	-5.6	~	~	~
312.5 mL A22773A/ha	7.5	-2.8	~	~	~
Toxic reference	97.5 *	97.2	~	~	~

^a The individual test item treatments were compared to the control using multiple sequentially-rejective Fisher test after Bonferroni-Holm and the toxic reference treatment was compared to the control using Fisher’s exact binomial test (one-sided, $> \text{control}$, $\alpha = 0.05$); an asterisk (*) indicates where there were significant statistical differences.

^b Derived using Abbott’s formula.

- ^c The results were compared using Dunnett's multiple t-test (one-sided, < control, $\alpha = 0.05$). No treatments differed significantly.
- ^d Percentage change in reproduction, relative to the control. A positive value indicates a decrease, a negative value an increase.
- ~ Treatment not assessed.

From the mortality data, the LR₅₀ value was > 5000 mL A22773A/ha. Based on the statistical outcome, the NOER value with respect to survival was 5000 mL test item/ha, the highest rate tested.

From the fecundity data, the ER₅₀ value was > 5000 mL test item/ha. Based on the statistical outcome, the NOER value with respect to reproduction was 5000 mL test item/ha.

Validity criteria

The test was considered valid;

- Mortality within the control treatment at 48 hours was 10.0% (should not exceed 13%, i.e. 5 wasps from 40).
- Corrected mortality within the toxic-reference treatment at 48 hours was 97.2% (should exceed 50%).
- In the reproduction assessment, the mean number of mummies/female in the control treatment was 66.2, with no zero values (should be > 5.0 mummies/female and no more than two zero values).

Conclusions

In a laboratory test to determine the effects of fresh residues of A22773A on the parasitic wasp *Aphidius rhopalosiphi*, the 48-h LR₅₀ value was > 5000 mL A22773A/ha. Based on statistical comparison with the control, the NOER value for wasp survival was 5000 mL test item/ha, the highest rate tested.

In terms of effect on the reproductive performance of surviving wasps, the ER₅₀ value for A22773A was > 5000 mL test item/ha and the NOER value for reproduction was 5000 mL test item/ha.

The overall NOER value was 5000 mL test item/ha.

(Stevens J, 2020)

A 2.3.2.2 KCP 10.3.2.2 Extended laboratory testing, aged residue studies with non-target arthropods

A 2.3.2.3 KCP 10.3.2.3 Semi-field studies with non-target arthropods

A 2.3.2.4 KCP 10.3.2.4 Field studies with non-target arthropods

A 2.3.2.5 KCP 10.3.2.5 Other routes of exposure for non-target arthropods

A 2.4 KCP 10.4 Effects on non-target soil meso- and macrofauna

A 2.4.1 KCP 10.4.1 Earthworms

Comments of zRMS:	The study was conducted to OECD guidance 207 and according to the principles of GLP. All validity criteria were met. The study is considered to be reliable.
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Reference:	KCP 10.4.1
Report:	Friedrich, S. (2020), Oxathiapiprolin/Azoxystrobin SC (A22773A) - Acute Toxicity to the Earthworm <i>Eisenia andrei</i> in Artificial Soil, Report Number 20 48 TEA 0018, BioChem agrar GmbH, Kupferstraße 6, 04827 Machern OT Gerichshain, Germany, Syngenta File No. VV-884611
Guideline(s):	OECD 207 (1984)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Executive Summary

In an acute toxicity test, in which earthworms (*Eisenia andrei*) were exposed to A22773A at concentrations of 62.5, 125, 250, 500 and 1000 mg test item/kg soil d.w. alongside a control, the 14-day NOEC for biomass was determined to be 500 mg test item/kg soil d.w. The NOEC for mortality was determined to be 1000 mg test item/kg soil d.w. The 14-d LC₅₀ could not be calculated, but it can be concluded that the LC₅₀ is greater than 1000 mg test item/kg soil d.w., the highest concentration tested.

Materials

Test Material	Oxathiapiprolin/Azoxystrobin SC (A22773A)
Lot/Batch #:	SFI003-174-001
Actual content of active ingredients:	Azoxystrobin 22.5 % w/w corresponding to 247 g/ oxathiapiprolin 1.02 % w/w corresponding to 11.2 g/L
Description:	beige liquid
Stability of test compound:	stable under recommended handling and storage conditions (< 30 °C)
Reanalysis/Expiry date:	end of February 2023
Density:	1096 kg/m ³
Treatments	
Test concentrations:	62.5, 125, 250, 500, 1000 mg test item/kg soil dry weight
Control:	Untreated (quartz sand only) 2-chloroacetamide in deionised water at concentrations of 14.1, 18.3, 23.8, 31.0 and 40.3 mg/kg soil d.w. (separate GLP study).
Toxic standard:	
Test organisms	
Species:	<i>Eisenia andrei</i> (BOUCHÉ, 1972)
Age and weight range at test start:	adult worms, 4 months old with clitellum 307 – 498 mg/worm W. Neudorff GmbH KG, An der Mühle 3, 31860 Emmerthal, Germany; reared under ambient laboratory conditions in the test facility
Source:	
Feeding:	none
Test design	
Vessels:	1 L glass jars with clear lids
Substrate:	artificial soil comprising 10 % sphagnum peat, 20 % kaolin clay (kaolinite content > 30 %), 69.5 % industrial quartz sand (> 50 % of the particles between 50 and 200 µm) and 0.5 % calcium carbonate. 751 g soil wet weight, corresponding to 556 g dry weight of artificial soil was added to each test vessel.
Replication:	4
No. of worms/vessel:	10
Duration of test:	14 days (14 days adult mortality)
Environmental test conditions	
Temperature:	19.8 – 21.2 °C
pH of soil:	test start: 6.01 – 6.05 test end: 5.80 – 5.85
Water content of soil:	test start: 55.6 – 55.7 % of max. WHC test end: 54.8 – 55.3 % of max. WHC

Photoperiod: continuous light (approximately 650 lux)

Study Design and Methods

Experimental dates: 09 September 2020 to 23 September 2020

Approximately 24 hours prior to test start, the dry artificial soil was pre-moistened by adding deionised water to obtain approximately half of the final water content. Earthworms were acclimatised in a separate batch of the untreated artificial soil for approximately 24 hours before test start. On the day of the test start, the test item was introduced by dispersing the quantity of test item required to obtain the desired test concentration in the volume of water required to hydrate the soil to 40-60 % of its WHC. The control substrate contained the corresponding amount of deionised water only. The test vessels were then filled with the treated soil. The acclimatised test animals were washed, gently dried on a paper towel, weighed and randomly placed onto the test substrate.

Assessments were performed after 7 and 14 days. The final number of surviving adult earthworms, the behaviour and pathological symptoms as well as their biomass change was recorded on day 14. Williams-t-test were used to compare the control biomass with the independent test item groups. The LC₅₀ at could not be quantified due to the absence of a toxic effect of the test item at the tested concentrations.

Results

Mortality and fecundity are summarised in the table below.

Table A 45: Effects of A22773A on mortality of earthworms

Endpoint	Treatment group [mg test item/kg soil d.w.]					
	Control	62.5	125	250	500	1000
Mortality of adult worms after 14 days (%)	0.0	0.0	0.0	0.0	0.0	0.0
Mean biomass change at 14 days (mg/worm)	-46.9	-48.8	-54.7	-53.0	-52.8	-59.6*
Mean biomass change (0-14 d) (%)	-11.5	-11.9	-13.4	-12.9	-13.0	-14.7
LC ₅₀	> 1000					
NOEC (mortality)	1000					
NOEC (biomass)	500					

* statistically significantly different compared to the control (Williams-t-test for biomass change, $\alpha = 0.05$, one-sided greater)
d.w.: dry weight (of artificial soil)

Validity Criteria

The test was considered valid;

- There was 0.0% mortality in the control (must be $\leq 10\%$)

Conclusion

In an acute toxicity test, in which earthworms (*Eisenia andrei*) were exposed to A22773A at concentrations of 62.5, 125, 250, 500 and 1000 mg test item/kg soil d.w. alongside a control, the 14-day NOEC for biomass was determined to be 500 mg test item/kg soil d.w. The NOEC for mortality was determined to be 1000 mg test item/kg soil d.w. The 14-d LC₅₀ could not be calculated, but it can be concluded that the LC₅₀ is greater than 1000 mg test item/kg soil d.w., the highest concentration tested.

(Friedrich S, 2020)

A 2.4.1.1 KCP 10.4.1.1 Earthworms - sub-lethal effects

Comments of zRMS:	The study was conducted to OECD guidance 222 and according to the principles of GLP. All validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.
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Reference:	KCP 10.4.1.1
Report:	Friedrich, S. (2020), Oxathiapiprolin/Azoxystrobin SC (A22773A) – Sublethal Effects on the Reproduction of the Earthworm <i>Eisenia andrei</i> in Artificial Soil, Report Number 20 48 TEC 0052, BioChem agrar GmbH, Kupferstraße 6, 04827 Machern OT Gerichshain, Germany. Syngenta File No. VV-883029
Guideline(s):	OECD 222 (2016)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Executive Summary

In an earthworm reproduction study, in which earthworms (*Eisenia andrei*) were exposed to A22773A, the NOEC for mortality was determined to be 1000 mg test item/kg soil d.w. The NOEC for biomass change and reproduction was determined to be 309 and 95.3 mg test item/kg soil d.w., respectively. The EC₁₀, EC₂₀ and EC₅₀ values for reproduction were calculated to be 96.8, 146 and 271 mg test item/kg soil d.w., respectively.

