

# REGISTRATION REPORT

## Part B

### Section 9

#### Ecotoxicology

Detailed summary of the risk assessment

Product code: CA3642

Product name(s): Joust Pro

Chemical active substances: prothioconazole and azoxystrobin

Prothioconazole, 150 g/L

Azoxystrobin, 150 g/L

Central Zone

Zonal Rapporteur Member State: Poland

#### CORE ASSESSMENT

New Authorisation (Art.33)

Applicant: Nufarm Crop Products UK Limited

Applicant: Nufarm Polska Sp. z o. o.

Submission date: 23/02/2023, update August 2023, May 2024

MS Finalisation date: August 2023, update May 2024

(initial Core Assessment)

October 2024, update December 2024 (final Core Assessment)

### Version history

When	What
February 2023	Original applicant version
August 2023	Update to include risk assessment for the use on sunflower further to zRMS PL request
August 2023	<p>Initial zRMS assessment</p> <p>The report in the dRR format has been prepared by the Applicant, therefore all comments, additional evaluations and conclusions of the zRMS are presented in grey commenting boxes. Minor changes are introduced directly in the text and highlighted in grey. Not agreed or not relevant information are <del>struck through</del> and shaded for transparency.</p> <p>Following the evaluation and before sending the document for commenting, all colored highlighting was removed, from the parts updated by the Applicant, for better legibility.</p>
May 2024	Update from the applicant with study report: CA3642 (Prothioconazole 150 g/L + Azoxystrobin 150 g/L SC): Nitrogen Transformation Test (report number: 3203658).
May 2024	<p>Update to Initial zRMS assessment following the submitted study (report number: 3203658).</p> <p>The report in the dRR format has been prepared by the Applicant, therefore all comments, additional evaluations and conclusions of the zRMS are presented in grey commenting boxes. Minor changes are introduced directly in the text and highlighted in grey. Not agreed or not relevant information are <del>struck through</del> and shaded for transparency.</p> <p>Following the evaluation and before sending the document for commenting, all colored highlighting was removed, from the parts updated by the Applicant, for better legibility.</p>
October 2024	<p>Final report (Core Assessment updated following the commenting period)</p> <p>Additional information/assessments included by the zRMS in the report in response to comments received from the Applicant are highlighted in yellow. Not agreed or not relevant information are <del>struck through</del> and shaded for transparency.</p>
December 2024	<p>Final report (Core Assessment updated following the second commenting period)</p> <p>Additional information/assessments included by the zRMS in the report in response to comments received from the Applicant are highlighted in yellow. Not agreed or not relevant information are <del>struck through</del> and shaded for transparency.</p>

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## 9 Ecotoxicology (KCP 10)

This application is in support of the registration of the new product CA3642, which is an SC formulation containing the active substances prothioconazole (150 g a.s./L) and azoxystrobin (150 g a.s./L), in the Central Zone (Article 33 application). This document reviews the ecotoxicological studies for the product, CA3642. Azoxystrobin was approved in Reg. (EU) No 703/2011 of 20 July 2011 and prothioconazole was approved in Dir. 2008/44/EC of 4 April 2008. Both active substances are in the process of renewal under Reg. (EC) No 1107/2009.

Prothioconazole and azoxystrobin are fungicides, which are used to treat winter and spring varieties of cereals and oilseed rape. These major crop groups, along with minor crops (sunflower, flax, linseeds, poppy, mustard and Gold of Pleasure) are included in the proposed GAP (exposures and risk assessments from the minor crop uses are covered by the proposed major crop uses on oilseed rape). CA3642 was not the representative formulation for the EU review of prothioconazole and azoxystrobin, and since the intended uses cannot be completely covered by the evaluations performed for the single active substances in the respective EU reviews, new risk assessments are therefore required.

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

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

For the environment, this includes consideration of the following as specified in the Commission Directive 2008/44/EC for prothioconazole:

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

For the environment this includes consideration of the following as specified in the Commission Implementing Regulation (EU) No 703/2011 for azoxystrobin:

- *The protection of aquatic organisms.*

These concerns have been addressed within the current submission.

Note: this Part B document only reviews data (active substance or product) and additional information that has not previously been considered within the EU review process, as part of the EU review of prothioconazole and azoxystrobin. New active-substance data are only included if they are considered essential for the evaluation and, in this case, a full study summary is provided. Note, in some cases, these new data include studies that have been submitted as part of the active-substance renewal dossier and currently under EU review. However, it is intended that this product registration is evaluated prior to the EU renewal of the active substances; existing EU-agreed endpoints, therefore, apply, unless further justification has been provided.

For the implementation of the uniform principles of Annex VI, this document follows the conclusions of the review report on prothioconazole (SANCO/3923/07 - final), the EFSA Conclusion on the peer review of the pesticide risk assessment of the active substance prothioconazole (EFSA Scientific Report (2007) 106, 1-98), the EFSA Conclusion on the peer review of the pesticide risk assessment of the active substance azoxystrobin (EFSA Journal 2010; 8(4):1542), EFSA supporting publication 2014:EN-718 on the confirmatory data for azoxystrobin, and the associated DAR documents.

Appendix 1 of this document contains the list of references included in this document for support of the evaluation.

Appendix 2 of this document details any new studies submitted for this evaluation.

Information on the detailed composition of CA3642 can be found in the confidential dossier of this submission (Registration Report - Part C).

## 9.1 Critical GAP and overall conclusions

**Table 9.1-1: Table of critical GAPs**

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms
Zonal uses (field or outdoor uses, certain types of protected crops)																			
1.	AT	Wheat (winter & spring)  Spelt  Einkorn wheat  Emmer Wheat  Tritordeum	F	Septoria leaf spot <i>Zymoseptoria tritici</i> <i>Mycosphaerella graminicola</i> (SEPTTR) Glume blotch <i>Stagonospora nodorum</i> (LEPTNO) Brown Rust <i>Puccinia recondita</i> <i>Puccinia triticina</i> (PUCCRT) Yellow Rust <i>Puccinia striiformis</i> (PUCST) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Eyespot <i>Oculimacula acuformis/Pseudocercospora herpotrichoides</i> (PSDCHE) Tan Spot <i>Pyrenophora tritici-repentis</i> (PYRNTR) Head blight of cereals <i>Fusarium spp.</i> (FUSASP) <i>Microdochium spp.</i> (MICDSP)	foliar spray	BBCH 30 – 69 (spring)	a) 2 b) 2	14-21	a) 1.2-1.4 b) 2.4-2.8	a) 360-420 (180+180 – 210+210)  b) 720-840 (360+360 – 420+420)	100-400	35		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms
2.	BE	Wheat (winter & spring)  Spelt  Einkorn wheat  Emmer Wheat  Tritordeum	F	Septoria leaf spot <i>Zymoseptoria tritici</i> <i>Mycosphaerella graminicola</i> (SEPTTR) Glume blotch <i>Stagonospora nodorum</i> (LEPTNO) Brown Rust <i>Puccinia recondita</i> <i>Puccinia triticina</i> (PUCCRT) Yellow Rust <i>Puccinia striiformis</i> (PUCGST) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Eyespot <i>Oculimacula</i> <i>acuformis/Pseudocercospora</i> <i>herpotrichoides</i> (PSDCHE) Tan Spot <i>Pyrenophora tritici-repentis</i> (PYRNTR) Head blight of cereals <i>Fusarium spp.</i> (FUSASP) <i>Microdochium spp.</i> (MICDSP)	foliar spray	BBCH 30 – 69 (spring)	a) 2 b) 2	14-21	a) 1.2-1.4 b) 2.4-2.8	a) 360-420 (180+180 – 210+210)  b) 720-840 (360+360 – 420+420)	100-400	35		A	A	R	A	A	A



1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms
3	CZ	Wheat (winter & spring)  Spelt  Einkorn wheat  Emmer Wheat  Tritordeum	F	Septoria leaf spot <i>Zymoseptoria tritici</i> <i>Mycosphaerella graminicola</i> (SEPTTR) Glume blotch <i>Stagonospora nodorum</i> (LEPTNO) Brown Rust <i>Puccinia recondita</i> <i>Puccinia triticina</i> (PUCCRT) Yellow Rust <i>Puccinia striiformis</i> (PUCGST) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Eyespot <i>Oculimacula</i> <i>acuformis/Pseudocercospora</i> <i>herpotrichoides</i> (PSDCHE) Tan Spot <i>Pyrenophora tritici-repentis</i> (PYRNTR) Head blight of cereals <i>Fusarium spp.</i> (FUSASP) <i>Microdochium spp.</i> (MICDSP)	foliar spray	BBCH 30 – 69 (spring)	a) 2 b) 2	14-21	a) 1.2-1.4 b) 2.4-2.8	a) 360-420 (180+180 – 210+210)  b) 720-840 (360+360 – 420+420)	100-400	35		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms
4	DE	Wheat (winter & spring) (within the group of wheat included: spelt, einkorn wheat, emmer wheat, durum wheat)  Tritordeum	F	Septoria leaf spot <i>Zymoseptoria tritici</i> (SEPTTR) Glume blotch <i>Septoria nodorum</i> (LEPTNO) Brown Rust <i>Puccinia recondita f. sp. tritici</i> (PUCCRT) Yellow Rust <i>Puccinia striiformis</i> (PUCST) Powdery mildew <i>Erysiphe graminis</i> (ERYSGR) Tan Spot <i>Drechslera tritici-repentis</i> (PYRNTR) Head blight of cereals <i>Microdochium spp.</i> (MICDSP)	foliar spray	BBCH 30 – 69 (spring)	a) 2 b) 2	14-21	a) 1.4 b) 2.8	a) 420 (210+210)  b) 840 (420+420)	150-400	35		A	A	R	A	A	A
5.	DE	Wheat (winter & spring) (within the group of wheat included: spelt, einkorn wheat, emmer wheat, durum wheat)  Tritordeum	F	Fusarium ear blight <i>Fusarium spp.</i> (FUSASP)	foliar spray	BBCH 61 – 69 (spring)	a) 1 b) 2	N/A	a) 1.4 b) 2.8	a) 420 (210+210)  b) 840 (420+420)	150-400	35		A	A	R	A	A	A
6.	DE	Wheat (winter & spring) (within the group of wheat included: spelt, einkorn wheat, emmer wheat, durum wheat)  Tritordeum	F	<i>Pseudocercospora</i> <i>herpotrichoides</i> (PSDCHE)	foliar spray	BBCH 30 – 32 (spring)	a) 1 b) 2	N/A	a) 1.4 b) 2.8	a) 420 (210+210)  b) 840 (420+420)	150-400	35		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
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					
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7.	HU	Wheat (winter & spring)  Spelt  Einkorn wheat  Emmer Wheat  Tritordeum	F	Septoria leaf spot <i>Zymoseptoria tritici</i> <i>Mycosphaerella graminicola</i> (SEPTTR) Glume blotch <i>Stagonospora nodorum</i> (LEPTNO) Brown Rust <i>Puccinia recondita</i> <i>Puccinia triticina</i> (PUCCRT) Yellow Rust <i>Puccinia striiformis</i> (PUCGST) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Eyespot <i>Oculimacula acufomis</i> / <i>Pseudocercospora</i> <i>herpotrichoides</i> (PSDCHE) Tan Spot <i>Pyrenophora tritici-repentis</i> (PYRNTR) Head blight of cereals <i>Fusarium spp.</i> (FUSASP) <i>Microdochium spp.</i> (MICDSP)	foliar spray	BBCH 30 – 69 (spring)	a) 2 b) 2	14-21	a) 1.2-1.4 b) 2.4-2.8	a) 360-420 (180+180 – 210+210)  b) 720-840 (360+360 – 420+420)	100-400	35		A	A	R	A	A	C

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
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					
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8.	IE	Wheat (winter & spring)  Spelt  Einkorn wheat  Emmer Wheat  Tritordeum	F	Septoria leaf spot <i>Zymoseptoria tritici</i> <i>Mycosphaerella graminicola</i> (SEPTTR) Glume blotch <i>Stagonospora nodorum</i> (LEPTNO) Brown Rust <i>Puccinia recondita</i> <i>Puccinia triticina</i> (PUCCRT) Yellow Rust <i>Puccinia striiformis</i> (PUCGST) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Eyespot <i>Oculimacula acufiformis</i> / <i>Pseudocercosporella</i> <i>herpotrichoides</i> (PSDCHE) Tan Spot <i>Pyrenophora tritici-repentis</i> (PYRNTR) Head blight of cereals <i>Fusarium spp.</i> (FUSASP) <i>Microdochium spp.</i> (MICDSP)	foliar spray	BBCH 30 – 69 (spring)	a) 2 b) 2	14-21	a) 1.2-1.4 b) 2.4-2.8	a) 360-420 (180+180 – 210+210)  b) 720-840 (360+360 – 420+420)	100-400	35		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms
9.	LU	Wheat (winter & spring)  Spelt  Einkorn wheat  Emmer Wheat  Tritordeum	F	Septoria leaf spot <i>Zymoseptoria tritici</i> <i>Mycosphaerella graminicola</i> (SEPTTR) Glume blotch <i>Stagonospora nodorum</i> (LEPTNO) Brown Rust <i>Puccinia recondita</i> <i>Puccinia triticina</i> (PUCCRT) Yellow Rust <i>Puccinia striiformis</i> (PUCGST) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Eyespot <i>Oculimacula acuformis</i> / <i>Pseudocercosporella</i> <i>herpotrichoides</i> (PSDCHE) Tan Spot <i>Pyrenophora tritici-repentis</i> (PYRNTR) Head blight of cereals <i>Fusarium spp.</i> (FUSASP) <i>Microdochium spp.</i> (MICDSP)	foliar spray	BBCH 30 – 69 (spring)	a) 2 b) 2	14-21	a) 1.2-1.4 b) 2.4-2.8	a) 360-420 (180+180 – 210+210)  b) 720-840 (360+360 – 420+420)	100-400	35		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms
10.	NL	Wheat (winter & spring)  Spelt  Einkorn wheat  Emmer Wheat  Tritordeum	F	Septoria leaf spot <i>Zymoseptoria tritici</i> <i>Mycosphaerella graminicola</i> (SEPTTR) Glume blotch <i>Stagonospora nodorum</i> (LEPTNO) Brown Rust <i>Puccinia recondita</i> <i>Puccinia triticina</i> (PUCCRT) Yellow Rust <i>Puccinia striiformis</i> (PUCGST) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Eyespot <i>Oculimacula acuformis</i> / <i>Pseudocercosporella</i> <i>herpotrichoides</i> (PSDCHE) Tan Spot <i>Pyrenophora tritici-repentis</i> (PYRNTR) Head blight of cereals <i>Fusarium spp.</i> (FUSASP) <i>Microdochium spp.</i> (MICDSP)	foliar spray	BBCH 30 – 69 (spring)	a) 2 b) 2	14-21	a) 1.2-1.4 b) 2.4-2.8	a) 360-420 (180+180 – 210+210)  b) 720-840 (360+360 – 420+420)	100-400	35		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms
11.	NI	Wheat (winter & spring)  Spelt  Einkorn wheat  Emmer Wheat  Tritordeum	F	Septoria leaf spot <i>Zymoseptoria tritici</i> <i>Mycosphaerella graminicola</i> (SEPTTR) Glume blotch <i>Stagonospora nodorum</i> (LEPTNO) Brown Rust <i>Puccinia recondita</i> <i>Puccinia triticina</i> (PUCCRT) Yellow Rust <i>Puccinia striiformis</i> (PUCGST) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Eyespot <i>Oculimacula acufiformis</i> / <i>Pseudocercosporella</i> <i>herpotrichoides</i> (PSDCHE) Tan Spot <i>Pyrenophora tritici-repentis</i> (PYRNTR) Head blight of cereals <i>Fusarium spp.</i> (FUSASP) <i>Microdochium spp.</i> (MICDSP)	foliar spray	BBCH 30 – 69 (spring)	a) 2 b) 2	14-21	a) 1.2-1.4 b) 2.4-2.8	a) 360-420 (180+180 – 210+210)  b) 720-840 (360+360 – 420+420)	100-400	35		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms
12.	PL	Wheat (winter & spring)  Spelt  Einkorn wheat  Emmer Wheat  Tritordeum	F	Septoria leaf spot <i>Zymoseptoria tritici</i> <i>Mycosphaerella graminicola</i> (SEPTTR) Glume blotch <i>Stagonospora nodorum</i> (LEPTNO) Brown Rust <i>Puccinia recondita</i> <i>Puccinia triticina</i> (PUCCRT) Yellow Rust <i>Puccinia striiformis</i> (PUCGST) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Eyespot <i>Oculimacula acufiformis</i> / <i>Pseudocercosporella</i> <i>herpotrichoides</i> (PSDCHE) Tan Spot <i>Pyrenophora tritici-repentis</i> (PYRNTR) Head blight of cereals <i>Fusarium spp.</i> (FUSASP) <i>Microdochium spp.</i> (MICDSP)	foliar spray	BBCH 30 – 69 (spring)	a) 2 b) 2	14-21	a) 1.2-1.4 b) 2.4-2.8	a) 360-420 (180+180 – 210+210)  b) 720-840 (360+360 – 420+420)	100-400	35		A	A	R	A	A	A



1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms
13.	RO	Wheat (winter & spring)  Spelt  Einkorn wheat  Emmer Wheat  Tritordeum	F	Septoria leaf spot <i>Zymoseptoria tritici</i> <i>Mycosphaerella graminicola</i> (SEPTTR) Glume blotch <i>Stagonospora nodorum</i> (LEPTNO) Brown Rust <i>Puccinia recondita</i> <i>Puccinia triticina</i> (PUCCRT) Yellow Rust <i>Puccinia striiformis</i> (PUCGST) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Eyespot <i>Oculimacula acufiformis</i> / <i>Pseudocercosporella</i> <i>herpotrichoides</i> (PSDCHE) Tan Spot <i>Pyrenophora tritici-repentis</i> (PYRNTR) Head blight of cereals <i>Fusarium spp.</i> (FUSASP) <i>Microdochium spp.</i> (MICDSP)	foliar spray	BBCH 30 – 69 (spring)	a) 2 b) 2	14-21	a) 1.2-1.4 b) 2.4-2.8	a) 360-420 (180+180 – 210+210)  b) 720-840 (360+360 – 420+420)	100-400	35		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms
14.	SK	Wheat (winter & spring)  Spelt  Einkorn wheat  Emmer Wheat  Tritordeum	F	Septoria leaf spot <i>Zymoseptoria tritici</i> <i>Mycosphaerella graminicola</i> (SEPTTR) Glume blotch <i>Stagonospora nodorum</i> (LEPTNO) Brown Rust <i>Puccinia recondita</i> <i>Puccinia triticina</i> (PUCCRT) Yellow Rust <i>Puccinia striiformis</i> (PUCGST) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Eyespot <i>Oculimacula acufomis</i> / <i>Pseudocercosporella</i> <i>herpotrichoides</i> (PSDCHE) Tan Spot <i>Pyrenophora tritici-repentis</i> (PYRNTR) Head blight of cereals <i>Fusarium spp.</i> (FUSASP) <i>Microdochium spp.</i> (MICDSP)	foliar spray	BBCH 30 – 69 (spring)	a) 2 b) 2	14-21	a) 1.2-1.4 b) 2.4-2.8	a) 360-420 (180+180 – 210+210)  b) 720-840 (360+360 – 420+420)	100-400	35		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms
15.	AT	Durum Wheat	F	Septoria leaf spot <i>Zymoseptoria tritici</i> <i>Mycosphaerella graminicola</i> (SEPTTR) Brown Rust <i>Puccinia recondita</i> <i>Puccinia triticina</i> (PUCCRT) Yellow/stripe Rust <i>Puccinia striiformis</i> (PUCGST) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Head blight of cereals <i>Fusarium spp.</i> (FUSASP) <i>Microdochium spp.</i> (MICDSP)	foliar spray	BBCH 30 – 69 (spring)	a) 2 b) 2	14-21	a) 1.2-1.4 b) 2.4-2.8	a) 360-420 (180+180 – 210+210)  b) 720-840 (360+360 – 420+420)	100-400	35		A	A	R	A	A	A
16.	BE	Durum Wheat	F	Septoria leaf spot <i>Zymoseptoria tritici</i> <i>Mycosphaerella graminicola</i> (SEPTTR) Brown Rust <i>Puccinia recondita</i> <i>Puccinia triticina</i> (PUCCRT) Yellow/stripe Rust <i>Puccinia striiformis</i> (PUCGST) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Head blight of cereals <i>Fusarium spp.</i> (FUSASP) <i>Microdochium spp.</i> (MICDSP)	foliar spray	BBCH 30 – 69 (spring)	a) 2 b) 2	14-21	a) 1.2-1.4 b) 2.4-2.8	a) 360-420 (180+180 – 210+210)  b) 720-840 (360+360 – 420+420)	100-400	35		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms
17.	CZ	Durum Wheat	F	Septoria leaf spot <i>Zymoseptoria tritici</i> <i>Mycosphaerella graminicola</i> (SEPTTR) Brown Rust <i>Puccinia recondita</i> <i>Puccinia triticina</i> (PUCCRT) Yellow/stripe Rust <i>Puccinia striiformis</i> (PUC CST) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Head blight of cereals <i>Fusarium spp.</i> (FUSASP) <i>Microdochium spp.</i> (MICDSP)	foliar spray	BBCH 30 – 69 (spring)	a) 2 b) 2	14-21	a) 1.2-1.4 b) 2.4-2.8	a) 360-420 (180+180 – 210+210)  b) 720-840 (360+360 – 420+420)	100-400	35		A	A	R	A	A	A
18.	HU	Durum Wheat	F	Septoria leaf spot <i>Zymoseptoria tritici</i> <i>Mycosphaerella graminicola</i> (SEPTTR) Brown Rust <i>Puccinia recondita</i> <i>Puccinia triticina</i> (PUC CRT) Yellow/stripe Rust <i>Puccinia striiformis</i> (PUC CST) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Head blight of cereals <i>Fusarium spp.</i> (FUSASP) <i>Microdochium spp.</i> (MICDSP)	foliar spray	BBCH 30 – 69 (spring)	a) 2 b) 2	14-21	a) 1.2-1.4 b) 2.4-2.8	a) 360-420 (180+180 – 210+210)  b) 720-840 (360+360 – 420+420)	100-400	35		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms
19.	IE	Durum Wheat	F	Septoria leaf spot <i>Zymoseptoria tritici</i> <i>Mycosphaerella graminicola</i> (SEPTTR) Brown Rust <i>Puccinia recondita</i> <i>Puccinia triticina</i> (PUCCRT) Yellow/stripe Rust <i>Puccinia striiformis</i> (PUCGST) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Head blight of cereals <i>Fusarium spp.</i> (FUSASP) <i>Microdochium spp.</i> (MICDSP)	foliar spray	BBCH 30 – 69 (spring)	a) 2 b) 2	14-21	a) 1.2-1.4 b) 2.4-2.8	a) 360-420 (180+180 – 210+210)  b) 720-840 (360+360 – 420+420)	100-400	35		A	A	R	A	A	A
20.	LU	Durum Wheat	F	Septoria leaf spot <i>Zymoseptoria tritici</i> <i>Mycosphaerella graminicola</i> (SEPTTR) Brown Rust <i>Puccinia recondita</i> <i>Puccinia triticina</i> (PUCCRT) Yellow/stripe Rust <i>Puccinia striiformis</i> (PUCGST) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Head blight of cereals <i>Fusarium spp.</i> (FUSASP) <i>Microdochium spp.</i> (MICDSP)	foliar spray	BBCH 30 – 69 (spring)	a) 2 b) 2	14-21	a) 1.2-1.4 b) 2.4-2.8	a) 360-420 (180+180 – 210+210)  b) 720-840 (360+360 – 420+420)	100-400	35		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms
21.	NL	Durum Wheat	F	Septoria leaf spot <i>Zymoseptoria tritici</i> <i>Mycosphaerella graminicola</i> (SEPTTR) Brown Rust <i>Puccinia recondita</i> <i>Puccinia triticina</i> (PUCCRT) Yellow/stripe Rust <i>Puccinia striiformis</i> (PUC CST) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Head blight of cereals <i>Fusarium spp.</i> (FUSASP) <i>Microdochium spp.</i> (MICDSP)	foliar spray	BBCH 30 – 69 (spring)	a) 2 b) 2	14-21	a) 1.2-1.4 b) 2.4-2.8	a) 360-420 (180+180 – 210+210)  b) 720-840 (360+360 – 420+420)	100-400	35		A	A	R	A	A	A
22.	NI	Durum Wheat	F	Septoria leaf spot <i>Zymoseptoria tritici</i> <i>Mycosphaerella graminicola</i> (SEPTTR) Brown Rust <i>Puccinia recondita</i> <i>Puccinia triticina</i> (PUC CRT) Yellow/stripe Rust <i>Puccinia striiformis</i> (PUC CST) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Head blight of cereals <i>Fusarium spp.</i> (FUSASP) <i>Microdochium spp.</i> (MICDSP)	foliar spray	BBCH 30 – 69 (spring)	a) 2 b) 2	14-21	a) 1.2-1.4 b) 2.4-2.8	a) 360-420 (180+180 – 210+210)  b) 720-840 (360+360 – 420+420)	100-400	35		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms
23.	PL	Durum Wheat	F	Septoria leaf spot <i>Zymoseptoria tritici</i> <i>Mycosphaerella graminicola</i> (SEPTTR) Brown Rust <i>Puccinia recondita</i> <i>Puccinia triticina</i> (PUCCRT) Yellow/stripe Rust <i>Puccinia striiformis</i> (PUC CST) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Head blight of cereals <i>Fusarium spp.</i> (FUSASP) <i>Microdochium spp.</i> (MICDSP)	foliar spray	BBCH 30 – 69 (spring)	a) 2 b) 2	14-21	a) 1.2-1.4 b) 2.4-2.8	a) 360-420 (180+180 – 210+210)  b) 720-840 (360+360 – 420+420)	100-400	35		A	A	R	A	A	A
24.	RO	Durum Wheat	F	Septoria leaf spot <i>Zymoseptoria tritici</i> <i>Mycosphaerella graminicola</i> (SEPTTR) Brown Rust <i>Puccinia recondita</i> <i>Puccinia triticina</i> (PUC CRT) Yellow/stripe Rust <i>Puccinia striiformis</i> (PUC CST) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Head blight of cereals <i>Fusarium spp.</i> (FUSASP) <i>Microdochium spp.</i> (MICDSP)	foliar spray	BBCH 30 – 69 (spring)	a) 2 b) 2	14-21	a) 1.2-1.4 b) 2.4-2.8	a) 360-420 (180+180 – 210+210)  b) 720-840 (360+360 – 420+420)	100-400	35		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I**	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms
25.	SK	Durum Wheat	F	Septoria leaf spot <i>Zymoseptoria tritici</i> <i>Mycosphaerella graminicola</i> (SEPTTR) Brown Rust <i>Puccinia recondita</i> <i>Puccinia triticina</i> (PUCCRT) Yellow/stripe Rust <i>Puccinia striiformis</i> (PUC CST) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Head blight of cereals <i>Fusarium spp.</i> (FUSASP) <i>Microdochium spp.</i> (MICDSP)	foliar spray	BBCH 30 – 69 (spring)	a) 2 b) 2	14-21	a) 1.2-1.4 b) 2.4-2.8	a) 360-420 (180+180 – 210+210)  b) 720-840 (360+360 – 420+420)	100-400	35		A	A	R	A	A	A
26.	AT	Triticale (winter & spring)	F	Septoria leaf spot <i>Zymoseptoria tritici</i> <i>Mycosphaerella graminicola</i> (SEPTTR) Brown Rust <i>Puccinia recondita</i> <i>Puccinia triticina</i> (PUC CRT) Leaf blotch <i>Rhynchosporium secalis</i> (RHYNSE) Yellow Rust <i>Puccinia striiformis</i> (PUC CST) Glume blotch <i>Stagonospora nodorum</i> (LEPTNO) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Head blight of cereals <i>Fusarium spp.</i> (FUSASP) <i>Microdochium spp.</i> (MICDSP)	foliar spray	BBCH 30 – 69 (spring)	a) 2 b) 2	14-21	a) 1.2-1.4 b) 2.4-2.8	a) 360-420 (180+180 – 210+210)  b) 720-840 (360+360 – 420+420)	100-400	35		A	A	R	A	A	A



1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms
27.	BE	Triticale (winter & spring)	F	Septoria leaf spot <i>Zymoseptoria tritici</i> <i>Mycosphaerella graminicola</i> (SEPTTR) Brown Rust <i>Puccinia recondita</i> <i>Puccinia triticina</i> (PUCCRT) Leaf blotch <i>Rhynchosporium secalis</i> (RHYNSE) Yellow Rust <i>Puccinia striiformis</i> (PUC CST) Glume blotch <i>Stagonospora nodorum</i> (LEPTNO) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Head blight of cereals <i>Fusarium spp.</i> (FUSASP) <i>Microdochium spp.</i> (MICDSP)	foliar spray	BBCH 30 – 69 (spring)	a) 2 b) 2	14-21	a) 1.2-1.4 b) 2.4-2.8	a) 360-420 (180+180 – 210+210)  b) 720-840 (360+360 – 420+420)	100-400	35		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms
28.	CZ	Triticale (winter & spring)	F	Septoria leaf spot <i>Zymoseptoria tritici</i> <i>Mycosphaerella graminicola</i> (SEPTTR) Brown Rust <i>Puccinia recondita</i> <i>Puccinia triticina</i> (PUCCRT) Leaf blotch <i>Rhynchosporium secalis</i> (RHYNSE) Yellow Rust <i>Puccinia striiformis</i> (PUC CST) Glume blotch <i>Stagonospora nodorum</i> (LEPTNO) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Head blight of cereals <i>Fusarium spp.</i> (FUSASP) <i>Microdochium spp.</i> (MICDSP)	foliar spray	BBCH 30 – 69 (spring)	a) 2 b) 2	14-21	a) 1.2-1.4 b) 2.4-2.8	a) 360-420 (180+180 – 210+210)  b) 720-840 (360+360 – 420+420)	100-400	35		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms
29.	DE	Triticale (winter & spring)	F	Septoria leaf spot <i>Zymoseptoria tritici</i> <i>Mycosphaerella graminicola</i> (SEPTTR) Brown Rust <i>Puccinia recondita</i> <i>Puccinia triticina</i> (PUCCRT) Leaf blotch <i>Rhynchosporium secalis</i> (RHYNSE) Yellow Rust <i>Puccinia striiformis</i> (PUCGST) Glume blotch <i>Stagonospora nodorum</i> (LEPTNO) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Head blight of cereals <i>Fusarium spp.</i> (FUSASP) <i>Microdochium spp.</i> (MICDSP)	foliar spray	BBCH 30 – 69 (spring)	a) 2 b) 2	14-21	a) 1.4 b) 2.8	a) 420 (210+210)  b) 840 (420+420)	150-400	35		A	A	R	A	A	A
30.	DE	Triticale (winter & spring)	F	<i>Fusarium spp.</i> (FUSASP) <i>Microdochium spp.</i> (MICDSP)	foliar spray	BBCH 61 – 69 (spring)	a) 1 b) 2		a) 1.4 b) 2.8	a) 420 (210+210)  b) 840 (420+420)	150-400	35		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms
31.	HU	Triticale (winter & spring)	F	Septoria leaf spot <i>Zymoseptoria tritici</i> <i>Mycosphaerella graminicola</i> (SEPTTR) Brown Rust <i>Puccinia recondita</i> <i>Puccinia triticina</i> (PUCCRT) Leaf blotch <i>Rhynchosporium secalis</i> (RHYNSE) Yellow Rust <i>Puccinia striiformis</i> (PUC CST) Glume blotch <i>Stagonospora nodorum</i> (LEPTNO) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Head blight of cereals <i>Fusarium spp.</i> (FUSASP) <i>Microdochium spp.</i> (MICDSP)	foliar spray	BBCH 30 – 69 (spring)	a) 2 b) 2	14-21	a) 1.2-1.4 b) 2.4-2.8	a) 360-420 (180+180 – 210+210)  b) 720-840 (360+360 – 420+420)	100-400	35		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms
32.	IE	Triticale (winter & spring)	F	Septoria leaf spot <i>Zymoseptoria tritici</i> <i>Mycosphaerella graminicola</i> (SEPTTR) Brown Rust <i>Puccinia recondita</i> <i>Puccinia triticina</i> (PUCCRT) Leaf blotch <i>Rhynchosporium secalis</i> (RHYNSE) Yellow Rust <i>Puccinia striiformis</i> (PUC CST) Glume blotch <i>Stagonospora nodorum</i> (LEPTNO) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Head blight of cereals <i>Fusarium spp.</i> (FUSASP) <i>Microdochium spp.</i> (MICDSP)	foliar spray	BBCH 30 – 69 (spring)	a) 2 b) 2	14-21	a) 1.2-1.4 b) 2.4-2.8	a) 360-420 (180+180 – 210+210)  b) 720-840 (360+360 – 420+420)	100-400	35		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms
33.	LU	Triticale (winter & spring)	F	Septoria leaf spot <i>Zymoseptoria tritici</i> <i>Mycosphaerella graminicola</i> (SEPTTR) Brown Rust <i>Puccinia recondita</i> <i>Puccinia triticina</i> (PUCCRT) Leaf blotch <i>Rhynchosporium secalis</i> (RHYNSE) Yellow Rust <i>Puccinia striiformis</i> (PUC CST) Glume blotch <i>Stagonospora nodorum</i> (LEPTNO) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Head blight of cereals <i>Fusarium spp.</i> (FUSASP) <i>Microdochium spp.</i> (MICDSP)	foliar spray	BBCH 30 – 69 (spring)	a) 2 b) 2	14-21	a) 1.2-1.4 b) 2.4-2.8	a) 360-420 (180+180 – 210+210)  b) 720-840 (360+360 – 420+420)	100-400	35		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms
34.	NL	Triticale (winter & spring)	F	Septoria leaf spot <i>Zymoseptoria tritici</i> <i>Mycosphaerella graminicola</i> (SEPTTR) Brown Rust <i>Puccinia recondita</i> <i>Puccinia triticina</i> (PUCCRT) Leaf blotch <i>Rhynchosporium secalis</i> (RHYNSE) Yellow Rust <i>Puccinia striiformis</i> (PUC CST) Glume blotch <i>Stagonospora nodorum</i> (LEPTNO) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Head blight of cereals <i>Fusarium spp.</i> (FUSASP) <i>Microdochium spp.</i> (MICDSP)	foliar spray	BBCH 30 – 69 (spring)	a) 2 b) 2	14-21	a) 1.2-1.4 b) 2.4-2.8	a) 360-420 (180+180 – 210+210)  b) 720-840 (360+360 – 420+420)	100-400	35		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms
35.	NI	Triticale (winter & spring)	F	Septoria leaf spot <i>Zymoseptoria tritici</i> <i>Mycosphaerella graminicola</i> (SEPTTR) Brown Rust <i>Puccinia recondita</i> <i>Puccinia triticina</i> (PUCCRT) Leaf blotch <i>Rhynchosporium secalis</i> (RHYNSE) Yellow Rust <i>Puccinia striiformis</i> (PUC CST) Glume blotch <i>Stagonospora nodorum</i> (LEPTNO) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Head blight of cereals <i>Fusarium spp.</i> (FUSASP) <i>Microdochium spp.</i> (MICDSP)	foliar spray	BBCH 30 – 69 (spring)	a) 2 b) 2	14-21	a) 1.2-1.4 b) 2.4-2.8	a) 360-420 (180+180 – 210+210)  b) 720-840 (360+360 – 420+420)	100-400	35		A	A	R	A	A	A



1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms
36.	PL	Triticale (winter & spring)	F	Septoria leaf spot <i>Zymoseptoria tritici</i> <i>Mycosphaerella graminicola</i> (SEPTTR) Brown Rust <i>Puccinia recondita</i> <i>Puccinia triticina</i> (PUCCRT) Leaf blotch <i>Rhynchosporium secalis</i> (RHYNSE) Yellow Rust <i>Puccinia striiformis</i> (PUC CST) Glume blotch <i>Stagonospora nodorum</i> (LEPTNO) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Head blight of cereals <i>Fusarium spp.</i> (FUSASP) <i>Microdochium spp.</i> (MICDSP)	foliar spray	BBCH 30 – 69 (spring)	a) 2 b) 2	14-21	a) 1.2-1.4 b) 2.4-2.8	a) 360-420 (180+180 – 210+210)  b) 720-840 (360+360 – 420+420)	100-400	35		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms
37.	RO	Triticale (winter & spring)	F	Septoria leaf spot <i>Zymoseptoria tritici</i> <i>Mycosphaerella graminicola</i> (SEPTTR) Brown Rust <i>Puccinia recondita</i> <i>Puccinia triticina</i> (PUCCRT) Leaf blotch <i>Rhynchosporium secalis</i> (RHYNSE) Yellow Rust <i>Puccinia striiformis</i> (PUC CST) Glume blotch <i>Stagonospora nodorum</i> (LEPTNO) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Head blight of cereals <i>Fusarium spp.</i> (FUSASP) <i>Microdochium spp.</i> (MICDSP)	foliar spray	BBCH 30 – 69 (spring)	a) 2 b) 2	14-21	a) 1.2-1.4 b) 2.4-2.8	a) 360-420 (180+180 – 210+210)  b) 720-840 (360+360 – 420+420)	100-400	35		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms
38.	SK	Triticale (winter & spring)	F	Septoria leaf spot <i>Zymoseptoria tritici</i> <i>Mycosphaerella graminicola</i> (SEPTTR) Brown Rust <i>Puccinia recondita</i> <i>Puccinia triticina</i> (PUCCRT) Leaf blotch <i>Rhynchosporium secalis</i> (RHYNSE) Yellow Rust <i>Puccinia striiformis</i> (PUC CST) Glume blotch <i>Stagonospora nodorum</i> (LEPTNO) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Head blight of cereals <i>Fusarium spp.</i> (FUSASP) <i>Microdochium spp.</i> (MICDSP)	foliar spray	BBCH 30 – 69 (spring)	a) 2 b) 2	14-21	a) 1.2-1.4 b) 2.4-2.8	a) 360-420 (180+180 – 210+210)  b) 720-840 (360+360 – 420+420)	100-400	35		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms
39.	AT	Rye (winter & spring)	F	Septoria leaf spot <i>Zymoseptoria tritici</i> <i>Mycosphaerella graminicola</i> (SEPTTR) Leaf blotch <i>Rhynchosporium secalis</i> (RHYNSE) Brown rust <i>Puccinia recondita/ Puccinia recondita f. sp. recondita</i> (PUCCRE/PUCCRR) Eyespot <i>Pseudocercospora herpotrichoides</i> (PSDCHE) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Head blight of cereals <i>Fusarium spp.</i> (FUSASP) <i>Microdochium spp.</i> (MICDSP)	foliar spray	BBCH 30 – 69 (spring)	a) 2 b) 2	14-21	a) 1.2-1.4 b) 2.4-2.8	a) 360-420 (180+180 – 210+210)  b) 720-840 (360+360 – 420+420)	100-400	35		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms
40.	BE	Rye (winter & spring)	F	Septoria leaf spot <i>Zymoseptoria tritici</i> <i>Mycosphaerella graminicola</i> (SEPTTR) Leaf blotch <i>Rhynchosporium secalis</i> (RHYNSE) Brown rust <i>Puccinia recondita/ Puccinia recondita f. sp. recondita</i> (PUCCRE/PUCCRR) Eyespot <i>Pseudocercospora herpotrichoides</i> (PSDCHE) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Head blight of cereals <i>Fusarium spp.</i> (FUSASP) <i>Microdochium spp.</i> (MICDSP)	foliar spray	BBCH 30 – 69 (spring)	a) 2 b) 2	14-21	a) 1.2-1.4 b) 2.4-2.8	a) 360-420 (180+180 – 210+210)  b) 720-840 (360+360 – 420+420)	100-400	35		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, G, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms
41.	CZ	Rye (winter & spring)	F	Septoria leaf spot <i>Zymoseptoria tritici</i> <i>Mycosphaerella graminicola</i> (SEPTTR) Leaf blotch <i>Rhynchosporium secalis</i> (RHYNSE) Brown rust <i>Puccinia recondita/ Puccinia recondita f. sp. recondita</i> (PUCCRE/PUCCRR) Eyespot <i>Pseudocercospora herpotrichoides</i> (PSDCHE) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Head blight of cereals <i>Fusarium spp.</i> (FUSASP) <i>Microdochium spp.</i> (MICDSP)	foliar spray	BBCH 30 – 69 (spring)	a) 2 b) 2	14-21	a) 1.2-1.4 b) 2.4-2.8	a) 360-420 (180+180 – 210+210)  b) 720-840 (360+360 – 420+420)	100-400	35		A	A	R	A	A	A
42.	DE	Rye (winter & spring)	F	Septoria leaf spot <i>Septoria tritici</i> (SEPTTR) Leaf blotch <i>Rhynchosporium secalis</i> (RHYNSE) Brown rust <i>Puccinia recondita/ Puccinia recondita f. sp. recondita</i> (PUCCRE/PUCCRR) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Head blight of cereals	foliar spray	BBCH 30 – 69 (spring)	a) 2 b) 2	14-21	a) 1.4 b) 2.8	a) 420 (210+210)  b) 840 (420+420)	150-400	35		A	A	R	A	A	A
43.	DE	Rye (winter & spring)	F	<i>Fusarium spp.</i> (FUSASP) <i>Microdochium spp.</i> (MICDSP)	foliar spray	BBCH 30 – 69 (spring)	a) 2 b) 2	14-21	a) 1.4 b) 2.8	a) 420 (210+210)  b) 840 (420+420)	150-400	35		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms
44.	DE	Rye (winter & spring)	F	<i>Pseudocercospora</i> <i>herpotrichoides</i> (PSDCHE)	foliar spray	BBCH 30 – 32 (spring)	a) 1 b) 2	N/A	a) 1.4 b) 2.8	a) 420 (210+210)  b) 840 (420+420)	150-400	35		A	A	R	A	A	A
45.	HU	Rye (winter & spring)	F	Septoria leaf spot <i>Zymoseptoria tritici</i> <i>Mycosphaerella graminicola</i> (SEPTTR) Leaf blotch <i>Rhynchosporium secalis</i> (RHYNSE) Brown rust <i>Puccinia recondita</i> / <i>Puccinia recondita f. sp. recondita</i> (PUCCRE/PUCCRR) Eyespot <i>Pseudocercospora</i> <i>herpotrichoides</i> (PSDCHE) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Head blight of cereals <i>Fusarium spp.</i> (FUSASP) <i>Microdochium spp.</i> (MICDSP)	foliar spray	BBCH 30 – 69 (spring)	a) 2 b) 2	14-21	a) 1.2-1.4 b) 2.4-2.8	a) 360-420 (180+180 – 210+210)  b) 720-840 (360+360 – 420+420)	100-400	35		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms
46.	IE	Rye (winter & spring)	F	Septoria leaf spot <i>Zymoseptoria tritici</i> <i>Mycosphaerella graminicola</i> (SEPTTR) Leaf blotch <i>Rhynchosporium secalis</i> (RHYNSE) Brown rust <i>Puccinia recondita/ Puccinia recondita f. sp. recondita</i> (PUCCRE/PUCCRR) Eyespot <i>Pseudocercospora herpotrichoides</i> (PSDCHE) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Head blight of cereals <i>Fusarium spp.</i> (FUSASP) <i>Microdochium spp.</i> (MICDSP)	foliar spray	BBCH 30 – 69 (spring)	a) 2 b) 2	14-21	a) 1.2-1.4 b) 2.4-2.8	a) 360-420 (180+180 – 210+210)  b) 720-840 (360+360 – 420+420)	100-400	35		A	A	R	A	A	A