Materials

Test Material	Oxathiapiprolin/Azoxystrobin SC (A22773A)
Lot/Batch #:	SFI003-174-001
Actual content of active ingredients:	Azoxystrobin 22.5 % w/w corresponding to 247 g/ oxathiapiprolin 1.02 % w/w corresponding to 11.2 g/L
Description:	beige liquid
Stability of test compound:	stable under recommended handling and storage conditions (< 30 °C)
Reanalysis/Expiry date:	end of February 2023
Density:	1096 kg/m ³
Treatments	
Test rates:	16.3, 29.4, 52.9, 95.3, 171, 309, 556, 1000 mg test item/kg soil dry weight (spacing factor: 1.8)
Control:	untreated
Toxic standard:	Maypon Flow (carbendazim, SC 500) was tested at concentrations of 2.2 and 4.3 mg a.i./kg soil dry weight (separate GLP study BioChem project No.: 20 48 TEC 0022).
Test organisms	
Species:	<i>Eisenia andrei</i> (BOUCHÉ, 1972)
Age and weight range at test start:	adult worms, 4 months old with clitellum 300 - 499 mg/worm
Source:	reared under ambient laboratory conditions in the test facility (originally purchased from W. Neudorff GmbH KG, An der Mühle 3, 31860 Emmerthal, Germany)
Feeding:	air-dried and finely ground horse manure
Test design	
Vessels:	plastic vessel (16.5 cm x 12 cm x 6 cm) with a lid pervious to air and light.
Substrate:	artificial soil comprising 5 % sphagnum peat, 20 % kaolin clay (kaolinite content > 30 %), 74.7 % industrial quartz sand (> 50 % of the particles between 50 and 200 µm) and 0.3 % calcium carbonate. 750 g soil wet weight, corresponding to 600 g dry weight of artificial soil was added to each test

Replication:	vessel. 8 replicates for the control group and 4 replicates for the treated groups
No. of worms/vessel :	10
Duration of test:	8 weeks (4 weeks adult mortality and biomass change; 8 weeks juvenile development)
Environmental test conditions	
Temperature:	19.0 – 21.3 °C
pH of soil:	test start: 6.18 – 6.23 test end: 5.47 – 6.07
Water content of soil:	test start: 57.2 – 57.5 % of max. WHC test end: 55.6 – 56.6 % of max. WHC
Photoperiod:	16 hours light : 8 hours dark (approximately 580 lux)

Study Design and Methods

Experimental dates: 02 September 2020 to 28 October 2020

Approximately 24 hours prior to test start, the artificial soil was prepared, and deionised water was added to the dry components to adjust the water content to approximately 40-60 % of its maximum water holding capacity (WHC). The worms were acclimatised in a separate batch of the untreated artificial substrate for approximately 24 hours before test start. On the day of the test start, the test item was mixed with a small quantity of finely ground quartz sand, such that the required test concentrations were achieved once mixed with the artificial soil. The acclimatised test animals were washed, gently dried on a paper towel, weighed and randomly placed onto the test substrate (10 animals per test vessel).

After four weeks, the adult worms were removed from the test vessels, and mortality and the body weight of the surviving worms were determined. After all of the adult worms had been removed, the soil in each vessel was mixed with 5 g horse manure. Four weeks later, the number of surviving juveniles and any morphological alterations were recorded. Observations of behavioural and pathological symptoms were observed weekly.

The endpoints were mortality, change of biomass (difference in fresh weight of surviving worms between test start and four weeks after treatment) and reproduction (the number of juveniles present). For identifying the NOEC values the Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm and the Williams-t-test were used to compare the control with the independent test item groups. The statistical analysis was performed with the software ToxRat Professional 3.3.0 (Ratte 2018). The EC_x values (number of juveniles) were calculated using the Weibull analysis.

Results

Mortality and reproduction are summarised in the table below.

Table A 46: Effect of A22773A on mortality, growth and reproduction of *Eisenia andrei*

Endpoint	Treatment group (mg test item/kg soil d.w.)								
	Control	16.3	29.4	52.9	95.3	171	309	556	1000
Mortality of adult worms after 4 weeks (%)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mean biomass change after 4 weeks (%)	29.8	29.0	27.3	31.3	28.4	30.4	27.9	19.7*	3.2*
Mean number of juveniles after 8 weeks	174.8	167.8	181.5	161.8	148.8	138.3*	71.8*	10.8*	0.0*
Change of reproduction compared to control (%)	-	4.0	-3.9	7.4	14.9	20.9	58.9	93.8	100.0
	Endpoint (mg test item/kg soil d.w.)								
NOEC (mortality)	1000								

Endpoint	Treatment group (mg test item/kg soil d.w.)								
	Control	16.3	29.4	52.9	95.3	171	309	556	1000
NOEC (biomass change)	309								
NOEC (reproduction)	95.3								
LC ₅₀ (mortality) ¹	> 1000								
EC ₁₀ (reproduction) ²	96.8 (95 % confidence limits 73.7 – 127)								
EC ₂₀ (reproduction) ²	146 (95 % confidence limits 121 – 176)								
EC ₅₀ (reproduction) ²	271 (95 % confidence limits 245 – 301)								

* statistically significantly different compared to control (Williams-t-test for biomass change and reproduction, $\alpha = 0.05$, one-sided smaller)

Negative % values for change of reproduction = increase, relative to control

¹ based on estimation of the data, ² Weibull analysis

Validity criteria

The test is considered valid:

- Adult mortality was 0 % in the control (must be $\leq 10\%$)
- The mean number of juveniles per control replicate was 131 to 216 (must be ≥ 30)
- The coefficient of variation for reproduction was 16.8 % (must be $\leq 30\%$)

Conclusion

In an earthworm reproduction study, in which earthworms (*Eisenia andrei*) were exposed to A22773A, the NOEC for mortality was determined to be 1000 mg test item/kg soil d.w. The NOEC for biomass change and reproduction was determined to be 309 and 95.3 mg test item/kg soil d.w., respectively. The EC₁₀, EC₂₀ and EC₅₀ values for reproduction were calculated to be 96.8, 146 and 271 mg test item/kg soil d.w., respectively.

(Friedrich S, 2020)

Comments of zRMS:	Study not evaluated.
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Reference:	KCA 8.4.1
Report:	Friedrich, S. (2010), R234886 - Sublethal Toxicity to the Earthworm <i>Eisenia fetida</i> in Artificial Soil with 5 % Peat. Report Number 10 10 48 078 S. BioChem agrar, Labor für biologische und chemische, Analytik GmbH, Kupferstraße 6, 04827 Gerichshain, Germany. Syngenta File No. VV-394786.
Guideline(s):	OECD Guideline for testing of chemicals No. 222 (adopted 13 April 2004): Earthworm Reproduction Test (<i>Eisenia fetida</i> / <i>Eisenia andrei</i>)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Executive Summary

In a chronic toxicity test in which earthworms (*Eisenia fetida*) were exposed to R234886 the NOEC based on both the biomass and reproductive performance of the worms was determined to be 16 mg R234886/kg soil dry weight, the highest concentration tested.

Since no concentration response was observed, the EC₅₀ could not be calculated, but it can be concluded that the EC₅₀ > 16 mg R234886/kg soil dry weight, this being the highest concentration tested.

Materials

Test Material	R234886
Parent:	Azoxystrobin
Lot/Batch #:	ASJ10063-01S
Purity:	100 % (estimated error: ± 2 %)
Description:	White solid
Stability of test compound:	Stable under standard conditions.
Reanalysis/Expiry date:	30 September 2012
Density:	Not applicable
Treatments	
Test rates:	1, 2, 4, 8, 16 mg R234886/kg soil d.w.
Control:	Untreated substrate irrigated with deionised water only
Toxic standard:	Nutdazim 50 FLOW (carbendazim, SC 500) was tested at concentrations of 5 and 10 mg product/kg soil dry weight (BioChem project No.: R 10 10 48 007 S, dated 05 August 2010).
Test organisms	
Species:	<i>Eisenia fetida</i> (Savigny) 1926 (subspecies <i>Eisenia fetida andrei</i> Bouché)
Age and weight range at test start:	Adult worms, approximately 3 months old with clitellum. 255 – 458 mg/worm.
Source:	Reared at test facility, original breeding animals from “W. Neudorff GmbH KG”, An der Mühle 3, 31860 Emmerthal, Germany
Feeding:	air-dried, finely ground horse manure
Test design	
Vessels:	Plastic vessels (about 16.5 × 12 × 6 cm) with lids pervious to air and light.
Substrate:	Artificial soil comprising 5 % sphagnum peat, 20 % kaolinite clay, 74.7 % industrial quartz sand (> 50 % of the particles between 0.05 mm and 0.2 mm) and 0.5 % calcium carbonate. 750 g wet weight soil, corresponding to 600 g dry weight, of artificial soil was added to each test vessel.
Replication:	4 per treatment group, 8 in control group
No. of worms/arena :	10

Duration of test:	8 weeks
Environmental conditions	test
Temperature:	18.8 – 21.7 °C
pH of soil:	test start: 5.71 – 5.92 test end: 5.58 – 5.77
Water content of soil:	test start: 24.9 – 25.0 % (equivalent to 57.8 – 58.0 % of WHC) test end: 24.8 – 24.9 % (equivalent to 57.5 – 57.8 % of WHC)
Photoperiod:	light : dark = 16 h : 8 hr (740 lx)

Study Design and Methods

Experimental dates: 05th August 2010 to 30th September 2010

Approximately 24 hours prior to test start, the artificial soil was prepared and deionised water was added to the dry components to adjust the water content to approximately 40-60 % of its maximum water holding capacity (WHC). The worms were acclimatised in a separate batch of the untreated artificial substrate (mixed with horse manure) for one day before test start.

The test item was mixed with 10 g (per vessel) of finely ground quartz sand such that the required test concentration was achieved once mixed with the artificial soil. The control substrate contained the corresponding amount of quartz sand only. The test vessels were filled with 750 g wet weight (600 g dry weight) of soil. The acclimatised test animals were weighed and randomly placed onto the test substrate (10 animals per test vessel).

One day after application, 5 g dried and ground horse manure was scattered on the soil surface of each test vessel. This was sprinkled with 5 mL deionised water. The feeding interval was weekly during the first four weeks of the test.

After four weeks, the adult worms were removed from the test vessels, and mortality and the body weight of the surviving worms were determined. After all of the adult worms had been removed, the soil in each vessel was mixed with 5 g horse manure. Four weeks later, the number of surviving juveniles and any morphological alterations were recorded. Observations of behavioural and pathological symptoms were observed weekly.

The mortality, biomass, and reproduction results were analysed with the software ToxRat Professional 2.10 (RATTE 2009). The Dunnett-test was used to compare the control with the independent test item groups. For statistical evaluation of the biomass change, the changed mean fresh weight of surviving worms per replicate was used.

Results and Discussion

Mortality and fecundity are summarised in the table below.

Table A 47: Effect of R234886 on mortality, growth and reproduction of *Eisenia fetida*

Endpoints	Treatment groups (mg R234886/kg soil dry weight)					
	Control	1	2	4	8	16
Mean adult mortality at 28 days (%)	0	0	0	0	0	0
Mean % biomass change of adults from 0-28 days	43.3	38.7	10.9	41.4	38.4	39.8
Mean number of juveniles/replicate after 8 weeks	61.6	58.0	62.0	57.0	64.0	57.8

Endpoints	Treatment groups (mg R234886/kg soil dry weight)					
	Control	1	2	4	8	16
Coefficient of variation for reproduction (cv %)	22.9	29.6	31.4	44.0	21.2	27.5
% difference in reproduction relative to the control*	-	5.9	-0.6	7.5	-3.9	6.3
NOEC (biomass/reproduction)	16 mg R234886/kg soil dry weight.					
EC ₅₀	> 16 mg R234886/kg soil dry weight ^A					

No results were statistically significant compared to control (DUNNETT-test, $p \leq 0.05$)

*Negative values indicate an increase relative to the control

^A based on reproduction

Validity criteria

The validity criteria for the control group were met.