1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms
47.	LU	Rye (winter & spring)	F	Septoria leaf spot <i>Zymoseptoria tritici</i> <i>Mycosphaerella graminicola</i> (SEPTTR) Leaf blotch <i>Rhynchosporium secalis</i> (RHYNSE) Brown rust <i>Puccinia recondita/ Puccinia recondita f. sp. recondita</i> (PUCCRE/PUCCRR) Eyespot <i>Pseudocercospora herpotrichoides</i> (PSDCHE) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Head blight of cereals <i>Fusarium spp.</i> (FUSASP) <i>Microdochium spp.</i> (MICDSP)	foliar spray	BBCH 30 – 69 (spring)	a) 2 b) 2	14-21	a) 1.2-1.4 b) 2.4-2.8	a) 360-420 (180+180 – 210+210)  b) 720-840 (360+360 – 420+420)	100-400	35		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms
48.	NL	Rye (winter & spring)	F	Septoria leaf spot <i>Zymoseptoria tritici</i> <i>Mycosphaerella graminicola</i> (SEPTTR) Leaf blotch <i>Rhynchosporium secalis</i> (RHYNSE) Brown rust <i>Puccinia recondita/ Puccinia recondita f. sp. recondita</i> (PUCCRE/PUCCRR) Eyespot <i>Pseudocercospora herpotrichoides</i> (PSDCHE) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Head blight of cereals <i>Fusarium spp.</i> (FUSASP) <i>Microdochium spp.</i> (MICDSP)	foliar spray	BBCH 30 – 69 (spring)	a) 2 b) 2	14-21	a) 1.2-1.4 b) 2.4-2.8	a) 360-420 (180+180 – 210+210)  b) 720-840 (360+360 – 420+420)	100-400	35		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms
49.	NI	Rye (winter & spring)	F	Septoria leaf spot <i>Zymoseptoria tritici</i> <i>Mycosphaerella graminicola</i> (SEPTTR) Leaf blotch <i>Rhynchosporium secalis</i> (RHYNSE) Brown rust <i>Puccinia recondita/ Puccinia recondita f. sp. recondita</i> (PUCCRE/PUCCRR) Eyespot <i>Pseudocercospora herpotrichoides</i> (PSDCHE) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Head blight of cereals <i>Fusarium spp.</i> (FUSASP) <i>Microdochium spp.</i> (MICDSP)	foliar spray	BBCH 30 – 69 (spring)	a) 2 b) 2	14-21	a) 1.2-1.4 b) 2.4-2.8	a) 360-420 (180+180 – 210+210)  b) 720-840 (360+360 – 420+420)	100-400	35		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms
50.	PL	Rye (winter & spring)	F	Septoria leaf spot <i>Zymoseptoria tritici</i> <i>Mycosphaerella graminicola</i> (SEPTTR) Leaf blotch <i>Rhynchosporium secalis</i> (RHYNSE) Brown rust <i>Puccinia recondita/ Puccinia recondita f. sp. recondita</i> (PUCCRE/PUCCRR) Eyespot <i>Pseudocercospora herpotrichoides</i> (PSDCHE) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Head blight of cereals <i>Fusarium spp.</i> (FUSASP) <i>Microdochium spp.</i> (MICDSP)	foliar spray	BBCH 30 – 69 (spring)	a) 2 b) 2	14-21	a) 1.2-1.4 b) 2.4-2.8	a) 360-420 (180+180 – 210+210)  b) 720-840 (360+360 – 420+420)	100-400	35		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms
51.	RO	Rye (winter & spring)	F	Septoria leaf spot <i>Zymoseptoria tritici</i> <i>Mycosphaerella graminicola</i> (SEPTTR) Leaf blotch <i>Rhynchosporium secalis</i> (RHYNSE) Brown rust <i>Puccinia recondita/ Puccinia recondita f. sp. recondita</i> (PUCCRE/PUCCRR) Eyespot <i>Pseudocercospora herpotrichoides</i> (PSDCHE) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Head blight of cereals <i>Fusarium spp.</i> (FUSASP) <i>Microdochium spp.</i> (MICDSP)	foliar spray	BBCH 30 – 69 (spring)	a) 2 b) 2	14-21	a) 1.2-1.4 b) 2.4-2.8	a) 360-420 (180+180 – 210+210)  b) 720-840 (360+360 – 420+420)	100-400	35		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms
52.	SK	Rye (winter & spring)	F	Septoria leaf spot <i>Zymoseptoria tritici</i> <i>Mycosphaerella graminicola</i> (SEPTTR) Leaf blotch <i>Rhynchosporium secalis</i> (RHYNSE) Brown rust <i>Puccinia recondita/ Puccinia recondita f. sp. recondita</i> (PUCCRE/PUCCRR) Eyespot <i>Pseudocercospora herpotrichoides</i> (PSDCHE) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Head blight of cereals <i>Fusarium spp.</i> (FUSASP) <i>Microdochium spp.</i> (MICDSP)	foliar spray	BBCH 30 – 69 (spring)	a) 2 b) 2	14-21	a) 1.2-1.4 b) 2.4-2.8	a) 360-420 (180+180 – 210+210)  b) 720-840 (360+360 – 420+420)	100-400	35		A	A	R	A	A	A
53.	AT	Oat (winter & spring)	F	Crown Rust <i>Puccinia coronata</i> (PUCCCO) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Leaf spot of oat <i>Pyrenophora chaetomioides</i> (PYRNAV) Eyespot <i>Oculimacula acutiformis/Pseudocercospora herpotrichoides</i> (PSDCHE)	foliar spray	BBCH 30 – 61 (spring)	a) 2 b) 2	14-21	a) 1.0 b) 2.0	a) 300 (150+150)  b) 600 (300+300)	100-400	35		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms
54.	BE	Oat (winter & spring)	F	Crown Rust <i>Puccinia coronata</i> (PUCCCO) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Leaf spot of oat <i>Pyrenophora chaetomioides</i> (PYRNAV) Eyespot <i>Oculimacula acutiformis/Pseudocercospora herpotrichoides</i> (PSDCHE)	foliar spray	BBCH 30 – 61 (spring)	a) 2 b) 2	14-21	a) 1.0 b) 2.0	a) 300 (150+150)  b) 600 (300+300)	100-400	35		A	A	R	A	A	A
55.	CZ	Oat (winter & spring)	F	Crown Rust <i>Puccinia coronata</i> (PUCCCO) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Leaf spot of oat <i>Pyrenophora chaetomioides</i> (PYRNAV) Eyespot <i>Oculimacula acutiformis/Pseudocercospora herpotrichoides</i> (PSDCHE)	foliar spray	BBCH 30 – 61 (spring)	a) 2 b) 2	14-21	a) 1.0 b) 2.0	a) 300 (150+150)  b) 600 (300+300)	100-400	35		A	A	R	A	A	A
56.	DE	Oat (winter & spring)	F	Crown Rust <i>Puccinia coronata</i> (PUCCCO) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Leaf spot of oat <i>Pyrenophora chaetomioides</i> (PYRNAV)	foliar spray	BBCH 30 – 61 (spring)	a) 2 b) 2	14-21	a) 1.0 b) 2.0	a) 300 (150+150)  b) 600 (300+300)	150-400	35		A	A	R	A	A	A
57.	DE	Oat (winter & spring)	F	Eyespot <i>Pseudocercospora herpotrichoides</i> (PSDCHE)	foliar spray	BBCH 30 – 32 (spring)	a) 1 b) 2		a) 1.0 b) 2.0	a) 300 (150+150)  b) 600 (300+300)	150-400	35		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
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			Min. interval between applications (days)	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		kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms
58.	HU	Oat (winter & spring)	F	Crown Rust <i>Puccinia coronata</i> (PUCCCO) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Leaf spot of oat <i>Pyrenophora chaetomioides</i> (PYRNAV) Eyespot <i>Oculimacula acufiformis/Pseudocercospora herpotrichoides</i> (PSDCHE)	foliar spray	BBCH 30 – 61 (spring)	a) 2 b) 2	14-21	a) 1.0 b) 2.0	a) 300 (150+150)  b) 600 (300+300)	100-400	35		A	A	R	A	A	A
59.	IE	Oat (winter & spring)	F	Crown Rust <i>Puccinia coronata</i> (PUCCCO) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Leaf spot of oat <i>Pyrenophora chaetomioides</i> (PYRNAV) Eyespot <i>Oculimacula acufiformis/Pseudocercospora herpotrichoides</i> (PSDCHE)	foliar spray	BBCH 30 – 61 (spring)	a) 2 b) 2	14-21	a) 1.0 b) 2.0	a) 300 (150+150)  b) 600 (300+300)	100-400	35		A	A	R	A	A	A
60.	LU	Oat (winter & spring)	F	Crown Rust <i>Puccinia coronata</i> (PUCCCO) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Leaf spot of oat <i>Pyrenophora chaetomioides</i> (PYRNAV) Eyespot <i>Oculimacula acufiformis/Pseudocercospora herpotrichoides</i> (PSDCHE)	foliar spray	BBCH 30 – 61 (spring)	a) 2 b) 2	14-21	a) 1.0 b) 2.0	a) 300 (150+150)  b) 600 (300+300)	100-400	35		A	A	R	A	A	A



1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
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			Min. interval between applications (days)	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		kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms
61.	NL	Oat (winter & spring)	F	Crown Rust <i>Puccinia coronata</i> (PUCCCO) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Leaf spot of oat <i>Pyrenophora chaetomioides</i> (PYRNAV) Eyespot <i>Oculimacula acufiformis/Pseudocercospora herpotrichoides</i> (PSDCHE)	foliar spray	BBCH 30 – 61 (spring)	a) 2 b) 2	14-21	a) 1.0 b) 2.0	a) 300 (150+150)  b) 600 (300+300)	100-400	35		A	A	R	A	A	A
62.	NI	Oat (winter & spring)	F	Crown Rust <i>Puccinia coronata</i> (PUCCCO) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Leaf spot of oat <i>Pyrenophora chaetomioides</i> (PYRNAV) Eyespot <i>Oculimacula acufiformis/Pseudocercospora herpotrichoides</i> (PSDCHE)	foliar spray	BBCH 30 – 61 (spring)	a) 2 b) 2	14-21	a) 1.0 b) 2.0	a) 300 (150+150)  b) 600 (300+300)	100-400	35		A	A	R	A	A	A
63.	PL	Oat (winter & spring)	F	Crown Rust <i>Puccinia coronata</i> (PUCCCO) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Leaf spot of oat <i>Pyrenophora chaetomioides</i> (PYRNAV) Eyespot <i>Oculimacula acufiformis/Pseudocercospora herpotrichoides</i> (PSDCHE)	foliar spray	BBCH 30 – 61 (spring)	a) 2 b) 2	14-21	a) 1.0 b) 2.0	a) 300 (150+150)  b) 600 (300+300)	100-400	35		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms
64.	RO	Oat (winter & spring)	F	Crown Rust <i>Puccinia coronata</i> (PUCCCO) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Leaf spot of oat <i>Pyrenophora chaetomioides</i> (PYRNAV) Eyespot <i>Oculimacula acuformis/Pseudocercospora herpotrichoides</i> (PSDCHE)	foliar spray	BBCH 30 – 61 (spring)	a) 2 b) 2	14-21	a) 1.0 b) 2.0	a) 300 (150+150)  b) 600 (300+300)	100-400	35		A	A	R	A	A	A
65.	SK	Oat (winter & spring)	F	Crown Rust <i>Puccinia coronata</i> (PUCCCO) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Leaf spot of oat <i>Pyrenophora chaetomioides</i> (PYRNAV) Eyespot <i>Oculimacula acuformis/Pseudocercospora herpotrichoides</i> (PSDCHE)	foliar spray	BBCH 30 – 61 (spring)	a) 2 b) 2	14-21	a) 1.0 b) 2.0	a) 300 (150+150)  b) 600 (300+300)	100-400	35		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms
66.	AT	Barley (winter & spring)	F	Leaf spot of Barley <i>Ramularia collo-cygni</i> (RAMUCC) Eyespot <i>Oculimacula acuformis/Pseudocercospora herpotrichoides</i> (PSDCHE) Brown Rust <i>Puccinia hordei</i> (PUCCHD) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Leaf Blotch <i>Rhynchosporium secalis</i> (RHYNSE) Net Blotch <i>Pyrenophora teres</i> (PYRNTE)	foliar spray	BBCH 30 – 61 (spring)	a) 2 b) 2	14-21	a) 1.0 b) 2.0	a) 300 (150+150)  b) 600 (300+300)	100-400	35		A	A	R	A	A	A
67.	BE	Barley (winter & spring)	F	Leaf spot of Barley <i>Ramularia collo-cygni</i> (RAMUCC) Eyespot <i>Oculimacula acuformis/Pseudocercospora herpotrichoides</i> (PSDCHE) Brown Rust <i>Puccinia hordei</i> (PUCCHD) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Leaf Blotch <i>Rhynchosporium secalis</i> (RHYNSE) Net Blotch <i>Pyrenophora teres</i> (PYRNTE)	foliar spray	BBCH 30 – 61 (spring)	a) 2 b) 2	14-21	a) 1.0 b) 2.0	a) 300 (150+150)  b) 600 (300+300)	100-400	35		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms
68.	CZ	Barley (winter & spring)	F	Leaf spot of Barley <i>Ramularia collo-cygni</i> (RAMUCC) Eyespot <i>Oculimacula acuformis/Pseudocercospora herpotrichoides</i> (PSDCHE) Brown Rust <i>Puccinia hordei</i> (PUCCHD) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Leaf Blotch <i>Rhynchosporium secalis</i> (RHYNSE) Net Blotch <i>Pyrenophora teres</i> (PYRNTE)	foliar spray	BBCH 30 – 61 (spring)	a) 2 b) 2	14-21	a) 1.0 b) 2.0	a) 300 (150+150)  b) 600 (300+300)	100-400	35		A	A	R	A	A	A
69.	DE	Barley (winter & spring)	F	Leaf spot of Barley <i>Ramularia collo-cygni</i> (RAMUCC) Brown Rust <i>Puccinia hordei</i> (PUCCHD) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Leaf Blotch <i>Rhynchosporium secalis</i> (RHYNSE ) Net Blotch <i>Pyrenophora teres</i> (PYRNTE)	foliar spray	BBCH 30 – 61 (spring)	a) 2 b) 2	14-21	a) 1.0 b) 2.0	a) 300 (150+150)  b) 600 (300+300)	150-400	35		A	A	R	A	A	A
70.	DE	Barley (winter & spring)	F	Eyespot <i>Pseudocercospora herpotrichoides</i> (PSDCHE)	foliar spray	BBCH 30 – 32 (spring)	a) 1 b) 2		a) 1.0 b) 2.0	a) 300 (150+150)  b) 600 (300+300)	150-400	35		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms
71.	HU	Barley (winter & spring)	F	Leaf spot of Barley <i>Ramularia collo-cygni</i> (RAMUCC) Eyespot <i>Oculimacula acutiformis</i> <i>Pseudocercospora herpotrichoides</i> (PSDCHE) Brown Rust <i>Puccinia hordei</i> (PUCCHD) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Leaf Blotch <i>Rhynchosporium secalis</i> (RHYNSE) Net Blotch <i>Pyrenophora teres</i> (PYRNTE)	foliar spray	BBCH 30 – 61 (spring)	a) 2 b) 2	14-21	a) 1.0 b) 2.0	a) 300 (150+150)  b) 600 (300+300)	100-400	35		A	A	R	A	A	A
72.	IE	Barley (winter & spring)	F	Leaf spot of Barley <i>Ramularia collo-cygni</i> (RAMUCC) Eyespot <i>Oculimacula acutiformis</i> <i>Pseudocercospora herpotrichoides</i> (PSDCHE) Brown Rust <i>Puccinia hordei</i> (PUCCHD) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Leaf Blotch <i>Rhynchosporium secalis</i> (RHYNSE) Net Blotch <i>Pyrenophora teres</i> (PYRNTE)	foliar spray	BBCH 30 – 61 (spring)	a) 2 b) 2	14-21	a) 1.0 b) 2.0	a) 300 (150+150)  b) 600 (300+300)	100-400	35		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, G, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms
73.	LU	Barley (winter & spring)	F	Leaf spot of Barley <i>Ramularia collo-cygni</i> (RAMUCC) Eyespot <i>Oculimacula acutiformis</i> <i>Pseudocercospora herpotrichoides</i> (PSDCHE) Brown Rust <i>Puccinia hordei</i> (PUCCHD) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Leaf Blotch <i>Rhynchosporium secalis</i> (RHYNSE) Net Blotch <i>Pyrenophora teres</i> (PYRNTE)	foliar spray	BBCH 30 – 61 (spring)	a) 2 b) 2	14-21	a) 1.0 b) 2.0	a) 300 (150+150)  b) 600 (300+300)	100-400	35		A	A	R	A	A	A
74.	NL	Barley (winter & spring)	F	Leaf spot of Barley <i>Ramularia collo-cygni</i> (RAMUCC) Eyespot <i>Oculimacula acutiformis</i> <i>Pseudocercospora herpotrichoides</i> (PSDCHE) Brown Rust <i>Puccinia hordei</i> (PUCCHD) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Leaf Blotch <i>Rhynchosporium secalis</i> (RHYNSE) Net Blotch <i>Pyrenophora teres</i> (PYRNTE)	foliar spray	BBCH 30 – 61 (spring)	a) 2 b) 2	14-21	a) 1.0 b) 2.0	a) 300 (150+150)  b) 600 (300+300)	100-400	35		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, G, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms
75.	NI	Barley (winter & spring)	F	Leaf spot of Barley <i>Ramularia collo-cygni</i> (RAMUCC) Eyespot <i>Oculimacula acutiformis</i> <i>Pseudocercospora</i> <i>herpotrichoides</i> (PSDCHE) Brown Rust <i>Puccinia hordei</i> (PUCCHD) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Leaf Blotch <i>Rhynchosporium secalis</i> (RHYNSE) Net Blotch <i>Pyrenophora teres</i> (PYRNTE)	foliar spray	BBCH 30 – 61 (spring)	a) 2 b) 2	14-21	a) 1.0 b) 2.0	a) 300 (150+150)  b) 600 (300+300)	100-400	35		A	A	R	A	A	A
76.	PL	Barley (winter & spring)	F	Leaf spot of Barley <i>Ramularia collo-cygni</i> (RAMUCC) Eyespot <i>Oculimacula acutiformis</i> <i>Pseudocercospora</i> <i>herpotrichoides</i> (PSDCHE) Brown Rust <i>Puccinia hordei</i> (PUCCHD) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Leaf Blotch <i>Rhynchosporium secalis</i> (RHYNSE) Net Blotch <i>Pyrenophora teres</i> (PYRNTE)	foliar spray	BBCH 30 – 61 (spring)	a) 2 b) 2	14-21	a) 1.0 b) 2.0	a) 300 (150+150)  b) 600 (300+300)	100-400	35		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, G, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms
77.	RO	Barley (winter & spring)	F	Leaf spot of Barley <i>Ramularia collo-cygni</i> (RAMUCC) Eyespot <i>Oculimacula acutiformis</i> <i>Pseudocercospora herpotrichoides</i> (PSDCHE) Brown Rust <i>Puccinia hordei</i> (PUCCHD) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Leaf Blotch <i>Rhynchosporium secalis</i> (RHYNSE) Net Blotch <i>Pyrenophora teres</i> (PYRNTE)	foliar spray	BBCH 30 – 61 (spring)	a) 2 b) 2	14-21	a) 1.0 b) 2.0	a) 300 (150+150)  b) 600 (300+300)	100-400	35		A	A	R	A	A	A
78.	SK	Barley (winter & spring)	F	Leaf spot of Barley <i>Ramularia collo-cygni</i> (RAMUCC) Eyespot <i>Oculimacula acutiformis</i> <i>Pseudocercospora herpotrichoides</i> (PSDCHE) Brown Rust <i>Puccinia hordei</i> (PUCCHD) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Leaf Blotch <i>Rhynchosporium secalis</i> (RHYNSE) Net Blotch <i>Pyrenophora teres</i> (PYRNTE)	foliar spray	BBCH 30 – 61 (spring)	a) 2 b) 2	14-21	a) 1.0 b) 2.0	a) 300 (150+150)  b) 600 (300+300)	100-400	35		A	A	R	A	A	A



1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion						
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms	
79.	AT	Winter Rape	Oilseed	F	Phoma leaf spot/stem canker <i>Leptosphaeria maculans</i> (LEPTMA) Sclerotinia stem rot <i>Sclerotinia sclerotiorum</i> (SCLESC) Powdery mildew <i>Erysiphe cruciferarum</i> (ERYSCR) Alternaria leaf spot <i>Alternaria brassicae</i> (ALTEBA) Light leaf spot <i>Pyrenopeziza brassicae</i> (PYRPBR) Grey mould <i>Botryotinia cinerea</i> (BOTRCI)	foliar spray	BBCH 14 – 18 (Autumn) or BBCH 20 – 69 (Spring)	a) 1 b) 1	N/A	a) 1.0-1.2 b) 1.0-1.2	a) 300 - 360 (150+150-180+180)  b) 300 - 360 (150+150-180+180)	100-400	56		A	A	R	A	A	A
80.	BE	Winter Rape	Oilseed	F	Phoma leaf spot/stem canker <i>Leptosphaeria maculans</i> (LEPTMA) Sclerotinia stem rot <i>Sclerotinia sclerotiorum</i> (SCLESC) Powdery mildew <i>Erysiphe cruciferarum</i> (ERYSCR) Alternaria leaf spot <i>Alternaria brassicae</i> (ALTEBA) Light leaf spot <i>Pyrenopeziza brassicae</i> (PYRPBR) Grey mould <i>Botryotinia cinerea</i> (BOTRCI)	foliar spray	BBCH 14 – 18 (Autumn) or BBCH 20 – 69 (Spring)	a) 1 b) 1	N/A	a) 1.0-1.2 b) 1.0-1.2	a) 300 - 360 (150+150-180+180)  b) 300 - 360 (150+150-180+180)	100-400	56		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms
81.	CZ	Winter Rape Oilseed	F	Phoma leaf spot/stem canker <i>Leptosphaeria maculans</i> (LEPTMA) Sclerotinia stem rot <i>Sclerotinia sclerotiorum</i> (SCLESC) Powdery mildew <i>Erysiphe cruciferarum</i> (ERYSCR) Alternaria leaf spot <i>Alternaria brassicae</i> (ALTEBA) Light leaf spot <i>Pyrenopeziza brassicae</i> (PYRPBR) Grey mould <i>Botryotinia cinerea</i> (BOTRCI)	foliar spray	BBCH 14 – 18 (Autumn) or BBCH 20 – 69 (Spring)	a) 1 b) 1	N/A	a) 1.0-1.2 b) 1.0-1.2	a) 300 - 360 (150+150-180+180)  b) 300 - 360 (150+150-180+180)	100-400	56		A	A	R	A	A	A
82.	DE	Winter Rape Oilseed	F	Phoma leaf spot/stem canker <i>Leptosphaeria maculans</i> (LEPTMA) Powdery mildew <i>Erysiphe cruciferarum</i> (ERYSCR) Light leaf spot <i>Cylindrosporium concentricum</i> (PYRPBR) Grey mould <i>Botryotinia cinera</i> (BOTRCI)	foliar spray	BBCH 14 – 18 (Autumn) or BBCH 20 – 69 (Spring)	a) 1 b) 1	N/A	a) 1.2 b) 1.2	a) 360 (180+180)  b) 360 (180+180)	150-400	56		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion						
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms	
83.	HU	Winter Rape	Oilseed	F	Phoma leaf spot/stem canker <i>Leptosphaeria maculans</i> (LEPTMA) Sclerotinia stem rot <i>Sclerotinia sclerotiorum</i> (SCLESC) Powdery mildew <i>Erysiphe cruciferarum</i> (ERYSCR) Alternaria leaf spot <i>Alternaria brassicae</i> (ALTEBA) Light leaf spot <i>Pyrenopeziza brassicae</i> (PYRPBR) Grey mould <i>Botryotinia cinera</i> (BOTRCI)	foliar spray	BBCH 14 – 18 (Autumn) or BBCH 20 – 69 (Spring)	a) 1 b) 1	N/A	a) 1.0-1.2 b) 1.0-1.2	a) 300 - 360 (150+150- 180+180)  b) 300 - 360 (150+150- 180+180)	100-400	56		A	A	R	A	A	A
84.	IE	Winter Rape	Oilseed	F	Phoma leaf spot/stem canker <i>Leptosphaeria maculans</i> (LEPTMA) Sclerotinia stem rot <i>Sclerotinia sclerotiorum</i> (SCLESC) Powdery mildew <i>Erysiphe cruciferarum</i> (ERYSCR) Alternaria leaf spot <i>Alternaria brassicae</i> (ALTEBA) Light leaf spot <i>Pyrenopeziza brassicae</i> (PYRPBR) Grey mould <i>Botryotinia cinera</i> (BOTRCI)	foliar spray	BBCH 14 – 18 (Autumn) or BBCH 20 – 69 (Spring)	a) 1 b) 1	N/A	a) 1.0-1.2 b) 1.0-1.2	a) 300 - 360 (150+150- 180+180)  b) 300 - 360 (150+150- 180+180)	100-400	56		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I**	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion						
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms	
85.	LU	Winter Rape	Oilseed	F	Phoma leaf spot/stem canker <i>Leptosphaeria maculans</i> (LEPTMA) Sclerotinia stem rot <i>Sclerotinia sclerotiorum</i> (SCLESC) Powdery mildew <i>Erysiphe cruciferarum</i> (ERYSCR) Alternaria leaf spot <i>Alternaria brassicae</i> (ALTEBA) Light leaf spot <i>Pyrenopeziza brassicae</i> (PYRPBR) Grey mould <i>Botryotinia cinera</i> (BOTRCI)	foliar spray	BBCH 14 – 18 (Autumn) or BBCH 20 – 69 (Spring)	a) 1 b) 1	N/A	a) 1.0-1.2 b) 1.0-1.2	a) 300 - 360 (150+150-180+180)  b) 300 - 360 (150+150-180+180)	100-400	56		A	A	R	A	A	A
86.	NL	Winter Rape	Oilseed	F	Phoma leaf spot/stem canker <i>Leptosphaeria maculans</i> (LEPTMA) Sclerotinia stem rot <i>Sclerotinia sclerotiorum</i> (SCLESC) Powdery mildew <i>Erysiphe cruciferarum</i> (ERYSCR) Alternaria leaf spot <i>Alternaria brassicae</i> (ALTEBA) Light leaf spot <i>Pyrenopeziza brassicae</i> (PYRPBR) Grey mould <i>Botryotinia cinera</i> (BOTRCI)	foliar spray	BBCH 14 – 18 (Autumn) or BBCH 20 – 69 (Spring)	a) 1 b) 1	N/A	a) 1.0-1.2 b) 1.0-1.2	a) 300 - 360 (150+150-180+180)  b) 300 - 360 (150+150-180+180)	100-400	56		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, G, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms
87.	NI	Winter Rape	Oilseed	F	Phoma leaf spot/stem canker <i>Leptosphaeria maculans</i> (LEPTMA) Sclerotinia stem rot <i>Sclerotinia sclerotiorum</i> (SCLESC) Powdery mildew <i>Erysiphe cruciferarum</i> (ERYSCR) Alternaria leaf spot <i>Alternaria brassicae</i> (ALTEBA) Light leaf spot <i>Pyrenopeziza brassicae</i> (PYRPBR) Grey mould <i>Botryotinia cinera</i> (BOTRCI)	foliar spray	BBCH 14 – 18 (Autumn) or BBCH 20 – 69 (Spring)	a) 1 b) 1	N/A	a) 1.0-1.2 b) 1.0-1.2	a) 300 - 360 (150+150- 180+180)  b) 300 - 360 (150+150- 180+180)	100-400	56						A
88.	PL	Winter Rape	Oilseed	F	Phoma leaf spot/stem canker <i>Leptosphaeria maculans</i> (LEPTMA) Sclerotinia stem rot <i>Sclerotinia sclerotiorum</i> (SCLESC) Powdery mildew <i>Erysiphe cruciferarum</i> (ERYSCR) Alternaria leaf spot <i>Alternaria brassicae</i> (ALTEBA) Light leaf spot <i>Pyrenopeziza brassicae</i> (PYRPBR) Grey mould <i>Botryotinia cinera</i> (BOTRCI)	foliar spray	BBCH 14 – 18 (Autumn) or BBCH 20 – 69 (Spring)	a) 1 b) 1	N/A	a) 1.0-1.2 b) 1.0-1.2	a) 300 - 360 (150+150- 180+180)  b) 300 - 360 (150+150- 180+180)	100-400	56						A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gpn or I**	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion						
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms	
89.	RO	Winter Rape	Oilseed	F	Phoma leaf spot/stem canker <i>Leptosphaeria maculans</i> (LEPTMA) Sclerotinia stem rot <i>Sclerotinia sclerotiorum</i> (SCLESC) Powdery mildew <i>Erysiphe cruciferarum</i> (ERYSCR) Alternaria leaf spot <i>Alternaria brassicae</i> (ALTEBA) Light leaf spot <i>Pyrenopeziza brassicae</i> (PYRPBR) Grey mould <i>Botryotinia cinera</i> (BOTRCI)	foliar spray	BBCH 14 – 18 (Autumn) or BBCH 20 – 69 (Spring)	a) 1 b) 1	N/A	a) 1.0-1.2 b) 1.0-1.2	a) 300 - 360 (150+150- 180+180)  b) 300 - 360 (150+150- 180+180)	100-400	56		A	A	R	A	A	A
90.	SK	Winter Rape	Oilseed	F	Phoma leaf spot/stem canker <i>Leptosphaeria maculans</i> (LEPTMA) Sclerotinia stem rot <i>Sclerotinia sclerotiorum</i> (SCLESC) Powdery mildew <i>Erysiphe cruciferarum</i> (ERYSCR) Alternaria leaf spot <i>Alternaria brassicae</i> (ALTEBA) Light leaf spot <i>Pyrenopeziza brassicae</i> (PYRPBR) Grey mould <i>Botryotinia cinera</i> (BOTRCI)	foliar spray	BBCH 14 – 18 (Autumn) or BBCH 20 – 69 (Spring)	a) 1 b) 1	N/A	a) 1.0-1.2 b) 1.0-1.2	a) 300 - 360 (150+150- 180+180)  b) 300 - 360 (150+150- 180+180)	100-400	56		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, G, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms
91.	AT	Spring Oilseed Rape	F	Phoma leaf spot/stem canker <i>Leptosphaeria maculans</i> (LEPTMA) Sclerotinia stem rot <i>Sclerotinia sclerotiorum</i> (SCLESC) Powdery mildew <i>Erysiphe cruciferarum</i> (ERYSCR) Alternaria leaf spot <i>Alternaria brassicae</i> (ALTEBA) Light leaf spot <i>Pyrenopeziza brassicae</i> (PYRPBR) Grey mould <i>Botryotinia cinera</i> (BOTRCI)	foliar spray	BBCH 20 – 69 (Spring)	a) 1 b) 1	N/A	a) 1.0-1.2 b) 1.0-1.2	a) 300 - 360 (150+150- 180+180)  b) 300 - 360 (150+150- 180+180)	100-400	56		A	A	R	A	A	A
92.	BE	Spring Oilseed Rape	F	Phoma leaf spot/stem canker <i>Leptosphaeria maculans</i> (LEPTMA) Sclerotinia stem rot <i>Sclerotinia sclerotiorum</i> (SCLESC) Powdery mildew <i>Erysiphe cruciferarum</i> (ERYSCR) Alternaria leaf spot <i>Alternaria brassicae</i> (ALTEBA) Light leaf spot <i>Pyrenopeziza brassicae</i> (PYRPBR) Grey mould <i>Botryotinia cinera</i> (BOTRCI)	foliar spray	BBCH 20 – 69 (Spring)	a) 1 b) 1	N/A	a) 1.0-1.2 b) 1.0-1.2	a) 300 - 360 (150+150- 180+180)  b) 300 - 360 (150+150- 180+180)	100-400	56		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms
93.	CZ	Spring Oilseed Rape	F	Phoma leaf spot/stem canker <i>Leptosphaeria maculans</i> (LEPTMA) Sclerotinia stem rot <i>Sclerotinia sclerotiorum</i> (SCLESC) Powdery mildew <i>Erysiphe cruciferarum</i> (ERYSCR) Alternaria leaf spot <i>Alternaria brassicae</i> (ALTEBA) Light leaf spot <i>Pyrenopeziza brassicae</i> (PYRPBR) Grey mould <i>Botryotinia cinera</i> (BOTRCI)	foliar spray	BBCH 20 – 69 (Spring)	a) 1 b) 1	N/A	a) 1.0-1.2 b) 1.0-1.2	a) 300 - 360 (150+150- 180+180)  b) 300 - 360 (150+150- 180+180)	100-400	56		A	A	R	A	A	A
94.	DE	Spring Oilseed Rape	F	Powdery mildew <i>Erysiphe cruciferarum</i> (ERYSCR) Light leaf spot <i>Cylindrosporium concentricum</i> (PYRPBR)	foliar spray	BBCH 20 – 69 (Spring)	a) 1 b) 1	N/A	a) 1.2 b) 1.2	a) 360 (180+180)  b) 360 (180+180)	150-400	56		A	A	R	A	A	A



1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms
95.	HU	Spring Oilseed Rape	F	Phoma leaf spot/stem canker <i>Leptosphaeria maculans</i> (LEPTMA) Sclerotinia stem rot <i>Sclerotinia sclerotiorum</i> (SCLESC) Powdery mildew <i>Erysiphe cruciferarum</i> (ERYSCR) Alternaria leaf spot <i>Alternaria brassicae</i> (ALTEBA) Light leaf spot <i>Pyrenopeziza brassicae</i> (PYRPBR) Grey mould <i>Botryotinia cinera</i> (BOTRCI)	foliar spray	BBCH 20 – 69 (Spring)	a) 1 b) 1	N/A	a) 1.0-1.2 b) 1.0-1.2	a) 300 - 360 (150+150- 180+180)  b) 300 - 360 (150+150- 180+180)	100-400	56		A	A	R	A	A	A
96.	IE	Spring Oilseed Rape	F	Phoma leaf spot/stem canker <i>Leptosphaeria maculans</i> (LEPTMA) Sclerotinia stem rot <i>Sclerotinia sclerotiorum</i> (SCLESC) Powdery mildew <i>Erysiphe cruciferarum</i> (ERYSCR) Alternaria leaf spot <i>Alternaria brassicae</i> (ALTEBA) Light leaf spot <i>Pyrenopeziza brassicae</i> (PYRPBR) Grey mould <i>Botryotinia cinera</i> (BOTRCI)	foliar spray	BBCH 20 – 69 (Spring)	a) 1 b) 1	N/A	a) 1.0-1.2 b) 1.0-1.2	a) 300 - 360 (150+150- 180+180)  b) 300 - 360 (150+150- 180+180)	100-400	56		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, G, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms
97.	LU	Spring Oilseed Rape	F	Phoma leaf spot/stem canker <i>Leptosphaeria maculans</i> (LEPTMA) Sclerotinia stem rot <i>Sclerotinia sclerotiorum</i> (SCLESC) Powdery mildew <i>Erysiphe cruciferarum</i> (ERYSCR) Alternaria leaf spot <i>Alternaria brassicae</i> (ALTEBA) Light leaf spot <i>Pyrenopeziza brassicae</i> (PYRPBR) Grey mould <i>Botryotinia cinera</i> (BOTRCI)	foliar spray	BBCH 20 – 69 (Spring)	a) 1 b) 1	N/A	a) 1.0-1.2 b) 1.0-1.2	a) 300 - 360 (150+150- 180+180)  b) 300 - 360 (150+150- 180+180)	100-400	56		A	A	R	A	A	A
98.	NL	Spring Oilseed Rape	F	Phoma leaf spot/stem canker <i>Leptosphaeria maculans</i> (LEPTMA) Sclerotinia stem rot <i>Sclerotinia sclerotiorum</i> (SCLESC) Powdery mildew <i>Erysiphe cruciferarum</i> (ERYSCR) Alternaria leaf spot <i>Alternaria brassicae</i> (ALTEBA) Light leaf spot <i>Pyrenopeziza brassicae</i> (PYRPBR) Grey mould <i>Botryotinia cinera</i> (BOTRCI)	foliar spray	BBCH 20 – 69 (Spring)	a) 1 b) 1	N/A	a) 1.0-1.2 b) 1.0-1.2	a) 300 - 360 (150+150- 180+180)  b) 300 - 360 (150+150- 180+180)	100-400	56		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, G, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms
99.	NI	Spring Oilseed Rape	F	Phoma leaf spot/stem canker <i>Leptosphaeria maculans</i> (LEPTMA) Sclerotinia stem rot <i>Sclerotinia sclerotiorum</i> (SCLESC) Powdery mildew <i>Erysiphe cruciferarum</i> (ERYSCR) Alternaria leaf spot <i>Alternaria brassicae</i> (ALTEBA) Light leaf spot <i>Pyrenopeziza brassicae</i> (PYRPBR) Grey mould <i>Botryotinia cinera</i> (BOTRCI)	foliar spray	BBCH 20 – 69 (Spring)	a) 1 b) 1	N/A	a) 1.0-1.2 b) 1.0-1.2	a) 300 - 360 (150+150- 180+180)  b) 300 - 360 (150+150- 180+180)	100-400	56		A	A	R	A	A	A
100.	PL	Spring Oilseed Rape	F	Phoma leaf spot/stem canker <i>Leptosphaeria maculans</i> (LEPTMA) Sclerotinia stem rot <i>Sclerotinia sclerotiorum</i> (SCLESC) Powdery mildew <i>Erysiphe cruciferarum</i> (ERYSCR) Alternaria leaf spot <i>Alternaria brassicae</i> (ALTEBA) Light leaf spot <i>Pyrenopeziza brassicae</i> (PYRPBR) Grey mould <i>Botryotinia cinera</i> (BOTRCI)	foliar spray	BBCH 20 – 69 (Spring)	a) 1 b) 1	N/A	a) 1.0-1.2 b) 1.0-1.2	a) 300 - 360 (150+150- 180+180)  b) 300 - 360 (150+150- 180+180)	100-400	56		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, G, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms
101.	RO	Spring Oilseed Rape	F	Phoma leaf spot/stem canker <i>Leptosphaeria maculans</i> (LEPTMA) Sclerotinia stem rot <i>Sclerotinia sclerotiorum</i> (SCLESC) Powdery mildew <i>Erysiphe cruciferarum</i> (ERYSCR) Alternaria leaf spot <i>Alternaria brassicae</i> (ALTEBA) Light leaf spot <i>Pyrenopeziza brassicae</i> (PYRPBR) Grey mould <i>Botryotinia cinera</i> (BOTRCI)	foliar spray	BBCH 20 – 69 (Spring)	a) 1 b) 1	N/A	a) 1.0-1.2 b) 1.0-1.2	a) 300 - 360 (150+150- 180+180)  b) 300 - 360 (150+150- 180+180)	100-400	56		A	A	R	A	A	A
102.	SK	Spring Oilseed Rape	F	Phoma leaf spot/stem canker <i>Leptosphaeria maculans</i> (LEPTMA) Sclerotinia stem rot <i>Sclerotinia sclerotiorum</i> (SCLESC) Powdery mildew <i>Erysiphe cruciferarum</i> (ERYSCR) Alternaria leaf spot <i>Alternaria brassicae</i> (ALTEBA) Light leaf spot <i>Pyrenopeziza brassicae</i> (PYRPBR) Grey mould <i>Botryotinia cinera</i> (BOTRCI)	foliar spray	BBCH 20 – 69 (Spring)	a) 1 b) 1	N/A	a) 1.0-1.2 b) 1.0-1.2	a) 300 - 360 (150+150- 180+180)  b) 300 - 360 (150+150- 180+180)	100-400	56		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Use- No. *	Member state(s)	Crop 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)	Applicatio n	Application rate	PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					Conclusion					
														Birds	Mammals	Aquatic	Bees	Non-target	Soil organisms
103.	PL	Sunflower	F	Sclerotinia Stem rot <i>Sclerotinia sclerotiorum</i> (SCLESC) Grey mould <i>Botryotinia cinera</i> (BOTRCI)Stalk rot of sunflower <i>Diaporthe helianthi</i> (DIAPHE) Black stem of Sunflower <i>Plenodomus lindquistii</i> (LEPTLI)	foliar spray	BBCH 16– 64 (spring)	a) 1 b) 1		a) 1.0-1.2 b) 1.0-1.2	a) 240-360 (120+120 180+180)  b) 240-360 (120+120 180+180)	100-400	56		A	A	R	A	A	A
104.	BE	Flax (for fiber production only)	F	Powdery mildew flax <i>Erysiphe spp</i> (ERYSP)	Foliar spray	BBCH 33 – 51	a) 1 b) 1	N/A	a) 1.0-1.2 b) 1.0-1.2	a) 300 - 360 (150+150- 180+180)  b) 300 - 360 (150+150- 180+180)	100-400	N/A		A	A	R	A	A	A
105.	AT	Linseeds, Poppy, Mustard and Gold of Pleasure	F	Phoma leaf spot/stem canker <i>Leptosphaeria maculans</i> (LEPTMA) Sclerotinia stem rot <i>Sclerotinia sclerotiorum</i> (SCLESC) Powdery mildew <i>Erysiphe cruciferarum</i> (ERYSCR) Alternaria leaf spot <i>Alternaria brassicae</i> (ALTEBA) Light leaf spot <i>Pyrenopeziza brassicae</i> (PYRPBR)	foliar spray	BBCH 14 – 18 (Autumn) or BBCH 20 – 69 (Spring)	a) 1 b) 1	N/A	a) 1.0-1.2 b) 1.0-1.2	a) 300 - 360 (150+150- 180+180)  b) 300 - 360 (150+150- 180+180)	100-400	56		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
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)	Applicatio n	Application rate	PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					Conclusion					
														Birds	Mammals	Aquatic	Bees	Non-target	Soil organisms
106.	BE	Linseeds, Poppy, Mustard and Gold of Pleasure	F	Phoma leaf spot/stem canker <i>Leptosphaeria maculans</i> (LEPTMA) Sclerotinia stem rot <i>Sclerotinia sclerotiorum</i> (SCLESC) Powdery mildew <i>Erysiphe cruciferarum</i> (ERYSCR) Alternaria leaf spot <i>Alternaria brassicae</i> (ALTEBA) Light leaf spot <i>Pyrenopeziza brassicae</i> (PYRPBR)	foliar spray	BBCH 14 – 18 (Autumn) or BBCH 20 – 69 (Spring)	a) 1 b) 1	N/A	a) 1.0-1.2 b) 1.0-1.2	a) 300 - 360 (150+150- 180+180)  b) 300 - 360 (150+150- 180+180)	100-400	56		A	A	R	A	A	A
107.	CZ	Linseeds, Poppy, Mustard and Gold of Pleasure	F	Phoma leaf spot/stem canker <i>Leptosphaeria maculans</i> (LEPTMA) Sclerotinia stem rot <i>Sclerotinia sclerotiorum</i> (SCLESC) Powdery mildew <i>Erysiphe cruciferarum</i> (ERYSCR) Alternaria leaf spot <i>Alternaria brassicae</i> (ALTEBA) Light leaf spot <i>Pyrenopeziza brassicae</i> (PYRPBR)	foliar spray	BBCH 14 – 18 (Autumn) or BBCH 20 – 69 (Spring)	a) 1 b) 1	N/A	a) 1.0-1.2 b) 1.0-1.2	a) 300 - 360 (150+150- 180+180)  b) 300 - 360 (150+150- 180+180)	100-400	56		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
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)	Applicatio n	Application rate	PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					Conclusion					
														Birds	Mammals	Aquatic	Bees	Non-target	Soil organisms
108.	DE	Seed bearing plants: Linseeds, Poppy, Mustard and Gold of Pleasure	F	Phoma leaf spot/stem canker <i>Leptosphaeria maculans</i> (LEPTMA) Sclerotinia stem rot <i>Sclerotinia sclerotiorum</i> (SCLESC) Powdery mildew <i>Erysiphe cruciferarum</i> (ERYSCR) Alternaria leaf spot <i>Alternaria brassicae</i> (ALTEBA) Light leaf spot <i>Cylindrosporium concentricum</i> (PYRPBR)	foliar spray	BBCH 14 – 18 (Autumn) or BBCH 20 – 69 (Spring)	a) 1 b) 1	N/A	a) 1.2 b) 1.2	a) 360 (180+180)  b) 360 (180+180)	150-400	56		A	A	R	A	A	A
109.	HU	Linseeds, Poppy, Mustard and Gold of Pleasure	F	Phoma leaf spot/stem canker <i>Leptosphaeria maculans</i> (LEPTMA) Sclerotinia stem rot <i>Sclerotinia sclerotiorum</i> (SCLESC) Powdery mildew <i>Erysiphe cruciferarum</i> (ERYSCR) Alternaria leaf spot <i>Alternaria brassicae</i> (ALTEBA) Light leaf spot <i>Pyrenopeziza brassicae</i> (PYRPBR)	foliar spray	BBCH 14 – 18 (Autumn) or BBCH 20 – 69 (Spring)	a) 1 b) 1	N/A	a) 1.0-1.2 b) 1.0-1.2	a) 300 - 360 (150+150- 180+180)  b) 300 - 360 (150+150- 180+180)	100-400	56		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
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)	Applicatio n	Application rate	PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					Conclusion					
														Birds	Mammals	Aquatic	Bees	Non-target	Soil organisms
110.	IE	Linseeds, Poppy, Mustard and Gold of Pleasure	F	Phoma leaf spot/stem canker <i>Leptosphaeria maculans</i> (LEPTMA) Sclerotinia stem rot <i>Sclerotinia sclerotiorum</i> (SCLESC) Powdery mildew <i>Erysiphe cruciferarum</i> (ERYSCR) Alternaria leaf spot <i>Alternaria brassicae</i> (ALTEBA) Light leaf spot <i>Pyrenopeziza brassicae</i> (PYRPBR)	foliar spray	BBCH 14 – 18 (Autumn) or BBCH 20 – 69 (Spring)	a) 1 b) 1	N/A	a) 1.0-1.2 b) 1.0-1.2	a) 300 - 360 (150+150- 180+180)  b) 300 - 360 (150+150- 180+180)	100-400	56		A	A	R	A	A	A
111.	LU	Linseeds, Poppy, Mustard and Gold of Pleasure	F	Phoma leaf spot/stem canker <i>Leptosphaeria maculans</i> (LEPTMA) Sclerotinia stem rot <i>Sclerotinia sclerotiorum</i> (SCLESC) Powdery mildew <i>Erysiphe cruciferarum</i> (ERYSCR) Alternaria leaf spot <i>Alternaria brassicae</i> (ALTEBA) Light leaf spot <i>Pyrenopeziza brassicae</i> (PYRPBR)	foliar spray	BBCH 14 – 18 (Autumn) or BBCH 20 – 69 (Spring)	a) 1 b) 1	N/A	a) 1.0-1.2 b) 1.0-1.2	a) 300 - 360 (150+150- 180+180)  b) 300 - 360 (150+150- 180+180)	100-400	56		A	A	R	A	A	A