- The adult mortality at 4 weeks was $\leq 10\%$ (observed: 0 %)
- The number of juveniles per replicate was ≥ 30 (observed 46 - 82)
- The coefficient of variation for reproduction was $\leq 30\%$ (observed 22.9 %)

Conclusions

In a chronic toxicity test in which earthworms (*Eisenia fetida*) were exposed to R234886 the NOEC based on both the biomass and reproductive performance of the worms was determined to be 16 mg R234886/kg soil dry weight, the highest concentration tested.

Since no concentration response was observed, the EC₅₀ could not be calculated, but it can be concluded that the EC₅₀ > 16 mg R234886/kg soil dry weight, this being the highest concentration tested.

(Friedrich S, 2010)

A 2.4.1.2 KCP 10.4.1.2 Earthworms - field studies

A 2.4.2 KCP 10.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

A 2.4.2.1 KCP 10.4.2.1 Species level testing

Comments of zRMS:	The study was conducted to OECD guidance 232 and according to the principles of GLP. All validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.
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Reference:	KCP 10.4.2.1
Report:	Friedrich, S. (2020) Oxathiapiprolin/Azoxystrobin SC (A22773A) - Effects on the Reproduction of the Collembolan <i>Folsomia candida</i> . Report Number 20 48 TCC 0049. BioChem agrar GmbH, Kupferstraße 6, 04827 Machern OT Gerichshain, Germany. Syngenta File No. VV-882647
Guideline(s):	OECD 232 (2016)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Executive Summary

In a Collembola reproduction study with A22773A, the NOEC for mortality of the parental collembolans was determined to be 1000 mg test item/kg soil dry weight. The LC₅₀ could not be calculated, but it can be concluded that the LC₅₀ is higher than 1000 mg test item/kg soil d.w., the highest concentration tested. The NOEC for reproduction was determined to be 1000 mg test item/kg soil dry weight. The EC₁₀, EC₂₀ and EC₅₀ values for reproduction could not be calculated, but it can be concluded that these values are higher than 1000 mg test item/kg soil d.w., the highest concentration tested.

Materials

Test Material	Oxathiapiprolin/Azoxystrobin SC (A22773A)
Lot/Batch #:	SFI003-174-001
Actual content of active ingredients:	azoxystrobin 22.5 % w/w corresponding to 247 g/L oxathiapiprolin 1.02 % w/w corresponding to 11.2 g/L
Description:	beige liquid
Stability of test compound:	stable under recommended handling and storage conditions (< 30 °C)
Reanalysis/Expiry date:	end of February 2023
Density:	1096 kg/m ³
Treatments	
Test rates:	16.3, 29.4, 52.9, 95.3, 171, 309, 556, 1000 mg test item/kg soil dry weight
Control:	untreated (deionised water only)
Toxic standard:	boric acid (separate GLP study BioChem project No.: 20 48 TCC 0064)
Application method:	Soil incorporation
Test organisms	
Species:	<i>Folsomia candida</i>
Age:	juvenile collembolans, 9-12 days old
Source:	originally purchased from "Biologische Bundesanstalt (BBA)", Berlin-Dahlem. reared under ambient laboratory conditions in the test facility
Feeding:	2 mg granulated dry yeast at the start of the test and after 14 days
Test design	
Vessels:	glass container (approximately 150 mL) covered with a lid

Substrate:	artificial soil comprising 5 % sphagnum peat, 20 % kaolin clay (kaolinite content > 30 %), 74.7 % industrial quartz sand (> 50 % of the particles between 50 and 200 μm) and 0.3 % calcium carbonate. 37.5 g wet weight soil, corresponding to 30 g dry weight of artificial soil was added to each test vessel.		
Replication:	8 replicates for the control group and 4 replicates for the treated groups		
No. of Collembola/vessel :	10		
Duration of test:	28 days		
Environmental test conditions			
Temperature:	18.1 – 21.6 °C		
pH of soil:	test start:	6.00 – 6.09	
	test end:	5.87 – 5.89	
Water content of soil:	test start:	57.2 – 57.5 % of max. WHC	
	test end:	55.9 – 56.8 % of max. WHC	
Photoperiod:	16 hours light : 8 hours dark photoperiod, approximately 620 lux		

Study Design and Methods

Experimental dates: 01 September 2020 to 29 September 2020

The test concentrations were prepared by dispersing an exactly weighed amount of the test item in deionised water to make a stock solution. This stock solution was diluted with deionised water for each test concentration and was thoroughly mixed with the artificial soil using a laboratory mixer, achieving a final nominal water content of 40-60 % of WHC. The control was treated with deionised water only.

After the application ten juvenile collembolans were transferred to the substrate surface of each test vessel using an aspirator. Four replicates were used per test item concentration and eight replicates were used for the control. The test organisms were fed twice during the test (at the start of the test and after 14 days) with approximately 2 mg of granulated dry yeast per test vessel. Four weeks after introducing the test organisms, the surviving parental collembolans and offspring (juveniles) were counted. All values presented throughout this report were calculated using the original raw data and were not based on rounded values.

The percentage mortality of the springtails was calculated for each treatment, both before and after correction for any control treatment losses. Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm and Williams-t-test were used to compare the control with the independent test item groups. The statistical analysis was performed with the software ToxRat Professional 3.3.0 (2018).

Results

Mortality and fecundity are summarised in the table below.

Table A 48: Effects of residues of A22773A on mortality and reproduction of *Collembola candida*

Endpoint	Treatment group (mg test item/kg soil dry weight)								
	Control	16.3	29.4	52.9	95.3	171	309	556	1000
% Mortality of parental collembolans after 4 weeks	3.8	2.5	0.0	2.5	5.0	5.0	2.5	2.5	5.0
% Corrected mortality (Abbott)	-	-1.3	-3.9	-1.3	1.3	1.3	-1.3	-1.3	1.3
Mean number of juveniles after 4 weeks	578	571	580	573	557	547	589	544	548
% Reduction of reproduction compared to control	-	1.2	-0.4	0.9	3.6	5.4	-2.0	5.9	5.1

	Endpoint (mg test item/kg soil dry weight)
NOEC (mortality)	1000
NOEC (reproduction)	1000
LC ₅₀ (mortality) ¹	> 1000
EC ₁₀ (reproduction) ¹	> 1000
EC ₂₀ (reproduction) ¹	> 1000
EC ₅₀ (reproduction) ¹	> 1000

Not statistically significantly different compared to control regarding mortality (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, $\alpha = 0.05$, one-sided greater) and reproduction (Williams-t-test, $\alpha = 0.05$, one-sided smaller)
Negative % values for change of reproduction = increase, relative to control

¹ based on estimation of the data

Validity criteria

The validity criteria are as follows:

- Control treatment mortality was 3.8 % (must be $\leq 20\%$)
- The mean number of juvenile recorded in the control treatment was 578 (must be ≥ 100 per replicate)
- The coefficient of variation of reproduction in the control was 9.4 % (must not be $< 30\%$)

Conclusion

In a Collembola reproduction study with A22773A, the NOEC for mortality of the parental collembolans was determined to be 1000 mg test item/kg soil dry weight. The LC₅₀ could not be calculated, but it can be concluded that the LC₅₀ is higher than 1000 mg test item/kg soil d.w., the highest concentration tested. The NOEC for reproduction was determined to be 1000 mg test item/kg soil dry weight. The EC₁₀, EC₂₀ and EC₅₀ values for reproduction could not be calculated, but it can be concluded that these values are higher than 1000 mg test item/kg soil d.w., the highest concentration tested.

(Friedrich S, 2020)

Comments of zRMS:	Study not evaluated.
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Reference:	KCA 8.4.2
Report:	Friedrich, S. (2019), R234886 - Effects on the Reproduction of the Collembolan <i>Folsomia candida</i> . Report Number 19 48 TCC 0011. BioChem agrar GmbH, Kupferstraße 6, 04827 Machern OT Gerichshain, Germany. Syngenta File No VV-471930
Guideline(s):	OECD 232 (2016)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Executive Summary

In a Collembola reproduction study with R234886, the NOEC for mortality of the parental collembolans was determined to be 250 mg test item/kg soil d.w. The LC₁₀, LC₂₀ and LC₅₀ for mortality were calculated to be 282, 436 and 922 mg test item/kg soil dry weight, respectively. The NOEC for reproduction was determined to be 250 mg test item/kg soil d.w. The EC₁₀, EC₂₀ and EC₅₀ values for reproduction were calculated to be 332, 477 and 955 mg test item/kg soil dry weight, respectively.

Materials

Test Material	R234886
Product code:	R234886
Substance type:	metabolite of azoxystrobin
Lot/Batch #:	MES 563/2
Actual content of active ingredients:	99 % w/w
Description:	off-white solid
Stability of test compound:	stable under recommended handling and storage conditions (< 10 °C)
Reanalysis/Expiry date:	end of March 2020
Treatments	
Test rates:	16, 31, 63, 125, 250, 500, 1000 mg test item/kg soil dry weight
Application method:	the test item was mixed with finely ground quartz sand and then the test item/sand mixtures were thoroughly mixed into the pre-moistened artificial soil
Control:	untreated
Toxic standard:	boric acid was tested at concentrations of 44, 67, 100, 150, 225 mg/kg soil dry weight (separate GLP study BioChem project No.: 18 48 TCC 0053).
Test organisms	
Species:	<i>Folsomia candida</i> (Willem)
Age:	juvenile collembolans, 9-12 days old
Source:	originally purchased from "Biologische Bundesanstalt (BBA)", Berlin-Dahlem. reared under ambient laboratory conditions in the test facility
Feeding:	2 mg granulated dry yeast at the start of the test and after 14 days
Test design	
Vessels:	glass container (approximately 150 mL) covered with a lid
Substrate:	artificial soil comprising 5 % sphagnum peat, 20 % kaolin clay (kaolinite content > 30 %), 74.7 % industrial quartz sand (> 50 % of the particles between 50 and 200 µm) and 0.3 % calcium carbonate. 37.5 g wet artificial soil (corresponding to 30 g dry weight) was added to each test vessel.
Replication:	8 replicates for the control and 4 replicates for the treated groups
No. of Collembola/vessel :	10
Duration of test:	28 days
Environmental test conditions	
Temperature:	19.7 – 21.4 °C

pH of soil:	test start: 5.88 – 6.02 test end: 5.80 – 5.89
Water content of soil:	test start: 58.9 – 59.1 % of max. WHC test end: 57.2 – 58.4 % of max. WHC
Photoperiod:	16 hours light : 8 hours dark photoperiod, approximately 510 lux

Study Design and Methods

Experimental dates: 18 March 2019 to 15 April 2019

Two days before the start of the test, the dry artificial soil was moistened by adding deionised water to adjust the water content to 40-60 % of WHC. On the day of test start, the test item was mixed with a small quantity of finely ground quartz sand, such that the required test concentration was achieved once mixed with the artificial soil (10 g treated sand per treatment group). The control was treated with untreated quartz sand only.

After the application ten juvenile collembolans were transferred to the substrate surface of each test vessel using an aspirator. Four replicates were used per test item concentration and eight replicates were used for the control. The test organisms were fed twice during the test (at the start of the test and after 14 days) with approximately 2 mg of granulated dry yeast per test vessel. Four weeks after introducing the test organisms, the surviving parental collembolans and offspring (juveniles) were counted.

The percentage mortality of the springtails was calculated for each treatment, both before and after correction for any control treatment losses using Abbott's formula. Step-down Cochran-Armitage test and Williams-t-test were used to compare the control with the independent test item groups for parent mortality and for reproduction, respectively. The LC_x -values for adult mortality was calculated by Logit analysis using linear maximum likelihood regression. The EC_x values for number of juveniles were calculated using the 3-parametric normal Cumulative Distribution Function (CDF).

Results and Discussion

Mortality and reproduction are summarised in the table below.

Table A 49: Effects of R234886 on mortality and reproduction of *Folsomia candida*

Endpoint	Treatment group (mg test item/kg soil d.w.)							
	Control	16	31	63	125	250	500	1000
% Mortality of parental collembolans after 4 weeks	3.8	2.5	5.0	5.0	2.5	7.5	35.0*	52.5*
% Corrected mortality (Abbott)	-	-1.3	1.3	1.3	-1.3	3.9	32.5	50.6
Mean number of juveniles after 4 weeks	1268	1281	1257	1291	1306	1241	979*	623*
% Reduction of reproduction compared to control	-	-1.0	0.8	-1.8	-3.0	2.1	22.8	50.9
Endpoint (mg test item/kg soil dry weight)								
NOEC (mortality)	250							
NOEC (reproduction)	250							
LC_{10} (mortality) ¹	282 (95 % confidence limits 203 - 391)							
LC_{20} (mortality) ¹	436 (95 % confidence limits 342 - 557)							
LC_{50} (mortality) ¹	922 (95 % confidence limits 696 - 1222)							

EC ₁₀ (reproduction) ²	332 (95 % confidence limits 296 - 368)
EC ₂₀ (reproduction) ²	477 (95 % confidence limits 444 - 512)
EC ₅₀ (reproduction) ²	955 (95 % confidence limits 923 - 991)

* statistically significant different compared to the control (Step-down Cochran-Armitage test for mortality, $p \leq 0.05$, one-sided greater; Williams-t-test for reproduction, $p \leq 0.05$, one-sided smaller)

¹ Logit analysis, ² 3-parametric normal CDF

Negative % values for change of reproduction = increase, relative to control

Validity criteria

The validity criteria for the control group were met:

- Mean adult mortality: $\leq 20 \%$ (observed: 3.8 %)
- Mean number of juveniles per test vessel: ≥ 100 (observed: average of 1268/vessel)
- Coefficient of variation for the mean number of juveniles: $< 30 \%$ (observed: 7.8 %)

Conclusions

In a Collembola reproduction study with R234886, the NOEC for mortality of the parental collembolans was determined to be 250 mg test item/kg soil d.w. The LC₁₀, LC₂₀ and LC₅₀ for mortality were calculated to be 282, 436 and 922 mg test item/kg soil dry weight, respectively. The NOEC for reproduction was determined to be 250 mg test item/kg soil d.w. The EC₁₀, EC₂₀ and EC₅₀ values for reproduction were calculated to be 332, 477 and 955 mg test item/kg soil dry weight, respectively.

(Friedrich S, 2019)

Comments of zRMS:	The study was conducted to OECD guidance 226 and according to the principles of GLP. All validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.
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Reference:	KCP 10.4.2.1
Report:	Schulz L., (2020), Oxathiapiprolin/Azoxystrobin SC (A22773A) - Effects on the Reproduction of the Predatory Mite <i>Hypoaspis aculeifer</i> . 20 48 THC 0042. BioChem agrar Labor für biologische und chemische Analytik GmbH, Kupferstraße 6, 04827 Machern OT Gerichshain, Germany. Syngenta file no VV-876276
Guideline(s):	OECD Guidelines No. 226 (2016)
Deviations:	No
GLP:	Yes

Executive Summary

In a 14-day *Hypoaspis aculeifer* reproduction study with A22773A, the LC₅₀ for mortality and the EC₁₀, EC₂₀ and EC₅₀ values for reproduction could not be calculated, but it can be concluded that these values are higher than 1000 mg test item/kg soil dry weight, the highest concentration tested. The NOEC for mortality and the NOEC for reproduction were determined to be equal or higher than 1000 mg test item/kg soil dry weight.

Materials

Test Material	Oxathiapiprolin/Azoxystrobin SC (A22773A)
Batch ID:	SFI003-174-001
Other batch ID:	1016797
Content of active ingredients nominal:	Oxathiapiprolin 12 g/L Azoxystrobin 250 g/L
Description:	beige liquid
Stability of test compound:	stable under the given conditions
Recertification date:	End of February 2023
Treatments	
Test rates:	16, 29, 53, 95, 171, 309, 556, 1000 mg test item/kg soil dry weight (spacing factor: 1.8)
Control:	prepared with deionised water
Toxic standard:	dimethoate (98.8 % ± 0.5 %, analysed) (separate GLP study)
Test organisms	
Species	<i>Hypoaspis aculeifer</i> (CANESTRINI)
Source:	Obtained synchronised from “Katz Biotech AG”, Baruth, Germany, on 11 August 2020 and hold in the test facility under ambient laboratory conditions until test start
Food:	<i>Tyrophagus putrescentiae</i> (SCHRANK); provided every 2-3 days
Age at test start:	Adult (30-32 days old)
Test design	
Vessels:	160 mL WECK-jar with glass lid
Substrate:	- 5.0 % sphagnum peat; origin: Torfwerk Moorkultur Ramsloh, 26683 Saterland, Germany, classified according to DIN 11540 (as close to pH 5.5-6.0 as possible, no visible plant remains, finely ground, dried to measured moisture content) - 20.0 % kaolin clay (kaolinite content > 30 %); type: Kaolin W, origin: ERBSLÖH Lohrheim GmbH, 65558 Lohrheim, Germany - 0.25 % calcium carbonate; origin: MERCK KGaA, 64271 Darmstadt, Germany - 74.75 % industrial quartz sand; type: Millisil W3, origin: Quarzwerke GmbH,

	50207 Frechen, Germany (predominantly fine sand with more than 50 % of the particles between 50 and 200 µm)
	- deionised water
Replication:	control: 8 (+ 2 replicates for determination of water content and pH-value; without predatory mites) treated group: 4 (+ 2 replicates for determination of water content and pH-value; without predatory mites)
No. of mites/arena :	10
Duration of test:	14 days
Environmental test conditions	
Temperature:	19.4 - 21.7 °C
pH:	test start: 6.2 - 6.4 test end: 6.4 - 6.5
Water content of soil:	test start: 16.75 - 17.10 (equivalent to 47.98 - 48.98 % of WHC) test end: 16.45 - 17.14 (equivalent to 47.12 - 49.10 % of WHC)
Photoperiod:	duration: light : dark = 16 h : 8 h intensity: 489 lux

Study Design and Methods

Experimental dates: 07 August 2020 - 28 August 2020

Adult females of the soil mite *Hypoaspis aculeifer* were exposed to different concentrations of Oxathiapiprolin/Mandipropamid SC (A22773A) incorporated into the test soil. An exactly weighed amount of the test item was mixed with purified water to make a stock solution, and appropriate volumes of this stock solution were further diluted with deionised water to obtain the test concentrations such that, when added to pre-moistened artificial soil, a final moisture content value of approximately 50% WHC was achieved.

Adult females were transferred to the test vessels which contained untreated (control) or test item treated artificial soil. Ten adult females were introduced to each test vessel. As a source of food, cheese mites (*Tyrophagus putrescentiae*) were added to the soil surface throughout the test. The test was carried out under controlled light-dark cycle. Fourteen days after introducing the test organisms, the surviving mites and the juveniles of *Hypoaspis aculeifer* were extracted by heat/light extraction and counted. From these data the mortality of the adult females and the reproductive output were calculated.

The statistical analysis was performed with the software ToxRat Professional 3.3.0 (RATTE, 2018). The Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm and Williams Multiple Sequential t-test Procedure were used to compare the control with the independent test item groups.

Results and Discussion

Mortality and reproduction are summarised in the table below.

Table A 50: Effects of A22773A on mortality and reproduction of *Hypoaspis aculeifer*

Endpoint	Treatment group (mg test item/kg soil d.w.)								
	Control	16	29	53	95	171	309	556	1000
	Mortality of adult mites after 14 days								
Mortality (%)	0.0	2.5	2.5	0.0	0.0	0.0	0.0	0.0	7.5
Corr. mortality (Abbott) (%)	-	2.5	2.5	0.0	0.0	0.0	0.0	0.0	7.5
	Number of juveniles after 14 days								
Mean	254.5	287.5	279.3	267.0	244.0	245.8	243.8	245.0	247.5
Standard deviation	13.2	9.3	24.9	28.6	11.1	6.3	13.5	21.9	21.0

Endpoint	Treatment group (mg test item/kg soil d.w.)								
	Control	16	29	53	95	171	309	556	1000
Coefficient of variation (%)	5.2	3.2	8.9	10.7	4.6	2.6	5.5	8.9	8.5
Reduction of reproduction compared to control (%)	-	-13.0	-9.7	-4.9	4.1	3.4	4.2	3.7	2.8
	Endpoint (mg test item/kg soil d.w.)								
NOEC (mortality)	≥ 1000								
LC ₅₀ (mortality)	> 1000								
NOEC (reproduction)	≥ 1000								
EC ₁₀ (reproduction)	> 1000								
EC ₂₀ (reproduction)	> 1000								
EC ₅₀ (reproduction)	> 1000								

Not statistically significantly different compared to the control (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm for mortality, $\alpha = 0.05$, one-sided greater and Williams Multiple Sequential t-test Procedure for reproduction, $\alpha = 0.05$, one-sided smaller)

Negative % values for reduction of reproduction = increase, relative to control

Validity Criteria

The validity criteria for the control group were met:

- Mean mortality of adult females: ≤ 20% (observed: 0.0 %)
- Mean number of juveniles per replicate: ≥ 50 (observed: 254.5)
- Coefficient of variation (mean number of juveniles per replicate): ≤ 30 % (observed: 5.2 %)

Conclusions

In a 14-day *Hypoaspis aculeifer* reproduction study with A22773A, the LC₅₀ for mortality and the EC₁₀, EC₂₀ and EC₅₀ values for reproduction could not be calculated, but it can be concluded that these values are higher than 1000 mg test item/kg soil dry weight, the highest concentration tested. The NOEC for mortality and the NOEC for reproduction were determined to be equal or higher than 1000 mg test item/kg soil dry weight.