1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
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)	Applicatio n	Application rate	PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					Conclusion					
														Birds	Mammals	Aquatic	Bees	Non-target	Soil organisms
112.	NL	Linseeds, Poppy, Mustard and Gold of Pleasure	F	Phoma leaf spot/stem canker <i>Leptosphaeria maculans</i> (LEPTMA) Sclerotinia stem rot <i>Sclerotinia sclerotiorum</i> (SCLESC) Powdery mildew <i>Erysiphe cruciferarum</i> (ERYSCR) Alternaria leaf spot <i>Alternaria brassicae</i> (ALTEBA) Light leaf spot <i>Pyrenopeziza brassicae</i> (PYRPBR)	foliar spray	BBCH 14 – 18 (Autumn) or BBCH 20 – 69 (Spring)	a) 1 b) 1	N/A	a) 1.0-1.2 b) 1.0-1.2	a) 300 - 360 (150+150- 180+180)  b) 300 - 360 (150+150- 180+180)	100-400	56		A	A	R	A	A	A
113.	NI	Linseeds, Poppy, Mustard and Gold of Pleasure	F	Phoma leaf spot/stem canker <i>Leptosphaeria maculans</i> (LEPTMA) Sclerotinia stem rot <i>Sclerotinia sclerotiorum</i> (SCLESC) Powdery mildew <i>Erysiphe cruciferarum</i> (ERYSCR) Alternaria leaf spot <i>Alternaria brassicae</i> (ALTEBA) Light leaf spot <i>Pyrenopeziza brassicae</i> (PYRPBR)	foliar spray	BBCH 14 – 18 (Autumn) or BBCH 20 – 69 (Spring)	a) 1 b) 1	N/A	a) 1.0-1.2 b) 1.0-1.2	a) 300 - 360 (150+150- 180+180)  b) 300 - 360 (150+150- 180+180)	100-400	56		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
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)	Applicatio n	Application rate	PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					Conclusion					
														Birds	Mammals	Aquatic	Bees	Non-target	Soil organisms
114.	PL	Linseeds, Poppy, Mustard and Gold of Pleasure	F	Phoma leaf spot/stem canker <i>Leptosphaeria maculans</i> (LEPTMA) Sclerotinia stem rot <i>Sclerotinia sclerotiorum</i> (SCLESC) Powdery mildew <i>Erysiphe cruciferarum</i> (ERYSCR) Alternaria leaf spot <i>Alternaria brassicae</i> (ALTEBA) Light leaf spot <i>Pyrenopeziza brassicae</i> (PYRPBR)	foliar spray	BBCH 14 – 18 (Autumn) or BBCH 20 – 69 (Spring)	a) 1 b) 1	N/A	a) 1.0-1.2 b) 1.0-1.2	a) 300 - 360 (150+150- 180+180)  b) 300 - 360 (150+150- 180+180)	100-400	56		A	A	R	A	A	A
115.	RO	Linseeds, Poppy, Mustard and Gold of Pleasure	F	Phoma leaf spot/stem canker <i>Leptosphaeria maculans</i> (LEPTMA) Sclerotinia stem rot <i>Sclerotinia sclerotiorum</i> (SCLESC) Powdery mildew <i>Erysiphe cruciferarum</i> (ERYSCR) Alternaria leaf spot <i>Alternaria brassicae</i> (ALTEBA) Light leaf spot <i>Pyrenopeziza brassicae</i> (PYRPBR)	foliar spray	BBCH 14 – 18 (Autumn) or BBCH 20 – 69 (Spring)	a) 1 b) 1	N/A	a) 1.0-1.2 b) 1.0-1.2	a) 300 - 360 (150+150- 180+180)  b) 300 - 360 (150+150- 180+180)	100-400	56		A	A	R	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
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)	Applicatio n	Application rate	PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion					Conclusion					
														Birds	Mammals	Aquatic	Bees	Non-target	Soil organisms
116.	SK	Linseeds, Poppy, Mustard and Gold of Pleasure	F	Phoma leaf spot/stem canker <i>Leptosphaeria maculans</i> (LEPTMA) Sclerotinia stem rot <i>Sclerotinia sclerotiorum</i> (SCLESC) Powdery mildew <i>Erysiphe cruciferarum</i> (ERYSCR) Alternaria leaf spot <i>Alternaria brassicae</i> (ALTEBA) Light leaf spot <i>Pyrenopeziza brassicae</i> (PYRPBR)	foliar spray	BBCH 14 – 18 (Autumn) or BBCH 20 – 69 (Spring)	a) 1 b) 1	N/A	a) 1.0-1.2 b) 1.0-1.2	a) 300 - 360 (150+150- 180+180)  b) 300 - 360 (150+150- 180+180)	100-400	56		A	A	R	A	A	A

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

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

#### Explanation for column 15 – 21 “Conclusion”

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

<b>Remarks table:</b>	<div> <div> (1) Numeration necessary to allow references</div> <div>(2) Use official codes/nomenclatures of EU</div> <div>(3) For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (<i>e.g.</i> fumigation of a structure)</div> <div>(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</div> <div>(5) Scientific names and EPPO-Codes of target pests/diseases/ weeds or when relevant the common names of the pest groups (<i>e.g.</i> 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</div> <div>(6) Method, <i>e.g.</i> high volume spraying, low volume spraying, spreading, dusting, drench  Kind, <i>e.g.</i> overall, broadcast, aerial spraying, row, individual plant, between the plants - type of equipment used must be indicated</div> <div>(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</div> <div>(8) The maximum number of application possible under practical conditions of use must be provided</div> <div>(9) Minimum interval (in days) between applications of the same product.</div> <div>(10) For specific uses other specifications might be possible, <i>e.g.</i>: g/m<sup>3</sup> in case of fumigation of empty rooms. See also EPPO-Guideline PP 1/239 Dose expression for plant protection products</div> <div>(11) The dimension (g, kg) must be clearly specified. (Maximum) dose of a.s. per treatment (usually g, kg or L product / ha).</div> <div>(12) If water volume range depends on application equipments (<i>e.g.</i> ULVA or LVA) it should be mentioned under “application: method/kind”.</div> <div>(13) PHI - minimum pre-harvest interval</div> <div>(14) Remarks may include: Extent of use/economic importance/restrictions</div> </div>
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## 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)

The risk assessment for birds and mammals was carried out according to EFSA/2009/1438.

The acute and long-term risks to birds and mammals were assessed from toxicity-exposure ratio (TER) values, between toxicity endpoints, estimated from studies with prothioconazole and azoxystrobin, the relevant metabolite prothioconazole-desthio, and maximum residues occurring on food items, following applications according to the proposed use pattern.

The acute and reproductive (long-term) risk for birds from dietary exposure to prothioconazole, azoxystrobin and the relevant metabolite prothioconazole-desthio is acceptable based on Screening Step and/or Tier 1 assessments, including considerations of combined exposure to the two actives (prothioconazole and azoxystrobin), and the metabolite prothioconazole-desthio and azoxystrobin.

The acute and reproductive (long-term) risk for mammals from dietary exposure to prothioconazole and azoxystrobin is acceptable based on Screening Step and/or Tier 1 assessments, including considerations of combined exposure to the two actives (prothioconazole and azoxystrobin). ~~and the metabolite prothioconazole-desthio and azoxystrobin.~~

For the relevant metabolite prothioconazole-desthio, the acute risk to mammals is acceptable at the screening step assessment. Also, most long-term first-tier risk assessments demonstrated acceptable risk according to the proposed use pattern. Only for the use on cereals, at BBCH  $\geq 40$ , and the generic focal species small herbivorous mammal “vole”, the TERLT value was below the relevant trigger of 5 for prothioconazole-desthio. Long-term risk from combination toxicity of prothioconazole-desthio and azoxystrobin for the Tier 1 generic focal species small herbivorous mammal “vole” is also not acceptable for uses in cereals, oilseed rape, and sunflower. Based on a refined risk assessment considering standard worst-case assumptions in combination with a refined deposition factor to grass under the crop, the reproductive risk to mammals from prothioconazole-desthio is considered acceptable for cereals and oilseed rape. **For the use in sunflower, the risk to small herbivorous mammal “vole” is refined by considering appropriate residue deposition to the grass under the crop as well as residue dissipation data. Based on refinements, no unacceptable risks to mammals in sunflower from the combined toxicity of azoxystrobin and prothioconazole-desthio is concluded.**

Acceptable risk for exposure of birds and mammals via drinking water and via secondary poisoning was shown from the proposed use of CA3642. No specific reptile or amphibian data are being submitted. However, the testing of these species and their appropriate risk assessment methodology are not yet agreed in Europe. An inherent level of safety has been demonstrated, based on the acceptable aquatic and terrestrial risk assessments that will cover the risk to these organisms.

### 9.1.1.2 Effects on aquatic organisms (KCP 10.2)

The risk assessment for aquatic organisms was carried out according to the Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (EFSA Journal 2013;11(7):3290).

Based on the available data and risk assessment for aquatic organisms including considerations on potential mixture toxicity, acceptable risk is indicated for the intended uses in winter and spring cereals, ~~and~~ winter and spring oilseed rape and sunflower based on data with the formulated product and when the following mitigation measures are respected:

**Based on the performed calculations for the worst-case scenario acceptable risk following application of CA3642 according to the Central Zone GAP may be concluded.**

Single use on spring and winter cereals: 10-m NSBZ+ 10-m VFS

Two-fold use on spring and winter cereals: 20-m NSBZ + 20-m VFS

Use on spring oilseed rape: 10-m NSBZ + 10-m VFS

Use on winter oilseed rape (spring application): 10-m NSBZ + 10-m VFS

Use on winter oilseed rape (autumn application): 20-m NSBZ + 20-m VFS

Use on sunflower: 10-m NSBZ + 10-m VFS

It should be noted that risk assessment minor uses included in the GAP table are covered by oilseed rape use (spring and winter).

Concerned Member States must decide on applicability of indicated risk mitigation measures in their countries at the product authorization.

Please note that additional aquatic risk assessment may be required by the concerned Member States that do not accept simulations performed according to FOCUS recommendations.

- For the use of 1 application to winter and spring cereals: 10 m no-spray buffer zone and 10-m vegetative filter strip.
- For the use of 2 applications to winter and spring cereals: 20 m no-spray buffer zone and a 20-m vegetative filter strip.
- For the use of 1 application to winter oilseed rape (spring application): 10 m no-spray buffer zone and 10-m vegetative filter strip.
- For the use of 1 application to winter oilseed rape (autumn application): 20 m no-spray buffer zone and 20-m vegetative filter strip.
- For the use of 1 application on spring oilseed rape: 10 m no-spray buffer zone and 10-m vegetative filter strip.
- For the use of 1 application on sunflower: 10 m no-spray buffer zone and 10-m vegetative filter strip.

#### 9.1.1.3 Effects on bees (KCP 10.2)

A first-tier risk assessment was conducted in accordance with SANCO/10329/2002 and indicated acceptable acute contact and oral risks to adult honey and bumble bees (hazard quotient values  $\leq 50$ ). For completeness, a risk assessment was also conducted in accordance with the EFSA bee guidance (EFSA/2013/3295), to determine the chronic risk to adult and larval honey bees. The screening risk assessment indicated acceptable acute oral and contact risks to honey bees and bumble bees, as well as an acceptable chronic oral risk to larval honey bees, but not to adult honey bees. The first-tier chronic oral risk assessment for adult honey bees indicated acceptable risks in cereals and oilseed rape, except for the scenario treated crop (all intended BBCH stages) and weeds (only BBCH 10-29) in oilseed rape, and sunflower except for the scenario treated crop (all intended BBCH stages) and weeds (only BBCH 10-29) in oilseed rape and sunflower.

Therefore, a honey bee semi-field tunnel study with CA3642 in winter oilseed rape was conducted. Overall, it was concluded that CA3642 had no significant effect on overall honey bee colony strength (mean number of adult honey bees), overall amount of brood or the development of the food storage area following two applications of 1.4 L/ha of CA3642. The two specific protection goals mentioned in EFSA (2014) i.e. no significant effects on forager mortality or honey bee colony strength, have been shown to be met for CA3642 when applied at 1.4 L/ha once pre flowering and once at flowering during daily bee flight under semi-field conditions in winter oilseed rape. It is considered appropriate to extrapolate results of this worst case oilseed rape semi-field study to refine the chronic risk to honey bees for the use in sunflower (1 x 1.2 L CA3642/ha). An acceptable risk to honey bees is therefore concluded for the proposed uses of CA3642 in cereals, oilseed rape, and sunflower.

#### 9.1.1.4 Effects on arthropods other than bees (KCP 10.3.2)

The risk assessment was conducted according to 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.

The risk from CA3642 to non-target arthropods was assessed in first-tier assessments, from hazard quotients, between toxicity endpoints that were estimated from laboratory studies with CA3642 and crop-specific use patterns. The assessment was conducted for the worst-case application pattern of 2 x 1.4 L/ha (14-d interval), covering the risk for non-target arthropods from all other intended uses.

An acceptable in-field and off-field risk (considering first tier testing) is indicated for exposure of terrestrial non-target arthropods other than bees towards the formulated product for the intended worst-case use of CA3642.

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

The risk to earthworms, *Folsomia candida* and *Hypoaspis aculeifer* from exposure to the active substances prothioconazole and azoxystrobin, and their metabolites was assessed and demonstrated to be acceptable based on the maximum predicted concentration in soil. PEC<sub>Soil</sub> values have been calculated for uses in cereals, spring oilseed rape, winter oilseed rape and sunflower. The worst-case use is application to sunflower. All TER<sub>LT</sub> values were above the trigger of 5.

No significant effects (<25%) on soil microorganisms for the both of a.s. and formulation CA3642 were shown for the proposed uses of CA3642 at concentrations greater than the predicted maximum soil concentrations. Therefore, the risk to soil micro-organisms was considered acceptable for these active substances and the formulation CA3642.

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

The risk assessment for non-target plants was considered acceptable using the maximum application rate of CA3642, using data from vegetative-vigour and seedling-emergence studies. No adverse effects are expected from the worst-case GAP (maximum 1.4 L CA3642/ha).

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

Further studies on other terrestrial organism are not required, as the risk to the standard organisms has been shown to be acceptable.

### **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 CA3642 grouped according to crop type**

Group	Intended uses	Maximum rate per application	Maximum number of applications	Minimum interval between applications	Maximum total rate per season
Winter or spring cereals	BBCH 30-61	210 g prothioconazole/ha  210 g azoxystrobin/ha	2	14 d	420 g prothioconazole/ha  420 g azoxystrobin/ha
Oilseed rape (covers minor uses on sunflower, flax, poppy, mustard and Gold of Pleasure, linseeds - except soil organism)	BBCH 14-18 (autumn use, first application)  BBCH 20-69 (spring use)	180 g prothioconazole/ha  180 g azoxystrobin/ha	1	-	180 g prothioconazole/ha  180 g azoxystrobin/ha
Sunflower (covers linseed in case of soil organism)	BBCH 16-64	180 g prothioconazole/ha  180 g azoxystrobin/ha	1	-	180 g prothioconazole/ha  180 g azoxystrobin/ha

**zRMS comments:**

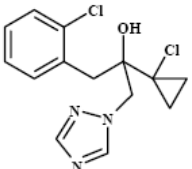
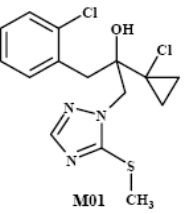
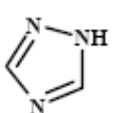
Critical use pattern of CA3642 grouped according to crop type has been validated by zRMS.

**It should be noted that risk assessment minor uses are covered by oilseed rape use (spring and winter) except use in linseed in case of risk for soil organism where PECs for sunflower is considered relevant according to e-fate expert comments in Section 8.**

### 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 CA3642 is indicated in the table.

**Table 9.1-3 Metabolites of prothioconazole**

Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
Prothioconazole-desthio (via soil and water/sediment)		312.2 g/mol	Soil (max. 57.1% at 7d) Water (max. 32.3% at 7d) Sediment (max. 26.95% at 14d) Water/sediment system (54.6% at 7d)	Yes (soil and aquatic)
Prothioconazole-S-methyl (via soil)	 M01	358.3 g/mol	Soil (max. 14.6% at 7d)	Yes (soil and aquatic)
1,2,4-triazole (via water/sediment)		69.1 g/mol	Water (max. 37.2% at 121d) Sediment (max. 6.1% at 121d)  Water/sediment system (max. 41.8% at 121d)	Yes (aquatic)



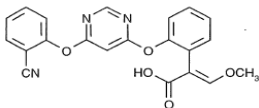
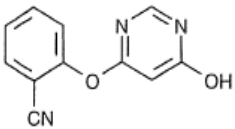
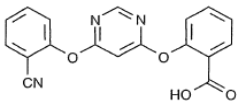
**zRMS comments:**

Metabolites relevant for soil and water compartment listed in Table 9.1-3 are the same as indicated in EFSA Scientific Report (2007) 106. It is noted that in the course of the EU review of prothioconazole metabolite JAU 6476-thiazocine was formed at >10% in photodegradation study in water, however according to EFSA Scientific Report (2007) 106, it was considered to be not relevant for evaluation in area of ecotoxicology.

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

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

**Table 9.1-4 Metabolites of azoxystrobin**

Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
R234886 (M 02) (via soil and water/sediment)		389.4 g/mol	<del>Soil (max. 28.8% at 360 d) Water (max 10.8% after 152d) Sediment (max 15.6% after 152d) Water/sediment system (max. 18.1 % at 152d*)</del>	Yes (soil and aquatic)
R401553 (M 28) (via soil and water)		213.2 g/mol	<del>Soil (max. 17% at unspecified time**) Water (photolysis, max. 8.9 % at unspecified time**)</del>	Yes (soil and aquatic)
R402173 (M 30) (via soil and water)		333.3 g/mol	<del>Soil (max. 17% at unspecified time**) Water (photolysis, max. 2.4 % at unspecified time**))</del>	Yes (soil and aquatic)

\* According to the EFSA conclusion, the maximum water/sediment concentration for R234886 was agreed to be 18.1% AR (derived by calculating the individual mean for each of 3 label positions from data from 3 TLC solvent systems prior to calculating an overall mean), however no timepoint is specified and no data on metabolite concentrations are provided in study summaries in the 2009 DAR or the 1997 monograph from the previous EU evaluation of azoxystrobin.

\*\* According to the 2009 DAR, the metabolites R401553 and R402173 were found in field studies and aqueous photolysis studies evaluated for the previous approval of azoxystrobin under Dir. 91/414/EEC, but the studies themselves have not been summarised in the DAR. These metabolites are not mentioned in the previous 1998 review report or the 1997 monograph and it was not possible to determine the time at which the peak was reached.

**zRMS comments:**

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

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

## 9.2 Effects on birds (KCP 10.1.1)

### 9.2.1 Toxicity data

Avian toxicity studies have been carried out with prothioconazole, azoxystrobin and their relevant metabolites. Full details of these studies are provided in the respective EU DARs and related documents.

Effects on birds exposed to CA3642 were not evaluated as part of the EU assessments of prothioconazole and azoxystrobin. Data on the formulation CA3642 is not considered essential, because the risk for terrestrial vertebrates is adequately addressed based on the data for the active substances and relevant metabolites. Therefore, no new data are submitted with this application.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review processes and the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438).

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

Species	Substance	Exposure system	Results	Reference
Active substance				
Northern bobwhite ( <i>Colinus virginianus</i> )	Prothioconazole	Acute, oral	LD <sub>50</sub> >2000 mg/kg bw	EFSA Sci. Report. 2007; 106, 1-98
Bobwhite quail ( <i>Colinus virginianus</i> )		Short-term dietary	LC <sub>50</sub> >5000 mg a.s./kg diet calc. LD <sub>50</sub> >1413 mg a.s./kd bw/day	
Mallard duck ( <i>Anas platyrhynchos</i> )		Reproductive, 22-week dietary	NOEL = 78 mg/kg bw/d	
Metabolite				
Northern bobwhite ( <i>Colinus virginianus</i> )	Prothioconazole-desthio	Acute	LD <sub>50</sub> >2000 mg/kg bw	EFSA Sci. Report. 2007; 106, 1-98
		Short-term, dietary	LD <sub>50</sub> >297 mg /kg bw/d	
		Reproductive, dietary	NOEL = 14.8 mg/kg bw/d	

**Table 9.2-2: Endpoints and effect values of azoxystrobin relevant for the risk assessment for birds**

Species	Substance	Exposure system	Results	Reference
Active substance				
Northern bobwhite ( <i>Colinus virginianus</i> )	Azoxystrobin	Acute, oral	<b>LD<sub>50</sub> &gt;2000 mg/kg bw</b>	EFSA Journal 2010; 8(4):1542
		Short-term dietary	<b>LDD<sub>50</sub> &gt;5200 mg/kg bw</b>	
		Reproductive, dietary	<b>NOEL = 1200 mg/kg food NOEL = 117 mg/kg bw/d</b>	

#### **zRMS comments:**

Avian toxicity data for azoxystrobin, prothioconazole and prothioconazole metabolite JAU 6476-desthio provided in Tables 9.2-1 and 9-2-2 above were confirmed by zRMS that they are in line with EU agreed endpoints reported in EFSA Journal 2010; 8(4):1542 and EFSA Scientific Report (2007) 106, respectively.

#### 9.2.1.1 Justification for new endpoints

Not relevant. No new data submitted.

## 9.2.2 Risk assessment for spray applications

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

The avian risk assessment has been carried out considering the critical GAP:

- Winter or spring cereals: 2 x 1.4 L product/ha, corresponding to 2 x 210 g prothioconazole/ha and 2 x 210 g azoxystrobin/ha (minimum interval of 14 days between applications); BBCH 30-69.
- Oilseed rape: 1 x 1.2 L product/ha, corresponding to 1 x 180 g prothioconazole/ha and 1 x 180 g azoxystrobin/ha; BBCH 14-18 (autumn) and BBCH 20-69 (spring).
- Sunflower: 1 x 1.2 L product/ha, corresponding to 1 x 180 g prothioconazole/ha and 1 x 180 g azoxystrobin/ha; BBCH 16-64.

For the risk assessment, the risk-envelope approach has been used for flax (1 x 1.2 L product/ha, at BBCH 33-51) and for ~~sunflower~~, linseeds, poppy, mustard and Gold of Pleasure (1 x 1.2 L product/ha, either in autumn, at BBCH 14-18, or spring, at BBCH 20-69). These crops are all listed within the oilseed rape crop group in the EFSA Guidance Document on Risk Assessment for Birds and Mammals (see Table 5, EFSA Journal 2009; 7(12): 1438). Therefore, the assessment for the use group oilseed rape also covers the risk to birds from all other intended uses in flax, linseeds, poppy, mustard and Gold of Pleasure (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.

#### Assessment of the risk from prothioconazole

**Table 9.2-2: Screening assessment of the acute and long-term/reproductive risk for birds due to the use of CA3642 in cereals, ~~and~~ oilseed rape and sunflower – prothioconazole and Tier 1 for long-term risk for birds in cereals, oilseed rape and sunflower.**

<b>Intended use</b>	Cereals. <del>and</del> oilseed rape, sunflower				
<b>Active substance</b>	Prothioconazole				
<b>Application rate</b>	2 x 210 g a.s./ha (14-d interval) – worst-case cereal GAP used in the screening step				
<b>Acute toxicity</b>	>2000 mg a.s./kg bw / >1413* mg a.s./kg bw (worst-case endpoint from 5 day dietary study)				
<b>TER criterion</b>	10				
<b>Crop scenario</b>	<b>Indicator/generic focal species</b>	<b>SV<sub>90</sub></b>	<b>MAF<sub>90</sub></b>	<b>DDD<sub>90</sub> (mg/kg bw/d)</b>	<b>TER<sub>A</sub></b>
<b>Growth stage</b>					
Screening step	Small omnivorous bird	158.8	1.2	40.0	>50.0 / >35.3
<b>Reprod. toxicity</b>	78 mg a.s./kg bw/d				
<b>TER criterion</b>	5				
<b>Crop scenario</b>	<b>Indicator/generic focal species</b>	<b>SV<sub>m</sub></b>	<b>MAF<sub>m</sub> × TWA</b>	<b>DDD<sub>m</sub> (mg/kg bw/d)</b>	<b>TER<sub>LT</sub></b>
<b>Growth stage</b>					
Screening step	Small omnivorous bird	64.8	1.4 x 0.53	10.1	7.7
<b>Tier 1** (cereals) 2 x 210 g a.s./ha, 14 d-interval</b>					
BBCH 30-39	Small omnivorous bird "lark"	5.4	1.4 x 0.53	0.84 1.59	92.8 49.05
BBCH ≥40	Small omnivorous bird "lark"	3.3	1.4 x 0.53	0.97	80.41
Cereals late season seed heads	Small granivorous/insectivorous bird "bunting"	15 1.25	1.4 x 0.53	2.33 1.04	33.5 50.51
<b>Tier 1** (oilseed rape) 1 x 180 g a.s./ha</b>					
BBCH 30-99	Small insectivorous bird "dunnock"	2.7	1 x 0.53	0.26	300

BBCH 10-29	Large herbivorous bird "goose"	15.9	1 x 0.53	1.52	51.31
BBCH 10-29	Small omnivorous bird "lark"	10.9	1 x 0.53	1.04	75.0
BBCH 30-39	Small omnivorous bird "lark"	3.3	1 x 0.53	0.31	251.61
BBCH ≥40	Small omnivorous bird "lark"	2.7	1 x 0.53	0.26	300
BBCH 10-19	medium herbivorous/granivorous bird "pigeon"	22.7	1 x 0.53	2.13	36.62
BBCH 20-29	medium herbivorous/granivorous bird "pigeon"	3.5	1 x 0.53	0.33	236.4
BBCH 30-39	medium herbivorous/granivorous bird "pigeon"	1.1	1 x 0.53	0.10	780
BBCH ≥40	medium herbivorous/granivorous bird "pigeon"	0.9	1 x 0.53	0.086	906.98
BBCH 10-19	Small insectivorous bird "wagtail"	5.9	1 x 0.53	0.56	139.30
BBCH 20-29	Small insectivorous bird "wagtail"	2.8	1 x 0.53	0.27	288.88
<b>Tier 1 (sunflower) 1 x 180 g a.s./ha</b>					
BBCH 00-19	Small omnivorous bird "lark"	10.9	1.0 x 0.53	1.0	78
BBCH 00-19	Small insectivorous bird "wagtail"	11.3	1.0 x 0.53	1.1	70.9
BBCH 61-92	Small granivorous/ insec-tivorous bird "bunting"	10	1.0 x 0.53	0.95	82.10

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

\*\*Tier 1 level added by zRMS as it needed in combined risk assessment

Acceptable acute and long-term risks to birds are concluded for the active substance prothioconazole, at the screening step, for the intended uses of CA3642, in cereals, and oilseed rape and sunflower.

For the screening and first-tier risk assessment for the metabolite prothioconazole-desthio (see tables below), it has been assumed that 100% of the parent becomes the metabolite, so the daily dietary doses of the metabolite are the same as for the active substance. This is a worst-case assumption.

#### Assessment of the risk from the metabolite prothioconazole-desthio

**Table 9.2-3: Screening and first-tier assessment of the acute and long-term/reproductive risk for birds due to the use of CA3642 in cereals – prothioconazole-desthio**

<b>Intended use</b>	Cereals				
<b>Metabolite</b>	Prothioconazole-desthio				
<b>Application rate</b>	2 x 210 g a.s./ha* (14-d interval); BBCH 30-69				
<b>Acute toxicity</b>	>297 mg metabolite/kg bw (worst-case, based on short-term dietary data)				
<b>TER criterion</b>	10				
<b>Crop scenario</b>	<b>Indicator/generic focal species</b>	<b>SV<sub>90</sub></b>	<b>MAF<sub>90</sub></b>	<b>DDD<sub>90</sub> (mg/kg bw/d)</b>	<b>TER<sub>A</sub></b>
Growth stage					
Screening step	Small omnivorous bird	158.8	1.2	40.0	>7.4
<b>Tier 1</b>					
BBCH 30-39	Small omnivorous bird "lark"	12.0	1.2	3.0	>98.2
BBCH ≥40	Small omnivorous bird "lark"	7.2	1.2	1.8	>163.7
Cereals late season seed heads	Small granivorous/insectivorous bird "bunting"	13	1.2	3.27	90.8

<b>Reprod. toxicity</b>	14.8 mg metabolite/kg bw/d				
<b>TER criterion</b>	5				
<b>Crop scenario</b>	<b>Indicator/generic focal species</b>	<b>SV<sub>m</sub></b>	<b>MAF<sub>m</sub> × TWA</b>	<b>DDD<sub>m</sub> (mg/kg bw/d)</b>	<b>TER<sub>LT</sub></b>
Screening step	Small omnivorous bird	64.8	1.4 x 0.53	10.1	<b>1.5</b>
<b>Tier 1</b>					
BBCH 30-39	Small omnivorous bird "lark"	5.4	1.4 x 0.53	0.8	17.6
BBCH ≥40	Small omnivorous bird "lark"	3.3		0.5	28.8
Cereals late season seed heads	Small granivorous/insectivorous bird "bunting"	<b>15</b> <del>12.5</del>	<b>1.4 x 0.53</b>	<b>2.33</b> <del>1.94</del>	<b>6.35</b> <del>5.15</del>

TER values shown in **bold** fall below the relevant trigger. \*It is assumed that 100% of the parent becomes the metabolite (if the molecular weight of the metabolite is considered, the amount of prothioconazole-desthio will be lower, therefore, this assumption represents a very conservative worst case). SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity-to-exposure ratio.

Acceptable acute and long-term risks to birds are concluded for the metabolite prothioconazole-desthio, at the first tier (acute and long-term), for the intended uses CA3642, in cereals.

**Table 9.2-4: Screening and first-tier assessment of the acute and long-term/reproductive risk for birds due to the use of CA3642 in oilseed rape – prothioconazole-desthio**

<b>Intended use</b>	Oilseed rape				
<b>Metabolite</b>	Prothioconazole-desthio				
<b>Application rate</b>	1 x 180 g a.s./ha*; BBCH 14-69				
<b>Acute toxicity</b>	>297 mg metabolite/kg bw (worst-case based on short-term dietary data)				
<b>TER criterion</b>	10				
<b>Crop scenario</b>	<b>Indicator/generic focal species</b>	<b>SV<sub>90</sub></b>	<b>MAF<sub>90</sub></b>	<b>DDD<sub>90</sub> (mg/kg bw/d)</b>	<b>TER<sub>A</sub></b>
Growth stage					
Screening step	Small omnivorous bird	158.8	1.0	28.6	>10.4
<b>Reprod. toxicity</b>	14.8 mg metabolite/kg bw/d				
<b>TER criterion</b>	5				
<b>Crop scenario</b>	<b>Indicator/generic focal species</b>	<b>SV<sub>m</sub></b>	<b>MAF<sub>m</sub> × TWA</b>	<b>DDD<sub>m</sub> (mg/kg bw/d)</b>	<b>TER<sub>LT</sub></b>
Growth stage					
Screening step	Small omnivorous bird	64.8	1.0 x 0.53	6.2	<b>2.4</b>
<b>Tier 1</b>					
BBCH 30-99	Small insectivorous bird "dunnoek"	2.7	1.0 x 0.53	0.3	57.5
BBCH 10-29	Large herbivorous bird "goose"	15.9		1.5	9.8
BBCH 10-29	Small omnivorous bird "lark"	10.9		1.0	14.2
BBCH 30-39	Small omnivorous bird "lark"	3.3		0.3	47.0
BBCH ≥40	Small omnivorous bird "lark"	2.7		0.3	57.5
BBCH 10-19	medium herbivorous/granivorous bird "pigeon"	22.7		2.2	6.8
BBCH 20-29	medium herbivorous/granivorous bird "pigeon"	3.5		0.3	44.3
BBCH 30-39	medium herbivorous/granivorous bird "pigeon"	1.1		0.1	141.0
BBCH ≥40	medium herbivorous/granivorous bird "pigeon"	0.9		0.1	172.4
BBCH 10-19	Small insectivorous bird "wagtail"	5.9		0.6	26.3
BBCH 20-29	Small insectivorous bird "wagtail"	2.8		0.3	55.4

TER values shown in **bold** fall below the relevant trigger. \*It is assumed that 100% of the parent becomes the metabolite (if the molecular weight of the metabolite is considered, the amount of prothioconazole-desthio will be lower, therefore, this assumption represents a very conservative worst case). SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity-to-exposure ratio.

Acceptable acute and long-term risks to birds are concluded for the metabolite, prothioconazole-desthio, at the first tier (acute and long-term), for the intended uses of CA3642, in oilseed rape. No further avian risk assessment or data are necessary.

**Table 9.2-5: Screening and first-tier assessment of the acute and long-term/reproductive risk for birds due to the use of CA3642 in sunflower – prothioconazole-desthio**

<b>Intended use</b>	Sunflower				
<b>Metabolite</b>	Prothioconazole-desthio				
<b>Application rate</b>	1 x 180 g a.s./ha*; BBCH 16-64				
<b>Acute toxicity</b>	>297 mg metabolite/kg bw (worst-case based on short-term dietary data)				
<b>TER criterion</b>	10				
<b>Crop scenario</b>	<b>Indicator/generic focal species</b>	<b>SV<sub>90</sub></b>	<b>MAF<sub>90</sub></b>	<b>DDD<sub>90</sub> (mg/kg bw/d)</b>	<b>TER<sub>A</sub></b>
Growth stage					

Screening step	Small omnivorous bird	158.8	1.0	28.6	>10.4
<b>Reprod. toxicity</b>	14.8 mg metabolite/kg bw/d				
<b>TER criterion</b>	5				
<b>Crop scenario</b>	<b>Indicator/generic focal species</b>	<b>SV<sub>m</sub></b>	<b>MAF<sub>m</sub> × TWA</b>	<b>DDD<sub>m</sub> (mg/kg bw/d)</b>	<b>TER<sub>LT</sub></b>
Growth stage					
Screening step	Small omnivorous bird	64.8	1.0 x 0.53	6.2	<b>2.4</b>
<b>Tier 1</b>					
BBCH 00-19	Small omnivorous bird "lark"	10.9	1.0 x 0.53	1.0	14.2
BBCH 00-19	Small insectivorous bird "wagtail"	11.3		1.1	13.7
BBCH 61-92	Small granivorous/ insectivorous bird "bunting"	10.0		1.0	15.5

TER values shown in **bold** fall below the relevant trigger. \*It is assumed that 100% of the parent becomes the metabolite (if the molecular weight of the metabolite is considered, the amount of prothioconazole-desthio will be lower, therefore, this assumption represents a very conservative worst case). SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity-to-exposure ratio.

Acceptable acute and long-term risks to birds are concluded for the metabolite, prothioconazole-desthio, at the screening step (acute) and at the first tier (long-term), for the intended uses of CA3642, in sunflower. No further avian risk assessment or data are necessary.

#### Assessment of the risk from azoxystrobin

**Table 9.2-6:** Screening and first-tier assessment of the acute and long-term/reproductive risk for birds due to the use of CA3642 in cereals – azoxystrobin

<b>Intended use</b>	Cereals				
<b>Active substance</b>	Azoxystrobin				
<b>Application rate</b>	2 x 210 g a.s./ha (14-day interval); BBCH 30-69				
<b>Acute toxicity</b>	>2000 mg a.s./kg bw				
<b>TER criterion</b>	10				
<b>Crop scenario</b>	<b>Indicator/generic focal species</b>	<b>SV<sub>90</sub></b>	<b>MAF<sub>90</sub></b>	<b>DDD<sub>90</sub> (mg/kg bw/d)</b>	<b>TER<sub>A</sub></b>
Growth stage					
Screening step	Small omnivorous bird	158.8	1.2	40.0	>50.0
<b>Reprod. toxicity</b>	117 mg a.s./kg bw/day				
<b>TER criterion</b>	5				
<b>Crop scenario</b>	<b>Indicator/generic focal species</b>	<b>SV<sub>m</sub></b>	<b>MAF<sub>m</sub> × TWA</b>	<b>DDD<sub>m</sub> (mg/kg bw/d)</b>	<b>TER<sub>LT</sub></b>
Growth stage					
Screening step	Small omnivorous bird	64.8	1.4 x 0.53	10.1	11.6
<b>Tier 1*</b>					
BBCH 30-39	Small omnivorous bird "lark"	5.4	1.4 x 0.53	0.84	139.0
BBCH ≥40	Small omnivorous bird "lark"	3.3		0.51	227.5
<b>Cereals late season seed heads</b>	<b>Small granivorous/insectivorous bird "bunting"</b>	<b>15</b>	<b>1.4 x 0.53</b>	<b>2.33</b>	<b>50.21</b>

TER values shown in **bold** fall below the relevant trigger. SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity-to-exposure ratio. \* The first-tier reproductive assessment is included to allow for combined simultaneous assessments for prothioconazole-desthio and azoxystrobin, which are presented further below.

An acceptable acute and long-term risk to birds is concluded for the active substance azoxystrobin, at the screening step, for intended uses of CA3642, in cereals.

**Table 9.2-76: Screening and first-tier assessment of the acute and long-term/reproductive risk for birds due to the use of CA3642 in oilseed rape – azoxystrobin**

<b>Intended use</b>	Oilseed rape				
<b>Active substance</b>	Azoxystrobin				
<b>Application rate</b>	1 x 180 g a.s./ha; BBCH 14-69				
<b>Acute toxicity</b>	>2000 mg a.s./kg bw				
<b>TER criterion</b>	10				
<b>Crop scenario</b>	<b>Indicator/generic focal species</b>	<b>SV<sub>90</sub></b>	<b>MAF<sub>90</sub></b>	<b>DDD<sub>90</sub> (mg/kg bw/d)</b>	<b>TER<sub>A</sub></b>
<b>Growth stage</b>					
Screening step	Small omnivorous bird	158.8	1	28.6	>70.0
<b>Reprod. toxicity</b>	117 mg a.s./kg bw/day				
<b>TER criterion</b>	5				
<b>Crop scenario</b>	<b>Indicator/generic focal species</b>	<b>SV<sub>m</sub></b>	<b>MAF<sub>m</sub> × TWA</b>	<b>DDD<sub>m</sub> (mg/kg bw/d)</b>	<b>TER<sub>LT</sub></b>
<b>Growth stage</b>					
Screening step	Small omnivorous bird	64.8	1.0 x 0.53	6.18	18.9
<b>Tier 1</b>					
Late, BBCH 30-99	Small insectivorous bird "dunnock"	2.7	1.0 x 0.53	0.3	454.2
Early, BBCH 10-29	Large herbivorous bird "goose"	15.9		1.5	77.1
BBCH 10-29	Small omnivorous bird "lark"	10.9		1.1	107.6
BBCH 30-39	Small omnivorous bird "lark"	3.3		0.31	371.6
BBCH ≥40	Small omnivorous bird "lark"	2.7		0.26	454.2
BBCH 10-19	Medium herbivorous/granivorous bird "pigeon"	22.7		2.17	54.0
BBCH 20-29	Medium herbivorous/granivorous bird "pigeon"	3.5		0.33	350.4
BBCH 30-39	Medium herbivorous/granivorous bird "pigeon"	1.1		0.10	1114.9
BBCH ≥40	Medium herbivorous/granivorous bird "pigeon"	0.9		0.09	1362.7
BBCH 10-19	Small insectivorous bird "wagtail"	5.9		0.56	207.9
BBCH 20-29	Small insectivorous bird "wagtail"	2.8		0.27	438.0

TER values shown in **bold** fall below the relevant trigger. SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity-to-exposure ratio. \*The first-tier reproductive assessment is included to allow for combined simultaneous assessments for prothioconazole-desthio and azoxystrobin, which are presented further below.



**Table 9.2-8: Screening and first-tier assessment of the acute and long-term/reproductive risk for birds due to the use of CA3642 in sunflower – azoxystrobin**

<b>Intended use</b>	Sunflower				
<b>Active substance</b>	Azoxystrobin				
<b>Application rate</b>	1 x 180 g a.s./ha; BBCH 16-64				
<b>Acute toxicity</b>	>2000 mg a.s./kg bw				
<b>TER criterion</b>	10				
<b>Crop scenario</b>	<b>Indicator/generic focal species</b>	<b>SV<sub>90</sub></b>	<b>MAF<sub>90</sub></b>	<b>DDD<sub>90</sub> (mg/kg bw/d)</b>	<b>TER<sub>A</sub></b>
<b>Growth stage</b>					
Screening step	Small omnivorous bird	158.8	1	28.6	>70.0
<b>Reprod. toxicity</b>	117 mg a.s./kg bw/day				
<b>TER criterion</b>	5				
<b>Crop scenario</b>	<b>Indicator/generic focal species</b>	<b>SV<sub>m</sub></b>	<b>MAF<sub>m</sub> TWA</b>	<b>DDD<sub>m</sub> (mg/kg bw/d)</b>	<b>TER<sub>LT</sub></b>
<b>Growth stage</b>					
Screening step	Small omnivorous bird	64.8	1.0 x 0.53	6.18	18.9
<b>Tier 1</b>					
BBCH 00-19	Small omnivorous bird "lark"	10.9	1.0 x 0.53	1.0	112.5
BBCH 00-19	Small insectivorous bird "wagtail"	11.3		1.1	108.5
BBCH 61-92	Small granivorous/ insec-tivorous bird "bunting"	10		1.0	122.6

TER values shown in **bold** fall below the relevant trigger. SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity-to-exposure ratio. \*The first-tier reproductive assessment is included to allow for combined simultaneous assessments for prothioconazole-desthio and azoxystrobin, which are presented further below.

An acceptable acute and long-term risk to birds is concluded for the active substance azoxystrobin, at the screening step, for intended uses of CA3642, in oilseed rape.

#### **zRMS comments:**

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

TER<sub>A</sub> and TER<sub>LT</sub> values for the exposure to prothioconazole and azoxystrobin are above the trigger of 10 and 5 for acute and long-term exposure, indicating acceptable risk for birds for all intended uses.

However, the Tier 1 level calculations of long-term risk for prothioconazole have been added separately for cereals, oilseed rape and sunflower uses as they needed for combined risk assessment.

In reference to acute risk for metabolite JAU 6476-desthio it should be noted that LD<sub>50</sub> >297mg pm/kg value and 100% conversion into JAU-desthio from parent as a worst-case approach was assumed.

Based on this results TER<sub>A</sub> and TER<sub>LT</sub> values for the exposure to prothioconazole-desthio are below the trigger of 10 and 5 for acute and long-term exposure for uses in cereals, indicating an unacceptable risk for birds. Therefore, further refinements has been provided at Tier-1 level and an acceptable risk has been concluded

#### **Commenting period process:**

During commenting period process ( The 1<sup>st</sup> and 2<sup>nd</sup> tour ) it was indicated that:

The TER<sub>A</sub> and TER<sub>LT</sub> calculations for the generic focal species “small granivorous/insectivorous bird” for the intended use in cereals are not correct. The issue of the SV value to be used for this scenario was already discussed (commenting phase on the document of HSE on errors and clarifications) but it was concluded not to change the SV given in the EFSA GD.

The RUD used to calculate the SV presented in Appendix A (4.0 and 4.7 fpr the 90th and 50th percentile) are based on the residues on grains/ears. These residue per unit dose (RUD) values of 13 and 15 for the 90th and mean are considered to be relevant for the scenario being assessed. The values in Table I.1 (27.0 and 12.5 for the 90th and

50th percentile) appear to have been based on the RUD value for seeds from Table 1 Appendix F; these data are not relevant for the scenario being assessed, i.e. the consumption of seeds from the seed head of cereals. The zRMS amended the risk considering this comment for cereals.

The Applicant comments also that for scenario Cereals late season seed heads, the focal species, small granivorous/insectivorous bird with diet consisting of 100% cereal seeds.

Joust Pro intended GAP for cereals is for applications until BBCH 69. BBCH 69 stage corresponds to the end of flowering (all spikelets have completed flowering but some -dehydrated anthers may remain), a stage at which the seed development (ripening) has not been initiated.

Considering the focal species corresponding to the scenario has a diet of 100% of cereal seeds and that at BBCH 69 no cereal seeds have actually been formed.

As further weight of evidence, the DT50 value of metabolite PTZ-desthio in cereals is 3.2 days according to residue decline study in section B.9.1.4.1(b) of the DAR. At the stage of "late season seed heads" the residues of PTZ-desthio will likely not be present. Seeds are not present until at least BBCH 83 (beginning of seed ripening phase). The period from end of flowering (BBCH 69) latest application stage in the GAP and beginning of seed ripening is between 19 and 35 days which correspond to the min-max number of days according to AppDate and focus scenarios Chateaudun and Hamburg respectively (scenarios relevant for Poland) and thus at the time that seeds are formed and present for consumption by birds there will be no residues of PTZ-desthio present.

zRMS agrees that the scenario is not relevant for current use in cereals for PL.

For the other MSs the decision of using this scenario in cereals is left at their national level.

In case of use in oilseed rape and sunflower TER<sub>A</sub> values for the exposure to prothioconazole-desthio are above the trigger of 10 for acute risk assessment, indicating an acceptable risk for birds. In the same time, TER<sub>LT</sub> for at screening step indicating an unacceptable risk for these uses. Therefore, further refinements has been provided at Tier-1 level and an acceptable risk has been concluded.

Overall, acceptable acute and long-term risk may be concluded for birds exposed to prothioconazole, azoxystrobin and metabolite JAU 6476-desthio in CA3642 for all intended uses in the GAP.

## Combined effects

### Acute toxicity

In the absence of avian toxicity studies with the formulated product, a surrogate acute LD<sub>50</sub> value is calculated for the mixture, in line with Appendix B of EFSA/2009/1438, Step 1, assuming additive toxicity of the two active substances, using the following equation:

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

Where:

$X(\text{a.s.}_i)$   $x_{\text{a.s.}_i}$  = the fraction of active substance [i] in the mixture

$LD_{50}(\text{a.s.}_i)$  = the acute toxicity value of the active substance [i]

Combined acute toxicity of prothioconazole and azoxystrobin:

$$LD_{50, \text{Mix}} = \left( \frac{0.5}{2000} + \frac{0.5}{2000} \right)^{-1} = 2000 \text{ mg/kg bw}$$

Since it is assumed that 100% of the parent prothioconazole is quickly transformed to the metabolite prothioconazole-desthio, a calculation of the combined acute toxicity of prothioconazole-desthio and azoxystrobin is additionally performed:

$$LD_{50, \text{Mix}} = \left( \frac{0.5}{297} + \frac{0.5}{2000} \right)^{-1} = 517.2 \text{ mg/kg bw}$$

To allow for comparison of single active substance and mixture toxicity, a "tox per fraction" quotient is calculated for each active substance and compared to the corresponding quotient for the mixture, to demonstrate whether one single active substance/relevant metabolite contributes >90% to the mixture toxicity, using the following equation:

$$\text{tox per fraction (a.s.)} = \frac{\text{LD}_{50}(\text{a.s.}_i)}{X(\text{a.s.}_i)}$$

$$\text{tox per fraction (mix)} = \frac{\text{LD}_{50}(\text{mix})}{\sum_i X(\text{a.s.}_i)}$$

Where:

$X(\text{a.s.}_i)$  = the fraction of active substance  $[i]$  in the mixture

$\text{LD}_{50}(\text{a.s.}_i)$  = the acute toxicity value of the active substance  $[i]$

The “tox per fraction” quotients for prothioconazole, prothioconazole-desthio and azoxystrobin compared to the “tox per fraction” quotient for the mixture, are detailed in the following tables.

**Table 9.2-9<sup>7</sup>:** Comparison of single active substance toxicity and mixture toxicity using “tox per fraction” quotients - prothioconazole and azoxystrobin

Substance	Acute endpoint $\text{LD}_{50}$ [mg a.s./kg bw]	Fraction of each a.s. in mixture	Total fraction of a.s. in mixture	Fraction/ $\text{LD}_{50}$	“Tox per fraction (a.s.)” quotient	“Tox per fraction (mixture)” quotient	Contribution to toxicity [%] <sup>1</sup>
Prothioconazole	>2000	0.5	1	$0.5/2000 = 0.00025$	$2000/0.5 = 4000$	-	50
Azoxystrobin	>2000	0.5		$0.5/2000 = 0.00025$	$2000/0.5 = 4000$	-	50
Calculated mixture	>2000 <sup>2</sup>	-	-	$1/0005 = 2000$	-	$2000/1 = 2000$	-

<sup>1</sup>A contribution  $\geq 90\%$  to mixture toxicity indicates a single driver of toxicity

<sup>2</sup>Calculated mixture toxicity

**Table 9.2-9-1:** Comparison of single active substance toxicity and mixture toxicity using “tox per fraction” quotients - prothioconazole and azoxystrobin.

Substance	Acute endpoint $\text{LD}_{50}$ [mg a.s./kg bw]	Fraction of each a.s. in mixture	Total fraction of a.s. in mixture	Fraction/ $\text{LD}_{50}$	“Tox per fraction (a.s.)” quotient	“Tox per fraction (mixture)” quotient	Contribution to toxicity [%] <sup>1</sup>
Prothioconazole	>1413	0.5	1	$0.5/1413 = 0.000356$	$1413/0.5 = 2830$	-	58
Azoxystrobin	>2000	0.5		$0.5/2000 = 0.00025$	$2000/0.5 = 4000$	-	42
Calculated mixture	>1650.2 <sup>2</sup>	-	-	0.000606	-	1650.2/1	-

<sup>1</sup>A contribution  $\geq 90\%$  to mixture toxicity indicates a single driver of toxicity

<sup>2</sup>Calculated mixture toxicity

**Table 9.2-10<sup>8</sup>:** Comparison of single active substance toxicity and mixture toxicity using “tox per fraction” quotients – prothioconazole-desthio and azoxystrobin

Substance	Acute endpoint $\text{LD}_{50}$ [mg a.s./kg bw]	Fraction of a.s. in mixture	Total fraction of a.s. in mixture	“Tox per fraction (a.s.)” quotient	“Tox per fraction (mixture)” quotient	Contribution to toxicity [%]
Prothioconazole-desthio	>297	0.5	1	$297/0.5 = 594$	-	87
Azoxystrobin	>2000	0.5		$2000/0.5 = 4000$	-	13
Calculated mixture	>517.2 <sup>2</sup>	-	-	-	$517.2/1 = 517.2$	-

<sup>1</sup>A contribution  $\geq 90\%$  to mixture toxicity indicates a single driver of toxicity

<sup>2</sup>Calculated mixture toxicity

The acute 'toxicity per fraction' analysis for both combinations, prothioconazole and azoxystrobin, and prothioconazole-desthio and azoxystrobin, indicates that, based on the assumption of Concentration Addition (CA), no single active substance or metabolite contributes  $\geq 90\%$  to the acute mixture toxicity. Therefore, in accordance with Appendix B of EFSA/2009/1438, Step 4, the predicted LD<sub>50</sub> value for the mixture is used in the risk assessment, below, based on the total application rate of the active substances.

**Table 9.2-11 9:** Screening assessment of the acute risk to birds due to the use of CA3642, in cereals, and oilseed rape and sunflower – combined simultaneous toxicity of prothioconazole + azoxystrobin

<b>Intended use</b>	Cereals, and oilseed rape and sunflower				
<b>Product</b>	CA3642				
<b>Application rate</b>	2 x 420 g total a.s./ha (14-d interval); BBCH 14-69- worst-case cereal GAP used in the screening step				
<b>Acute toxicity</b>	>2000 mg/kg bw (calculated LD <sub>50,MIX</sub> value) >1650.2 mg/kg bw (calculated LD <sub>50,MIX</sub> value with consideration of LD <sub>50</sub> >1413 mg prothioconazole/kg bw)				
<b>TER criterion</b>	10				
<b>Crop scenario</b>	<b>Indicator/generic focal species</b>	<b>SV<sub>90</sub></b>	<b>MAF<sub>90</sub></b>	<b>DDD<sub>90</sub> (mg/kg bw/d)</b>	<b>TER<sub>A</sub></b>
Screening	Small omnivorous bird	158.8	1.2	80.0	>25.0 >20.62

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity-to-exposure ratio.

An acceptable acute risk to birds is concluded for the combined effects of simultaneous exposure to prothioconazole and azoxystrobin, at the screening step, for intended uses of the product, CA3642, in cereals, and oilseed rape and sunflower.

**Table 9.2- 12 10:** Screening and first-tier assessment of the acute risk to birds due to the use of CA3642 in cereals, and oilseed rape and sunflower – combined simultaneous toxicity of prothioconazole-desthio + azoxystrobin

Acute toxicity	>517.2 mg/kg bw (calculated LD <sub>50,Mix</sub> value)				
TER criterion	10				
Cereals					
Intended use	Cereals				
Product	CA3642 (considering combined toxicity of prothioconazole-desthio + azoxystrobin)				
Application rate	2 x 420 g total a.s./ha (14-d interval); BBCH 30-69				
Crop scenario	Indicator/generic focal species	SV <sub>90</sub>	MAF <sub>90</sub>	DDD <sub>90</sub> (mg/kg bw/d)	TER <sub>A</sub>
Growth stage					
Screening step	Small omnivorous bird	158.8	1.2	80.0	>6.5
Tier 1					
BBCH 30-39	Small omnivorous bird "lark"	12.0	1.2	6.0	>85.5
BBCH ≥40	Small omnivorous bird "lark"	7.2		3.6	>142.5
Oilseed rape					
Intended use	Oilseed rape				
Product	CA3642 (considering combined toxicity of prothioconazole-desthio + azoxystrobin)				
Application rate	1 x 360 g total a.s./ha; BBCH 14-69				
Crop scenario	Indicator/generic focal species	SV <sub>90</sub>	MAF <sub>90</sub>	DDD <sub>90</sub> (mg/kg bw/d)	TER <sub>A</sub>
Growth stage					
Screening step	Small omnivorous bird	158.8	1.0	57.2	>9.0
Tier 1					

BBCH 30-99	Small insectivorous bird “dunnock”	7.4	1.0	2.7	>194.1
BBCH 10-29	Large herbivorous bird “goose”	39.0		14.0	>36.8
BBCH 10-29	Small omnivorous bird "lark"	24.0		8.6	>59.9
BBCH 30-39	Small omnivorous bird "lark"	7.2		2.6	>199.5
BBCH ≥40	Small omnivorous bird "lark"	6.0		2.2	>239.4
BBCH 10-19	medium herbivorous/granivorous bird "pigeon"	55.6		20.0	>25.8
BBCH 20-29	medium herbivorous/granivorous bird "pigeon"	4.0		1.4	>359.2
BBCH 30-39	medium herbivorous/granivorous bird "pigeon"	2.4		0.9	>598.6
BBCH ≥40	medium herbivorous/granivorous bird "pigeon"	2.0		0.7	>718.3
BBCH 10-19	Small insectivorous bird "wagtail"	10.9		3.9	>131.8
BBCH 20-29	Small insectivorous bird "wagtail"	7.7		2.8	>186.6
Sunflower					
Intended use	Sunflower				
Product	CA3642 (considering combined toxicity of prothioconazole-desthio + azoxystrobin), BBCH 16-64				
Application rate	1 x 360 g total a.s./ha*				
Crop scenario Growth stage	Indicator/generic focal species	SV <sub>90</sub>	MAF <sub>90</sub>	DDD <sub>90</sub> (mg/kg bw/d)	TER <sub>A</sub>
Screening step	Indicator/generic focal species	SV <sub>90</sub>	1.0	57.2	>9.0
Tier 1					
BBCH 00-19	Small omnivorous bird "lark"	24	1.0	8.6	>59.8
BBCH 00-19	Small insectivorous bird "wagtail"	26.8		9.6	>53.6
BBCH 61-92	Small granivorous/ insectivorous bird "bunting"	21.7		7.8	>66.2

TER values shown in bold fall below the relevant trigger. SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity-to-exposure ratio. \*It is assumed that 100% of the parent becomes the metabolite (if the molecular weight of the metabolite is considered, the amount of prothioconazole-desthio will be lower, therefore, this assumption represents a very conservative worst case).

An acceptable acute risk to birds is concluded for the combined effects of the metabolite prothioconazole-desthio and active substance azoxystrobin, for the intended uses of the product, CA3642, in cereals, and oilseed rape and sunflower.

#### **zRMS comments:**

##### Combined acute risk assessment

It is noted that for the combined acute risk assessment from exposure of active substances the Applicant selected acute toxicity endpoints LD<sub>50</sub>> 2000 mg a.s./kg for both active compounds (prothioconazole and azoxystrobin), which is considered generally acceptable by zRMS although that for the a.s.- prothioconazole lower endpoint from short-term study with LD<sub>50</sub> of 1413 mg p.m./kg is available.

In zRMS's opinion as no treatment related mortalities were observed in the short-term toxicity study for the a.s prothioconazole indicating that the dietary exposure has not resulted with increased mortality of tested birds and the acute LD<sub>50</sub>>2000 kg a.s./kg bw is sufficiently protective to use in the risk assessment.

However, for some MSs that prefers endpoint from dietary studies with LD<sub>50</sub>>1413 mg prothioconazole /kg bw in the acute combined risk assessment, the relevant calculations have been added by zRMS in the Tables 9.2-9-1 and 9.2-11.

In case of combined acute risk assessment considering combined toxicity of prothioconazole-desthio + azoxystrobin, the lowest endpoints for each compound have been used.

Overall, TER<sub>A</sub> values for acute combined risk assessment is above the trigger of 10 indicating an acceptable for all proposed uses in the GAP.

### Reproductive toxicity

EFSA/2009/1438, Appendix B, step 3 states:

‘...it is currently not recommended to consider the use of predicted toxicity [from more than one a.s.] values as surrogates in the [reproduction] risk assessment. Although it would be, in principle, possible to apply the concept of dose or concentration additivity of toxicity also to effect data for biological endpoints from long-term and reproductive toxicity testing, reliable results would only be expected for combinations of EC<sub>x</sub> values for the same biological endpoint. Moreover, additional bias would be introduced in the calculations if the values applied do not represent EC<sub>x</sub> values with defined x, but NOAELs, since these may represent varying risk or response levels for different compounds, depending on dose-spacing.’ However, for completeness a check of the worst-case chronic risk is provided below:

**Table 9.2.13 11: Comparison of single active substance toxicity and mixture toxicity using “tox per fraction” quotients – prothioconazole-desthio and azoxystrobin**

Substance	NOEL [mg a.s./kg bw]	Fraction of a.s. in mixture	Total fraction of a.s. in mixture	“Tox per fraction (a.s.)” quotient	“Tox per fraction (mixture)” quotient	Contribution to toxicity [%] <sup>1</sup>
Prothioconazole- desthio	14.8	0.5	1	14.8/0.5 = 29.6	-	89
Azoxystrobin	117	0.5		117/0.5 = 234	-	11
Calculated mixture	26.3 <sup>2</sup>	-	-	-	26.3	-

<sup>1</sup>A contribution of ≥90% to mixture toxicity indicates a single driver of toxicity; <sup>2</sup>Calculated mixture toxicity

The above toxic unit comparison demonstrates there is formally no clear driver (i.e. >90% contribution by one active substance to the mixture toxicity) of the reproductive toxicity to birds. However, the metabolite prothioconazole-desthio, contributes with 89% to the reproductive mixture toxicity, which is very close to the 90% cut-off. Additionally, it is very quickly formed from prothioconazole and is therefore likely to drive the reproductive risk assessment, based on its toxicity (NOEL = 14.8 mg/kg bw/day), relative to azoxystrobin (NOEL = 117 mg/kg bw/day). The reproductive risk assessments performed for prothioconazole-desthio alone assumed worst-case exposure (i.e., that 100% of the parent compound, prothioconazole, has transformed into the metabolite). Even with this assumption, the first-tier risk assessment for prothioconazole-desthio, for all proposed uses of CA3642, are acceptable (TER<sub>LT</sub> ≥ 17.6 for cereals and TER<sub>LT</sub> ≥ 6.8 for oilseed rape, TER<sub>LT</sub> ≥ 13.7 for sunflower). Therefore, it is considered that there should be no unacceptable risk, even when considering the combined toxicity of prothioconazole-desthio and azoxystrobin. This is confirmed when the value of the assessment factor divided by the TER, for each compound, is calculated and summed up, as noted in the bird and mammal section on combined risk assessment (page 36) of the GB’s HSE ‘Formulation studies and combined risk assessment in ecotoxicology’ (January, 2022). These calculations are presented in the table below:

**Table 9.2-14 12: Combined long-term risk assessment for birds due to the use of CA3642 in cereals and oilseed rape – combined simultaneous toxicity of prothioconazole-desthio + azoxystrobin**

Crop	Scenario	TER <sub>LT</sub> *		AF	ΣAF/TER
		Prothioconazole-desthio	Azoxystrobin		
Cereals	BBCH 30-39 ‘lark’	17.6	139	5	0.32
Oilseed rape	BBCH 10-19 ‘pigeon’	6.8	54	5	0.83
Sunflower	BBCH 10-19 ‘wagtail’	13.7	108.5	5	0.41

\*The lowest/worst-case, first-tier TER value is used in the assessment, in accordance with the HSE (Pers. Comm., 06/07/22, as there is an error in the guidance document “Formulation studies and combined risk assessment in ecotoxicology”).

As the value of ΣAF/TER is less than 1, for the proposed uses of CA3642 on cereals, and on oilseed rape and sunflower, no unacceptable risk from the combined toxicity of the metabolite prothioconazole-desthio and azoxystrobin is expected to birds, even when assuming the most conservative toxicity endpoints and

most conservative exposure.

#### zRMS comments:

##### Combined long-term toxicity

zRMS agrees that for the approach assuming dose additivity of the active substances, reliable results would only be expected for combinations of effect levels with a defined x, e.g. a LD<sub>50</sub>, but not for NOELs since the latter effect indicators may represent varying risk or response levels for different compounds depending on dose-spacing according to GD B&M, EFSA Journal 2009; 7(12): 1438, Appendix B. Therefore, for the risk assessment based on long-term effects it is not recommended to consider the use of predicted toxicity values.

Therefore, the calculated NOEL<sub>mix</sub> provided by the Applicant was not considered by zRMS in the current risk assessment.

In the same time, it should be stressed ~~out that~~ (TER<sub>mix</sub>) approach for combined risk assessment is agreed by among of MSs in CZ and for this reason, the respective zRMS's calculations of it based on the lowest TER<sub>LT</sub> values for each compound are presented below **despite of the TER<sub>combi</sub> presented by the Applicant in the Table 9.2-14.**

**The combined risk assessment presented by zRMS (full presence 100% prothioconazole and 100% prothioconazole-desthio occurring simultaneously without taking into account the degradation of the parent molecule for the formation of the metabolite) is an unrealistic extreme worst case and overestimates the exposure to prothioconazole and prothioconazole-desthio**

Cereals						Σ1/TER	Σ1/TER <sup>-1</sup>	Trigger
Azoxystrobin		Prothioconazole		JAU 6476-desthio				
139	0.0072	92.8	0.01	17.6	0.057	0.742	13.5	5
			0.02	5.2	0.19	0.21	4.8	
50.21*	0.02	33.5	0.029	6.35	0.15	1.17	5.0	5

<sup>1)</sup> Lowest Tier 1 TER<sub>LT</sub>

**\* small granivorous/insectivorous bird" scenario**

The TER<sub>mix</sub> for cereals is above ~~closed above to~~ trigger value of 5., indicating an acceptable risk It is ~~considered as acceptable by zRMS as for metabolite JAU 6476 desthio~~ the worst-case scenario ~~was assumed~~ in TER<sub>LT</sub> calculation at tier 1 (100% conversion from parent and the lowest LD<sub>50</sub> value from dietary study).

Oilseed rape						Σ1/TER	Σ1/TER <sup>-1</sup>	Trigger
Azoxystrobin		Prothioconazole		JAU 6476-desthio				
54	0.0185	36.62	0.027	6.8	0.147	0.19	5.26	5

<sup>1)</sup> Lowest Tier 1 TER<sub>LT</sub>

Sunflower						Σ1/TER	Σ1/TER <sup>-1</sup>	Trigger
Azoxystrobin		Prothioconazole		JAU 6476-desthio				
108.5	0.0092	70.9	0.014	13.7	0.073	0.0962	10.39	5
13.7	0.073	108.5	0.0092	14.2	0.07	0.15	6.66	

<sup>1)</sup> Lowest Tier 1 TER<sub>LT</sub>

The TER<sub>mix</sub> for OSR and sunflower is also above trigger value of 5, indicating an acceptable risk

Overall, the combined long-term risk assessment to birds is considered as acceptable for all proposed uses in the GAP.

### 9.2.2.2 Higher-tier risk assessment

No higher tier risk assessments are 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 CA3642 is not intended to be applied on leafy vegetables forming heads or crop plants with comparable water-collecting structures, at principal growth stage 4 or later, the leaf scenario does not have to be considered.

#### Puddle scenario

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

With a  $K_{fOC}$  of 1765 L/kg (EFSA Sci. Report. 2007; 106, 1-98), prothioconazole belongs to the group of more sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group cereals also covers the risk for birds from all other intended uses (see 9.1.2).

Effective application rate (g/ha)	420*	Quotient
Acute toxicity (mg/kg bw)	>2000 / 1413 <sup>a</sup>	<0.21 / 0.297 <sup>a</sup>
Reproduction toxicity (mg/kg bw/d)	78	5.38

\*Total maximum seasonal application rate, for worst-case use on cereals used.

<sup>a</sup>: Acute toxicity value based on worst case 5 dietary endpoint

Since the quotients are less than the trigger of 3000, quantitative risk assessments for prothioconazole in drinking water are not required.

With a  $K_{fOC}$  of 573.5 (geometric mean; see dRR B8, Table 8.5-3), prothioconazole-desthio belongs to the group of more sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group cereals also covers the risk for birds from all other intended uses (see 9.1.2).

Effective application rate (g/ha)	420* <sup>#</sup>	Quotient
Acute toxicity (mg/kg bw)	>297	<1.41
Reproduction toxicity (mg/kg bw/d)	14.8	28.38

\*Total maximum seasonal application rate of the a.s., for worst-case use on cereals used. <sup>#</sup>It is assumed that 100% of the parent becomes the metabolite.

Since the quotients are less than the trigger of 3000, quantitative risk assessments for prothioconazole-desthio in drinking water are not required.

Since the ratio of effective application rate to relevant endpoint does not exceed the trigger of 3000 for

With a  $K_{fOC}$  of 392 L/kg (geometric mean; see dRR B8, Table 8.5-5), azoxystrobin belongs to the group of less sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group cereals also covers the risk for birds from all other intended uses (see 9.1.2).



<b>Effective application rate (g/ha)</b>	420*	<b>Quotient</b>
<b>Acute toxicity (mg/kg bw)</b>	>2000	<0.21
<b>Reproduction toxicity (mg/kg bw/d)</b>	117	3.58

\*Total maximum seasonal application rate of the a.s. for worst-case use on cereals used.

Since the quotients are less than the trigger of 50, quantitative risk assessments for azoxystrobin in drinking water are not required.

Accordingly, an acceptable acute and reproductive risk for birds is indicated for exposure towards prothioconazole, prothioconazole-desthio and azoxystrobin via drinking water for the proposed uses of CA3642.

#### zRMS comments:

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

The calculations for active substances and prothioconazole-desthio has been validated by zRMS.

In order to apply consistent approach, the drinking water risk assessment was performed also for metabolite JAU 6476-S-methyl and is presented below. zRMS's calculations were performed with assumption of 10 times toxicity of the parent.

JAU 6476-S-methyl effective application rate 1 x 420 g/ha

Acute toxicity (mg/kg bw)	>200/143	quotient = 2.1/2.93	Trigger: 3000
Reprod. toxicity (mg/kg bw/d)	7.8	quotient=53.50	

The evaluation of the risk resulting from uptake of contaminated water in Puddle scenario was not required since ratio between effective application rate and endpoint relevant for acute risk and long-term assessment is <3000.

#### 9.2.2.4 Effects of secondary poisoning

The log P<sub>OW</sub> values for prothioconazole, prothioconazole-desthio, and prothioconazole-S-methyl are 4.16, 3.04, and 4.19, respectively, and thus exceed the trigger value triggering a secondary poisoning risk assessment. The log P<sub>OW</sub> value of 1,2,5-triazole is <3 and, therefore, bioconcentration is not expected for this metabolite. An assessment is not triggered for azoxystrobin (log K<sub>OW</sub> = 2.5) or its metabolites (EFSA Journal 2010; 8(4):1542).

#### Risk assessment for earthworm-eating birds via secondary poisoning

According to EFSA/2009/1438, the risk to 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.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group winter oilseed rape (worst-case soil PEC value,) also covers the risk to birds from all other intended uses.

**Table 9.2-15**: Assessment of the risk for earthworm-eating birds due to exposure to prothioconazole via bioaccumulation in earthworms (secondary poisoning) for the intended use of CA3642 in cereals, and oilseed rape and sunflower.

Parameter	Prothioconazole	Comments
<b>PEC<sub>Soil</sub> (mg/kg soil)</b>	0.192 0.144	Worst-case initial PEC <sub>Soil</sub> for sunflower (see Section 8.7) Worst case initial PEC <sub>Soil</sub> for winter oilseed rape (see Section 8.7, autumn application)
<b>log P<sub>OW</sub> / P<sub>OW</sub></b>	4.16 / 14454	Worst-case log P <sub>OW</sub> value at pH 4; EFSA Sci. Report. 2007; 106, 1-98
<b>K<sub>oc</sub></b>	1765	EFSA Sci. Report. 2007; 106, 1-98

Parameter	Prothioconazole	Comments
foc	0.02	Default
BCF <sub>Worm</sub>	4.94	$BCF_{Worm/Soil} = (PEC_{Worm,ww}/PEC_{Soil,dw}) = (0.84 + 0.012P_{ow}) / foc \times K_{oc}$
PEC <sub>Worm</sub>	0.95 0.711	$PEC_{Worm} = PEC_{Soil} \times BCF_{Worm/Soil}$
Daily dietary dose (mg/kg bw/d)	0.995 0.747	$DDD = PEC_{Worm} \times 1.05$
NOEL (mg/kg bw/d)	78.0	EFSA Sci. Report. 2007; 106, 1-98
TER <sub>LT</sub>	78 104.5	Acceptable risk demonstrated

TER values shown in **bold** fall below the relevant trigger (<5).

**Table 9.2-16 14:** Assessment of the risk for earthworm-eating birds due to exposure to prothioconazole-desthio via bioaccumulation in earthworms (secondary poisoning) for the intended use of CA3642 in cereals ,and oilseed rape and sunflower

Parameter	Prothioconazole-desthio	Comments
PEC <sub>Soil</sub> (mg/kg soil)	0.0994 0.0746	Worst-case initial PEC <sub>Soil</sub> for sunflower (see Section 8.7) Worst-case initial PEC <sub>Soil</sub> for winter oilseed rape (see Section 8.7, autumn application)
log Pow / Pow	3.04 / 1100	Worst-case log Pow value at pH 4; EFSA Sci. Report. 2007; 106, 1-98
K <sub>oc</sub>	573.5	Geometric mean; see dRR B8, Environmental Fate, Table 8.5-3
foc	0.02	Default
BCF <sub>Worm</sub>	1.22	$BCF_{Worm/Soil} = (PEC_{Worm,ww}/PEC_{Soil,dw}) = (0.09 + 0.0746 P_{ow}) / foc \times K_{oc}$
PEC <sub>Worm</sub>	0.12 0.09	$PEC_{Worm} = PEC_{Soil} \times BCF_{Worm/Soil}$
Daily dietary dose (mg/kg bw/d)	0.128 0.096	$DDD = PEC_{Worm} \times 1.05$
NOEL (mg/kg bw/d)	14.8	EFSA Sci. Report. 2007; 106, 1-98
TER <sub>LT</sub>	115.9 154.16	Acceptable risk demonstrated

TER values shown in **bold** fall below the relevant trigger (<5).

**Table 9.2-17 15:** Assessment of the risk for earthworm-eating birds due to exposure to prothioconazole-S-methyl via bioaccumulation in earthworms (secondary poisoning) for the intended use of CA3642 in cereals, and oilseed rape and sunflower

Parameter	Prothioconazole-S-methyl	Comments
PEC <sub>Soil</sub> (mg/kg soil)	0.0292 0.0219	Worst-case initial PEC <sub>Soil</sub> for sunflower, using FOCUS 1997 (see Section 8.7) Worst-case initial PEC <sub>Soil</sub> for winter oilseed rape, using FOCUS 1997 (see Section 8.7)
log Pow / Pow	4.19 / 15500	EFSA Sci. Report. 2007; 106, 1-98
K <sub>oc</sub>	2525.9	Table 8.5-2 for SEU dRR part B8 (CA3642), based on geometric mean
foc	0.02	Default
BCF <sub>Worm</sub>	3.7	$BCF_{Worm/Soil} = (PEC_{Worm,ww}/PEC_{Soil,dw}) = (0.08 + 0.0219P_{ow}) / foc \times K_{oc}$
PEC <sub>Worm</sub>	0.11	$PEC_{Worm} = PEC_{Soil} \times BCF_{Worm/Soil}$

Parameter	Prothioconazole-S-methyl	Comments
	0.08	
Daily dietary dose (mg/kg bw/d)	0.113 0.085	DDD = PEC <sub>Worm</sub> × 1.05
NOEL (mg/kg bw/d)	78.0/10 = 7.8	Conservative estimate of parent NOEL/10
TER <sub>LT</sub>	68.8 91.8	Acceptable risk demonstrated

TER values shown in **bold** fall below the relevant trigger (<5).

Using worst-case, conservative inputs and initial PEC<sub>Soil</sub> values (as opposed to 21-d TWA values), acceptable risks (i.e. >trigger of 5) are concluded for earthworm-eating birds, due to exposure to prothioconazole, prothioconazole-desthio, and prothioconazole-S-methyl, with large margins of safety.

### Risk assessment for fish-eating birds via secondary poisoning

According to EFSA/2009/1438, the risk to 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 for aquatic organisms and BCF values for prothioconazole, prothioconazole-desthio, and prothioconazole-S-methyl.

**Table 9.2-18~~16~~:** Assessment of the risk for fish-eating birds due to exposure to prothioconazole via bioaccumulation in fish (secondary poisoning) for the intended use of CA3642 in cereals ~~and~~ oilseed rape and sunflower

Parameter	Prothioconazole	Comments
PEC <sub>sw</sub> (mg/L)	0.02281	Worst-case FOCUS Step 1 PEC <sub>sw</sub> for 2 x 210 g a.s./ha in cereals (Table 8.9-16 and Table 8.9-17)
BCF <sub>Fish</sub>	19.7	EFSA Sci. Report. 2007; 106, 1-98
BMF	Not applicable	biomagnification factor (relevant to BCF ≥ 2000)
PEC <sub>Fish</sub>	0.45	PEC <sub>Fish</sub> = PEC <sub>Water</sub> × BCF <sub>Fish</sub>
Daily dietary dose (mg/kg bw/d)	0.071	DDD = PEC <sub>Fish</sub> × 0.159
NOEL (mg/kg bw/d)	78	EFSA Sci. Report. 2007; 106, 1-98
TER <sub>LT</sub>	1091.71	Acceptable risk demonstrated

TER values shown in **bold** fall below the relevant trigger (<5).

**Table 9.2-19~~17~~:** Assessment of the risk for fish-eating birds due to exposure to prothioconazole-desthio via bioaccumulation in fish (secondary poisoning) for the intended use of CA3642 in cereals ~~and~~ oilseed rape and sunflower

Parameter	Prothioconazole-desthio	Comments
PEC <sub>sw</sub> (mg/L)	0.08228	Worst-case FOCUS Step 1 PEC <sub>sw</sub> for 2 x 210 g a.s./ha in cereals (Table 8.9-31 and 8.9-31)
BCF <sub>Fish</sub>	65	EFSA Sci. Report. 2007; 106, 1-98
BMF	Not applicable	biomagnification factor (relevant to BCF ≥ 2000)
PEC <sub>Fish</sub>	5.35	PEC <sub>Fish</sub> = PEC <sub>Water</sub> × BCF <sub>Fish</sub>
Daily dietary dose (mg/kg bw/d)	0.850	DDD = PEC <sub>Fish</sub> × 0.159
NOEL (mg/kg bw/d)	14.8	EFSA Sci. Report. 2007; 106, 1-98
TER <sub>LT</sub>	17.40	Acceptable risk demonstrated

TER values shown in **bold** fall below the relevant trigger (<5).

**Table 9.2-2018:** Assessment of the risk for fish-eating birds due to exposure to prothioconazole-S-methyl via bioaccumulation in fish (secondary poisoning) for the intended use of CA3642 in cereals and oilseed rape and sunflower

Parameter	Prothioconazole-S-methyl	Comments
PEC <sub>sw</sub> (mg/L)	<b>0.00824</b> 0.03365 0.00487	Worst-case FOCUS Step 1 PEC <sub>sw</sub> for 2 x 210 g a.s./ha in cereals (Table 8.9- 26 ) <sup>24)</sup>
BCF <sub>Fish</sub>	5.08	Estimated with EPI Suite*
BMF	Not applicable	biomagnification factor (relevant to BCF ≥ 2000)
PEC <sub>Fish</sub>	<b>0.041</b> 0.17 0.02	PEC <sub>Fish</sub> = PEC <sub>Water</sub> × BCF <sub>Fish</sub>
Daily dietary dose (mg/kg bw/d)	<b>0.0065</b> 0.027 0.004	DDD = PEC <sub>Fish</sub> × 0.159
NOEL (mg/kg bw/d)	7.8	Conservative estimate of parent NOEL/10
TER <sub>LT</sub>	<b>1200</b> 288.88 1982.92	Acceptable risk demonstrated

TER values shown in bold fall below the relevant trigger. \*US EPA. 2021. Estimation Programs Interface Suite™ for Microsoft® Windows, v 4.11. United States Environmental Protection Agency, Washington, DC, USA.

Using worst-case, conservative inputs and FOCUS Step-1 initial PEC<sub>sw</sub> values, acceptable risks are concluded for fish-eating birds, due to exposure to prothioconazole, prothioconazole-desthio, and prothioconazole-S-methyl, with large margins of safety (TER values well above the trigger of 5).

#### **zRMS comments:**

The Applicants' approach in evaluation of the risk of secondary poisoning is in line with EFSA (2009). Compounds selected for this assessment are agreed by the zRMS. Evaluation was not triggered for remaining metabolites of active substance due to their log Pow <3.

Overall, acceptable risk of secondary exposure from all relevant compounds could be concluded for birds.

### **9.2.2.5 Biomagnification in terrestrial food chains**

Not relevant, as mammalian toxicity and goat metabolism data did not show the potential for accumulation of prothioconazole (EFSA Scientific Report. 2007, 106, 1-98) nor azoxystrobin (EFSA Journal, 2010; 8(4):1542), respectively.

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

Not relevant, as the product is proposed for use as foliar spray.

### **9.2.4 Overall conclusions**

Acceptable risks to birds from dietary, drinking water and secondary poisoning exposure routes can be concluded for all intended uses of CA3642, at the screening or first-tier assessments. No unacceptable risks to birds from the combined toxicity of prothioconazole and azoxystrobin or prothioconazole-desthio and azoxystrobin is also concluded.

The additional intended minor uses of CA3642 in sunflower, flax, linseeds, poppy, mustard and Gold of Pleasure are considered to be covered by the risk envelope for use in spring and winter oilseed rape respectively.

## 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 prothioconazole, azoxystrobin and their relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents as well as in Section 6 (Mammalian Toxicology) of this report.

Effects on mammals exposed to CA3642, were not evaluated as part of the EU assessment of prothioconazole or azoxystrobin. No new data are submitted with this application.

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

mammals				
Species	Substance	Exposure system	Results	Reference
Active substance				
Rat	Prothioconazole	Acute, oral	LD <sub>50</sub> >6200 mg/kg bw	EFSA Sci. Report. 2007; 106, 1-98
		Reproductive toxicity, dietary multiple-generation study	NOAEL = 95.6 mg/kg bw/d  (reduced pup weight gain, reduced litter size)	
Metabolite				
Mouse	Prothioconazole-desthio	Acute, oral	LD <sub>50</sub> = 2235 mg/kg bw (males)	EFSA Sci. Report. 2007; 106, 1-98
Rat		Reproductive, dietary, multiple-generation study	NOAEL = 10 mg/kg bw/d (reproductive effects)	

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

Species	Substance	Exposure system	Results	Reference
<b>Active substance</b>				
Rat	Azoxystrobin	Acute, oral	<b>LD<sub>50</sub> &gt;5000 mg/kg bw</b>	EFSA Journal 2010; 8(4):1542
Rat		Reproductive toxicity	<b>NOAEL = 32 mg/kg bw/d</b>	

#### **zRMS comments:**

Mammalian toxicity data for azoxystrobin, prothioconazole and prothioconazole metabolite JAU 6476-desthio provided in Tables 9.2-1 and 9.2-2 above were confirmed by zRMS that they are in line with EU agreed endpoints reported in EFSA-Journal 2010; 8(4):1542 and EFSA Scientific Report (2007) 106, respectively.

#### 9.3.1.1 Justification for new endpoints

Not relevant. No new mammalian toxicity data are submitted.

### 9.3.2 Risk assessment for spray applications

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

The mammalian risk assessment has been carried out considering the critical GAP:

- Winter or spring cereals: 2 x 1.4 L product/ha, corresponding to 2 x 210 g prothioconazole/ha and 2 x 210 g azoxystrobin/ha (minimum interval of 14 days between applications); BBCH 30-61.

- Oilseed rape: 1 x 1.2 L product/ha, corresponding to 1 x 180 g prothioconazole/ha and 1 x 180 g azoxystrobin/ha; BBCH 14-18 (autumn) and BBCH 20-69 (spring).
- Sunflower: 1 x 1.2 L product/ha, corresponding to 1 x 180 g prothioconazole/ha and 1 x 180 g azoxystrobin/ha; BBCH 16-64.

To achieve a concise risk assessment, the risk envelope approach has been used for flax 1 x 1.2 L product/ha, at BBCH 33-51) and for ~~sunflower~~, linseeds, poppy, mustard and Gold of Pleasure (1 x 1.2 L product/ha, either in autumn, at BBCH 14-18, or spring, at BBCH 20-69). These crops are all listed within the oilseed rape crop group in the EFSA Guidance Document on Risk Assessment for Birds and Mammals (see Table 5, EFSA Journal 2009; 7(12): 1438). Therefore, the assessment for the use group oilseed rape also covers the risk for mammals from all other intended uses in ~~sunflower~~, flax, linseed, poppy, mustard and Gold of Pleasure (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.