(Schulz L, 2020)

Comments of zRMS:	Study not evaluated.
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Reference:	KCA 8.4.2.1
Report:	Schulz L., (2019), R234886 - Effects on the Reproduction of the Predatory Mite <i>Hypoaspis aculeifer</i> . 19 48 THC 0004. BioChem agrar Labor für biologische und chemische Analytik GmbH, Kupferstraße 6, 04827 Machern OT Gerichshain, Germany. Syngenta file No VV-471883
Guideline(s):	OECD Guidelines No. 226 (2016)
Deviations:	No
GLP:	Yes

Executive Summary

In a 14-day *Hypoaspis aculeifer* reproduction study with R234886, the LC₅₀ for mortality and the EC₁₀, EC₂₀ and EC₅₀ values for reproduction could not be calculated, but it can be concluded that these values are greater than 1000 mg test item/kg soil dry weight, the highest concentration tested. The NOEC for mortality and the NOEC for reproduction were determined to be 1000 mg test item/kg soil dry weight.

Materials

Test Material	R234886
Lot/Batch #:	MES 563/2
Analysed purity:	99 % w/w
Description:	off-white solid
Stability of test compound:	stable under the given conditions
Reanalysis/Expiry date:	End of March 2020
Treatments	
Test rates:	16, 31, 63, 125, 250, 500, 1000 mg test item/kg soil dry weight (spacing factor: 2)
Application method:	the test item was mixed with finely ground quartz sand and then the test item/sand mixtures were thoroughly mixed into the pre-moistened artificial soil
Control:	quartz sand was added in the same amount as to the test item-treated soils
Toxic standard:	dimethoate (98.8 % ± 0.5 %, analysed) (separate GLP study, BioChem project No.: 18 48 THC 0063)
Test organisms	
Species	<i>Hypoaspis aculeifer</i> (CANESTRINI)
Source:	Originally obtained from “Bayer CropScience AG”, Monheim am Rhein, Germany; reared in the test facility
Food:	<i>Tyrophagus putrescentiae</i> (SCHRANK); by daily checking of the food mites and replenishing if necessary
Age at test start:	Adult (30-33 days old)
Test design	
Vessels:	160 mL WECK-jar with glass lid (inside dimensions: 4.7 cm diameter, 8 cm high, height of soil: approximately 1.7 cm)
Substrate:	- 5 % sphagnum peat; origin: Torfwerk Moorkultur Ramsloh, 26683 Saterland, Germany, classified according to DIN 11540 (as close to pH 5.5-6.0 as possible, no visible plant remains, finely ground, dried to measured moisture content) - 20.1 % kaolin clay (kaolinite content > 30 %); type: Kaolin W, origin: ERBSLÖH Lohrheim GmbH, 65558 Lohrheim, Germany - 0.2 % calcium carbonate; origin: MERCK KGaA, 64271 Darmstadt, Germany - 74.7 % industrial quartz sand; type: Millisil W3, origin: Quarzwerke GmbH, 50207 Frechen, Germany (predominantly fine sand with more than 50 % of the particles between 50 and 200 µm) - deionised water

Replication:	control: 8 (+ 2 replicates for determination of water content and pH-value; without predatory mites) treated group: 4 (+ 2 replicates for determination of water content and pH-value; without predatory mites)
No. of mites/arena:	10
Duration of test:	14 days
Environmental test conditions	
Temperature:	20.0 - 22.0 °C
pH:	test start: 5.8 - 6.0 test end: 5.5 - 5.6
Water content of soil:	test start: 18.48 - 19.37 (equivalent to 47.30 - 49.57 % of WHC) test end: 17.91 - 18.30 (equivalent to 45.83 - 46.84 % of WHC)
Photoperiod:	duration: light : dark = 16 h : 8 h intensity: 529 lux

Study Design and Methods

Experimental dates: 16 January 2019 to 28 February 2019

Adult females of the soil mite *Hypoaspis aculeifer* were exposed to different concentrations of R234886 incorporated into the test soil. Since data on the water solubility of the test item are not available, the test item was mixed with finely ground quartz sand (= stock mixture) using mortar and pestle. Subsequently, the obtained stock mixture was stepwise diluted with quartz sand to prepare 6 further test item mixtures (serial dilution, spacing factor 2) containing the amount of test item which was required to adjust the test item concentration of the respective treatment group. Afterwards the test item mixtures were thoroughly mixed by intensive stirring (about 1-2 minutes) with the pre-moistened artificial soil with a final moisture content of approximately 50% WHC by means of a hand stirrer. Adult females were transferred to the test vessels which contained untreated (control) or test item treated artificial soil. Ten adult females were introduced to each test vessel. As a source of food, cheese mites (*Tyrophagus putrescentiae*) were regularly added to the soil surface throughout the test. The test was carried out under controlled light-dark cycle. Fourteen days after introducing the test organisms, the surviving mites and the juveniles of *Hypoaspis aculeifer* were extracted by heat/light extraction and counted. From these data the mortality of the adult females and the reproductive output were calculated.

The statistical analysis was performed with the software ToxRat Professional 3.2.1 (RATTE, 2015). The Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm and the Dunnett-t-test were used to compare the control with the independent test item groups.

Results and Discussion

Mortality and reproduction are summarised in the table below.

Table A 51: Effects of R234886 on mortality and reproduction of *Hypoaspis aculeifer*

Endpoint	Treatment group (mg test item/kg soil d.w.)							
	Control	16	31	63	125	250	500	1000
	Mortality of adult mites after 14 days							
Mortality (%)	1.3	0.0	5.0	5.0	5.0	7.5	10.0	0.0
Corr. mortality (Abbott) (%)	-	-1.3	3.8	3.8	3.8	6.3	8.9	-1.3
	Number of juveniles after 14 days							
Mean	332.0	307.5	316.3	335.8	308.3	322.5	320.0	321.0
Standard deviation	24.2	28.9	39.7	26.6	41.3	54.2	30.3	36.6
Coefficient of variation (%)	7.3	9.4	12.6	7.9	13.4	16.8	9.5	11.4

Endpoint	Treatment group (mg test item/kg soil d.w.)							
	Control	16	31	63	125	250	500	1000
Reduction of reproduction compared to control (%)	-	7.4	4.7	-1.1	7.2	2.9	3.6	3.3
	Endpoint (mg test item/kg soil d.w.)							
NOEC (mortality)	1000							
LC ₅₀ (mortality)	> 1000							
NOEC (reproduction)	1000							
EC ₁₀ (reproduction)	> 1000							
EC ₂₀ (reproduction)	> 1000							
EC ₅₀ (reproduction)	> 1000							

Not statistically significantly different compared to the control (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm for mortality, $p > 0.05$, one-sided greater and Dunnett-t-test for reproduction, $p > 0.05$, one-sided smaller)
Negative % values for reduction of reproduction = increase, relative to control

Validity Criteria

The validity criteria for the control group were met:

- Mean mortality of adult females: $\leq 20\%$ (observed: 1.3 %)
- Mean number of juveniles per replicate: ≥ 50 (observed: 332)
- Coefficient of variation (mean number of juveniles per replicate): $\leq 30\%$ (observed: 7.3 %)

Conclusions

In a 14-day *Hypoaspis aculeifer* reproduction study with R234886, the LC₅₀ for mortality and the EC₁₀, EC₂₀ and EC₅₀ values for reproduction could not be calculated, but it can be concluded that these values are greater than 1000 mg test item/kg soil dry weight, the highest concentration tested. The NOEC for mortality and the NOEC for reproduction were determined to be 1000 mg test item/kg soil dry weight.

(Schulz, L., 2019)

Comments of zRMS:	Study not evaluated.
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Reference:	KCA 8.4.2.1
Report:	Schulz, L. (2017) Azoxystrobin SC (A12705B) - Effects on the Reproduction of the Predatory Mite <i>Hypoaspis aculeifer</i> , Report Number 17 48 THC 0019. BioChem agrar, Labor für biologische und chemische, Analytik GmbH, Kupferstraße 6, 04827 Gerichshain, Germany. Syngenta file No. VV-467698
Guideline(s):	OECD Guideline 226: Predatory mite (<i>Hypoaspis (Geolaelaps) aculeifer</i>) reproduction test in soil (2016)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Executive Summary

In a 14-day *Hypoaspis aculeifer* reproduction study with A12705B, the LC50 for mortality and the EC10, EC20 and EC50 values for reproduction could not be calculated, but it can be concluded that these values are greater than 1 000 mg test item/kg soil dry weight, the highest concentration tested. The NOEC for mortality and the NOEC for reproduction were determined to be 1 000 mg test item/kg soil dry weight.