#### Assessment of the risk from prothioconazole

**Table 9.3-2: Screening assessment of the acute and long-term/reproductive risk and Tier 1 long-term risk for mammals due to the use of CA3642 in cereals and oilseed rape and sunflower–prothioconazole**

<b>Intended use</b>	Cereals, <del>and</del> oilseed rape and sunflower				
<b>Active substance</b>	Prothioconazole				
<b>Application rate</b>	2 x 210 g a.s./ha (14-d interval) – worst-case cereal GAP used in screening step				
<b>Acute toxicity</b>	>6200 mg a.s./kg bw				
<b>TER criterion</b>	10				
<b>Crop scenario</b>	<b>Indicator/generic focal species</b>	<b>SV<sub>90</sub></b>	<b>MAF<sub>90</sub></b>	<b>DDD<sub>90</sub> (mg/kg bw/d)</b>	<b>TER<sub>A</sub></b>
<b>Growth stage</b>					
Screening step	Small herbivorous mammal	118.4	1.2	29.8	>207.8
<b>Reprod. Toxicity</b>	95.6 mg a.s./kg bw/d				
<b>TER criterion</b>	5				
<b>Crop scenario</b>	<b>Indicator/generic focal species</b>	<b>SV<sub>m</sub></b>	<b>MAF<sub>m</sub> × TWA</b>	<b>DDD<sub>m</sub> (mg/kg bw/d)</b>	<b>TER<sub>LT</sub></b>
<b>Growth stage</b>					
Screening step	Small herbivorous mammal	48.3	1.4 x 0.53	7.5	12.7
<b>Tier 1, cereals*, 2 x 210 g a.s./ha (14-d interval)</b>					
BBCH ≥20	Small insectivorous mammal “shrew”	1.9	1.4 x 0.53	0.3	318.66
BBCH ≥40	Small herbivorous mammal “vole”	21.7	1.4 x 0.53	3.38	28.28
BBCH 30-39	Small omnivorous mammal “mouse”	3.9	1.4 x 0.53	0.60	159.33
BBCH ≥40	Small omnivorous mammal “mouse”	2.3	1.4 x 0.53	0.36	265.5 <del>26.55</del>
<b>Tier 1, oilseed rape* 1 x 180 g a.s./ha</b>					
BBCH 10-19	Small insectivorous mammal “shrew”	4.2	1 x 0.53	0.40	239.0
BBCH≥20	Small insectivorous mammal “shrew”	1.9	1 x 0.53	0.18	531.1
BBCH≥40	Small herbivorous mammal “vole”	18.1	1 x 0.53	1.7	56.23
All season	Large herbivorous mammal “lagomorph”	14.3	1 x 0.53	1.4	68.28 <del>70.3</del>

BBCH 10-29	Small omnivorous mammal "mouse"	7.8	1 x 0.53	0.74	129.2
BBCH 30-39	Small omnivorous mammal "mouse"	2.3	1 x 0.53	0.22	434.54
BBCH ≥ 40	Small omnivorous mammal "mouse"	1.9	1 x 0.53	0.18	531.1
<b>Tier 1, sunflower 1 x 180 g a.s./ha</b>					
BBCH 10-19	Small insectivorous mammal "shrew"	4.2	1.0 x 0.53	0.4	239.0
BBCH ≥ 20	Small insectivorous mammal "shrew"	1.9		0.2	478.0
BBCH ≥ 40	Small herbivorous mammal "vole"	18.1		1.7 0.43*	56.23 222.32**
BBCH 10-19	Large herbivorous mammal "lagomorph"	14.3		1.4	68.28
BBCH 20-39	Large herbivorous mammal "lagomorph"	7.2		0.7	136.6
BBCH ≥ 40	Large herbivorous mammal "lagomorph"	3.6		0.3	318.6
BBCH 10-19	Small omnivorous mammal "mouse"	7.8 3.9		0.74 0.4	129.2 239.0
BBCH 20-39	Small omnivorous mammal "mouse"	3.9 1.9		0.37 0.2	258.37 478.0
BBCH ≥ 40	Small omnivorous mammal "mouse"	1.9 7.8		0.18 0.7	531.1 136.6

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

\*added by zRMS for combined long-term risk assessment

\*\*DF = 0.25

Acceptable acute and long-term risks to mammals are concluded for the active substance prothioconazole, at the screening step for intended uses of CA3642 in cereals and oilseed rape and sunflower. No further mammalian risk assessment is necessary.

For the screening and first-tier risk assessment for the metabolite prothioconazole-desthio (see tables below), it has been assumed that 100% of the parent becomes the metabolite, so the daily dietary doses of the metabolite are the same as for the active substance. This is a worst-case scenario.

**Table 9.3-3: Screening and first tier assessment of the acute and long-term/reproductive risk for mammals due to the use of CA3642, in cereals – prothioconazole-desthio**

<b>Intended use</b>	Cereals				
<b>Metabolite</b>	Prothioconazole-desthio				
<b>Application rate</b>	2 x 210 g a.s./ha* (14-d interval); BBCH 30-69				
<b>Acute toxicity</b>	2235 mg metabolite/kg bw				
<b>TER criterion</b>	10				
<b>Crop scenario</b>	<b>Indicator/generic focal species</b>	<b>SV<sub>90</sub></b>	<b>MAF<sub>90</sub></b>	<b>DDD<sub>90</sub> (mg/kg bw/d)</b>	<b>TER<sub>A</sub></b>
<b>Growth stage</b>					
Screening step	Small herbivorous mammal	118.4	1.2	29.8	74.9
<b>Reprod. Toxicity</b>	10 mg metabolite/kg bw/d				
<b>TER criterion</b>	5				
<b>Crop scenario</b>	<b>Indicator/generic focal species</b>	<b>SV<sub>m</sub></b>	<b>MAF<sub>m</sub> × TWA</b>	<b>DDD<sub>m</sub> (mg/kg bw/d)</b>	<b>TER<sub>LT</sub></b>
<b>Growth stage</b>					

Screening step	Small herbivorous mammal	48.3	1.4 x 0.53	7.5	<b>1.3</b>
<b>Tier 1</b>					
BBCH $\geq 20$	Small insectivorous mammal “shrew”	1.9	1.4 x 0.53	0.296	33.8
BBCH $\geq 40$	Small herbivorous mammal “vole”	21.7		3.381	<b>3.0</b>
BBCH 30-39	Small omnivorous mammal “mouse”	3.9		0.608	16.5
BBCH $\geq 40$	Small omnivorous mammal “mouse”	2.3		0.358	27.9

TER values shown in **bold** fall below the relevant trigger. \*It is assumed that 100% of the parent becomes the metabolite. SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity-to-exposure ratio.

The acute risk to mammals is shown to be acceptable at the screening stage (TER values > trigger 10) for the proposed use of CA3642, in cereals.

The reproductive risk assessment at the first tier is acceptable (i.e., TER values >5), except for the small herbivorous mammal “vole” scenario. Therefore, a higher-tier risk assessment is required for this scenario to address the long-term reproductive risk to mammals from the metabolite prothioconazole-desthio. See further discussion, Section 9.3.2.2, below.

**Table 9.3-4: Screening and first-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of CA3642 in oilseed rape – prothioconazole-desthio**

Intended use	Oilseed rape				
Metabolite	Prothioconazole-desthio				
Application rate	1 x 180 g a.s./ha* (14-d interval); BBCH 14-69				
Acute toxicity	2235 mg metabolite/kg bw				
TER criterion	10				
Crop scenario	Indicator/generic focal species	SV <sub>90</sub>	MAF <sub>90</sub>	DDD <sub>90</sub> (mg/kg bw/d)	TER <sub>A</sub>
Growth stage					
Screening step	Small herbivorous mammal	118.4	1.0	21.3	104.9
Reprod. Toxicity	10 mg metabolite/kg bw/d				
TER criterion	5				
Crop scenario	Indicator/generic focal species	SV <sub>m</sub>	MAF <sub>m</sub> × TWA	DDD <sub>m</sub> (mg/kg bw/d)	TER <sub>LT</sub>
Growth stage					
Screening step	Small herbivorous mammal	48.3	1.0 x 0.53	4.6	<b>2.2</b>
<b>Tier 1</b>					
BBCH 10-19	Small insectivorous mammal “shrew”	4.2	1.0 x 0.53	0.4	25.0
BBCH $\geq 20$	Small insectivorous mammal “shrew”	1.9		0.2	55.2
BBCH $\geq 40$	Small herbivorous mammal “vole”	18.1		1.7	5.8
All season	Large herbivorous mammal “lagomorph”	14.3		1.4	7.3
BBCH 10-29	Small omnivorous mammal “mouse”	7.8		0.7	13.4
BBCH 30-39	Small omnivorous mammal “mouse”	2.3		0.2	45.6
BBCH $\geq 40$	Small omnivorous mammal “mouse”	1.9		0.2	55.2

TER values shown in **bold** fall below the relevant trigger. \* It is assumed that 100% of the parent becomes the metabolite (if the molecular weight of the metabolite is considered, the amount of prothioconazole-desthio will be lower, therefore, this assumption represents a very conservative worst case). SV: shortcut value; MAF: multiple application factor; TW: time-weighted average factor; DDD: daily dietary dose; TER: toxicity-to-exposure ratio

The acute and reproductive risks to mammals are shown to be acceptable at the screening and first-tier assessments, respectively, for the proposed use of CA3642, in oilseed rape.



**Table 9.3-5: Screening and first-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of CA3642 in sunflower – prothioconazole-desthio**

<b>Intended use</b>	Sunflower				
<b>Metabolite</b>	Prothioconazole-desthio				
<b>Application rate</b>	1 x 180 g a.s./ha*; BBCH 16-64				
<b>Acute toxicity</b>	2235 mg metabolite/kg bw				
<b>TER criterion</b>	10				
<b>Crop scenario</b>	<b>Indicator/generic focal species</b>	<b>SV<sub>90</sub></b>	<b>MAF<sub>90</sub></b>	<b>DDD<sub>90</sub> (mg/kg bw/d)</b>	<b>TER<sub>A</sub></b>
<b>Growth stage</b>					
Screening step	Small herbivorous mammal	118.4	1.0	21.3	104.9
<b>Reprod. Toxicity</b>	10 mg metabolite/kg bw/d				
<b>TER criterion</b>	5				
<b>Crop scenario</b>	<b>Indicator/generic focal species</b>	<b>SV<sub>m</sub></b>	<b>MAF<sub>m</sub> × TWA</b>	<b>DDD<sub>m</sub> (mg/kg bw/d)</b>	<b>TER<sub>LT</sub></b>
<b>Growth stage</b>					
Screening step	Small herbivorous mammal	48.3	1.0 x 0.53	4.6	<b>2.2</b>
<b>Tier 1</b>					
BBCH 10-19	Small insectivorous mammal "shrew"	4.2	1.0 x 0.53	0.4	25.0
BBCH ≥ 20	Small insectivorous mammal "shrew"	1.9		0.2	55.2
BBCH ≥ 40	Small herbivorous mammal "vole"	18.1		<b>1.7</b> <del>0.43</del>	<b>5.8</b> <del>23.25</del>
BBCH 10-19	Large herbivorous mammal "lagomorph"	14.3		1.4	7.3
BBCH 20-39	Large herbivorous mammal "lagomorph"	7.2		0.7	14.6
BBCH ≥ 40	Large herbivorous mammal "lagomorph"	3.6		0.3	29.1
BBCH 10-19	Small omnivorous mammal "mouse"	<b>7.8</b> <del>3.9</del>		<b>0.744</b> <del>0.4</del>	<b>13.44</b> <del>26.9</del>
BBCH 20-39	Small omnivorous mammal "mouse"	<b>3.9</b> <del>1.9</del>		<b>0.37</b> <del>0.2</del>	<b>27.02</b> <del>55.2</del>
BBCH ≥ 40	Small omnivorous mammal "mouse"	<b>1.9</b> <del>0.8</del>		<b>0.18</b> <del>0.7</del>	<b>55.5</b> <del>13.4</del>

TER values shown in **bold** fall below the relevant trigger. \* It is assumed that 100% of the parent becomes the metabolite (if the molecular weight of the metabolite is considered, the amount of prothioconazole-desthio will be lower, therefore, this assumption represents a very conservative worst case). SV: shortcut value; MAF: multiple application factor; TW: time-weighted average factor; DDD: daily dietary dose; TER: toxicity-to-exposure ratio

The acute and reproductive risks to mammals from prothioconazole-desthio are shown to be acceptable at the screening and first-tier assessments, respectively, for the proposed use of CA3642, in sunflower.

#### Assessment of the risk from azoxystrobin

**Table 9.3- 6: Screening and first-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of CA3642 in cereals – azoxystrobin**

<b>Intended use</b>	Cereals				
<b>Active substance</b>	Azoxystrobin				
<b>Application rate</b>	2 x 210 g a.s./ha (14-d interval); BBCH 30-69				
<b>Acute toxicity</b>	>5000 mg a.s./kg bw				
<b>TER criterion</b>	10				
<b>Crop scenario</b>	<b>Indicator/generic focal species</b>	<b>SV<sub>90</sub></b>	<b>MAF<sub>90</sub></b>	<b>DDD<sub>90</sub> (mg/kg bw/d)</b>	<b>TER<sub>A</sub></b>
<b>Growth stage</b>					
Screening step	Small herbivorous mammal	118.4	1.2	29.8	>167.6

<b>Reprod. Toxicity</b>	32 mg a.s./kg bw/d				
<b>TER criterion</b>	5				
<b>Crop scenario</b>	<b>Indicator/generic focal species</b>	<b>SV<sub>m</sub></b>	<b>MAF<sub>m</sub> × TWA</b>	<b>DDD<sub>m</sub> (mg/kg bw/d)</b>	<b>TER<sub>LT</sub></b>
<b>Growth stage</b>					
Screening step	Small herbivorous mammal	48.3	1.4 x 0.53	7.5	<b>4.3</b>
Tier 1					
BBCH ≥20	Small insectivorous mammal “shrew”	1.9	1.4 x 0.53	0.3	108.1
BBCH ≥40	Small herbivorous mammal “vole”	21.7		3.4	9.5
BBCH 30-39	Small omnivorous mammal “mouse”	3.9		0.6	52.7
BBCH ≥40	Small omnivorous mammal “mouse”	2.3		0.4	89.3

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

An acceptable acute and long-term risk to mammals is concluded for the active substance azoxystrobin, at the screening step, for intended use of CA3642, in cereals.

**Table 9.3-7 6:** Screening and first-tier assessment of the acute and long-term/reproductive risk for mammals, due to the use of CA3642, in oilseed rape – azoxystrobin

<b>Intended use</b>	Oilseed rape				
<b>Active substance</b>	Azoxystrobin				
<b>Application rate</b>	1 x 180 g a.s./ha; BBCH 14-69				
<b>Acute toxicity</b>	>5000 mg a.s./kg bw				
<b>TER criterion</b>	10				
<b>Crop scenario</b>	<b>Indicator/generic focal species</b>	<b>SV<sub>90</sub></b>	<b>MAF<sub>90</sub></b>	<b>DDD<sub>90</sub> (mg/kg bw/d)</b>	<b>TER<sub>A</sub></b>
<b>Growth stage</b>					
Screening step	Small herbivorous mammal	118.4	1.0	21.3	234.6
<b>Reprod. Toxicity</b>	32 mg a.s./kg bw/d				
<b>TER criterion</b>	5				
<b>Crop scenario</b>	<b>Indicator/generic focal species</b>	<b>SV<sub>m</sub></b>	<b>MAF<sub>m</sub> × TWA</b>	<b>DDD<sub>m</sub> (mg/kg bw/d)</b>	<b>TER<sub>LT</sub></b>
<b>Growth stage</b>					
Screening step	Small herbivorous mammal	48.3	1.0 x 0.53	4.6	6.9
Tier 1					
BBCH 10-19	Small insectivorous mammal “shrew”	4.2	1.0 x 0.53	0.4	79.9
BBCH ≥20	Small insectivorous mammal “shrew”	1.9		0.2	176.5
BBCH ≥40	Small herbivorous mammal “vole”	18.1		1.7	18.5
All season	Large herbivorous mammal “lagomorph”	14.3		1.4	23.5
BBCH 10-29	Small omnivorous mammal “mouse”	7.8		0.7	43.0
BBCH 30-39	Small omnivorous mammal “mouse”	2.3		0.2	145.8
BBCH ≥40	Small omnivorous mammal “mouse”	1.9		0.2	176.5

TER values shown in **bold** fall below the relevant trigger. SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity-to-exposure ratio. \* The first-tier reproductive assessment is included to allow for combined simultaneous assessments for prothioconazole-desthio and azoxystrobin, which are presented further below.

An acceptable acute and long-term risk to mammals is concluded for the active substance azoxystrobin, at the screening step, for intended use of CA3642 in oilseed rape.

**Table 9.3-8: Screening and first-tier assessment of the acute and long-term/reproductive risk for mammals, due to the use of CA3642, in sunflower – azoxystrobin**

<b>Intended use</b>	Sunflower				
<b>Active substance</b>	Azoxystrobin				
<b>Application rate</b>	1 x 180 g a.s./ha; BBCH 16-64				
<b>Acute toxicity</b>	>5000 mg a.s./kg bw				
<b>TER criterion</b>	10				
<b>Crop scenario</b>	<b>Indicator/generic focal species</b>	<b>SV<sub>90</sub></b>	<b>MAF<sub>90</sub></b>	<b>DDD<sub>90</sub> (mg/kg bw/d)</b>	<b>TER<sub>A</sub></b>
Growth stage					
Screening step	Small herbivorous mammal	118.4	1.0	21.3	234.6
<b>Reprod. Toxicity</b>	32 mg a.s./kg bw/d				
<b>TER criterion</b>	5				
<b>Crop scenario</b>	<b>Indicator/generic focal species</b>	<b>SV<sub>m</sub></b>	<b>MAF<sub>m</sub> × TWA</b>	<b>DDD<sub>m</sub> (mg/kg bw/d)</b>	<b>TER<sub>LT</sub></b>
Growth stage					
Screening step	Small herbivorous mammal	48.3	1.0 x 0.53	4.6	6.9
Tier 1					
BBCH 10-19	Small insectivorous mammal "shrew"	4.2	1.0 x 0.53	0.4	79.9
BBCH ≥ 20	Small insectivorous mammal "shrew"	1.9		0.2	176.5
BBCH ≥ 40	Small herbivorous mammal "vole"	18.1		1.7 0.43	18.5 74.41**
BBCH 10-19	Large herbivorous mammal "lagomorph"	14.3		1.4	23.5
BBCH 20-39	Large herbivorous mammal "lagomorph"	7.2		0.7	46.6
BBCH ≥ 40	Large herbivorous mammal "lagomorph"	3.6		0.3	93.2
BBCH 10-19	Small omnivorous mammal "mouse"	7.8 3.9		0.744 0.4	43.0 86.0
BBCH 20-39	Small omnivorous mammal "mouse"	3.9 1.9		0.37 0.2	86.48 176.5
BBCH ≥ 40	Small omnivorous mammal "mouse"	1.9 7.8		0.18 0.7	177.7 43.0

TER values shown in **bold** fall below the relevant trigger. SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity-to-exposure ratio. \* The first-tier reproductive assessment is included to allow for combined simultaneous assessments for prothioconazole-desthio and azoxystrobin, which are presented further below.

\*\*DF=0.25

An acceptable acute and long-term risk to mammals is concluded for the active substance azoxystrobin, at the screening step, for intended use of CA3642 in sunflower.

**zRMS comments:**

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

TER<sub>A</sub> and TER<sub>LT</sub> values for the exposure to prothioconazole and azoxystrobin are above the trigger of 10 and 5 for acute and long-term exposure, respectively, indicating acceptable risk for mammals for all intended uses. However, the Tier 1 level calculations of long-term risk for prothioconazole and azoxystrobin have been added separately for cereals, oilseed rape and sunflower uses as they are needed for combined risk assessment.

In the same time TER<sub>A</sub> values for acute exposure for prothioconazole metabolite JAU 6476-desthio at screening step is above trigger value of 10 indicating an acceptable acute risk but the TER<sub>LT</sub> values indicating an unacceptable risk for all intended uses.

Based on the tier 1 level risk assessment the acceptable risk has been concluded for all uses except cereals for small herbivorous mammals-vole at BBCH>40.

For this reason, the refined long-term risk assessment for cereals from exposure from this metabolite has been provided at Point 9.3.2.2.

## Combined effects

### Acute toxicity

In the absence of mammalian toxicity studies with the formulated product, a surrogate acute LD<sub>50</sub> value is calculated for the mixture, in line with Appendix B of EFSA/2009/1438, Step 1, assuming additive toxicity of the two active substances, using the following equation:

$$LD_{50,Mix} = \sum_i \left( \frac{X_{a.s.i}}{LD_{50,a.s.i}} \right)^{-1}$$

Where:

X(a.s.<sub>i</sub>)  $X_{a.s.i}$  = the fraction of active substance [i] in the mixture

LD<sub>50</sub>(a.s.<sub>i</sub>) = the acute toxicity value of the active substance [i]

Combined acute toxicity of prothioconazole and azoxystrobin:

$$LD_{50,Mix} = \left( \frac{0.5}{6200} + \frac{0.5}{5000} \right)^{-1} = 5536 \text{ mg/kg bw}$$

Since it is assumed that 100% of the parent prothioconazole is quickly transformed to the metabolite prothioconazole-desthio, a calculation of the combined acute toxicity of prothioconazole-desthio and azoxystrobin is performed:

$$LD_{50,Mix} = \left( \frac{0.5}{2235} + \frac{0.5}{5000} \right)^{-1} = 3089.2 \text{ mg/kg bw}$$

To allow for comparison of single active substance and mixture toxicity, a “tox per fraction” quotient is calculated for each active substance and compared to the corresponding quotient for the mixture, to demonstrate whether one single active substance/relevant metabolite contributes >90% to the mixture toxicity, using the following equation:

$$\begin{aligned} \text{tox per fraction (a.s.)} &= \frac{LD_{50}(a.s._i)}{X(a.s._i)} \\ \text{tox per fraction (mix)} &= \frac{LD_{50}(\text{mix})}{\sum_i X(a.s._i)} \end{aligned}$$

Where:

X(a.s.<sub>i</sub>)  $X_{a.s.i}$  = the fraction of active substance [i] in the mixture

LD<sub>50</sub>(a.s.<sub>i</sub>) = the acute toxicity value of the active substance [i]

The “tox per fraction” quotients for prothioconazole, prothioconazole-desthio and azoxystrobin compared to the “tox per fraction” quotient for the mixture, are detailed in the following tables.

**Table 9.3- 9 :** Comparison of single active substance acute toxicity and mixture acute toxicity using “tox per fraction” quotients – prothioconazole and azoxystrobin

Substance	Acute endpoint LD <sub>50</sub> [mg/kg bw]	Fraction of each a.s. in mixture	Total fraction of a.s. in mixture	“Tox per fraction (a.s.)” quotient	“Tox per fraction (mixture)” quotient	Contribution to toxicity [%] <sup>1</sup>
Prothioconazole	>6200	0.5	1	6200/0.5 = 12400	-	55

<b>Azoxystrobin</b>	5000	0.5		$5000/0.5 = 10000$	-	45
<b>Calculated mixture</b>	5536 <sup>2</sup>	-	-	-	$5536/1 = 5536$	-

<sup>1</sup>A contribution  $\geq 90\%$  to mixture toxicity indicates a single driver of toxicity

<sup>2</sup>Calculated mixture toxicity

**Table 9.3-10:** Comparison of single active substance acute toxicity and acute mixture toxicity using “tox per fraction” quotients – prothioconazole-desthio and azoxystrobin

Substance	Acute endpoint LD <sub>50</sub> [mg/kg bw]	Fraction of a.s. in mixture	Total fraction of a.s. in mixture	“Tox per fraction (a.s.)” quotient	“Tox per fraction (mixture)” quotient	Contribution to toxicity [%] <sup>1</sup>
<b>Prothioconazole-desthio</b>	2235	0.5	1	$2235/0.5 = 4407$	-	31
<b>Azoxystrobin</b>	5000	0.5		$5000/0.5 = 10000$	-	69
<b>Calculated mixture</b>	3089 <sup>2</sup>	-	-	-	$3089/1 = 3089$	-

<sup>1</sup>A contribution  $\geq 90\%$  to mixture toxicity indicates a single driver of toxicity

<sup>2</sup>Calculated mixture toxicity

The acute ‘toxicity per fraction’ analysis for both combinations, prothioconazole and azoxystrobin, and prothioconazole-desthio and azoxystrobin, indicates that based on the assumption of Concentration Addition (CA) no single active substance or metabolite contributes  $\geq 90\%$  to the acute mixture toxicity. Therefore, in accordance with Appendix B of EFSA/2009/1438, Step 4, the predicted LD<sub>50</sub> value for the mixture is used in the risk assessment, below, based on the total application rate of the active substances.

**Table 9.3-11:** Screening assessment of the acute risk to mammals due to the use of CA3642 on cereals and oilseed rape and sunflower– combined simultaneous toxicity of prothioconazole + azoxystrobin

<b>Intended use</b>	Cereals, oilseed rape and sunflower				
<b>Product</b>	CA3642				
<b>Application rate</b>	2 x 420 g total a.s./ha (14-d interval) – worst-case cereal GAP used in the screening step				
<b>Acute toxicity</b>	5536 mg/kg bw (calculated LD <sub>50, MIX</sub> value)				
<b>TER criterion</b>	10				
<b>Crop scenario</b>	<b>Indicator/generic focal species</b>	<b>SV<sub>90</sub></b>	<b>MAF<sub>90</sub></b>	<b>DDD<sub>90</sub> (mg/kg bw/d)</b>	<b>TER<sub>A</sub></b>
Screening	Small herbivorous mammal	118.4	1.2	59.7	92.8

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

An acceptable acute risk to mammals is concluded for the combined effects of simultaneous exposure to prothioconazole and azoxystrobin at the screening step for intended uses of CA3642 in cereals and oilseed rape.

**Table 9.3-12:** Screening assessment of the acute risk to mammals, due to the use of CA3642 on cereals, and oilseed rape and sunflower – combined simultaneous toxicity of prothioconazole-desthio + azoxystrobin.

<b>Intended use</b>	Cereals, and oilseed rape and sunflower				
<b>Product</b>	CA3642 (considering combined toxicity of prothioconazole-desthio + azoxystrobin)				
<b>Application rate</b>	2 x 420 g total a.s./ha (14-d interval); BBCH 14-69				
<b>Acute toxicity</b>	3089 mg/kg bw (calculated LD <sub>50, MIX</sub> value)				
<b>TER criterion</b>	10				
<b>Crop scenario</b>	<b>Indicator/generic focal species</b>	<b>SV<sub>90</sub></b>	<b>MAF<sub>90</sub></b>	<b>DDD<sub>90</sub> (mg/kg bw/d)</b>	<b>TER<sub>A</sub></b>
Screening	Small herbivorous mammal	118.4	1.2	59.7	51.8

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

An acceptable acute risk to mammals is concluded for the combined effects of simultaneous exposure to prothioconazole-desthio and azoxystrobin at the screening step for intended uses of CA3642 on cereals and oilseed rape.

#### zRMS comments:

##### Acute combined risk assessment:

LD<sub>0</sub> mix values calculated with consideration of relevant toxicity endpoints for both of active substances and active substance – azoxystrobin and metabolite prothioconazole-desthio has been validated by zRMS.

Overall, based on calculations provided in the Table 9.3-11 and 9.3-12 an acceptable acute risk to mammals is concluded for the combined effects of simultaneous exposure to prothioconazole and azoxystrobin or prothioconazole-desthio and azoxystrobin at the screening step for intended uses of CA3642 on cereals and oilseed rape.

#### Reproductive toxicity

EFSA/2009/1438, Appendix B, step 3 states:

‘...it is currently not recommended to consider the use of predicted toxicity [from more than one a.s.] values as surrogates in the [reproduction] risk assessment. Although it would be, in principle, possible to apply the concept of dose or concentration additivity of toxicity also to effect data for biological endpoints from long-term and reproductive toxicity testing, reliable results would only be expected for combinations of EC<sub>x</sub> values for the same biological endpoint. Moreover, additional bias would be introduced in the calculations if the values applied do not represent EC<sub>x</sub> values with defined x, but NOAELs, since these may represent varying risk or response levels for different compounds, depending on dose-spacing.’

Therefore, the chronic risk from the combined exposure of the two active substances or from the combined exposure to the metabolite prothioconazole-desthio and azoxystrobin is not required according to the current guidance. However, for completeness a check of the worst-case chronic risk is provided below:

**Table 9.3- 13 9:** Comparison of single active substance toxicity and mixture toxicity using “tox per fraction” quotients – prothioconazole-desthio and azoxystrobin

Substance	NOEL [mg a.s./kg bw]	Fraction of a.s. in mixture	Total fraction of a.s. in mixture	“Tox per fraction (a.s.)” quotient	“Tox per fraction (mixture)” quotient	Contribution to toxicity [%] <sup>1</sup>
Prothioconazole- desthio	10	0.5	1	10/0.5 = 20	-	76
Azoxystrobin	32	0.5		32/0.5 = 64	-	24
Calculated mixture	15.2 <sup>2</sup>	-	-	-	15.2	-

<sup>1</sup>A contribution of ≥90% to mixture toxicity indicates a single driver of toxicity; <sup>2</sup>Calculated mixture toxicity

The above comparison demonstrates there is no clear driver (i.e. >90% contribution of one substance to the mixture toxicity) of the reproductive toxicity to mammals. However, the metabolite, prothioconazole-desthio (NOAEL = 10 mg/kg bw/day), is very quickly formed from prothioconazole (NOAEL = 95.6 mg/kg bw/day) and is likely to have a greater influence on the reproductive risk assessment, based on its toxicity, relative to azoxystrobin (NOAEL = 32 mg/kg bw/day). The reproductive risk assessments performed for prothioconazole-desthio alone assumed worst-case exposure (i.e., that 100% of the parent compound, prothioconazole, has transformed into the metabolite). Even with this worst-case assumption, the first- and higher-tier risk assessments for prothioconazole-desthio, for all proposed uses of CA3642, are acceptable (lowest TER<sub>LT</sub> = 8.9 (refined, focal species ‘vole’ – Section 9.3.2.2) for cereals and lowest TER<sub>LT</sub> = 5.8 (tier 1, focal species ‘vole’) for oilseed rape and sunflower). Therefore, it is considered that

there should be no unacceptable risk, even when considering the combined toxicity of prothioconazole-desthio and azoxystrobin. This is confirmed when the value of the assessment factor divided by the TER<sub>LT</sub>, for each compound, is calculated and summed up, as noted in the bird and mammal section on combined risk assessment (page 36) of the HSE ‘Formulation studies and combined risk assessment in ecotoxicology’ (January, 2022). These calculations are presented in the table below (note that refined TER<sub>LT</sub> values for prothioconazole-desthio and azoxystrobin are derived from higher tier risk assessments, presented in Section 9.3.2.2, below).

**Table 9.3-14:** Combined long-term risk assessment for mammals, due to the use of CA3642 in cereals and oilseed rape – combined simultaneous toxicity of prothioconazole-desthio + azoxystrobin

Crop	Scenario	TER <sub>LT</sub> <sup>*</sup>		AF	ΣAF/TER
		Prothioconazole-desthio	Azoxystrobin		
Based on the first-tier risk assessment					
Cereals	BBCH ≥40 ‘vole’	3.0	9.5	5	2.19
Oilseed rape	BBCH ≥40 ‘vole’	5.8	18.5	5	1.13
Sunflower	BBCH ≥40 ‘vole’	5.8	18.5	5	1.13
Based on a refined, higher-tier assessment, with deposition values of 10% (cereals) and 20% (oilseed rape)					
Cereals	BBCH ≥40 ‘vole’	8.9	28.5	5	0.74
Oilseed rape	BBCH ≥40 ‘vole’	7.3	23.3	5	0.90
Based on refined residues of prothioconazole-desthio on plant material for the use in sunflower					
Sunflower	BBCH ≥40 ‘vole’	14.0	18.5	5	0.63
Based on updated RUD for monocotyledons*					
Sunflower	BBCH >40 ‘vole’	6.7	18.5	5	1.0

Values in **bold** are greater than the trigger of 1. \*The lowest/worst-case, first-tier TER value is used in the assessment, in accordance with the HSE (Pers. Comm., 06/07/22, as there is an error in the guidance document “Formulation studies and combined risk assessment in ecotoxicology”).

\* Based on RUD data for monocotyledons is not considered in the refined risk assessment by zRMS (B&M guidance (EFSA Journal 2023;21(2):7790)).

Based on the first-tier combined risk assessment for the worst-case focal species ‘vole’, the values of ΣAF/TER are greater than 1 for the proposed uses of CA3642 in cereals and oilseed rape. However, as discussed in section 9.3.2.2, below, the standard TER values for the use in cereals and oilseed rape.

The deposition onto the grass under the crop, based on Table 1.5 of “Generic Guidance for Tier 1 FOCUS Ground Water Assessments” (Version 2.2; May 2014) can be used to determine refined TER values. The deposition onto cereals and oilseed rape at BBCH ≥40 is 10% and 20%, respectively.

With these deposition refinements in combination with the standard, worst-case values for the other parameters, the ΣAF/TER values are less than the trigger of 1 and an acceptable combined risk to mammals is demonstrated from the proposed uses of CA3642 in cereals and oilseed rape.

For the use in sunflower, DT50 of 3.2 days based on residue data on wheat for the metabolite prothioconazole-desthio available in the Section B.9.1.4.1(b) of the DAR for prothioconazole (2005) and EFSA Conclusions for prothioconazole (EFSA Scientific Report, 2007, 106, 1-98) is used to refine long-term risk to small herbivorous mammal “vole” (see section 9.3.2.2 below).

Because the diet of small herbivorous mammal “vole” consists 100% of grass (monocots) according to Appendix A EFSA/2009/1438, it is considered appropriate to use residue decline study data on wheat in the refined risk assessment. This is in agreement with EFSA Supporting publication 2019:EN-1673<sup>1</sup>, stating that extrapolation of residue decline data within the group of monocot plants (green parts and roots) is possible.

As an additional approach, an updated RUD data for monocotyledons is considered in the refined risk assessment based on a database developed by Lahr et al. (2018)<sup>2</sup>, used in the updated B&M guidance (EFSA Journal 2023;21(2):7790).

<sup>1</sup> EFSA (European Food Safety Authority), 2019. Technical report on the outcome of the Pesticides Peer Review Meeting on general recurring issues in ecotoxicology. EFSA supporting publication 2019:EN-1673. 117pp. doi:10.2903/sp.efsa.2019.EN-1673

<sup>2</sup> Lahr J, Kramer W, Mazerolles V, Poulsen V, Jolli D, Muller M, McVey E, Wassenberg J, Derkx R, Brouwer A, Deneer D, Beltman W, Lammertsma D, Jansman H and Buij R, 2018. Data collection for the estimation of ecological data (specific focal species, time spent in treated areas collecting food, composition of diet), residue level and residue decline on food items to be used in the risk assessment for birds and mammals. EFSA supporting publication 2018:EN-1513, 155 pp.

**zRMS comments:**

zRMS agrees with the calculations based on Applicant's approach. However, zRMS calculated TER<sub>mix</sub> with consideration of all compounds: azoxystrobin, prothioconazole and prothioconazole-desthio as the worst-case scenario. As an additional approach with TER<sub>LT</sub> calculated with updated RUD data for monocotyledons is not considered in the refined risk assessment by zRMS. TER<sub>LT</sub> presented in the Table 9.3-14 are taken from updated version of B&M guidance (EFSA Journal 2023;21(2):7790).

### 9.3.2.2 Higher-tier risk assessment

#### Prothioconazole-desthio, refinement of long-term risk to mammals for the use in cereals and oilseed rape

The first-tier mammalian risk assessment used a worst-case, default deposition factor (DF) of 0.3 (cereals, at BBCH ≥40), from Appendix A of EFSA/2009/1438, for the vole food item of 100% grass. This default DF value is based on the interception values for cereals taken from the FOCUS groundwater guidance (2001). However, a refined DF values of 0.1 (BBCH ≥40) **for grass under cereal crop and 0.2 (BBCH>30) for grass under OSR crop**, can be used based on the interception values in the more recent FOCUS groundwater guidance (version 2.2; May 2014).

A higher-tier risk assessment is presented below using this refined DF value.

**Table 9.3-15** **Higher-tier assessment of the long-term/reproductive risk for mammals due to the use of CA3642 in cereals – prothioconazole-desthio – refined parameters (deposition factor (DF)\*) are further described and justified in the text**

<b>Intended use</b>	Cereals							
<b>Metabolite</b>	Prothioconazole-desthio							
<b>Application rate</b>	2 x 210 g a.s./ha <sup>#</sup> (14-d interval); BBCH 30-69							
<b>Reprod. Toxicity</b>	10 mg metabolite/kg bw/day							
<b>TER criterion</b>	5							
<b>Generic focal species</b>	<b>Food category (% diet)</b>	<b>FIR/bw</b>	<b>RUD<sub>m</sub></b>	<b>DF*</b>	<b>MAF<sub>m</sub> × TWA</b>	<b>PT</b>	<b>DDD<sub>m</sub> (mg/kg bw/d)</b>	<b>TER<sub>LT</sub></b>
Small herbivorous mammal "vole" BBCH >40	Grass, 100%	1.33	54.2	0.1	1.4 x 0.53	1	1.12	8.90

TER values shown in **bold** fall below the relevant trigger. <sup>#</sup>It is assumed that 100% of the parent becomes the metabolite. \*Deposition factor (DF) is refined based on an interception value of 90% in spring and winter cereals, at BBCH 40-69, from Table 1.5 of "Generic Guidance for Tier 1 FOCUS Ground Water Assessments" (Version 2.2; May 2014). 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; DDD: daily dietary dose; TER: toxicity-to-exposure ratio

The same approach was used by zRMS for a.s.-prothioconazole as it is needed for combined risk.

The relevant calculations are provided below:

**Table 9.3-15 -1: Higher-tier assessment of the long-term/reproductive risk for mammals due to the use of CA3642 in cereals – prothioconazole– refined parameters (deposition factor (DF)\*) are further described and justified in the text**

<b>Intended use</b>	Cereals							
<b>Metabolite</b>	Prothioconazole*							
<b>Application rate</b>	2 x 210 g a.s./ha <sup>#</sup> (14-d interval); BBCH 30-69							
<b>Reprod. Toxicity</b>	95.6 /kg bw/day							
<b>TER criterion</b>	5							
<b>Generic focal species</b>	<b>Food category (% diet)</b>	<b>FIR/bw</b>	<b>RUD<sub>m</sub></b>	<b>DF*</b>	<b>MAF<sub>m</sub> × TWA</b>	<b>PT</b>	<b>DDD<sub>m</sub> (mg/kg bw/d)</b>	<b>TER<sub>LT</sub></b>
Small herbivorous mammal "vole" BBCH >40	Grass, 100%	1.33	54.2	0.1	1.4 x 0.53	1	1.12	85.35

TER values shown in **bold** fall below the relevant trigger. <sup>#</sup>It is assumed that 100% of the parent becomes the metabolite.



\*Deposition factor (DF) is refined based on an interception value of 90% in spring and winter cereals, at BBCH 40-69, from Table 1.5 of “Generic Guidance for Tier 1 FOCUS Ground Water Assessments” (Version 2.2; May 2014). 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; DDD: daily dietary dose; TER: toxicity-to-exposure ratio

Using a refined DF value of 0.1, the TER<sub>LT</sub> value is greater than the trigger of 5, concluding an acceptable long-term risk to the small herbivorous mammal “vole” from the metabolite prothioconazole-desthio, prothioconazole, following the intended use of CA3642, in cereals.

Although an acceptable long-term risk to mammals for exposure to prothioconazole-desthio (oilseed rape), azoxystrobin and to prothioconazole (cereals and oilseed rape) was demonstrated based on Tier 1 calculations, higher-tier risk assessments are presented below to derive refined TER<sub>LT</sub> values, which are used in the combined risk assessment, presented above in Table 9.3-9.

**Table 9.3 16 742: Higher-tier assessment of the long-term/reproductive risk for mammals due to the use of CA3642 in oilseed rape – prothioconazole-desthio – refined parameters (deposition factor (DF)\*) are further described and justified in the text**

<b>Intended use</b>	Oilseed rape							
<b>Metabolite</b>	Prothioconazole-desthio							
<b>Application rate</b>	1 x 180 g a.s./ha <sup>#</sup> ; BBCH 14-69							
<b>Reprod. Toxicity</b>	10 mg metabolite/kg bw/day							
<b>TER criterion</b>	5							
<b>Generic focal species</b>	<b>Food category (% diet)</b>	<b>FIR/bw</b>	<b>RUD<sub>m</sub></b>	<b>DF*</b>	<b>MAF<sub>m</sub> × TWA</b>	<b>PT</b>	<b>DDD<sub>m</sub> (mg/kg bw/d)</b>	<b>TER<sub>LT</sub></b>
Small herbivorous mammal “vole” BBCH >40	Grass, 100%	1.33	54.2	0.2	1.0 x 0.53	1	1.375	7.3

TER values shown in **bold** fall below the relevant trigger. <sup>#</sup>It is assumed that 100% of the parent becomes the metabolite. \*Deposition factor (DF) is refined based on an interception value of 80% in spring and winter oilseed rape, at BBCH 14-69, from Table 1.5 of “Generic Guidance for Tier 1 FOCUS Ground Water Assessments” (Version 2.2; May 2014). 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; DDD: daily dietary dose; TER: toxicity-to-exposure ratio

**Table 9.3-17: Higher-tier assessment of the long-term/reproductive risk for mammals due to the use of CA3642 in oilseed rape – prothioconazole— refined parameters (deposition factor (DF)\*) are further described and justified in the text**

<b>Intended use</b>	Oilseed rape							
<b>Metabolite</b>	Prothioconazole							
<b>Application rate</b>	1 x 180 g a.s./ha <sup>#</sup> ; BBCH 14-69							
<b>Reprod. Toxicity</b>	95.6 kg bw/day							
<b>TER criterion</b>	5							
<b>Generic focal species</b>	<b>Food category (% diet)</b>	<b>FIR/bw</b>	<b>RUD<sub>m</sub></b>	<b>DF*</b>	<b>MAF<sub>m</sub> × TWA</b>	<b>PT</b>	<b>DDD<sub>m</sub> (mg/kg bw/d)</b>	<b>TER<sub>LT</sub></b>
Small herbivorous mammal “vole” BBCH >40	Grass, 100%	1.33	54.2	0.2	1.0 x 0.53	1	1.375	69.52

TER values shown in **bold** fall below the relevant trigger. <sup>#</sup>It is assumed that 100% of the parent becomes the metabolite. \*Deposition factor (DF) is refined based on an interception value of 80% in spring and winter oilseed rape, at BBCH 14-69, from Table 1.5 of “Generic Guidance for Tier 1 FOCUS Ground Water Assessments” (Version 2.2; May 2014). 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; DDD: daily dietary dose; TER: toxicity-to-exposure ratio

**Table 9.3- 18 7 43: Higher-tier assessment of the long-term/reproductive risk for mammals due to the use of CA3642 in cereals – azoxystrobin – refined parameters (deposition factor (DF)\*) are further described and justified in the text**

<b>Intended use</b>	Cereals
<b>Active substance</b>	Azoxystrobin

<b>Application rate</b>	2 x 210 g a.s./ha <sup>#</sup> (14-d interval); BBCH 30-69							
<b>Reprod. Toxicity</b>	32 mg a.s./kg bw/day							
<b>TER criterion</b>	5							
<b>Generic focal species</b>	<b>Food category (% diet)</b>	<b>FIR/bw</b>	<b>RUD<sub>m</sub></b>	<b>DF*</b>	<b>MAF<sub>m</sub> × TWA</b>	<b>PT</b>	<b>DDD<sub>m</sub> (mg/kg bw/d)</b>	<b>TER<sub>LT</sub></b>
Small herbivorous mammal “vole” BBCH >40	Grass, 100%	1.33	54.2	0.1	1.4 x 0.53	1	1.12	28.5

TER values shown in **bold** fall below the relevant trigger. \*Deposition factor (DF) is refined based on an interception value of 90% in spring and winter cereals, at BBCH 40-69, from Table 1.5 of “Generic Guidance for Tier 1 FOCUS Ground Water Assessments” (Version 2.2; May 2014). 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; DDD: daily dietary dose; TER: toxicity-to-exposure ratio

**Table 9.3-19<sup>814</sup>: Higher-tier assessment of the long-term/reproductive risk for mammals due to the use of CA3642 in oilseed rape – azoxystrobin – refined parameters (deposition factor (DF)\*) are further described and justified in the text**

<b>Intended use</b>	Oilseed rape							
<b>Active substance</b>	Azoxystrobin							
<b>Application rate</b>	1 x 180 g a.s./ha <sup>#</sup> ; BBCH 14-69							
<b>Reprod. Toxicity</b>	32 mg a.s./kg bw/day							
<b>TER criterion</b>	5							
<b>Generic focal species</b>	<b>Food category (% diet)</b>	<b>FIR/bw</b>	<b>RUD<sub>m</sub></b>	<b>DF*</b>	<b>MAF<sub>m</sub> × TWA</b>	<b>PT</b>	<b>DDD<sub>m</sub> (mg/kg bw/d)</b>	<b>TER<sub>LT</sub></b>
Small herbivorous mammal “vole” BBCH >40	Grass, 100%	1.33	54.2	0.2	1.0 x 0.53	1	1.375	23.3

TER values shown in **bold** fall below the relevant trigger. \*Deposition factor (DF) is refined based on an interception value of 80% in spring and winter oilseed rape, at BBCH 14-69, from Table 1.5 of “Generic Guidance for Tier 1 FOCUS Ground Water Assessments” (Version 2.2; May 2014). 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; DDD: daily dietary dose; TER: toxicity-to-exposure ratio

#### Prothioconazole-desthio, refinement of long-term risk to mammals for the use in sunflower

Although an acceptable long-term risk to mammals for exposure to prothioconazole-desthio and to azoxystrobin was demonstrated for sunflower based on Tier 1 calculations, higher-tier risk assessments are presented below to derive refined TER<sub>LT</sub> values, which are used in the combined risk assessment. Experimentally determined residue values for prothioconazole-desthio on feed available in the Section B.9.1.4.1(b) of the DAR for prothioconazole (2005) and EFSA Conclusions for prothioconazole (EFSA Scientific Report, 2007, 106, 1-98) have been used to calculate an appropriate TWA value. A total of 8 trials were conducted to determine the residue decline of prothioconazole-desthio metabolite in wheat and its DT<sub>50</sub> value after a spray application of 200 g a.s./ha. A maximum residue value of 3.7 mg/kg was measured. The DT<sub>50</sub> values in the individual studies were in the range of 2.47 to 5.04 days. The overall mean DT<sub>50</sub> value for prothioconazole-desthio in wheat from the 8 studies was 3.2 days.). Based on a DT<sub>50</sub> value of 3.2 days, TWA equals 0.22 (calculated according to Appendix H of EFSA/2009/1438). Refined risk assessment is shown in the Table below:

**Table 9.3-20<sup>820</sup>: Higher-tier assessment of the long-term/reproductive risk for mammals due to the use of CA3642 in sunflower – prothioconazole-desthio – refined deposition to grass and refined TWA value**

<b>Intended use</b>	Sunflower
<b>Active substance</b>	Prothioconazole-desthio
<b>Application rate</b>	1 x 180 g a.s./ha <sup>#</sup> ; BBCH 16-64
<b>Reprod. Toxicity</b>	10 mg a.s./kg bw/day

<b>TER criterion</b>	5							
<b>Generic focal species</b>	<b>Food category (% diet)</b>	<b>FIR/bw</b>	<b>RUD<sub>m</sub></b>	<b>DF*</b>	<b>MAF<sub>m</sub> × TWA**</b>	<b>PT</b>	<b>DDD<sub>m</sub> (mg/kg bw/d)</b>	<b>TER<sub>LT</sub></b>
Small herbivorous mammal “vole” BBCH >40	Grass, 100%	1.33	54.2	0.25	0.22	1	0.71	14.0

TER values shown in **bold** fall below the relevant trigger. \*Deposition factor (DF) is according to Appendix A of EFSA/2009/1438. \*\*Refined based on DT50 of 3.2 days according to the DAR (2005); 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; DDD: daily dietary dose; TER: toxicity-to-exposure ratio

Refined combination toxicity risk assessment based on DT50 value of 3.2 days for prothioconazole-desthio is shown in Table 9.3-10.

As an additional approach, an updated RUD data for monocotyledons is considered in the refined risk assessment based on a database developed by Lahr et al. (2018), summarised in updated B&M guidance (EFSA Journal 2023;21(2):7790). RUD for monocotyledons of 47.2 used in updated B&M guidance (EFSA Journal 2023;21(2):7790) is based on 218 samples, in comparison with RUD of 54.2 based on 132 samples used in EFSA/2009/1438.

**Table 9.3-20-1:** Higher-tier assessment of the long-term/reproductive risk for mammals due to the use of CA3642 in sunflower – prothioconazole-desthio – updated RUD for monocotyledons based on EFSA Journal 2023;21(2):7790

<b>Intended use</b>	Sunflower							
<b>Active substance</b>	Prothioconazole-desthio							
<b>Application rate</b>	1 x 180 g a.s./ha <sup>#</sup> ; BBCH 16-64							
<b>Reprod. Toxicity</b>	10 mg a.s./kg bw/day							
<b>TER criterion</b>	5							
<b>Generic focal species</b>	<b>Food category (% diet)</b>	<b>FIR/bw</b>	<b>RUD<sub>m</sub></b>	<b>DF*</b>	<b>MAF<sub>m</sub> × TWA</b>	<b>PT</b>	<b>DDD<sub>m</sub> (mg/kg bw/d)</b>	<b>TER<sub>LT</sub></b>
Small herbivorous mammal “vole” BBCH >40	Grass, 100%	1.33	47.2**	0.25	0.53	1	1.50	6.7

TER values shown in **bold** fall below the relevant trigger. \*Deposition factor (DF) is according to Appendix A of EFSA/2009/1438. \*\*Updated RUD for monocotyledons based on EFSA Journal 2023;21(2):7790 ; 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; DDD: daily dietary dose; TER: toxicity-to-exposure ratio

**zRMS comments:**

The additional calculations presented above in the Table 9.3-20 based on the new RUD value for monocotyledons of 47.2 used in updated B&M guidance (EFSA Journal 2023;21(2):7790) is not taken into account by zRMS-PL.

Refined combination toxicity risk assessment based on updated RUD for monocotyledons is shown in Table 9.3-10.

**zRMS comments:**

Combined long-term toxicity

zRMS agrees that for the approach assuming dose additivity of the active substances, reliable results would only be expected for combinations of effect levels with a defined x, e.g. a LD<sub>50</sub>, but not for NOELs since the latter effect indicators may represent varying risk or response levels for different compounds depending on dose-spacing according to GD B&M, EFSA Journal 2009; 7(12): 1438, Appendix B. Therefore, for the risk assessment based on long-term effects it is not recommended to consider the use of predicted toxicity values. Therefore, the calculated NOEL<sub>mix</sub> provided by the Applicant was not considered by zRMS in the current risk assessment.

It should be noted that according to recommendation given in Appendix B of the Guidance Document 2009 for the evaluation of sublethal effects the use of the lowest NO(A)EL of the actives in the formulation, along with the combined exposure estimate from both active substances provides a conservative representation of long-term risks to mammals.

The Applicant presented TERmix approach in the Table 9.3-14 which has been accepted by zRMS-PL. However, it should be stressed ~~out~~ that (TER<sub>mix</sub>) approach ~~was~~ agreed by among of MSs in CZ is calculated for all compounds by zRMS and for this reason, the respective zRMS's calculations based on the lowest TER<sub>LT</sub> values for each compound for the same species are presented below. It should be noted the worst -case scenario where 100% parent prothioconazole plus 100% prothioconazole-desthio and azoxystrobin were considered by zRMS in the Tables below despite of the TERcombi presented by the Applicant in the Table 9.2-14.

The combined risk assessment presented by zRMS (full presence 100% prothioconazole and 100% prothioconazole-desthio occurring simultaneously without taking into account the degradation of the parent molecule for the formation of the metabolite) is an unrealistic extreme worst case and overestimates the exposure to prothioconazole and prothioconazole-desthio.

Cereals, vole BBCH>40 – Tier 1						Σ1/TER	Σ1/TER <sup>-1</sup>	Trigger
Azoxystrobin		Prothioconazole		JAU 6476-desthio				
9.5	0.1	265.5	0.0037	3.0	0.33	0.43	2.32	5
		<del>28.28</del>	<del>0.035</del>			<del>0.33</del>	<del>3.03</del>	

<sup>1)</sup> Lowest Tier 1 TER<sub>LT</sub>

Oilseed rape vole BBCH>40-Tier 1						Σ1/TER	Σ1/TER <sup>-1</sup>	Trigger
Azoxystrobin		Prothioconazole		JAU 6476-desthio				
18.5	0.054	56.23	0.017	5.8	0.172	0.24	4.16	5
			<del>0.018</del>			<del>0.244</del>	<del>4.09</del>	

<sup>1)</sup> Lowest Tier 1 TER<sub>LT</sub>

Sunflower, vole BBCH>40-Tier 1						Σ1/TER	Σ1/TER <sup>-1</sup>	Trigger
Azoxystrobin		Prothioconazole		JAU 6476-desthio				
18.5	0.054	56.23	0.017	5.8	0.17	0.24	4.16	5

<sup>1)</sup> Lowest Tier 1 TER<sub>LT</sub>

The refinement of combined long-term risk assessment for azoxystrobin, prothioconazole and prothioconazole metabolite JAU 6476-desthio together with consideration of fdep of 0.1 (reported in the latest ‘Generic Guidance for Tier-1 FOCUS Ground Water Assessment’ (vers. 2.2; May 2014)) for cereals crop stages at BBCH 40-69 and fdep 0.2 for oilseed rape at BBCH 40-69 and fdep 0.25 for sunflower at BBCH 40-69–has been validated by zRMS.

~~The refinement of the long term risk assessment for sunflower for sunflower is not appropriate. In zRMS's opinion it is not possible to extrapolate the amount of the metabolite JAU desthio from the residue study carried out for cereals (monocots) to dicots such as sunflower. Therefore, a calculation of the estimated dose of the metabolite JAU desthio was crossed out in the Table 9.3 19 and 9.3 20.~~ According to the GD B&M, EFSA Journal 2009; 7(12): 1438 the diet of the generic focal species Small herbivorous mammal “vole” at BBCH≥40 consists of 100% grass (grass and cereals - monocots).

Considering this, the generic focal species consume grass and cereals (overall monocots) potentially growing beneath the crop. Consequently, the Wheat residue decline study which is included in Prothioconazole DAR of 2005 is used to refine the risk for use in sunflower. Experimentally determined residue values for prothioconazole-desthio on feed available in the Section B.9.1.4.1(b) of the DAR for prothioconazole (2005) and EFSA Conclusions for prothioconazole (EFSA Scientific Report, 2007, 106, 1-98) have been used to calculate an appropriate TWA value. A total of 8 trials were conducted to determine the residue decline of prothioconazole-desthio metabolite in wheat and its DT50 value after a spray application of 200 g a.s./ha. A maximum residue value of 3.7 mg/kg was measured. The DT50 values in the individual studies were in the range of 2.47 to 5.04 days. The overall mean DT50 value for prothioconazole-desthio in wheat from the 8 studies was 3.2 days.).

The relevant zRMS's calculations based on refined parameter DF and are provided below:

**Refined long-term combined risk assessment with consideration DF values for BBCH 40-69**

Cereals, vole BBCH>40 – Tier 1, DF=0.1						Σ1/TER	Σ1/TER <sup>-1</sup>	Trigger
Azoxystrobin		Prothioconazole		JAU 6476-desthio				
28.5	0.035	85.35	0.0118	8.9	0.11	0.16	6.25	5

<sup>1)</sup>Lowest Tier 1 TER<sub>LT</sub>

Oilseed rape, vole BBCH>40-Tier 1, DF=0.2						Σ1/TER	Σ1/TER <sup>-1</sup>	Trigger
Azoxystrobin		Prothioconazole		JAU 6476-desthio				
23.3	0.043	69.52	0.014	7.3	0.136	0.193	5.18	5

<sup>1)</sup>Lowest Tier 1 TER<sub>LT</sub>

Sunflower, vole BBCH>40-Tier 1, including refined residues on grass (vole diet)						Σ1/TER	Σ1/TER <sup>-1</sup>	Trigger
Azoxystrobin		Prothioconazole		JAU 6476-desthio				
18.5 <sup>1</sup>	0.054	56.23 <sup>1</sup>	0.017	14 <i>5.8</i>	0.071 <i>0.17</i>	0.12 <i>0.24</i>	7.04 <i>4.16</i>	5

<sup>1)</sup>Lowest Tier 1 TER<sub>LT</sub>

For completeness purpose, long-term mixture toxicity risk assessment is also conducted for generic focal species “lagomorph” based on Tier 1 TER<sub>LT</sub> for use in sunflower.

**Combined long-term risk assessment for mammals, due to the use of CA3642 in sunflower – combined simultaneous toxicity of prothioconazole-desthio + azoxystrobin +prothioconazole to generic focal species “lagomorph”**

Sunflower, lagomorph-Tier 1						Σ1/TER	Σ1/TER <sup>-1</sup>	Trigger
Azoxystrobin		Prothioconazole		JAU 6476-desthio				
23.5	0.042	68.28	0.01	7.3	0.137	0.189	5.3	5

<sup>1)</sup>Lowest Tier 1 TER<sub>LT</sub>

Based on the first-tier combined risk assessment for the generic focal species “lagomorph” (including worst-case unrealistic assumption that 100% parent prothioconazole and 100% prothioconazole-desthio occur simultaneously), the value of ΣAF/TER<sup>-1</sup> is above 5 for the proposed uses of CA3642 in sunflower indicating acceptable long-term reproductive risk.

Overall, the combined long-term risk assessment is considered as acceptable for all proposed uses in the GAP.

### 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).

#### Leaf scenario

Since the product, CA3642, is not intended to be applied to leafy vegetables forming heads or crop plants with comparable water-collecting structures, at principal growth stage 4 or later, the leaf scenario does not need consideration.

#### 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  $K_{fOC}$  of 1765 L/kg (EFSA Sci. Report. 2007; 106, 1-98), prothioconazole belongs to the group of more sorptive substances.

<b>Effective application rate (g/ha)</b>	420*	<b>Quotient</b>
<b>Acute toxicity (mg/kg bw)</b>	>6200	<0.07
<b>Reprod. Toxicity (mg/kg bw/d)</b>	95.6	4.39

\*Total maximum seasonal application rate used here as a worst-case.

Since the quotients are less than the trigger of 3000, quantitative risk assessments for prothioconazole in drinking water are not required.

With a  $K_{fOC}$  of 573.5 (geometric mean; see dRR B8, Table 8.5-3), the metabolite, prothioconazole-desthio, belongs to the group of more sorptive substances.

<b>Effective application rate (g/ha)</b>	420* <sup>#</sup>	<b>Quotient</b>
<b>Acute toxicity (mg/kg bw)</b>	2235	0.19
<b>Reprod. Toxicity (mg/kg bw/d)</b>	10	42.0

\*Total maximum seasonal application rate of the a.s. used here as a worst-case. <sup>#</sup> It is assumed that 100% of the parent becomes the metabolite.

Since the quotients are less than the trigger of 3000, quantitative risk assessments for prothioconazole-desthio in drinking water are not required.

With a  $K_{fOC}$  of 392 L/kg (geometric mean; see dRR B8), azoxystrobin belongs to the group of less sorptive substances.

<b>Effective application rate (g/ha)</b>	420*	<b>Quotient</b>
<b>Acute toxicity (mg/kg bw)</b>	>5000	<0.08
<b>Reprod. Toxicity (mg/kg bw/d)</b>	32	13.13

\*Total maximum seasonal application rate of the a.s. for worst-case use on cereals used.

Since the quotients are less than the trigger of 50, quantitative risk assessments for azoxystrobin in drinking water are not required.

Based on their respective drinking water assessments, above, the resulting quotients derived for prothioconazole, prothioconazole-desthio and azoxystrobin, are between three and four orders of magnitude lower than the relevant trigger values. Since such a large margin of safety has been demonstrated for the individual substances, it is considered highly unlikely that a potential combined toxicity will pose an unacceptable risk to mammals from contaminated drinking water.

#### zRMS comments:

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

The calculations for active substances and prothioconazole-desthio has been validated by zRMS.

In order to apply consistent approach, the drinking water risk assessment was performed also for metabolite JAU 6476-S-methyl and is presented below. zRMS's calculations were performed with assumption of 10 times toxicity of the parent.

JAU 6476-S-methyl effective application rate 1 x 420 g/ha:

Acute toxicity (mg/kg bw)	620 quotient	= 0.67	Trigger: 3000
Reprod. toxicity (mg/kg bw/d)	9.56 quotient	= 43.93	

The evaluation of the risk resulting from uptake of contaminated water in Puddle scenario was not required since ratio between effective application rate and endpoint relevant for acute risk and long-term assessment is <3000.

### 9.3.2.4 Effects of secondary poisoning

The log  $P_{OW}$  values for prothioconazole, prothioconazole-desthio, and prothioconazole-S-methyl are 4.16, 3.04, and 4.19, respectively, and thus exceed the trigger value of 3 and a risk assessment for effects due to secondary poisoning is required. The log  $P_{OW}$  value for 1,2,5-triazole is <3 and, therefore, bioconcentration is not expected for this metabolite. An assessment is not triggered for azoxystrobin (log  $K_{OW}$  = 2.5) or its metabolites (EFSA Journal 2010; 8(4):1542).

#### Risk assessment for earthworm-eating mammals via secondary poisoning

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

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the oilseed rape with worst case initial soil PEC value) also covers the risk to mammals from all other intended uses (see 9.1.2).

**Table 9.3-21**: Assessment of the risk for earthworm-eating mammals due to exposure to prothioconazole via bioaccumulation in earthworms (secondary poisoning) for the intended uses of CA3642 in cereals and oilseed rape and sunflower

Parameter	Prothioconazole	Comments
PEC <sub>Soil</sub> (mg/kg soil)	0.192 0.1440	Worst-case initial PEC <sub>Soil</sub> for sunflower (see section Part B, Section 8.7) Worst-case initial PEC <sub>Soil</sub> for winter oilseed rape (see section Part B, Section 8.7)
log $P_{OW}$ / $P_{OW}$	4.16 / 14454	Worst-case log $P_{OW}$ value at pH 4; EFSA Sci. Report. 2007; 106, 1-98
$K_{oc}$	1765	EFSA Sci. Report. 2007; 106, 1-98
f <sub>oc</sub>	0.02	Default
BCF <sub>Worm</sub>	4.94	$BCF_{Worm/Soil} = (PEC_{Worm,ww}/PEC_{Soil,dw}) = (0.84 + 0.012P_{OW}) / f_{oc} \times K_{oc}$
PEC <sub>Worm</sub>	0.95 0.711	$PEC_{Worm} = PEC_{Soil} \times BCF_{Worm/Soil}$
Daily dietary dose (mg/kg bw/d)	1.213 0.91	$DDD = PEC_{Worm} \times 1.28$
NOEL (mg/kg bw/d)	95.6	EFSA Sci. Report. 2007; 106, 1-98
TER <sub>LT</sub>	78.78 105.05	Acceptable risk demonstrated

TER values shown in **bold** fall below the relevant trigger (<5).

**Table 9.3-22**: Assessment of the risk for earthworm-eating mammals due to exposure to prothioconazole-desthio via bioaccumulation in earthworms (secondary poisoning) for the intended uses of CA3642 in cereals and oilseed rape and sunflower

Parameter	Prothioconazole-desthio	Comments
PEC <sub>Soil</sub> (mg/kg soil)	0.0994 0.0746	Worst-case initial PEC <sub>Soil</sub> for sunflower (see Part B, Section 8.7) Worst-case initial PEC <sub>Soil</sub> for winter oilseed rape (see Part B, Section 8.7)
log $P_{OW}$ / $P_{OW}$	3.04 / 1100	Worst-case log $P_{OW}$ value at pH 4; EFSA Sci. Report. 2007; 106, 1-98
$K_{oc}$	573.5	Geometric mean; see dRR B8, Environmental Fate, Table 8.5-3
f <sub>oc</sub>	0.02	Default

Parameter	Prothioconazole-desthio	Comments
<b>BCF<sub>Worm</sub></b>	1.22	$BCF_{Worm/Soil} = (PEC_{Worm,ww}/PEC_{Soil,dw}) = (0.84 + 0.012P_{ow}) / f_{oc} \times K_{oc}$
<b>PEC<sub>Worm</sub></b>	0.12 <del>0.09</del>	$PEC_{Worm} = PEC_{Soil} \times BCF_{Worm/Soil}$
<b>Daily dietary dose (mg/kg bw/d)</b>	0.156 <del>0.117</del>	$DDD = PEC_{Worm} \times 1.28$
<b>NOEL (mg/kg bw/d)</b>	10.0	EFSA Sci. Report. 2007; 106, 1-98
<b>TER<sub>LT</sub></b>	64.21 <del>85.56</del>	Acceptable risk demonstrated

TER values shown in **bold** fall below the relevant trigger. (<5)

**Table 9.3-23~~17~~**: Assessment of the risk for earthworm-eating mammals due to exposure to prothioconazole-S-methyl via bioaccumulation in earthworms (secondary poisoning) for the intended uses of CA3642 in cereals ~~and~~ oilseed rape and sunflower

Parameter	Prothioconazole-S-methyl	Comments
<b>PEC<sub>Soil</sub> (mg/kg soil)</b>	0.0292 <del>0.0219</del>	Worst-case initial PEC <sub>Soil</sub> for sunflower, using FOCUS 1997 (see Section 8.7) <del>Worst case initial PEC<sub>Soil</sub> for winter cereals oilseed rape (see Part B, Section 8.7)</del>
<b>log Pow / Pow</b>	4.19 / 15500	EFSA Sci. Report. 2007; 106, 1-98
<b>K<sub>oc</sub></b>	2525.9	Table 8.5-2 for SEU dRR part B8 (CA3642), based on geometric mean
<b>f<sub>oc</sub></b>	0.02	Default
<b>BCF<sub>Worm</sub></b>	3.70	$BCF_{Worm/Soil} = (PEC_{Worm,ww}/PEC_{Soil,dw}) = (0.84 + 0.012P_{ow}) / f_{oc} \times K_{oc}$
<b>PEC<sub>Worm</sub></b>	0.11 <del>0.08</del>	$PEC_{Worm} = PEC_{Soil} \times BCF_{Worm/Soil}$
<b>Daily dietary dose (mg/kg bw/d)</b>	0.138 <del>0.104</del>	$DDD = PEC_{Worm} \times 1.28$
<b>NOEL (mg/kg bw/d)</b>	9.56	Conservative estimate of parent NOEL/10
<b>TER<sub>LT</sub></b>	69.2 <del>92.21</del>	Acceptable risk demonstrated

TER values shown in **bold** fall below the relevant trigger (<5).

Using worst-case, conservative initial PEC<sub>Soil</sub> values (as opposed to 21-d TWA values), acceptable risks are concluded for earthworm-eating mammals, due to exposure to prothioconazole, prothioconazole-desthio, and prothioconazole-S-methyl, with large margins of safety (i.e., TER values well above the trigger of 5).

### Risk assessment for fish-eating mammals via secondary poisoning

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

**Table 9.3-24~~18~~**: Assessment of the risk for fish-eating mammals due to exposure to prothioconazole via bioaccumulation in fish (secondary poisoning) for the intended uses of CA3642 in cereals, ~~and~~ oilseed rape and sunflower

Parameter	Prothioconazole	Comments
<b>PEC<sub>sw</sub> (mg/L)</b>	0.02281	Worst-case FOCUS Step 1 PEC <sub>sw</sub> for 2 x 210 g a.s./ha in cereals (Part B, Section 8, Table 8.9-16)
<b>BCF<sub>Fish</sub></b>	19.7	EFSA Sci. Report. 2007; 106, 1-98



Parameter	Prothioconazole	Comments
<b>BMF</b>	Not applicable	biomagnification factor (relevant to $BCF \geq 2000$ )
<b>PEC<sub>Fish</sub></b>	0.45	$PEC_{Fish} = PEC_{Water} \times BCF_{Fish}$
<b>Daily dietary dose (mg/kg bw/d)</b>	0.0638	$DDD = PEC_{Fish} \times 0.142$
<b>NOEL (mg/kg bw/d)</b>	95.6	EFSA Sci. Report. 2007; 106, 1-98
<b>TER<sub>LT</sub></b>	1498.23	Acceptable risk demonstrated

TER values shown in **bold** fall below the relevant trigger (<5).

**Table 9.3-25 19:** Assessment of the risk for fish-eating mammals due to exposure to prothioconazole-desthio via bioaccumulation in fish (secondary poisoning) for the intended uses of CA3642 in cereals, and oilseed rape and sunflower

Parameter	Prothioconazole-desthio	Comments
<b>PEC<sub>sw</sub> (mg/L)</b>	0.08228	Worst-case FOCUS Step 1 PEC <sub>sw</sub> for 2 x 210 g a.s./ha in cereals (Part B, Section 8, Table 8.9-31)
<b>BCF<sub>Fish</sub></b>	65	EFSA Sci. Report. 2007; 106, 1-98
<b>BMF</b>	Not applicable	biomagnification factor (relevant to $BCF \geq 2000$ )
<b>PEC<sub>Fish</sub></b>	5.35	$PEC_{Fish} = PEC_{Water} \times BCF_{Fish}$
<b>Daily dietary dose (mg/kg bw/d)</b>	0.759	$DDD = PEC_{Fish} \times 0.142$
<b>NOEL (mg/kg bw/d)</b>	10.0	EFSA Sci. Report. 2007; 106, 1-98
<b>TER<sub>LT</sub></b>	13.17	Acceptable risk demonstrated

TER values shown in **bold** fall below the relevant trigger (<5).

**Table 9.3-26 20:** Assessment of the risk for fish-eating mammals due to exposure to prothioconazole-S-methyl via bioaccumulation in fish (secondary poisoning) for the intended uses of CA3642 in cereals and oilseed rape and sunflower

Parameter	Prothioconazole-S-methyl	Comments
<b>PEC<sub>sw</sub> (mg/L)</b>	0.00824 0.03365 0.00487	Worst-case FOCUS Step 1 PEC <sub>sw</sub> for 2 x 210 g a.s./ha in cereals (Part B, Section 8, Table 8.9-24)
<b>BCF<sub>Fish</sub></b>	5.08	Estimated using EPI Suite <sup>3</sup>
<b>BMF</b>	Not applicable	biomagnification factor (relevant to $BCF \geq 2000$ )
<b>PEC<sub>Fish</sub></b>	0.041 0.17 0.025	$PEC_{Fish} = PEC_{Water} \times BCF_{Fish}$
<b>Daily dietary dose (mg/kg bw/d)</b>	0.0059 0.024 0.004	$DDD = PEC_{Fish} \times 0.142$
<b>NOEL (mg/kg bw/d)</b>	9.56	Conservative estimate of parent NOEL/10
<b>TER<sub>LT</sub></b>	1620.34 398.3 2721.3	Acceptable risk demonstrated

TER values shown in **bold** fall below the relevant trigger (<5).

Using worst-case, conservative inputs and FOCUS Step-1 actual PEC<sub>sw</sub> values, acceptable risks are concluded for fish-eating mammals, due to exposure to prothioconazole, prothioconazole-desthio, and prothioconazole-S-methyl, with large margins of safety (i.e., TER values well above the trigger of 5).

**zRMS comments:**

<sup>3</sup>US EPA. 2021. Estimation Programs Interface Suite™ for Microsoft® Windows, v 4.11. United States Environmental Protection Agency, Washington, DC, USA.

The Applicants' approach in evaluation of the risk of secondary poisoning is in line with EFSA (2009). Compounds selected for this assessment are agreed by the zRMS. Evaluation was not triggered for remaining metabolites of active substance due to their log Pow <3.

Overall, acceptable risk of secondary exposure from all relevant compounds could be concluded for mammals.

### **9.3.2.5 Biomagnification in terrestrial food chains**

Not relevant, as mammalian toxicity and goat metabolism data did not show the potential for accumulation of prothioconazole (EFSA Scientific Report. 2007, 106, 1-98) nor azoxystrobin (EFSA Journal, 2010; 8(4):1542), respectively.

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

Not relevant, as the product is proposed for use as foliar spray.

### **9.3.4 Overall conclusions**

Acceptable risks to mammals from dietary, drinking water and secondary poisoning exposure routes can be concluded for all intended uses of CA3642 at the screening and first-tier assessments apart from the long-term risk from prothioconazole-desthio at BBCH  $\geq 40$  for cereals and the scenario *small herbivorous mammal "vole"*.

Long-term risk from combination toxicity of prothioconazole-desthio and azoxystrobin at Tier 1 for the generic focal species small herbivorous mammal "vole" is also not acceptable for uses in cereals, oilseed rape, and sunflower.

However, after applying a robust, conservative higher-tier risk assessment (refined deposition factor and 100% formation of the metabolite from the parent), an acceptable risk to this generic focal species is concluded in cereals. Based on refinements no unacceptable risks to mammals from the combined toxicity of prothioconazole and azoxystrobin or prothioconazole-desthio and azoxystrobin is also concluded.

For the use in sunflower, the combined risk to small herbivorous mammal "vole" is refined by considering appropriate deposition to grass under the crop as well as residue dissipation data.

The additional intended minor uses of CA3642 in sunflower, flax, linseed, poppy, mustard and Gold of pleasure are considered to be covered by the risk assessment envelope for use in spring and winter oilseed rape respectively.

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

No specific reptile or amphibian data are being submitted. However, the testing of these species and their appropriate risk assessment methodology are not yet agreed in Europe. An inherent level of safety has been demonstrated, based on the acceptable aquatic and terrestrial risk assessments that will cover the risk to these organisms.

## **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 prothioconazole, azoxystrobin and their relevant metabolites. Full details of these studies are provided in the respective EU DARs and related documents.

Effects on aquatic organisms of CA3642 were not evaluated as part of the EU assessment of prothioconazole and azoxystrobin. New data for this product submitted with this application are listed in Appendix 1 and summarised in **Appendix 2**.

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

Species	Substance	Exposure System	Results	RAC (µg/L)	Reference
Active substance					
<i>Oncorhynchus mykiss</i>	Prothioconazole	96-h, s	LC <sub>50</sub> = 1.83 mg a.s./L <sub>mm</sub>	18.3	EFSA Sci. Report. 2007; 106, 1-98
		97-d ELS	NOEC = 0.308 mg a.s./L <sub>mm</sub>	30.8	
<i>Daphnia magna</i>		48-h, s	EC <sub>50</sub> = 1.3 mg a.s./L <sub>mm</sub>	13	
		21-d, ss	NOEC = 0.56 mg a.s./L <sub>nom</sub>	56	
<i>Chironomus riparius</i>		28-d, s, spiked water	NOEC = 9.14 mg/L <sub>nom</sub>	914	
<i>Pseudokirchneriella subcapitata</i>		96-h, s	72 h ErC <sub>50</sub> = 2.18 mg a.s./L <sub>im</sub>	218	
Prothioconazole-desthio					
<i>Oncorhynchus mykiss</i>	Prothioconazole-desthio	96-h, s	LC <sub>50</sub> = 6.63 mg/L <sub>nom</sub>	66.3	EFSA Sci. Report. 2007; 106, 1-98
		96-d ELS	NOEC (deformities) = 0.00334 mg/L <sub>mm</sub>	0.334	
<i>Daphnia magna</i>		48-h, s	EC <sub>50</sub> >10 mg/L <sub>mm</sub>	>100	
		21-d, ss	NOEC = 0.10 mg/L <sub>nom</sub>	10	
<i>Chironomus riparius</i>		28-d, s, spiked water	NOEC = 2.0 mg/L <sub>nom</sub>	200	
<i>Scenedesmus subspicatus</i>		96-h, s	ErC <sub>50</sub> = 0.55 mg/L <sub>nom</sub>	55	
Prothioconazole-S-methyl (not in surface water or sediment residue definition in EFSA Sci. Report. 2007; 106, 1-98)					
<i>Oncorhynchus mykiss</i>	Prothioconazole-S-methyl	96-h, ss	LC <sub>50</sub> = 1.8 mg/L <sub>mm</sub>	18.0	EFSA Sci. Report. 2007; 106, 1-98
<i>Daphnia magna</i>		48-h, s	EC <sub>50</sub> = 2.8 mg/L <sub>nom</sub>	28	
<i>Pseudokirchneriella subcapitata</i>		72 h, s	ErC <sub>50</sub> = 47.4 mg/L <sub>im</sub>	4740	
1,2,4-triazole					
<i>Oncorhynchus mykiss</i>	1,2,4-triazole	96-h, s	LC <sub>50</sub> = 498 mg/L	4980	EFSA Sci. Report. 2007; 106, 1-98
		97-d ELS	NOEC = 3.2 mg/L	320	
<i>Daphnia magna</i>		48-h, s	EC <sub>50</sub> = 900 mg/L	9000	
		48-h, s	EC <sub>50</sub> >100 mg/L	>1000	
<i>Pseudokirchneriella subcapitata</i>		72-h, s	ErC <sub>50</sub> = 22.5 mg/L	2250	
Fish bioconcentration					
<i>Lepomis macrochirus</i>	Prothioconazole	Bioconcentration	BCF 19.7 (Whole fish wet weight) Clearance time (CT <sub>50</sub> days):0.8 Level of residues (%) after 14 days depuration phase: 9%		EFSA Scientific Report (2007) 106, 1- 98
<i>Lepomis macrochirus</i>	JAU 6476-desthio	Bioconcentration	BCF 65 (Whole fish wet weight) Clearance time (CT <sub>50</sub> days):0.4-0.5		EFSA Scientific Report (2007) 106, 1- 98

			Level of residues (%) after 14 days depuration phase: 4%		
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RAC value in bold is the critical RAC (regulatory acceptable concentration) for each a.s. or metabolite. mm: mean measured; nom: nominal; im: initial measured.

**zRMS comments:**

Aquatic toxicity data for prothioconazole and prothioconazole -desthio provided in Tables 9.5-1 above are in line with EU agreed endpoints reported in EFSA Scientific Report (2007) 106.

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

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

**Table 9.5-2: Critical endpoints and effect values relevant for the risk assessment for aquatic organisms – azoxystrobin and relevant metabolites**

– azoxystrobin and relevant metabolites					
Species	Substance	Exposure System	Results	RAC (µg/L)	Reference
Active substance					
<i>Oncorhynchus mykiss</i>	Azoxystrobin	96-h, f	EC <sub>50</sub> = 0.47 mg a.s./L <sub>mm</sub>	4.7	EFSA Journal 2010; 8(4):1542
<i>Pimephales promelas</i>		33 d ELS	NOEC (growth) = 0.147 mg a.s./L <sub>mm</sub>	14.7	
<i>Daphnia magna</i>		48-h, s	EC <sub>50</sub> = 0.23 mg a.s./L <sub>mm</sub>	2.3	
<i>Macrocyclus fuscus</i>		48-h, s	EC <sub>50</sub> = 0.13 mg a.s./L <sub>nom</sub>	1.3	
<i>Mysidopsis bahia</i>		96-h, s	EC <sub>50</sub> = 0.055 mg a.s./L <sub>nom</sub>	0.55	
<i>Crassostrea gigas</i>		48-h, s	EC <sub>50</sub> = 1.3 mg a.s./L <sub>nom</sub>	13.0	
<i>Daphnia magna</i>		21-d, s	NOEC = 0.044 mg a.s./L <sub>nom</sub>	4.4	
<i>Chironomus riparius</i>		28-d, s	NOEC = 0.8 mg a.s./L	80	
<i>Selenastrum capricornutum</i>		72-h, s	EC <sub>50</sub> = 0.36 mg a.s./L <sub>mm</sub>	36.0	
<i>Skeletonema costatum</i>		72-h, s	E <sub>r</sub> C <sub>50</sub> = 0.3 mg a.s./L <sub>nom</sub>	30.0	
<i>Navicula pelliculosa</i>		120-h, s	E <sub>r</sub> C <sub>50</sub> = 0.146 mg a.s./L <sub>nom</sub>	14.6	
<i>Anabaena flos-aquae</i>		120-h, s	E <sub>r</sub> C <sub>50</sub> = 13.9 mg a.s./L <sub>mm</sub>	139	
<i>Lemna gibba</i> †		14-d, s	EC <sub>50</sub> = 3.2 mg a.s./L <sub>nom</sub>	320	
Higher-tier invertebrate data		-	-	<b>3.3*</b>	
Metabolites					
<i>Oncorhynchus mykiss</i>	R234886	96-h, f	EC <sub>50</sub> >150 mg/L <sub>mm</sub>	<b>&gt;1500</b>	EFSA Journal 2010; 8(4):1542
<i>Daphnia magna</i>		48-h, s	EC <sub>50</sub> >180 mg a.s./L <sub>mm</sub>	>1800	
<i>Selenastrum capricornutum</i>		72-h, s	EC <sub>50</sub> = 47.0 mg a.s./L <sub>mm</sub>	4700	
<i>Oncorhynchus mykiss</i>	R402173	96-h, s	EC <sub>50</sub> = 62 mg/L <sub>nom</sub>	<b>620</b>	EFSA Journal 2010; 8(4):1542
<i>Daphnia magna</i>		48-h, s	EC <sub>50</sub> >100 mg a.s./L <sub>nom</sub>	>1000	
<i>Selenastrum capricornutum</i>		72-h, s	E <sub>r</sub> C <sub>50</sub> = 67.0 mg a.s./L <sub>mm</sub>	6700	
<i>Oncorhynchus mykiss</i>	R401553	96-h, s	EC <sub>50</sub> >120 mg/L <sub>nom</sub>	<b>&gt;1200</b>	

<i>Daphnia magna</i>		48-h, s	EC <sub>50</sub> >120 mg a.s./L <sub>nom</sub>	>1200	EFSA Journal 2010; 8(4):1542
<i>Selenastrum capricornutum</i>		72-h, s	ErC <sub>50</sub> >120 mg a.s./L <sub>nom</sub>	>12000	

RAC value in **bold** is the critical RAC (regulatory acceptable concentration) for each a.s. or metabolite. mm: mean measured; nom: nominal; im: initial measured. #The worst case algal endpoint covers the risk to aquatic macrophytes. \*Higher-tier RAC value, based on data from a mesocosm study, first-tier toxicity data, and the lower limit of a calculated HC<sub>5</sub> – see EFSA conclusion on azoxystrobin (EFSA Journal 2010; 8(4):1542), for full details.

**zRMS comments:**

Aquatic toxicity data (critical endpoints) for provided in Tables 9.5-2 above are in line with EU agreed endpoints reported in EFSA Scientific Report 2010; 8(4):1542.

**Table 9.5-3: Critical endpoints and effect values relevant for the risk assessment for aquatic organisms – product CA3642**

Species	Substance	Exposure System	Results	RAC (µg/L)	Reference
<b>CA3642</b>					
<i>Oncorhynchus mykiss</i>	CA3642	96 h, s	LC <sub>50</sub> >2.04 mg CA3642/L mm  LC <sub>50</sub> >0.57 mg sum a.s./L (sum geometric mm )	>20.4  5.7	KCP 10.2.1/01
<i>Daphnia magna</i>	CA3642	48 h, s	EC <sub>50</sub> = 0.97 mg CA3642/L mm  EC <sub>50</sub> = 0.27 mg sum a.s./L (sum geometric mm)	9.7  2.7	KCP 10.2.1/02
<i>Skeletonema costatum</i>	CA3642	72 h, s	ErC <sub>50</sub> = 0.487 mg CA3642/L mm  ErC <sub>50</sub> = 0.136 mg sum a.s./L (sum geometric mm)  EyC <sub>50</sub> = 0.222 mg CA3642/L mm  NOEC =0.068 mg CA3642/L (mm) ErC <sub>10</sub> = 0.172 mg CA3642/L (mm) EyC <sub>10</sub> = 0.086 mg CA3642/L (mm)	48.7  13.6	KCP 10.2.1/03

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

**zRMS comments:**

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

### 9.5.1.1 Justification for new endpoints

To comply with data requirements of Regulation (EU) 284/2013, three new studies with fish, aquatic invertebrates, and algae were conducted with an SC fungicide containing two active substances. The choice of algal species for testing was based on the draft renewal information for prothioconazole and azoxystrobin. As there are two active substances in the product CA3642 and the combined toxicity is not known, studies on all three taxonomic groups (fish, aquatic invertebrates and algae) have been conducted,

in accordance with “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”.

Based on the available data, it is considered that the new data for CA3642, do not show any significant increase in aquatic toxicity compared to the expected additive toxicity of the two active substances.

A formulation study with aquatic macrophytes is not considered necessary as CA3642 is not a herbicide and the available data for both active substances demonstrate a lower toxicity to *Lemna gibba* than to aquatic invertebrates.

## 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).

Worst case endpoints are used in the aquatic risk assessment and compared to the relevant maximum FOCUS Step 1, 2 and 3 PEC<sub>SW</sub> values for risk assessments covering the proposed use pattern (see Part B8 for full details of the PEC<sub>SW</sub> values) and the resulting PEC/RAC ratios are presented in the tables below.

The following text is added due to agreements during the Central Zone harmonisation meetings. It should be noted that this text has no impact on the outcome of zonal evaluation of formulation CA3642/ Joust Pro which was performed in line with the EU agreed methodology.

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

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the oilseed rape group also covers the risk for aquatic organisms from all other intended uses ~~in sunflower~~, flax, linseeds, poppy, mustard and Gold of Pleasure (see 9.1.2).

The aquatic risk assessments presented below, unless otherwise stated, are based on PEC<sub>SW</sub> values for the intended uses of the product, CA3642, in cereals and oilseed rape, which are considered worst-case calculations. Note that all D1, D2, D6 and R2 scenarios are not relevant to the Central EU zone according to the Working Document of the Central Zone in the Authorisation of PPPs (v1r1 June 2018).

The ratios between predicted environmental concentrations in surface water bodies (PEC<sub>SW</sub>, PEC<sub>SED</sub>) and regulatory acceptable concentrations (RAC) for aquatic organisms are given per intended use for each FOCUS scenario and each organism group.