Materials

Test Material	Azoxystrobin SC (A12705B)
Lot/Batch #:	GRA5J052A/1
Actual content of active ingredients:	Azoxystrobin 22.7 % w/w corresponding to 248 g/L
Description:	off-white to yellow liquid
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	End of November 2018
Density:	1 094 kg/m ³
Treatments	
Test rates:	16, 29, 53, 95, 171, 309, 556, 1 000 mg test item/kg soil dry weight
Control:	Untreated.
Toxic standard:	Dimethoate
Test organisms	
Species	<i>Hypoaspis aculeifer</i> (CANESTRINI)
Source:	Cultured in test facility (originally: "Bayer CropScience AG" Monheim, Germany)
Food:	Cheese mites, <i>Tyrophagus putrescentiae</i> at 2 spatulas on day 0, 1 and 5, 1 spatula on day 7 and 9, ½ a spatula on day 12.
Age at test start:	adults from a synchronised culture with an age difference of 3 days
Test design	
Vessels:	100 mL SCHOTT-bottles with screw cap (inside dimensions: 4 cm in diameter, 11 cm high)
Substrate:	Artificial soil comprising 5 % sphagnum peat, 20 % kaolinite clay, 74.8 % industrial quartz sand and 0.2 % calcium carbonate.
Replication:	Control group: 8 (+ 2 replicates for determination of water content and pH-value; without predatory mites) Treated group: 4 (+ 2 replicates for determination of water content and pH-value; without predatory mites)
No. of mites/arena :	10
Duration of test:	14 days

Environmental test conditions

Temperature:	20.1 - 20.5 °C
pH:	test start: 6.1 - 6.3 test end: 5.9 - 6.1
Water content of soil:	test start: 19.46 - 19.82 (equivalent to 48.69 - 49.59 % of WHC) test end: 17.56 - 19.42 (equivalent to 43.94 - 48.58 % of WHC)
Photoperiod:	source: artificial light (Lumilux L58W) intensity: 597 lx duration: light : dark = 16 h : 8 h

Study Design and Methods

Experimental dates: 1st March 2017 to 26th April 2017

Adult females of the soil mite *Hypoaspis aculeifer* were exposed to concentrations of 16, 29, 53, 95, 171, 309, 556, 1 000 mg test item/kg soil dry weight incorporated into the test soil. An exactly weighed amount of the test item was mixed with purified water to make a stock solution, and appropriate volumes of this stock solution were further diluted with purified water to obtain the test concentrations such that, when added to pre-moistened artificial soil, a final moisture content value of 50 % WHC was achieved. Adult females were transferred to the test vessels which contained untreated (control), reference item or test item treated artificial soil. Ten adult females were introduced to each test vessel. As a source of food, cheese mites (*Tyrophagus putrescentiae*) were added to the soil surface throughout the test. The test was carried out under controlled light-dark cycle. Fourteen days after introducing the test organisms, the surviving mites and the juveniles of *Hypoaspis aculeifer* were extracted by heat/light extraction. From these data the mortality of the adult females and the reproductive output were calculated.

Mortality (number of dead adults) in % for the treatment groups was calculated. Missing mites were counted as dead. Observations in the treatment group were expressed relative to the control group. The corrected mortality in the treatment groups was calculated according to ABBOTT (1925). The reduction of reproductive output for the treatment groups was calculated in comparison to the control group.

The statistical analysis was performed with the software ToxRat Professional 3.2.1 (RATTE, 2015). The Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm and the Welch-t-test after Bonferroni-Holm were used to compare the control with the independent test item groups.

Results and Discussion

Mortality and fecundity are summarised in the table below.

Table A 52: Effects of residues of A12705B on mortality and reproduction of *Hypoaspis aculeifer*

Endpoint	Treatment group (mg A12705B/kg soil d.w.)								
	Control	16	29	53	95	171	309	556	1 000
	Mortality of adult mites after 14 days								
% mortality	0	0	0	5	0	0	0	0	2.5
% corrected mortality	-	0	0	5	0	0	0	0	2.5
	Number of juveniles after 14 days								
Mean no. progeny per replicate	304.3	329.3	332.5	332.3	266.5	336.8	306.5	304.3	300.3
standard deviation	51.5	40.6	23.2	31.6	79.1	11.7	34.3	10.3	22.4
% reduction compared to control	-	-8.2	-9.3	-9.2	12.4	-10.7	-0.7	0	1.3

NOEC (mortality)	1 000
LC₅₀ (mortality)	> 1 000
NOEC (reproduction)	1 000
EC₅₀ (reproduction)	> 1 000
EC₂₀ (reproduction)	> 1 000
EC₁₀ (reproduction)	> 1 000

Not statistically significantly different compared to the control (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm for mortality, $p > 0.05$, one-sided greater and Welch-t-test after Bonferroni-Holm for reproduction, $p > 0.05$, one-sided smaller)

Validity Criteria

The validity criteria for the control group were met:

- Mean mortality of adult females: $\leq 20\%$ (observed: 0 %)
- Mean number of juveniles per replicate: ≥ 50 (calculated: 304.3)
- Coefficient of variation (mean number of juveniles per replicate): $\leq 30\%$ (calculated: 16.9 %)

Conclusions

In a 14-day *Hypoaspis aculeifer* reproduction study with A12705B, the LC₅₀ for mortality and the EC₁₀, EC₂₀ and EC₅₀ values for reproduction could not be calculated, but it can be concluded that these values are greater than 1 000 mg test item/kg soil dry weight, the highest concentration tested. The NOEC for mortality and the NOEC for reproduction were determined to be 1 000 mg test item/kg soil dry weight.

(Schulz L, 2017)

A 2.4.2.2 KCP 10.4.2.2 Higher tier testing

A 2.5 KCP 10.5 Effects on soil nitrogen transformation

Comments of zRMS:	The study was conducted to OECD guidance and according to the principles of GLP. All validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.
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Reference:	KCP 10.5
Report:	Schulz, L. (2020), Oxathiapiprolin/Azoxystrobin SC (A22773A) - Effects on the Activity of Soil Microflora (Nitrogen and Carbon Transformation Tests). Report Number 20 48 SMO 0017. BioChem agrar GmbH, Kupferstraße 6, 04827 Machern OT Gerichshain, Germany. Syngenta file no VV-885459
Guideline(s):	OECD Guidelines No. 216 / 217 (2000)
Deviations:	No
GLP:	Yes

Executive Summary

The test item A22773A, tested at 2.92 mg/kg soil dry weight and 14.61 mg/kg soil dry weight (corresponding

to 2 L test item/ha and 10 L test item/ha, respectively) caused no adverse effects (deviation from control < 25 %, OECD 216/217) on soil nitrogen transformation (measured as NO₃-N-production) and on soil carbon transformation (measured as O₂-consumption) at the end of the 28-day incubation period.

Materials

Test Material	Oxathiapiprolin/Azoxystrobin SC (A22773A)
Lot/Batch #:	SFI003-174-001
Actual content of active ingredients:	Oxathiapiprolin 1.02 % w/w corresponding to 11.2 g/L Azoxystrobin 22.5 % w/w corresponding to 247 g/L
Description:	beige liquid
Reanalysis/Expiry date:	End of February 2023
Density:	1096 kg/m ³
Treatments	
Test rates:	2.92 mg test item/kg soil dry weight (2 L test item/ha) 14.61 mg test item/kg soil dry weight (10 L test item/ha)
Control:	Deionised water
Toxic standard:	Dinoterb (tested in separate GLP study)
Test design	
Soil type:	loamy sand (DIN ISO 11277; DIN 4220)
Test units:	Nitrogen transformation test: wide-mouth glass flasks (500 mL) Carbon transformation test: stainless steel vessels (4 L)
Replication:	3
Sampling intervals:	0 (3 hours after application), 7, 14 and 28 days
Duration of test:	28 days
Environmental test conditions	
Temperature:	19.4 - 20.7 °C
pH of soil:	Nitrogen transformation test: 6.2, Carbon transformation test: 6.2 - 6.3
Soil moisture content:	Approximately 45% of maximum water holding capacity
Photoperiod:	Continuous darkness

Study Design and Methods

Experimental dates: 18 September 2020 to 16 October 2020.

Soil samples were treated with A22773A at two doses, 2.92 and 14.61 mg A22773A/kg dry soil (corresponding to 2 L test item/ha and 10 L test item/ha, respectively). Test concentrations related to a soil depth of 5 cm and a soil density of 1.5 g/cm³.

The test item was mixed with deionised water and the test solution was subsequently mixed with the soil (carbon transformation test: laboratory mixer, nitrogen transformation test: hand stirrer). Water was added to the soil to achieve a water content of approximately 45 % of WHC. The water content of the soil in each test vessel was determined at test start (after application) and adjusted once a week to the required range of 40 - 50 % of WHC.

Three replicate soil samples were prepared for each treatment rate and the control for the nitrogen transformation test and carbon transformation test.

Mean nitrogen content (mg NO₃/kg soil d.w.), standard deviation and coefficient of variation as well as the mean nitrogen content/day (mg NO₃/kg soil d.w./day) were calculated for each treatment group and sampling date.

For the evaluation of the results the relative deviations (%) of the test item treatment groups from the control were calculated (based on the mean nitrogen content/day) for each sampling date.

The cumulative O₂ consumption after 12 hours was calculated (using regression analysis; the goodness of fit (R²) was > 0.99 in all replicates and on all days). Furthermore, standard deviation and coefficient of variation were calculated for each treatment group and sampling dates.

For evaluation of the results the relative deviations (%) of the test item treatment groups from the control were calculated for each sampling date. A 2-sided Student-t-test at 5 % significance level was performed

for statistical evaluation.

Results and Discussion

Results from the Nitrogen transformation test and the Carbon transformation test are summarised in the tables below.

Table A 53: Effects on Nitrogen Transformation in Soil after Treatment with A22773A

Time Interval (days)	Control		2.92 mg test item/kg soil dry weight			14.61 mg test item/kg soil dry weight		
	NO ₃ -N [mg/kg soil d.w.]	NO ₃ -N [mg/kg soil d.w./day]	NO ₃ -N [mg/kg soil d.w.]	NO ₃ -N [mg/kg soil d.w./day]	Deviation from control [%] ¹⁾	NO ₃ -N [mg/kg soil d.w.]	NO ₃ -N [mg/kg soil d.w./day]	Deviation from control [%] ¹⁾
0 - 7	90.3	6.82	91.5	7.19	+5.4	86.8	6.37	-6.6
0 - 14	99.1	4.04	99.1	4.14	+2.5	99.7	4.10	+1.7
0 - 28	126.7	3.00	125.7	3.02	+0.5	128.7	3.09	+2.9

¹⁾ based on NO₃-nitrogen-production; - = inhibition; + = stimulation

Not statistically significant difference to the control (Student-t-test for homogeneous variances, 2-sided, $\alpha = 0.05$).

The calculations were performed with non-rounded values.

Table A 54: Effects on Carbon Transformation in Soil after Treatment with A22773A

Days after application	Control	2.92 mg test item/kg soil dry weight		14.61 mg test item/kg soil dry weight	
	O ₂ -consumption [mg/kg soil d.w./h]	O ₂ -consumption [mg/kg soil d.w./h]	Deviation from control [%] ¹⁾	O ₂ -consumption [mg/kg soil d.w./h]	Deviation from control [%] ¹⁾
0	13.42	13.11	-2.3	13.60	+1.4
7	13.33	13.37	+0.3	13.22	-0.8
14	12.82	12.83	+0.1	12.34	-3.7
28	11.50	11.32	-1.5	11.22	-2.4

¹⁾ based on O₂-consumption; - = inhibition; + = stimulation

Not statistically significant difference to the control (Student-t-test for homogeneous variances, 2-sided, $\alpha = 0.05$).