### Assessment of the risk from the active substance, prothioconazole

**Table 9.5-4: Acceptability of risk (PEC/RAC <1) for the active substance prothioconazole for each organism group, based on PEC<sub>SW</sub> values from FOCUS Steps 1 and 2, for the use of CA3642 in spring and winter cereals (2 x 210 g a.s./ha)**

Group	Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Sediment dweller	Algae
Test species	<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>C. riparius</i>	<i>P. subcapitata</i>
Endpoint	LC <sub>50</sub>	NOEC	EC <sub>50</sub>	NOEC	NOEC	E <sub>r</sub> C <sub>50</sub>
(µg a.s./L)	1830	308	1300	560	9140	2180
AF	100	10	100	10	10	10

<b>RAC (µg a.s./L)</b>		18.3	30.8	13	56	914	218
<b>FOCUS Scenario</b>	<b>PEC<sub>sw</sub> (µg a.s./L)</b>	<b>PEC/RAC</b>					
	<b>Step 1</b>						
Worst case	22.81	<b>1.25</b>	0.74	<b>1.75</b>	0.41	0.025	0.10
	<b>Step 2</b>						
N-EU‡	1.93	0.11	-	0.15	-	-	-

PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**. PEC<sub>sw</sub> values selected from the worst-case FOCUS Step-1 and 2 values, either for single or double applications. ‡FOCUS Step 2 N-EU PEC<sub>sw</sub> values are relevant for all central zone member states. AF: assessment factor; PEC: predicted environmental concentration; RAC: regulatory acceptable concentration

**Table 9.5-5: Acceptability of risk (PEC/RAC <1) for the active substance, prothioconazole for each organism group based on PEC<sub>sw</sub> values from FOCUS Steps 1 and 2, for the use of CA3642 in spring and winter oilseed rape (1 x 180 g a.s./ha)**

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Sediment dweller	Algae
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>C. riparius</i>	<i>P. subcapitata</i>
Endpoint		LC <sub>50</sub>	NOEC	EC <sub>50</sub>	NOEC	NOEC	E <sub>r</sub> C <sub>50</sub>
(µg a.s./L)		1830	308	1300	560	9140	2180
AF		100	10	100	10	10	10
RAC (µg a.s./L)		18.3	30.8	13	56	914	218
FOCUS Scenario	PEC <sub>sw</sub> (µg a.s./L)	PEC/RAC					
Step 1							
Worst case	19.55	1.07	0.63	1.50	0.35	0.021	0.09
Step 2							
N-EU‡	1.66	0.09	-	0.13	-	-	-

PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**. PEC<sub>sw</sub> values selected from the worst-case FOCUS Step-1 and 2 values. <sup>‡</sup>FOCUS Step 2 N-EU PEC<sub>sw</sub> values are relevant for all central zone member states. AF: assessment factor; PEC: predicted environmental concentration; RAC: regulatory acceptable concentration.

An acceptable risk from the active substance, prothioconazole, is concluded for all aquatic organisms based on FOCUS Step 1 and 2 PEC<sub>sw</sub> values for the intended uses of the product, CA3642.

**Table 9.5-6: Acceptability of risk (PEC/RAC <1) for the active substance, prothioconazole for each organism group based on PEC<sub>sw</sub> values from FOCUS Steps 1 and 2, for the use of CA3642 in sunflower (1 x 180 g a.s./ha)**

In sunflower (1 x 100 g a.s./ha)							
Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Sediment dweller	Algae
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>C. riparius</i>	<i>P. subcapitata</i>
Endpoint		LC <sub>50</sub>	NOEC	EC <sub>50</sub>	NOEC	NOEC	E <sub>r</sub> C <sub>50</sub>
(µg a.s./L)		1830	308	1300	560	9140	2180
AF		100	10	100	10	10	10
RAC (µg a.s./L)		18.3	30.8	13	56	914	218
FOCUS Scenario		PEC <sub>sw</sub> (µg a.s./L)	PEC/RAC				
Step 1							
Worst case		19.55	1.07	0.63	1.50	0.35	0.021
Step 2							
N-EU†		1.66	0.09	-	0.13	-	-

PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**. PEC<sub>sw</sub> values selected from the worst-case FOCUS Step-1 and 2 values. <sup>‡</sup>FOCUS Step 2 N-EU PEC<sub>sw</sub> values are relevant for all central zone member states. AF: assessment factor; PEC: predicted environmental concentration; RAC: regulatory acceptable concentration.

An acceptable risk from the active substance, prothioconazole, is concluded for all aquatic organisms based on FOCUS Step 1 and 2 PEC<sub>sw</sub> values for the intended uses of the product, CA3642.



### Assessment of the risk from the metabolite, prothioconazole-S-methyl

**Table 9.5-7-6:** Acceptability of risk (PEC/RAC <1) for the metabolite, prothioconazole-S-methyl, for each organism group, based on PEC<sub>sw</sub> values from FOCUS Step 1, for the use of CA3642 in spring and winter cereals (2 x 210 g a.s./ha)

Group		Fish acute	Inverteb. acute	Algae
Test species		<i>O. mykiss</i>	<i>D. magna</i>	<i>P. subcapitata</i>
Endpoint (µg metabolite/L)		LC <sub>50</sub> 1800	EC <sub>50</sub> 2800	ErC <sub>50</sub> 47400
AF		100	100	10
RAC (µg metabolite/L)		18	28	4740
FOCUS Scenario	PEC <sub>sw</sub> (µg metabolite/L)	PEC/RAC		
Step 1				
Worst case	8.24 33.65 4.87	0.45 1.87 0.27	0.29 1.2 0.17	0.001 0.0079 <0.01
Step 2				
Worst case	0.58 1.78	0.03 0.098	0.02 0.063	—

PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**. AF: assessment factor; PEC: predicted environmental concentration; RAC: regulatory acceptable concentration.

**Table 9.5-8 7:** Acceptability of risk (PEC/RAC <1) for the metabolite, prothioconazole-S-methyl, for each organism group, based on PEC<sub>sw</sub> values from FOCUS Steps 1, for the use of CA3642 in spring and winter oilseed rape (1 x 180 g a.s./ha)

Group		Fish acute	Inverteb. acute	Algae
Test species		<i>O. mykiss</i>	<i>D. magna</i>	<i>P. subcapitata</i>
Endpoint		LC <sub>50</sub>	EC <sub>50</sub>	ErC <sub>50</sub>
(µg metabolite/L)		1800	2800	47400
AF		100	100	10
RAC (µg metabolite/L)		18	28	4740
FOCUS Scenario	PEC <sub>sw</sub> (µg metabolite/L)	PEC/RAC		
Step 1				
Worst case	4.12	0.22	0.15	0.000087

PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**. AF: assessment factor; PEC: predicted environmental concentration; RAC: regulatory acceptable concentration.

An acceptable risk from the metabolite, prothioconazole-s-methyl, is concluded for all aquatic organisms based on FOCUS Step 1 PEC<sub>sw</sub> values for the intended uses of CA3642.

**Table 9.5-9:** Acceptability of risk (PEC/RAC <1) for the metabolite, prothioconazole-S-methyl, for each organism group, based on PEC<sub>sw</sub> values from FOCUS Step 1, for the use of CA3642 in sunflower (1 x 180 g a.s./ha)

Group		Fish acute	Inverteb. acute	Algae
Test species		<i>O. mykiss</i>	<i>D. magna</i>	<i>P. subcapitata</i>
Endpoint (µg metabolite/L)		LC <sub>50</sub> 1800	EC <sub>50</sub> 2800	ErC <sub>50</sub> 47400
AF		100	100	10
RAC (µg metabolite/L)		18	28	4740
FOCUS Scenario	PEC <sub>sw</sub> (µg metabolite/L)	PEC/RAC		
Step 1				
Worst case	4.12 14.42 2.09	0.22 0.18 0.12	0.15 0.51 0.07	0.000087 0.0036 0.01

PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**. AF: assessment factor; PEC: predicted environmental concentration; RAC: regulatory acceptable concentration.

An acceptable risk from the metabolite, prothioconazole-s-methyl, is concluded for all aquatic organisms based on FOCUS Step 1-2 PEC<sub>sw</sub> values for the intended uses of CA3642.

## Assessment of the risk from 1,2,4-triazole

**Table 9.5-10:** Acceptability of risk (PEC/RAC <1) for the metabolite, 1,2,4-triazole, for each organism group, based on PEC<sub>sw</sub> values from FOCUS Step 1, for the use of CA3642 in spring and winter cereals (2 x 210 g a.s./ha)

Winter cereals (2 x 210 g a.s./ha)					
Group		Fish acute	Fish prolonged	Inverteb. acute	Algae
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>P. subcapitata</i>
Endpoint		LC <sub>50</sub>	NOEC	EC <sub>50</sub>	ErC <sub>50</sub>
(µg metabolite/L)		498000	3200	900 000	22500
AF		100	10	100	10
RAC (µg metabolite/L)		4980	320	9000	2250
FOCUS Scenario		PEC <sub>sw</sub> (µg metabolite/L)	PEC/RAC		
Step 1					
Worst case		6.98	<0.01	0.02	<0.01

PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**. AF: assessment factor; PEC: predicted environmental concentration; RAC: regulatory acceptable concentration.

**Table 9.5- 11:** Acceptability of risk (PEC/RAC <1) for the metabolite, 1,2,4-triazole, for each organism group, based on PEC<sub>sw</sub> values from FOCUS Step 1, for the use CA3642 in spring and winter oilseed rape (1 x 180 g a.s./ha)

Group		Fish acute	Fish prolonged	Inverteb. acute	Algae
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>P. subcapitata</i>
Endpoint		LC <sub>50</sub>	NOEC	EC <sub>50</sub>	ErC <sub>50</sub>
(µg metabolite/L)		498000	3200	900000	22500
AF		100	10	100	10
RAC (µg metabolite/L)		4980	320	9000	2250
FOCUS Scenario	PEC <sub>sw</sub> (µg metabolite/L)	PEC/RAC			
Step 1					
Worst case	2.99	<0.01	<0.01	<0.01	<0.01

PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**. AF: assessment factor; PEC: predicted environmental concentration; RAC: regulatory acceptable concentration.

An acceptable risk from the metabolite 1,2,4-triazole is concluded for all aquatic organisms based on FOCUS Step 1 PEC<sub>sw</sub> values for the intended uses of the product CA3642.

**Table 9.5-12:** Acceptability of risk (PEC/RAC <1) for the metabolite, 1,2,4-triazole, for each organism group, based on PEC<sub>sw</sub> values from FOCUS Step 1, for the use of CA3642 in sunflower (1 x 180 g a.s./ha)

Group		Fish acute	Fish prolonged	Inverteb. acute	Algae
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>P. subcapitata</i>
Endpoint		LC <sub>50</sub>	NOEC	EC <sub>50</sub>	ErC <sub>50</sub>
(µg metabolite/L)		498000	3200	900 000 <del>1000000</del>	22500
AF		100	10	100	10
RAC (µg metabolite/L)		4980	320	9000 <del>10000</del>	2250
FOCUS Scenario	PEC <sub>sw</sub> (µg metabolite/L)	PEC/RAC			
Step 1					
Worst case	2.99	<0.01	<0.01	<0.01	<0.01

PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**. AF: assessment factor; PEC: predicted environmental concentration; RAC: regulatory acceptable concentration.

An acceptable risk from the metabolite 1,2,4-triazole is concluded for all aquatic organisms based on

FOCUS Step 1 PEC<sub>sw</sub> values for the intended uses of the product CA3642.

**zRMS comments:**

The calculations of the risk assessment for metabolite have been amended for metabolite prothioconazole-S-methyl with regard the PEC<sub>sw</sub> values agreed in Section 8.

Overall, an acceptable risk from the metabolites is concluded for all aquatic organisms based on FOCUS Step 1-2 PEC<sub>sw</sub> values for the intended uses of the product CA364.

Assessment of the risk from the metabolite prothioconazole-desthio to spring and winter cereals

**Table 9.5-13 10:** Acceptability of risk (PEC/RAC <1) for the metabolite prothioconazole-desthio for each organism group based on PEC<sub>sw</sub> values from FOCUS Steps 1-3, for the use of CA3642 in spring cereals (1 x and 2 x 210 g a.s./ha)

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Sediment dweller	Algae
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>C. riparius</i>	<i>S. subspicatus</i>
Endpoint (µg metabolite/L)		LC <sub>50</sub> 6630	NOEC 3.34	EC <sub>50</sub> >10000	NOEC 100	NOEC 2000	E <sub>r</sub> C <sub>50</sub> 550
AF		100	10	100	10	10	10
RAC (µg metabolite/L)		66.3	0.334	>100	10	200	55
FOCUS Scenario	PEC <sub>sw</sub> (µg metabolite/L)	PEC/RAC					
Step 1							
Worst case	82.28	1.24	246.35	<0.82	8.23	0.41	1.50
Step 2							
N-EU‡	6.23	0.09	18.65	-	1.14	-	0.21
Step-3, Single applications							
D3/Ditch	0.1279	-	0.38	-	0.01	-	-
D4/Pond	0.02946	-	0.09	-	0.00	-	-
D4/Stream	0.08644	-	0.26	-	0.01	-	-
D5/Pond	0.02774	-	0.08	-	0.00	-	-
D5/Stream	0.1227	-	0.37	-	0.01	-	-
R4/Stream	0.7175	-	2.15	-	0.07	-	-
Step-3, Two applications							
D3/Ditch	0.1158	-	0.35	-	0.01	-	-
D4/Pond	0.0468	-	0.14	-	0.005	-	-
D4/Stream	0.0812	-	0.24	-	0.01	-	-
D5/Pond	0.04524	-	0.14	-	0.005	-	-
D5/Stream	0.1107	-	0.33	-	0.01	-	-
R4/Stream	1.386	-	4.15	-	0.14	-	-

PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**. PEC<sub>sw</sub> values selected from the worst-case FOCUS Step-1-2 values, either for single or double applications. †FOCUS Step 2 N-EU PEC<sub>sw</sub> values are relevant for all central zone member states. AF: assessment factor; PEC: predicted environmental concentration; RAC: regulatory acceptable concentration.

For the intended uses of CA3642 in spring cereals an acceptable risk from the metabolite prothioconazole-desthio is concluded for all aquatic organisms for most FOCUS Step-3 scenarios (based on the critical tier-1 RAC<sub>sw</sub> value of 0.334 µg/L of chronic fish). The exception occurs with the FOCUS Step-3 scenario R4/stream for single and two applications of CA3642, therefore risk mitigation measures are required for this scenario.

**Table 9.5-14** Acceptability of risk (PEC/RAC <1) for the metabolite prothioconazole-desthio for each organism group based on PEC<sub>sw</sub> values from FOCUS Steps 1-3 for the use of CA3642 in winter cereals (1 x and 2 x 210 g a.s./ha)

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Sediment dweller	Algae
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>C. riparius</i>	<i>S. subspicatus</i>
Endpoint (µg metabolite/L)		LC <sub>50</sub> 6630	NOEC 3.34	EC <sub>50</sub> >10000	NOEC 100	NOEC 2000	E <sub>r</sub> C <sub>50</sub> 550
AF		100	10	100	10	10	10
RAC (µg metabolite/L)		66.3	0.334	>100	10	200	55
FOCUS Scenario	PEC <sub>sw</sub> (µg metabolite/L)	PEC/RAC					
Step 1							
Worst case	82.28	1.24	246.35	<0.82	8.23	0.41	1.50
Step 2							
N-EU‡	6.23	0.09	18.65	-	0.88	-	0.16
Step-3, Single applications							
D3/Ditch	0.06281	-	0.19	-	-	-	-
D4/Pond	0.02159	-	0.06	-	-	-	-
D4/Stream	0.0757	-	0.23	-	-	-	-
D5/Pond	0.02706	-	0.08	-	-	-	-
D5/Stream	0.1166	-	0.35	-	-	-	-
D6/Ditch	0.08564	-	0.26	-	-	-	-
R1/Pond	0.05745	-	0.17	-	-	-	-
R1/Stream	0.3729	-	1.12	-	-	-	-
R3/Stream	0.4835	-	1.45	-	-	-	-
R4/Stream	0.2592	-	0.78	-	-	-	-
Step-3, Two applications							
D3/Ditch	0.1108	-	0.33	-	-	-	-
D4/Pond	0.03718	-	0.11	-	-	-	-
D4/Stream	0.06775	-	0.20	-	-	-	-
D5/Pond	0.04599	-	0.14	-	-	-	-
D5/Stream	0.1125	-	0.34	-	-	-	-
D6/Ditch	0.2078	-	0.62	-	-	-	-
R1/Pond	0.1475	-	0.44	-	-	-	-
R1/Stream	1.1210	-	3.36	-	-	-	-
R3/Stream	1.2000	-	3.59	-	-	-	-
R4/Stream	0.7536	-	2.26	-	-	-	-

PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**. PEC<sub>sw</sub> values selected from the worst-case FOCUS Step-1 and 2 values, either for single or double applications. ‡FOCUS Step 2 N-EU PEC<sub>sw</sub> values are relevant for all central zone member states. AF: assessment factor; PEC: predicted environmental concentration; RAC: regulatory acceptable concentration.

For the intended uses of CA3642 in winter cereals an acceptable risk from the metabolite prothioconazole-desthio is concluded for all aquatic organisms for most FOCUS Step-3 scenarios (based on the critical tier-1 RAC<sub>sw</sub> value of 0.334 µg/L of chronic fish). The exceptions occur with the FOCUS Step-3 scenarios R1/stream,

R3/stream, and R4/stream for single and two applications of CA3642 therefore, risk mitigation measures are required for these scenarios.

**Table 9.5-15 12:** Acceptability of risk (PEC/RAC <1) for the metabolite prothioconazole-desthio for the critical organism group based on PEC<sub>sw</sub> values from FOCUS Step 4 for the use CA3642 in spring and winter cereals (2 x 210 g a.s./ha) – FOCUS scenarios relevant to the Central EU zone

Substance	Prothioconazole-desthio					
Critical aquatic RAC	0.334µg metabolite/L (chronic fish)					
FOCUS Step-4 scenario	5-m NSBZ		10-m NSBZ + 10-m VFS		20-m NSBZ + 20-m VFS	
	PEC <sub>sw</sub> (µg/L)	PEC/RAC	PEC <sub>sw</sub> (µg/L)	PEC/RAC	PEC <sub>sw</sub> (µg/L)	PEC/RAC
<b>Spring cereals (single applications)</b>						
R4/Stream	0.7175	<b>2.15</b>	0.3263	0.98	-	-
<b>Spring cereals (two applications)</b>						
R4/Stream	1.386	<b>4.15</b>	0.6237	<b>1.87</b>	0.3254	0.97
<b>Winter cereals (single applications)</b>						
R1/Stream	0.3729	<b>1.12</b>	0.1694	0.51	-	-
R3/Stream	0.4835	<b>1.45</b>	0.2206	0.66	-	-
<b>Winter cereals (two applications)</b>						
R1/Stream	1.1210	<b>3.36</b>	0.5091	<b>1.52</b>	0.2665	0.80
R3/Stream	1.2000	<b>3.59</b>	0.5477	<b>1.64</b>	0.2873	0.86
R4/Stream	0.7536	<b>2.26</b>	0.34	<b>1.02</b>	0.1775	0.53

PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**. NSBZ: no-spray buffer zone (to mitigate drift); VFS: vegetated filter strip (to mitigate spray drift and runoff); PEC: predicted environmental concentration; RAC: regulatory acceptable concentration.

Assessment of the risk from the metabolite prothioconazole-desthio to spring and winter oilseed rape

**Table 9.5-1613:** Acceptability of risk (PEC/RAC <1) for the metabolite prothioconazole-desthio for each organism group based on PEC<sub>sw</sub> values from FOCUS Steps 1-3 for the use of CA3642 in spring oilseed rape (1 x 180 g a.s./ha)

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Sediment dweller	Algae
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>C. riparius</i>	<i>S. subspicatus</i>
Endpoint (µg metabolite/L)		LC <sub>50</sub> 6630	NOEC 3.34	EC <sub>50</sub> 10000	NOEC 100	NOEC 2000	E <sub>r</sub> C <sub>50</sub> 550
AF		100	10	100	10	10	10
RAC (µg metabolite/L)		66.3	0.334	100	10	200	55
FOCUS Scenario	PEC <sub>sw</sub> (µg metabolite/L)	PEC/RAC					
Step 1							
Worst case	35.26	0.53	105.57	0.35	3.53	0.18	0.64
Step 2							
N-EU‡	1.58	-	4.73	-	0.16	-	-
Step-3, Single applications							
D3/Ditch	0.1147	-	0.34	-	-	-	-
D4/Pond	0.0252	-	0.08	-	-	-	-
D4/Stream	0.07435	-	0.22	-	-	-	-
D5/Pond	0.0229	-	0.07	-	-	-	-
D5/Stream	0.0994	-	0.30	-	-	-	-
R1/Pond	0.0539	-	0.16	-	-	-	-
R1/Stream	0.4218	-	1.26	-	-	-	-

PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**. PEC<sub>sw</sub> values selected from the worst-case FOCUS Step-1 and 2 values. ‡FOCUS Step 2 N-EU PEC<sub>sw</sub> values are relevant for all central zone member states. AF: assessment factor; PEC: predicted environmental concentration; RAC: regulatory acceptable concentration.

For the intended uses of the product, CA3642 in spring oilseed rape an acceptable risk from the metabolite prothioconazole-desthio is concluded for all aquatic organisms for most FOCUS Step-3 scenarios (based on the critical tier-1 RAC<sub>sw</sub> value of 0.334 µg/L of chronic fish). The exception occurs with the FOCUS Step-3 scenario R1/stream (single applications of CA3642) therefore risk mitigation measures are required for this scenario.

**Table 9.5-17~~14~~:** Acceptability of risk (PEC/RAC <1) for the metabolite prothioconazole-desthio for each organism group based on PEC<sub>sw</sub> values from FOCUS Steps 1-3 for the use of CA3642 in winter oilseed rape (1 x 180 g a.s./ha)

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Sediment dweller	Algae
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D.</i>	<i>C. riparius</i>	<i>S. subspicatus</i>
Endpoint (µg metabolite/L)		LC <sub>50</sub> 6630	NOEC 3.34	EC <sub>50</sub> 10000	NOEC 100	NOEC 2000	E <sub>r</sub> C <sub>50</sub> 550
AF		100	10	100	10	10	10
RAC (µg metabolite/L)		66.3	0.334	100	10	200	55
FOCUS Scenario	PEC <sub>sw</sub> (µg metabolite/L)	PEC/RAC					
Step 1							
Worst case	35.26	0.53	105.57	0.35	3.53	0.18	0.64
Step 2							
N-EU‡	5.72	-	17.13	-	0.57	-	-
Step-3, Autumn applications							
D3/Ditch	0.1575	-	0.47	-	-	-	-
D4/Pond	0.02497	-	0.07	-	-	-	-
D4/Stream	0.09069	-	0.27	-	-	-	-
D5/Pond	0.02609	-	0.08	-	-	-	-
D5/Stream	0.1452	-	0.43	-	-	-	-
R1/Pond	0.03418	-	0.10	-	-	-	-
R1/Stream	0.2791	-	0.84	-	-	-	-
R3/Stream	0.7631	-	2.28	-	-	-	-
Step-3, Spring applications							
D3/Ditch	0.03366	-	0.10	-	-	-	-
D4/Pond	0.01915	-	0.06	-	-	-	-
D4/Stream	0.06826	-	0.20	-	-	-	-
D5/Pond	0.021	-	0.06	-	-	-	-
D5/Stream	0.08112	-	0.24	-	-	-	-
R1/Pond	0.04732	-	0.14	-	-	-	-
R1/Stream	0.2881	-	0.86	-	-	-	-
R3/Stream	0.4957	-	1.48	-	-	-	-

PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**. PEC<sub>sw</sub> values selected from the worst-case FOCUS Step-1 and 2 values. †FOCUS Step 2 N-EU PEC<sub>sw</sub> values are relevant for all central zone member states. AF: assessment factor; PEC: predicted environmental concentration; RAC: regulatory acceptable concentration.

For the intended uses of CA3642 in winter oilseed rape an acceptable risk from the metabolite prothioconazole-desthio is concluded for all aquatic organisms for most FOCUS Step-3 scenarios (based on the critical tier-1 RAC<sub>sw</sub> value of 0.334 µg/L of chronic fish). The exception occurs with the FOCUS Step-3 scenario R3/stream for both autumn and spring applications of CA3642 therefore risk mitigation measures are required for this scenario.



**Table 9.5-18 15:** Acceptability of risk (PEC/RAC <1) for the metabolite prothioconazole-desthio for the critical organism group based on PEC<sub>sw</sub> values from FOCUS Step 4 for the use of CA3642 in spring and winter oilseed rape (1 x 180 g a.s./ha) – FOCUS scenarios relevant to the Central EU zone

Substance	Prothioconazole-desthio					
Critical aquatic RAC	0.334µg metabolite/L (chronic fish)					
FOCUS Step-4 scenario	5-m NSBZ		10-m NSBZ + 10-m VFS		20-m NSBZ + 10-m VFS	
	PEC <sub>sw</sub> (µg/L)	PEC/RAC	PEC <sub>sw</sub> (µg/L)	PEC/RAC	PEC <sub>sw</sub> (µg/L)	PEC/RAC
<b>Spring oilseed rape</b>						
R1/Stream	0.4218	<b>1.26</b>	0.1915	0.57	-	-
<b>Winter oilseed rape (spring application)*</b>						
R3/Stream	0.4957	<b>1.48</b>	0.2191	0.66	-	-
<b>Winter oilseed rape (autumn application)*</b>						
R3/Stream	0.7631	<b>2.28</b>	0.3473	<b>1.04</b>	0.1820	0.54

PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**. \*Only single applications of CA3642 are relevant, according to the GAP table. NSBZ: no-spray buffer zone (to mitigate drift); VFS: vegetated filter strip (to mitigate spray drift and runoff); PEC: predicted environmental concentration; RAC: regulatory acceptable concentration.

#### Assessment of the risk from the metabolite prothioconazole-desthio to sunflower

**Table 9.5-19:** Acceptability of risk (PEC/RAC <1) for the metabolite prothioconazole-desthio for each organism group based on PEC<sub>sw</sub> values from FOCUS Steps 1-3 for the use of CA3642 in sunflower (1 x 180 g a.s./ha)

F-5 for the use of CH3042 in sunflower (1 x 100 g a.s./ha)							
Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Sediment dweller	Algae
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D.</i>	<i>C. riparius</i>	<i>S. subspicatus</i>
Endpoint (µg metabolite/L)		LC <sub>50</sub> 6630	NOEC 3.34	EC <sub>50</sub> 10000	NOEC 100	NOEC 2000	ErC <sub>50</sub> 550
AF		100	10	100	10	10	10
RAC (µg metabolite/L)		66.3	0.334	100	10	200	55
FOCUS Scenario	PEC <sub>sw</sub> (µg metabolite/L)	PEC/RAC					
Step 1							
Worst case	35.26	0.53	105.57	0.35	3.53	0.18	0.64
Step 2							
N-EU‡	3.3	-	9.88	-	0.33	-	-
Step-3							
D3/Ditch	0.09735	-	0.29	-	-	-	-
D4/Pond	0.02443	-	0.07	-	-	-	-
D4/Stream	0.06438	-	0.19	-	-	-	-
R1/Pond	0.07567	-	0.23	-	-	-	-
R1/Stream	0.6933	-	2.08	-	-	-	-

PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**. PEC<sub>sw</sub> values selected from the worst-case FOCUS Step-1 and 2 values. ‡FOCUS Step 2 N-EU PEC<sub>sw</sub> values are relevant for all central zone member states. AF: assessment factor; PEC: predicted environmental concentration; RAC: regulatory acceptable concentration.

For the intended uses of CA3642 in sunflower an acceptable risk from the metabolite prothioconazole-desthio is concluded for all aquatic organisms for most FOCUS Step-3 scenarios (based on the critical tier-1 RAC<sub>sw</sub> value of 0.334 µg/L of chronic fish). The exception occurs with the FOCUS Step-3 scenario R1/stream, therefore risk mitigation measures are required for this scenario.

**Table 9.5-20: Acceptability of risk (PEC/RAC <1) for the metabolite prothioconazole-desthio for the critical organism group based on PEC<sub>sw</sub> values from FOCUS Step 4 for the use of CA3642 in sunflower (1 x 180 g a.s./ha) – FOCUS scenarios relevant to the Central EU zone**

Substance	Prothioconazole-desthio					
Critical aquatic RAC	0.334µg metabolite/L (chronic fish)					
FOCUS Step-4 scenario	5-m NSBZ		10-m NSBZ + 10-m VFS		20-m NSBZ + 10-m VFS	
	PEC <sub>sw</sub> (µg/L)	PEC/RAC	PEC <sub>sw</sub> (µg/L)	PEC/RAC	PEC <sub>sw</sub> (µg/L)	PEC/RAC
<b>Sunflower</b>						
R1/Stream	0.6933	<b>2.08</b>	0.3153	0.944	-	-

PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**. \*Only single applications of CA3642 are relevant, according to the GAP table. NSBZ: no-spray buffer zone (to mitigate drift); VFS: vegetated filter strip (to mitigate spray drift and runoff); PEC: predicted environmental concentration; RAC: regulatory acceptable concentration.

An acceptable risk for the FOCUS Step-3 scenario R1/stream is concluded using 10-m NSBZ and 10-m VFS mitigation measures for the use on sunflower.

### Overall conclusion for prothioconazole and its relevant metabolites

The D1, D2, D6 and R2 scenarios are not relevant to the Central EU zone according to the Working Document of the Central Zone in the Authorisation of PPPs (v1r1 June 2018). For the intended uses of CA3642 in cereals and oilseed rape acceptable aquatic risk from the metabolite prothioconazole-desthio is concluded, for all aquatic organisms (based on the critical tier-1 RAC<sub>sw</sub> value of 0.334 µg/L of chronic fish) with the following risk-mitigation measures in the Central zone:

FOCUS scenario	Mitigation required to conclude an acceptable aquatic risk to the metabolite, prothioconazole-desthio, for the intended uses			
	Spring cereals		Winter cereals	
	1 x application	2 x application	1 x application	2 x application
D3/Ditch	-	-	-	-
D4/Pond	-	-	-	-
D4/Stream	-	-	-	-
D5/Pond	-	-	-	-
D5/Stream	-	-	-	-
R1/Pond	-	-	-	-
R1/Stream	-	-	10-m NSBZ +10-m VFS	20-m NSBZ + 20-m VFS
R3/Stream	-	-	10-m NSBZ + 10-m VFS	20-m NSBZ + 20-m VFS
R4/Stream	10-m NSBZ + 10-m VFS	20-m NSBZ + 20-m VFS	-	20-m NSBZ + 20-m VFS
FOCUS scenario	Mitigation required to conclude an acceptable aquatic risk to the metabolite, prothioconazole-desthio, for the intended uses			
	Spring oilseed rape*		Winter oilseed rape*	

	Spring application	Spring application	Autumn application
D3/Ditch	-	-	-
D4/Pond	-	-	-
D4/Stream	-	-	-
D5/Pond	-	-	-
D5/Stream	-	-	-
R1/Pond	-	-	-
R1/Stream	10-m NSBZ + 10-m VFS	-	-
R3/Stream	-	10-m NSBZ + 10-m VFS	20-m NSBZ + 20-m VFS
R4/Stream	-	-	-

Dashes (-) indicate no required risk mitigation measures. \*Only one application is proposed for spring and winter oilseed rape. NSBZ: no-spray buffer zone (to mitigate drift); VFS: vegetated filter strip (to mitigate spray drift and runoff).

FOCUS scenario	Mitigation required to conclude an acceptable aquatic risk to the metabolite, prothioconazole-desthio, for the intended uses
	Sunflower, 1 x application
D3/Ditch	-
D4/Pond	-
D4/Stream	-
R1/Pond	-
R1/Stream	10-m NSBZ + 10-m VFS

#### zRMS comments:

The calculations of the risk assessment for the a.s.- prothioconazole and its metabolites provided in the Tables 9.5-4 to 9.5-15 have been validated by zRMS. The acceptable risk from the active substance prothioconazole is concluded for all aquatic organisms based on already FOCUS Step 1 - 2 PEC<sub>sw</sub> values.

In the same time for the intended uses of CA3642 in cereals, oilseed rape and sunflower, an acceptable aquatic risk for prothioconazole-desthio (based on the critical tier-1 RAC<sub>sw</sub> value of 0.334 µg a.s./L of chronic fish) is indicated for all aquatic organisms with the following risk-mitigation measures for scenarios in the Central zone:

FOCUS scenario	Mitigation required to conclude an acceptable aquatic risk to the metabolite, prothioconazole-desthio, for the intended uses			
	Spring cereals		Winter cereals	
	1 x application	2 x application	1 x application	2 x application
D3/Ditch	-	-	-	-
D4/Pond	-	-	-	-
D4/Stream	-	-	-	-
D5/Pond	-	-	-	-
D5/Stream	-	-	-	-

R1/Pond	-	-	-	-
R1/Stream	-	-	10-m NSBZ +10-m VFS	20-m NSBZ + 20-m VFS
R3/Stream	-	-	10-m NSBZ + 10-m VFS	20-m NSBZ + 20-m VFS
R4/Stream	10-m NSBZ + 10-m VFS	20-m NSBZ + 20-m VFS	-	20-m NSBZ + 20-m VFS
FOCUS scenario	Mitigation required to conclude an acceptable aquatic risk to the metabolite, prothioconazole-desthio, for the intended uses			
	Spring oilseed rape*		Winter oilseed rape*	
	Spring application		Spring application	Autumn application
D3/Ditch	-	-	-	-
D4/Pond	-	-	-	-
D4/Stream	-	-	-	-
D5/Pond	-	-	-	-
D5/Stream	-	-	-	-
R1/Pond	-	-	-	-
R1/Stream	10-m NSBZ + 10-m VFS	-	-	-
R3/Stream	-	10-m NSBZ + 10-m VFS	20-m NSBZ + 20-m VFS	-
R4/Stream	-	-	-	-

Dashes (-) indicate no required risk mitigation measures. \*Only one application is proposed for spring and winter oilseed rape. NSBZ: no-spray buffer zone (to mitigate drift); VFS: vegetated filter strip (to mitigate spray drift and runoff).

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

FOCUS scenario	Mitigation required to conclude an acceptable aquatic risk to the metabolite, prothioconazole-desthio, for the intended uses
	Sunflower, 1 x application
D3/Ditch	-
D4/Pond	-
D4/Stream	-
R1/Pond	-
R1/Stream	10-m NSBZ + 10-m VFS

Assessment of the risk from the active substance azoxystrobin to spring and winter cereals

**Table 9.5- 21 16:** Acceptability of risk (PEC/RAC <1) for the active substance azoxystrobin for each organism group based on PEC<sub>sw</sub> values from FOCUS Steps 1-3 for the use of CA3642 in spring cereals (2 x 210 g a.s./ha)

Group		Fish acute	Fish prolonged	Acute invertebrates	Acute invertebrates	Long-term invertebrates	Higher-tier data	Sediment dweller	Algae#	Macrophytes sub-chronic
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	M.bahia	Geomean	M.bahia	Invertebrates	<i>Chironomus riparius</i>	<i>Navicula pelliculosa</i>	<i>Lemna gibba</i>
Endpoint		LC <sub>50</sub>	NOEC	EC <sub>50</sub>	EC <sub>50</sub>	NOEC	See footnotes*	NOEC	ErC <sub>50</sub>	EC <sub>50</sub>
(µg a.s./L)		470	147				55	118	9.54	-
AF		100	10	100	10	10	-	10	10	10
RAC (µg a.s./L)		4.7	14.7	0.55	1.18	0.954	3.3	80	14.6	320
FOCUS Scenario	PEC <sub>sw</sub> (µg a.s./L)	PEC/RAC								
Step 1										
Worst case	95.81	20.39	6.52	174.2		100.43	29.03	1.20	6.56	0.3
Step 2										
N-EU‡	15.81	3.36	1.08	28.74		15.67	4.79	0.20	1.08	0.05
Step-3, Single applications										
D3/Ditch	1.3310	0.28	0.09	2.418	1.127	1.394	0.40	-	0.09	0.004
D4/Pond	0.467	0.10	0.03	0.849	0.396	0.490	0.14	-	0.03	0.001
D4/Stream	1.0890	0.23	0.07	1.980	0.923	1.142	0.33	-	0.07	0.003
D5/Pond	0.1391	0.03	0.01	0.253	0.118	0.146	0.04	-	0.01	0.000
D5/Stream	1.122	0.24	0.08	2.040	0.951	1.176	0.34	-	0.08	0.004
R4/Stream	2.569	0.55	0.17	4.671	2.177	2.693	0.78	-	0.18	0.008
Step-3, Two applications										
D3/Ditch	1.1640	0.25	0.08	2.116	0.986	1.220	0.35	-	0.08	0.004
D4/Pond	0.8872	0.19	0.06	1.613	0.752	0.930	0.27	-	0.06	0.003
D4/Stream	0.9725	0.21	0.07	1.768	0.824	1.019	0.29	-	0.07	0.003
D5/Pond	0.2767	0.06	0.02	0.503	0.234	0.290	0.08	-	0.02	0.001
D5/Stream	1.01	0.21	0.07	1.836	0.856	1.059	0.31	-	0.07	0.003
R4/Stream	4.321	0.92	0.29	7.856	3.662	4.529	1.31	-	0.30	0.014

PEC/RAC ratios above the relevant trigger of 1 are shown in bold. \*EU agreed Higher-tier RAC value, based on data from a mesocosm study, first-tier toxicity data, and the lower limit of an HC5 value – see the EFSA conclusion on azoxystrobin (EFSA Journal 2010; 8(4):1542), for full details. AF: assessment factor; PEC: predicted environmental concentration; RAC: regulatory acceptable concentration. PEC<sub>sw</sub> values selected from the worst-case FOCUS Step-1 and 2 values, either for single or double applications. #FOCUS Step 2 N-EU PEC<sub>sw</sub> values are relevant for all central zone member states. #The worst case algal endpoint covers the risk to aquatic macrophytes.

\*\* The geomean EC50 of 118 µg a.s./L value calculated by zRMS from data available for D. magna (230 µg a.s./L), M. fuscus (0.13 µg a.s./L) and M. bahia (55 µg a.s./L) for acute risk assessment

For the intended uses of CA3642 in spring cereals an acceptable risk from the active substance, azoxystrobin is concluded for all aquatic organisms for most FOCUS Step-3 scenarios (based on the higher tier RAC<sub>sw</sub> value of 3.3 µg/L for invertebrates). The exception occurs with the FOCUS Step-3 scenario R4/stream (two applications of CA3642) therefore risk mitigation measures are required for this scenario.

**Table 9.5- 2217: Acceptability of risk (PEC/RAC <1) for the active substance azoxystrobin for each organism group based on PEC<sub>sw</sub> values from FOCUS Steps 1-3 for the use of CA3642 in winter cereals (2 x 210 g a.s./ha)**

For the use of chemicals in water bodies (2012 to 2015 g annual)										
Group		Fish acute	Fish prolonged	Acute invertebrates	Acute invertebrates	Long-term invertebrates	Higher-tier data	Sediment dweller	Algae#	Macrophytes Sub-chronic
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	M.bahia	Geomean	M.bahia	Invertebrates	<i>Chironomus riparius</i>	<i>Navicula pelliculosa</i>	<i>Lemna gibba</i>
Endpoint		LC <sub>50</sub>	NOEC	EC <sub>50</sub>	EC <sub>50</sub>	NOEC	See footnotes*	NOEC	E <sub>r</sub> C <sub>50</sub>	EC <sub>50</sub>
(µg a.s./L)		470	147	55	118	9.54	-	800	146	3200
AF		100	10	100	10	10	-	10	10	10
RAC (µg a.s./L)		4.7	14.7	0.55	1.18	0.954	3.3	80	14.6	320
FOCUS Scenario	PEC <sub>sw</sub> (µg a.s./L)	PEC/RAC								
Step 1										
Worst case	95.81	20.39	6.52	174.2		100.43	29.03	1.20	6.56	0.3
Step 2										
N-EU‡	15.81	3.36	1.08	28.75		16.60	4.79	0.20	1.08	0.05
Step-3, Single applications										
D3/Ditch	1.3300	0.28	0.09	2.418	1.127	1.394	0.40	-	0.09	0.004
D4/Pond	0.4256	0.09	0.03	0.774	0.361	0.446	0.13	-	0.03	0.001
D4/Stream	0.9848	0.21	0.07	1.791	0.835	1.032	0.30	-	0.07	0.003
D5/Pond	0.1370	0.03	0.01	0.249	0.116	0.144	0.04	-	0.01	0.000
D5/Stream	1.0680	0.23	0.07	1.942	0.905	1.119	0.32	-	0.07	0.003
D6/Ditch	1.3320	0.28	0.09	2.422	1.129	1.396	0.40	-	0.09	0.004
R1/Pond	0.1501	0.03	0.01	0.273	0.127	0.157	0.05	-	0.01	0.000
R1/Stream	1.3990	0.30	0.10	2.544	1.186	1.466	0.42	-	0.10	0.004
R3/Stream	1.9650	0.42	0.13	3.573	1.665	2.060	0.60	-	0.13	0.006
R4/Stream	1.0150	0.22	0.07	1.845	0.860	1.064	0.31	-	0.07	0.003
Step-3, Two applications										
D3/Ditch	1.1640	0.25	0.08	2.116	0.986	1.220	0.35	-	0.08	0.004
D4/Pond	0.9800	0.21	0.07	1.782	0.831	1.027	0.30	-	0.07	0.003
D4/Stream	0.9963	0.21	0.07	1.811	0.844	1.044	0.30	-	0.07	0.003
D5/Pond	0.2858	0.06	0.02	0.520	0.242	0.300	0.09	-	0.02	0.001

D5/Stream	1.0230	0.22	0.07	<b>1.860</b>	0.867	<b>1.072</b>	0.31	-	0.07	0.003
D6/Ditch	1.1700	0.25	0.08	<b>2.127</b>	0.992	<b>1.226</b>	0.35	-	0.08	0.004
R1/Pond	0.4057	0.09	0.03	0.738	0.344	0.425	0.12	-	0.03	0.001
R1/Stream	4.1400	0.88	0.28	<b>7.527</b>	<b>3.508</b>	<b>4.340</b>	<b>1.25</b>	-	0.28	0.013
R3/Stream	4.5280	0.96	0.31	<b>8.233</b>	<b>3.837</b>	<b>4.746</b>	<b>1.37</b>	-	0.31	0.014
R4/Stream	2.8330	0.60	0.19	<b>5.151</b>	<b>2.401</b>	<b>2.970</b>	0.86	-	0.19	0.009

PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**. \*EU agreed higher-tier RAC value, based on data from a mesocosm study, first-tier toxicity data, and the lower limit of an HC5 value – see the EFSA conclusion on azoxystrobin (EFSA Journal 2010; 8(4):1542), for full details. AF: assessment factor; PEC: predicted environmental concentration; RAC: regulatory acceptable concentration. PEC<sub>sw</sub> values selected from the worst-case FOCUS Step-1 and 2 values, either for single or double applications. #FOCUS Step 2 N-EU PEC<sub>sw</sub> values are relevant for all central zone member states. #~~The worst case algal endpoint covers the risk to aquatic macrophytes.~~

\*\* The geomean EC50 of 118 µg a.s./L value calculated by zRMS from data available for D. magna (230 µg a.s./L), M. fuscus (0.13 µg a.s./L) and M. bahia (55 µg a.s./L) for acute risk assessment

For the intended uses of CA3642, in winter cereals an acceptable risk from the active substance azoxystrobin is concluded for all aquatic organisms for most FOCUS Step-3 scenarios (based on the higher tier RAC<sub>sw</sub> value of 3.3 µg/L for invertebrates). The exceptions occur with the FOCUS Step-3 scenarios R1/stream and R3/stream (two applications of CA3642) therefore risk mitigation measures are required only for these scenarios for two applications in winter cereals.