The calculations were performed with non-rounded values.

Validity criteria

The validity criteria are listed below:

- The coefficient of variation in the control group of the nitrogen and carbon transformation tests (for the whole test duration) were at maximum 3.8 and 3.6 % respectively (must be ≤ 15 %)
- The toxic standard caused effects of +28.3 % and +48.6 % at concentrations 6.80 and 13.60 mg/kg soil d.w. in the Nitrogen transformation test after a 28 day exposure, demonstrating the sensitivity of the test system (must be ≥ 25 %)
- The toxic standard caused effects of -31.4 %, -40.2 % and -39.8 % at concentrations 6.80, 13.60 and 27.20 mg/kg soil d.w., respectively in the Carbon transformation test after a 28 day exposure, demonstrating the sensitivity of the test system (must be ≥ 25 %)

Conclusions

The test item A22773A tested at 2.92 mg/kg soil dry weight and 14.61 mg/kg soil dry (corresponding to 2 L test item/ha and 10 L test item/ha, respectively) caused no adverse effects (deviation from control < 25 %, OECD 216/217) on soil nitrogen transformation (measured as NO₃-N-production) and on soil carbon transformation (measured as O₂-consumption) at the end of the 28-day incubation period.

(Schulz L, 2020)

A 2.6 KCP 10.6 Effects on terrestrial non-target higher plants

A 2.6.1 KCP 10.6.1 Summary of screening data

A 2.6.2 KCP 10.6.2 Testing on non-target plants

Comments of zRMS:	The study is considered to be reliable and suitable for the risk assessment (supplementary data).
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Reference:	KCP 10.6.2
Report:	Jones, K. (2020), Oxathiapiprolin/azoxystrobin SC (A22773A) – plus Adjuvant A12127R - Phytotoxicity to Non-Target Plants Screening Test. Report Number ACE-20-101. AgroChemex Ltd., Aldhams Farm Research Station, Dead Lane, Lawford, Manningtree, Essex, CO11 2NF, United Kingdom. Syngenta file no VV-880671
Guideline(s):	Study was carried out following a standardised study protocol based on Syngenta herbicide profiling test
Deviations:	No
GLP:	Yes

Executive Summary

The effects of A22773A plus adjuvant A12127R on the seedling emergence and vegetative vigour of six non-target plant species (onion, wheat, sugar beet, oilseed rape, cucumber and soybean) were assessed following a range of applications, (0 (deionised water only), 31.25, 62.5, 125, 250, 500, 1000 mL A22773A/ha plus 125, 250, 500, 1000, 2000 and 4000 mL A12127R/ha).

For seedling emergence none of the tested plant species showed any phytotoxic effects at any of the test rates. In the vegetative vigour test, onion and wheat did not show any phytotoxic effects up to and including the top dose of 1000 mL A22773A/ha plus 4000 mL A12127R/ha. Sugar beet, oilseed rape and cucumber showed phytotoxic effects starting at 250 mL A22773A/ha plus 1000 mL A12127R/ha up to and including 1000 mL A22773A/ha plus 4000 mL A12127R/ha and soybean showed phytotoxic effect starting at 500 mL A22773A/ha plus 2000 mL A12127R/ha up to and including 1000 mL A22773A/ha plus 4000 mL A12127R/ha.

Materials

Test material	Oxathiapiprolin/azoxystrobin SC
Lot/Batch #:	SF1003-174-001
Actual content of active ingredients:	Oxathiapiprolin 1.02% w/w corresponding to 11.2 g/L Azoxystrobin 22.5% w/w corresponding to 247 g/L
Description:	Beige liquid
Stability of test compound:	Stable under normal conditions
Reanalysis/expiry date:	End Feb 2023
Treatments	
Test concentrations:	31.25, 62.5, 125, 250, 500, 1000 mL/ha
Control:	Deionised water
Spray volume:	200 L/ha ± 10%
Application method:	Mardrive cabinet sprayer
Test organisms	
Species:	Onion (<i>Allium cepa</i>), Wheat (<i>Triticum aestivum</i>), Sugar beet (<i>Beta vulgaris</i>), Oilseed rape (<i>Brassica napus</i>), Cucumber (<i>Cucumis sativus</i>), Soybean (<i>Glycine max</i>).

Test soil:	Sandy loam mixed in a ratio of approximately 2 parts sterile loam and 1 part sand. 125 g slow release fertiliser (Osmocote® Pro) incorporated into 30 litres of soil mix prior to study start. Organic carbon content 0.9 %.
Test design	
Test vessels:	Non-porous plastic pots were used (9 x 9 x 10 cm; w x d x h).
Sampling interval:	Seedling emergence: Visual phytotoxicity assessment undertaken 28 days after the application of the test item. Vegetative vigour: Visual phytotoxicity assessment undertaken 21 days after the application of the test item.
Replication:	Three pots per treatment with four seeds/plants per pot (six seeds/plants for onion).
Duration:	28 days for seedling emergence test; 21 days for vegetative vigour test.
Environmental conditions	
Test temperature:	Min: 16.3 – Max: 28.2 °C (Mean: 21.3 °C)
Humidity:	Min: 38.6 – Max: 75.3% (Mean: 56.5%)
Soil pH:	7.4
Lighting:	Min: 0.4 – Max: 54.0 kilolux (Mean: 8.2 kilolux)

Study Design and Methods

Experimental dates: 17 September 2020 – 15 October 2020

Foliage of plants of two monocot species (*Allium cepa*, and *Triticum aestivum*) and four dicot species (*Beta vulgaris*, *Brassica napus*, *Cucumis sativus* and *Glycine max*) were sprayed with A22773A + A12127R to assess the vegetative vigour. For the seedling emergence test, A22773A + A12127R were applied directly to the soil.

For the application (seedling emergence and vegetative vigour) the highest concentration of spray solution was prepared by weighing 1.7276 g of A22773A and 5.8325 g of A12127R and diluting in 300 mL of deionised water. This solution served as the spray mixture for the 1000 mL A22773A + 4000 mL A12127R application rate. Lower dose rates were prepared by serial dilution.

The spray solutions were not analysed to determine the active ingredient concentration and no statistical analysis was required for this study.

At the final assessments for seedling emergence and vegetative vigour the level of phytotoxicity was recorded using a visual scale of 0 to 10:

Table A 55: Assessment of Injury Scale

Rating	Nominal % effect	Description of effects
0	0	Vigorous healthy plants, emergence of normal amount of seeds, indistinguishable from control
1	10	Vigorous, but with slight discoloration, malformation or stunting – slightly impaired emergence, growth or development
2	20	Less vigorous, with discoloration, malformation or stunting – slightly impaired growth and development, recovery likely, rate of emergence slightly reduced
3	30	Less vigorous, with obvious discoloration, malformation or stunting – impaired growth and development, recovery likely, rate of emergence reduced
4	40	Less vigorous, with more pronounced discoloration, malformation or stunting – recovery possible, clear reduction of rate of emergence
5	50	Poor vigour due to discoloration, malformation or stunting – recovery possible, emergence rate only about half of the control
6	60	Poor vigour due to discoloration, malformation or stunting and senescence – recovery doubtful, emergence of only a minor part of the seeds
7	70	Very poor vigour due to discoloration, malformation, stunting or senescence – still growing but recovery unlikely, emergence of few seeds only
8	80	Very poor vigour due to severe discoloration, malformation, stunting or senescence – recovery unlikely, emergence of very few seeds only

9	90	Very poor vigour – not all tissue dead but further growth unlikely, only some germination
10	100	Complete destruction of plant parts above ground, complete inhibition of germination

Results and Discussion

The results of the visual observation of phytotoxicity in the vegetative vigour and seedling emergence tests are given in the tables below.

Table A 56: Visual observation of phytotoxicity in the vegetative vigour test

Application rate (mL A22773A/ha)	0 (deionised water only)	31.25	62.5	125	250	500	1000
Onion	0	0	0	0	0	0	0
Wheat	0	0	0	0	0	0	0
Sugar beet	0	0	0	0	1	4	5
Oilseed rape	0	0	0	0	1	1	2
Cucumber	0	0	0	0	1	3	4
Soybean	0	0	0	0	0	1	3

Table A 57: Visual observation of phytotoxicity in the seedling emergence test

Application rate (mL A22773A/ha)	0 (deionised water only)	31.25	62.5	125	250	500	1000
Onion	0	0	0	0	0	0	0
Wheat	0	0	0	0	0	0	0
Sugar beet	0	0	0	0	0	0	0
Oilseed rape	0	0	0	0	0	0	0
Cucumber	0	0	0	0	0	0	0
Soybean	0	0	0	0	0	0	0

Validity criteria

The test was considered valid;

- The control plants did not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis, and wilting, leaf and stem deformation) and the plants exhibited only normal variation in growth and morphology for that particular species.
- Environmental conditions for a particular species were identical and growing media contained the same amount of soil matrix, support media or substrate from the same source.

Conclusions

Seedling emergence:

For seedling emergence none of the tested plant species showed any phytotoxic effects at any of the test rates.

Vegetative vigour:

In the vegetative vigour test, onion and wheat did not show any phytotoxic effects up to and including the top dose of 1000 mL A22773A/ha plus 4000 mL A12127R/ha. Sugar beet, oilseed rape and cucumber showed phytotoxic effects starting at 250 mL A22773A/ha plus 1000 mL A12127R/ha up to and including 1000 mL A22773A/ha and soybean showed phytotoxic effect starting at 500 mL A22773A/ha plus 2000 mL A12127R/ha up to and including 1000 mL A22773A/ha plus 4000 mL A12127R/ha.

(Jones K, 2020)

Comments of zRMS:	The study was conducted to OECD guidance and according to the principles of GLP. All validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.
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Reference:	KCP 10.6.2
Report:	Bützler, R. (2021), Oxathiapiprolin/azoxystrobin SC (A22773A) - Effects on Terrestrial (Non-Target) Plants: Vegetative Vigour Test. Report Number 159471087. ibacon GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany. Syngenta File no. VV-912999
Guideline(s):	OECD Guidelines No. 227 (2006)
Deviations:	No
GLP:	Yes

Executive Summary

The effects of oxathiapiprolin/azoxystrobin SC (A22773A) on the vegetative vigour, survival, height, dry weight and phytotoxicity of ten non-target plant species representing seven plant families were assessed following a single post-emergent application of a range of concentrations 4103, 2414, 1420, 835, 491, 289, 170, 100 mL test item/ha. No significant, adverse treatment related effects were seen in any of the ten species. The ER_{25/50} values for dry weight and height for all species were > 4103 mL A22773A/ha. The NOER values for all plant species were 4103 mL test item/ha. Phytotoxic effects observed were chlorosis (*Allium cepa*), necrosis (*Brassica napus*, *Allium cepa*) and deformation (*Allium cepa*). The ER_{10/20/50} values for phytotoxicity were > 4103 mL test item/ha.

Materials

Test material	Oxathiapiprolin/azoxystrobin SC (A22773A)
Lot/Batch #:	SFI003-174-001
Other batch ID:	1127290
Actual content of active ingredients:	Content of azoxystrobin: 22.5% w/w corresponding to 247 g/L Content of oxathiapiprolin: 1.02% w/w corresponding to 11.2 g/L According to certificate of analysis
Description:	Beige liquid
Stability of test compound:	Stable under standard conditions
Reanalysis/expiry date:	End of February 2023
Treatments	
Test concentrations:	4103, 2414, 1420, 835, 491, 289, 170, 100 mL test item/ha (corresponding to 22.5, 13.2, 7.78, 4.58, 2.69, 1.58, 0.932, 0.548 g test item/L)
Control:	Deionised water
Spray volume:	200 L/ha (corresponding to 2 mg/cm ²)
Application method:	Spray application (Calibrated Fa. Schachtner, D-71640 Ludwigsburg equipment with TeeJet 8002 EVS nozzles)
Test organisms	
Species:	<i>Brassica napus</i> , <i>Brassica oleracea</i> , <i>Glycine max</i> , <i>Beta vulgaris</i> , <i>Lactuca sativa</i> , <i>Cucumis sativus</i> , <i>Zea mays</i> , <i>Lolium perenne</i> , <i>Allium cepa</i> , <i>Avena sativa</i>
Test soil:	LUFA 2.3 (USDA: sandy loam)
Test design	
Test vessels:	Commercial plastic flower pots (Æ 15 cm)
Sampling interval:	Phytotoxicity and mortality 7, 14 and 21 days after application, dry weight and height 14 or 21 days after 50% emergence in the control.
Replication:	5, 7 or 10 pots per treatment group with 2, 3 or 4 seeds were tested for each treatment group. Each pot represented one replicate.

Duration:	21 days
Environmental conditions	
Test temperature:	16.6 °C to 23.6 °C (cultivation period), 16.8 °C to 24.1 °C (exposure period)
Humidity:	45% to 74% (cultivation period), 48% to 83%. (exposure period)
Soil pH:	6.2 ± 0.3 (for <i>Brassica napus</i> , <i>Brassica oleracea</i> , <i>Glycine max</i> , <i>Beta vulgaris</i> , <i>Lactuca sativa</i> , <i>Cucumis sativus</i> , <i>Zea mays</i> , <i>Lolium perenne</i> and <i>Avena sativa</i>) 6.1 ± 0.4 (for <i>Allium cepa</i>)
Lighting:	16 h light : 8 h dark, 300 to 400 µE/m ² /s (cultivation period), 300 to 400 µE/m ² /s (exposure period)

Study Design and Methods

Experimental dates: 28 April 2021 – 20 July 2021

The seeds were introduced manually into the soil (soil depth 5 to 15 mm). For a given test species, all seeds used in the test were from the same source and lot number. Only untreated seeds were used. To account for the different development speed of the species the sowing was done on different dates, to ensure that all species were in the 2 to 4 true leaf stage at the application day. After sowing the pots were placed on saucers and watered.

On the day of application, the spray mixture for the highest rate was prepared by diluting 44.97 g test item to 2000 mL with deionised water (22.48 g test item/L corresponding to 4103 mL test item/ha in 200 L/ha). This stock solution was checked analytically using LC-MS/MS. The subsequent concentrations were prepared by serial dilutions of 1150 g out of the next higher concentration made to 1955 g with deionised water to provide the spray mixture. Young plants (2-4 leaves) of four monocot species (*Zea mays*, *Lolium perenne*, *Allium cepa* and *Avena sativa*) and six dicot species (*Brassica napus*, *Brassica oleracea*, *Glycine max*, *Beta vulgaris*, *Lactuca sativa*, *Cucumis sativus*) were sprayed with 4103, 2414, 1420, 835, 491, 289, 170, 100 mL test item/ha; each rate sprayed in 200 L deionised water/ha.

Growth stages at the application day and 21 days after application were recorded according to BBCH-Monograph. Visual phytotoxicity (e.g. chlorosis, necrosis, deformation) was recorded 7, 14 and 21 days after application (DAA) according to EPPO Standard PP 1/135. The number of living and dead plants was recorded 7, 14 and 21 days after application. A plant was considered dead if no living tissue could be found on the leaves or shoots. All other plants were considered living. The height of the above ground part of each individual surviving plant of a pot (each pot is considered as a replicate) was recorded on day 21 after application. The height of each shoot was measured from the soil surface to the leaf apex or to the growing point. The dry weight of the above ground part of all survived plants of a pot (each pot was considered as a replicate) 21 days after application was determined. The plants were cut above the soil surface and transferred to a heat-resistant bag. The plant material was dried at 70 °C for minimum 24 hours until it has reached a constant weight.

Dry weight and height data were tested for normal distribution and homogeneity of variance using Shapiro-Wilk's test ($\alpha = 0.01$) and the Levene's test ($\alpha = 0.01$). If the data were normally distributed, homogeneous and the trend analysis of contrasts revealed a linear trend the Williams t-test (multiple comparison, one-sided smaller, $\alpha = 0.05$) was used for comparing treatment groups and control. If the analysis of contrasts did not result in a linear trend the Dunnett's-test (multiple comparison, one-sided smaller, $\alpha = 0.05$) was used. If the data were not normally distributed the Bonferroni-Holm-U-test was performed (multiple comparison, one-sided smaller, $\alpha = 0.05$). If the data were normally distributed but not homogeneous the Bonferroni-Welch t-test (multiple comparison, one-sided smaller, $\alpha = 0.05$) was used. For the data of the dry weight and height of *Allium cepa* an Outlier-test after Hampel ($\alpha = 0.05$) was performed and the minimum and/or maximum values of some test item rates were excluded from evaluation.

In order to determine the ER_{25/50} values for dry weight and height, a regression analysis with the mean values of the control and each test item group was performed (Probit-analysis). In order to determine the ER_{10/20/50}

values on phytotoxicity data a regression analysis for the species with phytotoxic effects was performed (Probit-Analysis).

Results and Discussion

The endpoints based on height and dry weight for each tested plant species are presented in table below:

Table A 58: Effect Rates of A22773A on 21-Day based on Height and Dry Weight

Test species (common name)	ER ₂₅ (mL test item/ha)	ER ₅₀ (mL test item/ha)	NOER (mL test item/ha)
<i>Brassica napus</i> (Oilseed rape)	> 4103	> 4103	4103
<i>Brassica oleracea</i> (Wild cabbage)	> 4103	> 4103	4103
<i>Glycine max</i> (Soybean)	> 4103	> 4103	4103
<i>Beta vulgaris</i> (Sugar beet)	> 4103	> 4103	4103
<i>Lactuca sativa</i> (Lettuce)	> 4103	> 4103	4103
<i>Cucumis sativus</i> (Cucumber)	> 4103	> 4103	4103
<i>Zea mays</i> (Corn)	> 4103	> 4103	4103
<i>Lolium perenne</i> (Perennial ryegrass)	> 4103	> 4103	4103
<i>Allium cepa</i> (Garden onion)	> 4103	> 4103	4103
<i>Avena sativa</i> (Oat)	> 4103	> 4103	4103

Table A 59: Effect Rates of A22773A based on Phytotoxicity

Test species (common name)	ER ₁₀ (mL test item/ha)	ER ₂₀ (mL test item/ha)	ER ₅₀ (mL test item/ha)
<i>Brassica napus</i> (Oilseed rape)	> 4103	> 4103	> 4103
<i>Brassica oleracea</i> (Wild cabbage)	> 4103	> 4103	> 4103
<i>Glycine max</i> (Soybean)	> 4103	> 4103	> 4103
<i>Beta vulgaris</i> (Sugar beet)	> 4103	> 4103	> 4103
<i>Lactuca sativa</i> (Lettuce)	> 4103	> 4103	> 4103
<i>Cucumis sativus</i> (Cucumber)	> 4103	> 4103	> 4103
<i>Zea mays</i> (Corn)	> 4103	> 4103	> 4103
<i>Lolium perenne</i> (Perennial Ryegrass)	> 4103	> 4103	> 4103
<i>Allium cepa</i> (Garden onion)	> 4103	> 4103	> 4103
<i>Avena sativa</i> (Oat)	> 4103	> 4103	> 4103

The concentration of the active ingredient azoxystrobin in the stock solution was verified analytically using LC-MS/MS and the analytical recovery rate in the stock solution was 99% of the nominal value.

Validity criteria

The test was considered valid;

- The seedling emergence was at least 70% (actual 81 - 100%)

- Control seedlings exhibited only normal variation in growth and morphology for that particular species (must not exhibit visible phytotoxic effects and exhibit only normal variation in growth and morphology for that particular species)
- Control plant survival was 100% for the duration of the study (must be at least 90%)
- Environmental conditions for a particular species were identical, and growing medium contained the same amount of soil matrix, support media, or substrate from the same source

Conclusions

The effects of oxathiapiprolin/azoxystrobin OD (A22773A) on the vegetative vigour, survival, height, dry weight and phytotoxicity of ten non-target plant species representing seven plant families were assessed following a single post-emergent application of a range of concentrations - 4103, 2414, 1420, 835, 491, 289, 170, 100 mL test item/ha. No significant, adverse treatment related effects were seen in any of the ten species. The ER_{25/50} values for dry weight and height for all species were > 4103 mL A22773A/ha. The NOER values for all plant species were 4103 mL test item/ha. Phytotoxic effects observed were chlorosis (*Allium cepa*), necrosis (*Brassica napus*, *Allium cepa*) and deformation (*Allium cepa*). The ER_{10/20/50} values for phytotoxicity were > 4103 mL test item/ha.

(Bützler K, 2020)

A 2.6.3 KCP 10.6.3 Extended laboratory studies on non-target plants

A 2.6.4 KCP 10.6.4 Semi-field and field tests on non-target plants

A 2.7 KCP 10.7 Effects on other terrestrial organisms (flora and fauna)

A 2.8 KCP 10.8 Monitoring data