**Table 9.5-23 18:** Acceptability of risk (PEC/RAC <1) for the active substance azoxystrobin for the critical organism group based on PEC<sub>sw</sub> values from FOCUS Step 4 for the use of CA3642 in spring and winter cereals (2 x 210 g a.s./ha) – FOCUS scenarios relevant to the Central EU zone

Substance Critical aquatic RAC	Azoxystrobin 3.3 µg a.s./L (higher-tier aquatic invertebrate data)					
	5-m NSBZ		10-m NSBZ + 10-m VFS		20-m NSBZ + 20-m VFS	
	PEC <sub>sw</sub> (µg/L)	PEC/RAC	PEC <sub>sw</sub> (µg/L)	PEC/RAC	PEC <sub>sw</sub> (µg/L)	PEC/RAC
<b>Spring cereals (two applications)</b>						
R4/Stream	4.321	<b>1.31</b>	1.942	0.59	-	-
<b>Winter cereals (two applications)</b>						
R1/stream	4.140	<b>1.25</b>	1.881	0.57	-	-
R3/Stream	4.528	<b>1.37</b>	2.067	0.63	-	-

PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**. NSBZ: no-spray buffer zone (to mitigate drift); VFS: vegetated filter strip (to mitigate spray drift and runoff); PEC: predicted environmental concentration; RAC: regulatory acceptable concentration.

#### Assessment of the risk from the active substance azoxystrobin to spring and winter oilseed rape

**Table 9.5- 24 19:** Acceptability of risk (PEC/RAC <1) for the active substance azoxystrobin for each organism group based on PEC<sub>sw</sub> values from FOCUS Steps 1-3 for the use of CA3642 in spring oilseed rape (1 x 180 g a.s./ha)

For the use of Chironomid spring-onseed rape (1 x 100 g a.s./ha)										
Group		Fish acute	Fish prolonged	Acute invertebrates	Acute invertebrates	Long-term invertebrates	Higher-tier data	Sediment dweller	Algae#	Macrophytes sub-chronic
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	M.bahia	Geomean**	M.bahia	Invertebrates	<i>Chironomus riparius</i>	<i>Navicula pelliculosa</i>	<i>Lemna gibba</i>
Endpoint		LC <sub>50</sub>	NOEC	EC <sub>50</sub>	EC <sub>50</sub>	NOEC	See footnotes*	NOEC	E <sub>r</sub> C <sub>50</sub>	EC <sub>50</sub>
(µg a.s./L)		470	147	55	118	9.54	-	800	146	3200
AF		100	10	100	10	10	-	10	10	10
RAC (µg a.s./L)		4.7	14.7	0.55	1.18	0.954	3.3	80	14.6	320
FOCUS Scenario	PEC <sub>sw</sub> (µg a.s./L)	PEC/RAC								
Step 1										
Worst case	41.06	8.74	2.79	74.65		76.04	12.44	0.51	2.81	0.128
Step 2										
N-EU†	3.49	0.74	0.24	6.35		3.65	1.06	-	0.40	0.010
Step-3, Single applications										
D3/Ditch	1.141	-	-	2.075	0.967	1.196	0.35	-	-	0.004
D4/Pond	0.3901	-	-	0.709	0.331	0.409	0.12	-	-	0.001
D4/Stream	0.9346	-	-	1.699	0.792	0.980	0.28	-	-	0.003
D5/Pond	0.1387	-	-	0.252	0.118	0.145	0.04	-	-	0.000



D5/Stream	0.9102	-	-	1.655	0.771	0.954	0.28	-	-	0.003
R1/Pond	0.1448	-	-	0.263	0.123	0.152	0.04	-	-	0.000
R1/Stream	1.566	-	-	2.847	1.327	1.642	0.47	-	-	0.005

PEC/RAC ratios above the relevant trigger of 1 are shown in bold. \*EU higher-tier RAC value, based on data from a mesocosm study, first-tier toxicity data, and the lower limit of an HC5 value – see the EFSA conclusion on azoxystrobin (EFSA Journal 2010; 8(4):1542), for full details. AF: assessment factor; PEC: predicted environmental concentration; RAC: regulatory acceptable concentration. PECSW values selected from the worst-case FOCUS Step-1 and 2 values. †FOCUS Step 2 N-EU PEC<sub>sw</sub> values are relevant for all central zone member states. #The worst case algal endpoint covers the risk to aquatic macrophytes.

\*\* The geomean EC<sub>50</sub> of 11.8 µg a.s./L value calculated by zRMS from data available for D. magna (230 µg a.s./L), M. fuscus (130 µg a.s./L) and M. bahia (55 µg a.s./L) for acute risk assessment

For the intended uses of CA3642 in spring oilseed rape an acceptable risk for all aquatic organisms from the active substance azoxystrobin is concluded for all FOCUS Step-3 scenarios (based on the higher-tier RAC<sub>sw</sub> value of 3.3 µg/L for invertebrates).

**Table 9.5- 25 20: Acceptability of risk (PEC/RAC <1) for the active substance azoxystrobin for each organism group based on PEC<sub>sw</sub> values from FOCUS Steps 1-3 for the use of CA364, in winter oilseed rape (1 x 180 g a.s./ha)**

Group		Fish acute	Fish prolonged	Acute invertebrates	Acute invertebrates	Long-term invertebrates	Higher-tier data	Sediment dweller	Algae#	Macrophytes sub-chronic
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	M.bahia	Geomean**	M.bahia	Invertebrates	<i>Chironomus riparius</i>	<i>Navicula pelliculosa</i>	<i>Lemna gibba</i>
Endpoint		LC <sub>50</sub>	NOEC	EC <sub>50</sub>	EC <sub>50</sub>	NOEC	See footnotes*	NOEC	E <sub>r</sub> C <sub>50</sub>	EC <sub>50</sub>
(µg a.s./L)		470	147	55	11.8	9.54	-	800	146	3200
AF		100	10	100	10	10	-	10	10	10
RAC (µg a.s./L)		4.7	14.7	0.55	1.18	0.954	3.3	80	14.6	320
FOCUS Scenario	PEC <sub>sw</sub> (µg a.s./L)	PEC/RAC								
Step 1										
Worst case	41.06	8.74	2.79	74.65		43.04	12.44	0.51	2.81	0.128
Step 2										
N-EU† autumn applications (Oct-Feb)	12.62	2.69	0.86	23		22.95	3.82	-	0.86	0.039
N-EU† spring applications (March-May/ June-Sept)	3.49	0.74	0.24	6.34		3.56	1.06	-	0.24	0.01
Step-3, Autumn applications										
D3/Ditch	1.145	0.24	-	2.082	0.970	1.200	0.35	-	-	0.004
D4/Pond	1.111	0.24	-	2.020	0.942	1.165	0.34	-	-	0.003
D4/Stream	1.263	0.27	-	2.296	1.070	1.324	0.38	-	-	0.004
D5/Pond	0.4981	0.11	-	0.906	0.422	0.522	0.15	-	-	0.002
D5/Stream	1.0640	0.23	-	1.935	0.902	1.115	0.32	-	-	0.003

\*\*The geomean EC<sub>50</sub> of 118 µg a.s./L value calculated by zRMS from data available for *D. magna* (230 µg a.s./L), *M. fuscus* (0.13 µg a.s./L) and *M. bahia* (55 µg a.s./L)

## Assessment of the risk from the active substance azoxystrobin to sunflower

Group		Fish acute	Fish prolonged	Acute invertebrates	Acute invertebrates	Long-term invertebrates	Higher-tier data	Sediment dweller	Algae#	Macrophytes sub-chronic
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	M.bahia	Geomean**	M.bahia	Invertebrates	<i>Chironomus riparius</i>	<i>Navicula pelliculosa</i>	<i>Lemna gibba</i>
Endpoint		LC <sub>50</sub>	NOEC	EC <sub>50</sub>	EC <sub>50</sub>	NOEC	See footnotes*	NOEC	ErC <sub>50</sub>	EC <sub>50</sub>
(µg a.s./L)		470	147	55	11.8	9.54	-	800	146	3200
AF		100	10	100	10	10	-	10	10	10
RAC (µg a.s./L)		4.7	14.7	0.55	1.18	0.954	3.3	80	14.6	320
FOCUS Scenario	PEC <sub>sw</sub> (µg a.s./L)	PEC/RAC								

Step 1										
Worst case	41.06	8.74	2.79				12.44	0.513	2.81	
Step 2										
N-EU‡	7.3	1.55	0.50				2.21	-	0.50	
Step-3, Single applications										
D3/Ditch	0.9443	0.20	-	1.717	0.8003	0.990	0.29	-	-	0.0030
D4/Pond	0.4766	0.10	-	0.867	0.4039	0.500	0.14	-	-	0.0015
D4/Stream	0.8109	0.17	-	1.474	0.6872	0.850	0.25	-	-	0.0025
R1/Pond	1.4681	0.31	-	2.669	1.2442	1.539	0.44	-	-	0.0046
R1/Stream	2.0891	0.44	-	3.798	1.7704	2.190	0.63	-	-	0.0065

PEC/RAC ratios above the relevant trigger of 1 are shown in bold. \*EU agreed Higher-tier RAC value, based on data from a mesocosm study, first-tier toxicity data, and the lower limit of an HC5 value – see the EFSA conclusion on azoxystrobin (EFSA Journal 2010; 8(4):1542), for full details. AF: assessment factor; PEC: predicted environmental concentration; RAC: regulatory acceptable concentration.. ‡FOCUS Step 2 N-EU PECsw values are relevant for all central zone member states. ~~‡The worst case algal endpoint covers the risk to aquatic macrophytes.~~

For the intended uses of CA3642 in sunflower an acceptable risk from the active substance azoxystrobin is concluded for all aquatic organisms for all FOCUS Step-3 scenarios (based on the worst case higher-tier  $RAC_{sw}$  value of 3.3 µg/L for aquatic invertebrates).

**zRMS comments:**

The risk assessment for a.s.- azoxystrobin provided in the Tables 9.5-16 to 9.5-20, has been amended by zRMS considering to acute and long-term endpoints agreed in EFSA Conclusion 2010 for for aquatic invertebrates and aquatic macrophytes.

In case of acute risk assessment for aquatic invertebrates the geomean value calculated from data available for *D. magna* (230 µg a.s./L), *M. fuscus* (0.13 µg a.s./L) and *M. bahia* (55 µg a.s./L) was used by zRMS as a refinement option of the acute risk assessment.

Based on the acute and long-term risk assessment with regard to endpoints from laboratory studies further refinement for acute and chronic risk for aquatic invertebrates was required for some scenarios. the RAC of 3.3 µg a.s./L agreed value at EU level still valid and used by some of MSs, was considered for the aquatic invertebrate risk assessment with STEP 3-4  $PEC_{sw}$  values calculated by FOCUS program.

Based on the performed calculations taking into account  $RAC=3.3 \text{ f } \mu\text{g a.s./L}$  following conclusions may be derived for the a.s.- azoxystrobin:

FOCUS scenario	Mitigation required to conclude an acceptable aquatic risk to the active substance, azoxystrobin, for the intended uses			
	Spring cereals		Winter cereals	
	1 x application	2 x application	1 x application	2 x application
D3/Ditch	-	-	-	-
D4/Pond	-	-	-	-
D4/Stream	-	-	-	-
D5/Pond	-	-	-	-
D5/Stream	-	-	-	-
D6/Ditch	-	-	-	-
R1/Pond	-	-	-	-
R1/Stream	-	-	-	10-m NSBZ + 10-m VFS
R3/Stream	-	-	-	10-m NSBZ + 10-m VFS
R4/Stream	-	10-m NSBZ + 10-m VFS	-	-

The uses in oilseed rape and sunflower no risk mitigation measures are required.

Based on the performed calculations for the worst-case scenario acceptable risk following application of CA3642 according to the Central Zone GAP may be concluded.

Concerned Member States must decide on applicability of indicated risk mitigation measures in their countries at the product authorisation.

Please note that additional aquatic risk assessment may be required by the concerned Member States that do not accept simulations performed according to FOCUS recommendations.

For MSs that need **PECsw** use the laboratory data for risk assessment for aquatic invertebrates for azoxystrobin based on RAC of 0.954 µg a.s./L values (lower than geomean RAC acute) it has been calculated by zRMS with risk mitigation using STEP 4 calculations for scenarios that did not pass the trigger value of 1 at STEP 3.

**Table 9.5-23-1: Acceptability of risk (PEC/RAC <1) for the active substance azoxystrobin for the critical organism group based on PEC<sub>sw</sub> values from FOCUS Step 4 for the use of CA3642 in spring and winter cereals (2 x 210 g a.s./ha) – FOCUS scenarios relevant to the Central EU zone**

Substance Critical aquatic RAC FOCUS Step-4 scenario	Azoxystrobin 0.954 µg a.s./L (Tier 1 for aquatic chronic invertebrate data as the worst case)					
	5-m NSBZ		10-m NSBZ + 10-m VFS		20-m NSBZ + 20-m VFS	
	PEC <sub>sw</sub> (µg/L)	PEC/RAC	PEC <sub>sw</sub> (µg/L)	PEC/RAC	PEC <sub>sw</sub> (µg/L)	PEC/RAC
<b>Spring cereals (one applications)</b>						
D3/Ditch	0.3607	0.3781	0.1913	0.201	0.09939	0.104
D4/Stream	0.5017	0.5259	0.5017	0.526	0.5017	0.526
D5/Stream	0.4129	0.4328	0.2211	0.232	0.2186	0.229
R4/Stream	2.569	<b>2.6929</b>	1.159	<b>1.215</b>	0.6052	0.634
<b>Spring cereals (two applications)</b>						
D3/Ditch	0.302	0.3166	0.1569	0.164	0.07975	0.084
D4/pond	0.8853	0.9280	0.8818	0.924	0.8789	0.921
D4/Stream	0.9051	0.9487	0.9051	0.949	0.9051	0.949
D5/Stream	0.4208	0.4411	0.4208	0.441	0.4208	0.441
R4/Stream	4.321	<b>4.5294</b>	<b>1.942</b>	<b>2.036</b>	<b>1.013</b>	<b>1.062</b>
<b>Winter cereals (one applications)</b>						
D3/ditch	0.3604	0.3778	0.1911	0.200	0.09929	0.104
D4/stream	0.4515	0.4733	0.4515	0.473	0.4515	0.473
D5/stream	0.3947	0.4137	0.2124	0.223	0.2070	0.217
R1/stream	1.399	<b>1.4665</b>	0.6355	0.666	0.3328	0.349
R3/Stream	1.965	<b>2.0597</b>	0.8966	0.940	0.4705-	0.493
R4/stream	1.015	1.0639	0.4583	0.480	0.2392	0.251
<b>Winter cereals (two applications)</b>						
D3/ditch	0.3019	0.316	0.1568	0.164	0.0797	0.084
D4/pond	0.9785	<b>1.026</b>	0.9759	<b>1.023</b>	0.9737	<b>1.021</b>
D4/stream	0.9963	<b>1.044</b>	0.9963	<b>1.044</b>	0.9963	<b>1.044</b>
D5/stream	0.4336	0.455	0.4336	0.455	0.4336	0.455
R1/stream	4.140	<b>4.340</b>	<b>1.881</b>	<b>1.972</b>	0.9849	<b>1.032</b>
R3/Stream	4.528	<b>4.746</b>	<b>2.067</b>	<b>2.167</b>	<b>1.084</b>	<b>1.136</b>
R4/stream	2.833	<b>2.970</b>	<b>1.279</b>	<b>1.341</b>	0.6675	0.6997

PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**. NSBZ: no-spray buffer zone (to mitigate drift); VFS: vegetated filter strip (to mitigate spray drift and runoff); PEC: predicted environmental concentration; RAC: regulatory acceptable concentration.

**Table 9.5-25-1: Acceptability of risk (PEC/RAC <1) for the active substance azoxystrobin for the critical organism group based on PEC<sub>sw</sub> values from FOCUS Step 4 for the use of CA3642 in spring and winter oilseed rape (1 x 180 g a.s./ha)**

Substance Critical aquatic RAC	Azoxystrobin 0.954 µg a.s./L (Tier 1 for aquatic chronic invertebrate data a.s. the worst case)					
	5-m NSBZ		10-m NSBZ + 10-m VFS		20-m NSBZ + 20-m VFS	
	PEC <sub>sw</sub> (µg/L)	PEC/RAC	PEC <sub>sw</sub> (µg/L)	PEC/RAC	PEC <sub>sw</sub> (µg/L)	PEC/RAC
<b>Spring oilseed rape (one applications)</b>						
D3/Ditch	0.3094	0.324	0.1640	0.172	0.08522	0.0893
D4/Stream	0.4336	0.455	0.4336	0.455	0.4336	0.4545
D5/Stream	0.3356	0.352	0.2114	0.222	0.2114	0.2216
R4/Stream	1.566	<b>1.642</b>	0.7110	0.745	0.3723	0.3903
<b>Winter oilseed rape (spring applications)</b>						
D3/ditch	0.3083	0.323	0.1635	0.171	0.08492	0.0890
D4/stream	0.3661	0.384	0.3661	0.384	0.3661	0.3838
D5/stream	0.2878	0.302	0.1612	0.169	0.1521	0.1594
R1/stream	1.104	<b>1.157</b>	0.5009	0.525	0.2623	0.2749
R3/Stream	1.860	<b>1.950</b>	0.8215	0.861	0.4256	0.4461
<b>Winter oilseed rape (autumn applications)</b>						
D3/ditch	0.3103	0.325	0.1646	0.173	0.08549	0.0896
D4/pond	1.110	<b>1.164</b>	<b>1.107</b>	<b>1.160</b>	<b>1.104</b>	<b>1.1572</b>
D4/stream	1.263	<b>1.324</b>	<b>1.263</b>	<b>1.324</b>	<b>1.263</b>	<b>1.3239</b>
D5/stream	0.9225	0.967	0.9225	0.967	0.9225	0.9670
R1/stream	1.104	<b>1.157</b>	0.4842	0.508	0.2501	0.2622
R3/Stream	2.760	<b>2.893</b>	<b>1.257</b>	<b>1.318</b>	0.6584	0.6901

PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**. NSBZ: no-spray buffer zone (to mitigate drift); VFS: vegetated filter strip (to mitigate spray drift and runoff); PEC: predicted environmental concentration; RAC: regulatory acceptable concentration.

**Table 9.5-26-1: Acceptability of risk (PEC/RAC <1) for the active substance azoxystrobin for the critical organism group based on PEC<sub>sw</sub> values from FOCUS Step 4 for the use of CA3642 in sunflower in spring and winter oilseed rape (1 x 180 g a.s./ha)**

Substance Critical aquatic RAC	Azoxystrobin 0.954 µg a.s./L (Tier 1 aquatic chronic invertebrate data a.s. the worst case)					
	5-m NSBZ		10-m NSBZ + 10-m VFS		20-m NSBZ + 20-m VFS	
	PEC <sub>sw</sub> (µg/L)	PEC/RAC	PEC <sub>sw</sub> (µg/L)	PEC/RAC	PEC <sub>sw</sub> (µg/L)	PEC/RAC
<b>Spring oilseed rape (one applications)</b>						
D3/Ditch	0.3095	0.3244	0.1641	0.1720	0.0852	0.0894
D4/pond	0.4759	0.4988	0.4743	0.4971	0.4729	0.4957
D4/stream	0.5271	0.5525	0.5271	0.5525	0.5271	0.5525
R1/pond	0.1771	0.1856	0.0801	0.0897	0.0435	0.0457
R1/Stream	1.885	<b>1.9759</b>	0.8572	0.8985	0.4491	0.4707

	0.6933	0.727	0.3153	0.331	0.1652	0.1732
<p>PEC/RAC ratios above the relevant trigger of 1 are shown in <b>bold</b>. NSBZ: no-spray buffer zone (to mitigate drift); VFS: vegetated filter strip (to mitigate spray drift and runoff); PEC: predicted environmental concentration; RAC: regulatory acceptable concentration.</p>						
<p><b>Commenting period process</b></p> <p>During the commenting period the Applicant commented the risk assessment based on the lowest endpoint for M.bahia as follows: <i>“during recent evaluation of products containing the active substance azoxystrobin from MS belonging to the Central EU Regulatory Zone, an additional pragmatic approach has been followed using a safety factor of at least 5 applied to the mesocosm NOEAEC (<u>hence the RAC is 2 µg a.s./L</u>) to cover the uncertainty arising from the mesocosm study itself. The effects observed in the mesocosm study at the NOEAEC level were only Class 1/2 effects, and therefore the risk from uses with multiple applications should potentially be covered by the safety factor of 5 for this application. This higher tier RAC is a 3.6 times higher concentration than the Tier 1 RAC based on acute endpoint for Mysisidopsis bahia (RAC 0.55 µg a.s./L).</i></p> <p><i>Consequently Nufarm believes that a worst case higher tier RAC value of 2 µg a.s./L that has already been considered appropriate and followed in the Central zone, would be more appropriate to be used in risk assessment for aquatic invertebrates than lower tier 1 RAC values, if the EU officially accepted RAC value of 3.3 µg a.s./L from the EFSA Journal 2010; 8(4):1542) is not accepted in particular concerned MS”.</i></p> <p>Nufarm considers the end point of 2 µg/L can further address concerns expressed by CMSes <i>“on shortcomings of the mesocosm study which make their use in a current risk assessment questionable”.</i></p>						
<p><b>zRMS:</b></p> <p>This approach is left for decision of MS on their national level.</p>						

### Assessment of the risk from the metabolite R234886

**Table 9.5- 27 21:** Acceptability of risk (PEC/RAC <1) for the metabolite R234886 for each organism group based on PEC<sub>sw</sub> values from FOCUS Step 1 for the use of CA3642 in spring and winter cereals (2 x 210 g a.s./ha)

Cereals (2 x 210 g a.s./ha)				
Group		Fish acute	Inverteb. acute	Algae
Test species		<i>O. mykiss</i>	<i>D. magna</i>	<i>S. capricornutum</i>
Endpoint		LC <sub>50</sub>	EC <sub>50</sub>	E <sub>r</sub> C <sub>50</sub>
(µg a.s./L)		>150000	>180000	47000
AF		100	100	10
RAC (µg a.s./L)		>1500	>1800	4700
FOCUS Scenario		PEC <sub>sw</sub> (µg a.s./L)	PEC/RAC	
Step 1				
Worst case		51.98	<0.03	<0.03
				0.01

AF: assessment factor; PEC: predicted environmental concentration; RAC: regulatory acceptable concentration.

**Table 9.5- 28 22:** Acceptability of risk (PEC/RAC <1) for the metabolite R234886 for each organism group based on PEC<sub>sw</sub> values from FOCUS Step 1 for the use of CA3642 in spring and winter oilseed rape (1 x 180 g a.s./ha)

Group		Fish acute	Inverteb. acute	Algae
Test species		<i>O. mykiss</i>	<i>D. magna</i>	<i>S. capricornutum</i>
Endpoint (µg a.s./L)		LC <sub>50</sub> >150000	EC <sub>50</sub> >180000	E <sub>r</sub> C <sub>50</sub> 47000
AF		100	100	10
RAC (µg a.s./L)		>1500	>1800	4700
FOCUS Scenario	PEC <sub>sw</sub> (µg a.s./L)	PEC/RAC		
Step 1				
Worst case	22.28	<0.015	<0.012	0.005

AF: assessment factor; PEC: predicted environmental concentration; RAC: regulatory acceptable concentration.

For the intended uses of CA3642 an acceptable risk from the metabolite R234886 is concluded.

**Table 9.5-29:** Acceptability of risk (PEC/RAC <1) for the metabolite R234886 for each organism group based on PEC<sub>sw</sub> values from FOCUS Step 1 for the use of CA3642 in sunflower (1 x 180 g a.s./ha)

Group		Fish acute	Inverteb. acute	Algae
Test species		<i>O. mykiss</i>	<i>D. magna</i>	<i>S. capricornutum</i>
Endpoint (µg a.s./L)		LC <sub>50</sub> >150000	EC <sub>50</sub> >180000	E <sub>r</sub> C <sub>50</sub> 47000
AF		100	100	10
RAC (µg a.s./L)		>1500	>1800	4700
FOCUS Scenario	PEC <sub>sw</sub> (µg a.s./L)	PEC/RAC		
Step 1				
Worst case	22.28	<0.015	<0.012	0.005

AF: assessment factor; PEC: predicted environmental concentration; RAC: regulatory acceptable concentration.

### Assessment of the risk from the metabolite, R401553

**Table 9.5- 30 23:** Acceptability of risk (PEC/RAC <1) for the metabolite R401553 for each organism group based on PEC<sub>sw</sub> values from FOCUS Step 1 for the use of CA3642 in spring and winter cereals (2 x 210 g a.s./ha)

Group	Fish acute	Inverteb. acute	Algae
Test species	<i>O. mykiss</i>	<i>D. magna</i>	<i>S. capricornutum</i>
Endpoint (µg a.s./L)	LC <sub>50</sub> >120000	EC <sub>50</sub> >120000	E <sub>r</sub> C <sub>50</sub> >120000
AF	100	100	10
RAC (µg a.s./L)	>1200	>1200	>12000
FOCUS Scenario	PEC <sub>sw</sub> (µg a.s./L)	PEC/RAC	



<b>Step 1</b>				
Worst case	16.28	<0.014	<0.014	<0.0014

AF: assessment factor; PEC: predicted environmental concentration; RAC: regulatory acceptable concentration.

**Table 9.5-31 24:** Acceptability of risk (PEC/RAC <1) for the metabolite, R401553, for each organism group, based on PEC<sub>sw</sub> values from FOCUS Step 1, for the use of the product, CA3642, in spring and winter oilseed rape (1 x 180 g a.s./ha)

Group		Fish acute	Inverteb. acute	Algae
Test species		<i>O. mykiss</i>	<i>D. magna</i>	<i>S. capricornutum</i>
Endpoint		LC <sub>50</sub>	EC <sub>50</sub>	ErC <sub>50</sub>
(µg a.s./L)		>120000	>120000	>120000
AF		100	100	10
RAC (µg a.s./L)		>1200	>1200	>12000
FOCUS Scenario	PEC <sub>Sw</sub> (µg a.s./L)	PEC/RAC		
Step 1				
Worst case	6.98	<0.006	<0.006	<0.0006

AF: assessment factor; PEC: predicted environmental concentration; RAC: regulatory acceptable concentration.

For the intended uses of CA3642an acceptable risk from the metabolite, R401553 is concluded.

**Table 9.5-32:** Acceptability of risk (PEC/RAC <1) for the metabolite R401553 for each organism group based on PEC<sub>sw</sub> values from FOCUS Step 1 for the use of CA3642 in sunflower (1 x 180 g a.s./ha)

Group		Fish acute	Inverteb. acute	Algae
Test species		<i>O. mykiss</i>	<i>D. magna</i>	<i>S. capricornutum</i>
Endpoint		LC <sub>50</sub>	EC <sub>50</sub>	ErC <sub>50</sub>
(µg a.s./L)		>120000	>120000	>120000
AF		100	100	10
RAC (µg a.s./L)		>1200	>1200	>12000
FOCUS Scenario	PEC <sub>sw</sub> (µg a.s./L)	PEC/RAC		
Step 1				
Worst case	6.98	<0.006	<0.006	<0.0006

AF: assessment factor; PEC: predicted environmental concentration; RAC: regulatory acceptable concentration.

#### Assessment of the risk from the metabolite, R402173

**Table 9.5-33 25:** Acceptability of risk (PEC/RAC <1) for the metabolite R402173 for each organism group based on PEC<sub>sw</sub> values from FOCUS Step 1 for the use of CA3642 in spring and winter cereals (2 x 210 g a.s./ha)

Group		Fish acute	Inverteb. acute	Algae
Test species		<i>O. mykiss</i>	<i>D. magna</i>	<i>S. capricornutum</i>
Endpoint (µg a.s./L)		LC <sub>50</sub> 62000	EC <sub>50</sub> >100000	ErC <sub>50</sub> 67000
AF		100	100	10
RAC (µg a.s./L)		620	>1000	6700
FOCUS Scenario	PEC <sub>SW</sub> (µg a.s./L)	PEC/RAC		
Step 1				
Worst case	21.79	0.035	<0.022	0.0033

AF: assessment factor; PEC: predicted environmental concentration; RAC: regulatory acceptable concentration.

**Table 9.5- 34 26:** Acceptability of risk (PEC/RAC <1) for the metabolite R402173 for each organism group based on PEC<sub>sw</sub> values from FOCUS Step 1 for the use of CA3642 in spring and winter oilseed rape (1 x 180 g a.s./ha)

Group		Fish acute	Inverteb. acute	Algae
Test species		<i>O. mykiss</i>	<i>D. magna</i>	<i>S. capricornutum</i>
Endpoint (µg a.s./L)		LC <sub>50</sub> 62000	EC <sub>50</sub> >100000	ErC <sub>50</sub> 67000
AF		100	100	10
RAC (µg a.s./L)		620	>1000	6700
FOCUS Scenario	PEC <sub>sw</sub> (µg a.s./L)	PEC/RAC		
Step 1				
Worst case	9.34	0.015	<0.009	0.0014

AF: assessment factor; PEC: predicted environmental concentration; RAC: regulatory acceptable concentration.

For the intended uses of CA3642 an acceptable risk from the metabolite R402173 is concluded.

**Table 9.5-35: Acceptability of risk (PEC/RAC <1) for the metabolite R402173 for each organism group based on PEC<sub>sw</sub> values from FOCUS Step 1 for the use of CA3642 in sunflower (1 x 180 g a.s./ha)**

Group		Fish acute	Inverteb. acute	Algae
Test species		<i>O. mykiss</i>	<i>D. magna</i>	<i>S. capricornutum</i>
Endpoint (µg a.s./L)		LC <sub>50</sub> 62000	EC <sub>50</sub> >100000	ErC <sub>50</sub> 67000
AF		100	100	10
RAC (µg a.s./L)		620	>1000	6700
FOCUS Scenario	PEC <sub>SW</sub> (µg a.s./L)	PEC/RAC		
Step 1				
Worst case	9.34	0.015	<0.009	0.0014

AF: assessment factor; PEC: predicted environmental concentration; RAC: regulatory acceptable concentration.

**zRMS comments:**

Since metabolites R234886, R402173 and R401553 are significantly less toxic than the parent azoxystrobin, a specific aquatic risk assessment was not deemed necessary for these compounds as it is covered by active substance. However, the Applicant's calculation have been presented and validated by zRMS.

### Overall conclusion, based on azoxystrobin and its relevant metabolites

For the intended uses of CA3642 in cereals and oilseed rape, acceptable risks from azoxystrobin are concluded for all aquatic organisms (based on the higher tier RAC<sub>SW</sub> value of 3.3 µg a.s./L for invertebrates), with the following risk-mitigation measures:

FOCUS scenario	Mitigation required to conclude an acceptable aquatic risk to the active substance, azoxystrobin, for the intended uses			
	Spring cereals		Winter cereals	
	1 x application	2 x application	1 x application	2 x application
D3/Ditch	-	-	-	-
D4/Pond	-	-	-	-
D4/Stream	-	-	-	-
D5/Pond	-	-	-	-
D5/Stream	-	-	-	-
D6/Ditch	-	-	-	-
R1/Pond	-	-	-	-
R1/Stream	-	-	-	10-m NSBZ + 10-m VFS
R3/Stream	-	-	-	10-m NSBZ + 10-m VFS
R4/Stream	-	10-m NSBZ + 10-m VFS	-	-
FOCUS scenario	Mitigation required to conclude an acceptable aquatic risk to the active substance, azoxystrobin, for the intended uses			
	Spring oilseed rape*		Winter oilseed rape*	
	Spring application		Spring application	Autumn application
D3/Ditch	-		-	-
D4/Pond	-		-	-
D4/Stream	-		-	-
D5/Pond	-		-	-
D5/Stream	-		-	-
D6/Ditch	-		-	-
R1/Pond	-		-	-
R1/Stream	-		-	-
R3/Stream	-		-	-
R4/Stream	-		-	-
FOCUS scenario	Mitigation required to conclude an acceptable aquatic risk to the metabolite, prothioconazole-desthio, for the intended uses			
	Sunflower, 1 x application			
D3/Ditch	-			
D4/Pond	-			
D4/Stream	-			
R1/Pond	-			
R1/Stream	-			

Dashes (-) indicate no risk mitigation measures are required. \*Only one application is proposed for oilseed rape. NSBZ: no-spray buffer zone (to mitigate drift); VFS: vegetated filter strip (to mitigate spray drift and runoff).

## Combined aquatic toxicity assessment

In accordance with the EFSA Journal 2013;11(7):3290 guidance document, and in line with the Regulation (EC) No. 1107/2009, the following stepwise mixture risk assessment is presented for the product, CA3542, whereby the interaction between the two active substances, prothioconazole and azoxystrobin are considered.

Due to the very fast degradation of prothioconazole to prothioconazole-desthio (DT<sub>50</sub> 0.8 -1.0 days in water at 20°C in the dark) and due to the metabolite being clearly more toxic than the parent, also the combination of prothioconazole-desthio and azoxystrobin is considered for the combined assessment. Chronic exposure of aquatic organisms to prothioconazole is therefore highly unlikely (and particularly unlikely for the indirect exposure routes of run-off and drainage). Therefore, the chronic risk assessment is sufficiently addressed by prothioconazole-desthio, however, in a comprehensive approach, assessments for both combinations of prothioconazole/azoxystrobin and prothioconazole-desthio/azoxystrobin are presented.

Initially, a detailed examination is conducted to determine whether the critical aquatic risk assessments (for acute fish, chronic fish and aquatic invertebrates) are covered by the relevant risk assessments for the single active substance or metabolite/s. Aquatic mixture toxicity values are calculated (based on Equation 13 from Section 10.3.3 of the EFSA Journal 2013;11(7):3290, below) and are compared to experimental measured aquatic toxicity data for CA3642.

$$\text{Equation 13: } ECx_{mix-CA} = \left( \sum_{i=1}^n \frac{p_i}{ECx_i} \right)^{-1}$$

where:

- n: number of mixture components
- i: index from 1...n mixture components
- p<sub>i</sub>: the i<sup>th</sup> component as a relative fraction of the mixture composition (note:  $\sum p_i$  must be 1)
- ECx<sub>i</sub>: concentration of component i provoking x % effect (pragmatically, NOEC<sub>i</sub> may be inserted, too).

$$\begin{aligned} \text{Acute fish } ECx_{mix-CA} &= [(0.5/1.83) + (0.5/0.47)]^{-1} \\ &= \mathbf{0.748 \text{ mg/L}} \end{aligned}$$

$$\begin{aligned} \text{Acute } Daphnia \text{ } ECx_{mix-CA} &= [(0.5/1.3) + (0.5/0.23)]^{-1} \\ &= \mathbf{0.391 \text{ mg/L}} \end{aligned}$$

$$\begin{aligned} \text{Algae (} S. \text{ costatum) }^* ECx_{mix-CA} &= [(0.5/0.03278) + (0.5/0.3)]^{-1} \\ &= \mathbf{0.059 \text{ mg/L}} \end{aligned}$$

\*Note that this endpoint was used to enable appropriate comparison with the product study, which was conducted with *S. costatum* (the most sensitive species in renewal active substance data).

The modelled deviation ratio (MDR) calculations are shown in the table below.

**Table 9.5-36 27: Comparison of measured formulation toxicity (EC<sub>xPPP</sub>) against calculated mixture toxicity EC<sub>xmix-CA</sub>**

	Fish ( <i>O. mykiss</i> )	Aquatic invertebrates ( <i>Daphnia magna</i> )	Algae ( <i>S. costatum</i> )
ECx mg total a.s./L	0.748	0.391	0.059
EC <sub>xmix-CA</sub> mg product/L	2.68	1.401	0.211
EC <sub>xPPP</sub> mg CA3642/L	>2.04	0.97	0.487
EC <sub>xPPP</sub> mg total of a.s./L	0.57	0.27	0.136

<b>MDR product</b>	<b>1.31</b>	<b>1.44</b>	<b>0.43</b>
<b>MDR sum a.s.</b>	<b>1.31</b>	<b>1.45</b>	<b>0.43</b>

$MDR = EC_{X_{mix-CA}}/EC_{X_{PPP}}$

If  $MDR = 0.2-5$ : concentration addition (CA) holds for the mixture; If  $MDR > 5$ : mixture is more toxic than CA; If  $MDR < 0.2$ : mixture is less toxic than CA.

The MDR values for CA3642, for the three aquatic taxonomic groups are all clearly within the range of 0.2 to 5 demonstrating that the formulation toxicity can be fully explained by the concentration addition model (EFSA Journal 2013;11(7):3290, Section 2.5, Step 2). There are no identified synergistic or antagonistic effects from the two active substances.

Given the relatively close agreement of the calculated mixture toxicity ( $EC_{X_{mix-CA}}$ ) with experimental mixture toxicity ( $EC_{X_{PPP}}$ ) for the product, CA3642, the active substance endpoints (or worst-case a.s. endpoint from a single a.s. formulation) are considered appropriate for use in the aquatic risk assessment.

Based on the FOCUS Step 1 and Step 2  $PEC_{sw}$  values for the active substances, prothioconazole and azoxystrobin and the metabolite prothioconazole-desthio, (dRR Part B8, Section 8.9), the composition of the active substances in the product (mixture) and the composition of the  $PEC_{mix}$  are not the same, as demonstrated in the table, below:

**Table 9.5-37<sup>28</sup>:  $PEC_{sw}$  values (µg/L) at FOCUS STEPS 1-2**

<b>Substance/Crop</b>	<b>STEP 1</b>	<b>STEP 2</b>
<b>Prothioconazole</b>		
Winter/Spring Cereals*	22.81	1.93
Winter/Spring OSR (Spring applications)	19.55	1.66
Winter OSR (Autumn applications)	19.55	1.66
<b>Prothioconazole-desthio</b>		
Winter/Spring Cereals*	82.28	11.35
Winter/Spring OSR (Spring applications)	35.26	2.61
Winter OSR (Autumn applications)	35.26	4.68
<b>Azoxystrobin</b>		
Winter/Spring Cereals*	95.81	29.17
Winter/Spring OSR (Spring applications)	41.06	5.77
Winter OSR (Autumn applications)	41.06	10.32

\*Worst-case  $PEC_{sw}$  values for each substance/crop combination, from calculations for all possible numbers of applications, seasons and pH-dependence.

CA3642 contains the active substances prothioconazole and azoxystrobin. Aquatic risk assessments have been conducted for both active substances and their metabolites. For full details of the PEC/RAC calculations please see Tables 9.5-4 to 9.5-26 above. Most of these assessments indicate an acceptable risk to aquatic organisms, using FOCUS surface water Steps 1, 2 or 3  $PEC_{sw}$  values. The only risk assessments that required risk mitigation measures, using FOCUS Step 4  $PEC_{sw}$  values, were for:

- The metabolite prothioconazole-desthio for chronic fish (based on the critical Tier-1 RAC value of 0.334 µg metabolite/L)
- The active substance, azoxystrobin, for acute fish (based on a RAC value of 4.7 µg/L)
- The active substance, azoxystrobin, for aquatic invertebrates (based on a RAC value of 3.3 µg/L)

It is noted that for metabolites formed in another compartment (e.g. when aquatic entry is via run-off), they are currently only considered in a separate risk assessment and no mixture toxicity assessment is required (see FAQ Aquatic MixTox Tool, v1 – Version 1: January 2021; Section M4. For toxic metabolites formed in another compartment – <https://zenodo.org/record/4593676>).

According to the EFSA Journal 2013;11(7):3290, Section 2.5, Step 5, a check on whether one of the components clearly drives the toxicity, by considering if one single active substance/relevant metabolite is responsible for >90% of the toxic units in the mixture, is conducted for the critical areas of the risk assessment, based on Equation 14 from Section 10.3.3 of the EFSA Journal 2013;11(7):3290, below:

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

Where:

$n$ : number of mixture components

$i$ : index from 1... $n$  mixture components

TU: toxic unit

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

Based on the critical areas of the risk assessment, identified above, a comparison of the toxic units (TU) for the potential mixture components is presented in the table below.

**Table 9.5-38<sup>29</sup>: Toxic unit (TU) comparison for the potential mixture components of CA3642 based on EU agreed endpoints for prothioconazole (EFSA Sci Report (2007) 106, 1-98) and azoxystrobin (EFSA Journal 2010: 8(4): 1542)**

Taxonom- ic group	Aquatic Organis- m	Time- scale	Test substance	Toxicity endpoint [µg/L]	Fraction of substance in the mixture	Toxic Unit (TU) <sup>1</sup> [%]
Fish	<i>O. mykiss</i>	Acute	Azoxystrobin	470	0.5	80
			Prothioconazole	1830	0.5	20
			Azoxystrobin	470	0.5	<b>93</b>
			Prothioconazole-desthio	6630	0.5	7
Fish	<i>P. promelas</i>	Chronic	Azoxystrobin	147	0.5	2
	<i>O. mykiss</i>		Prothioconazole-desthio	3.34	0.5	<b>98</b>
Aquatic inverte- brate	<i>D. magna</i>	Acute	Azoxystrobin	230	0.5	85
	<i>M. bahia</i> <sup>2</sup>		Prothioconazole	1300	0.5	15
	<i>D. magna</i>		Azoxystrobin	55	0.5	<b>96</b>
	<i>D. magna</i>		Prothioconazole	1300	0.5	4
Aquatic inverte- brate	<i>D. magna</i>	Acute	Azoxystrobin	230	0.5	<b>98</b>
	<i>D. magna</i>		Prothioconazole-desthio	10000	0.5	2
	<i>M. bahia</i> <sup>2</sup>		Azoxystrobin	55	0.5	<b>99</b>
	<i>D. magna</i>		Prothioconazole-desthio	10000	0.5	1
Aquatic inverte- brate	<i>D. magna</i>	Chronic	Azoxystrobin	44	0.5	<b>93</b>
	<i>D. magna</i>		Prothioconazole	560	0.5	7
Aquatic inverte- brate	<i>D. magna</i>	Chronic	Azoxystrobin	44	0.5	69
	<i>D. magna</i>		Prothioconazole-desthio	100	0.5	31

<sup>1</sup> Contribution to overall toxicity [%]. <sup>2</sup> Most sensitive aquatic invertebrate: NB The difference in sensitivity between freshwater and marine species is lower than one order of magnitude (10).

TU values in **bold** (i.e. >90%) indicate the substance drives the risk assessment.

### Acute fish

As demonstrated in Table 9.5-27, above, the MDR value for the product CA3642 for acute fish is 1.31. This value is clearly within the range of 0.2 to 5 required to conclude that the formulation toxicity is explained by the concentration addition model (EFSA Journal 2013;11(7):3290, Section 2.5).

The azoxystrobin acute fish risk assessment is based on a RAC value of 4.7 µg/L (LC<sub>50</sub> value of 470 µg azoxystrobin/L). As demonstrated in Table 9.5-29, above, azoxystrobin is the main driver for the acute fish toxicity risk assessment (contributing 93% of the toxicity) when compared to the toxicity of the metabolite, prothioconazole-desthio, with an LC<sub>50</sub> value of 6630 µg/L (contributing 7% of the toxicity). In addition, the aquatic mixture risk assessment identified worst case prothioconazole-desthio PEC<sub>sw</sub> values in FOCUS R scenarios due to run-off, therefore consideration of this metabolite for combined mixture toxicity assessment is not currently considered appropriate (see: FAQ Aquatic MixTox Tool, v1 – Version 1: January 2021, <https://zenodo.org/record/4593676>). Therefore, overall the combined acute fish toxicity of azoxystrobin and prothioconazole-desthio is not considered to be required.

Conversely, based on the proportion of the acute fish toxicity attributed to azoxystrobin compared to prothioconazole no clear driver (i.e. >90%) is identified despite azoxystrobin showing higher proportion of toxicity units (80%). Therefore, it is considered appropriate that a mixture toxicity assessment for the acute risk to fish from the two active substances, prothioconazole and azoxystrobin, is provided for completeness.

### Chronic fish

Following comparison of the toxic units for the metabolite prothioconazole-desthio and the active substance azoxystrobin relevant for the chronic fish risk assessment (based on NOEC values of 3.34 µg prothioconazole-desthio/L and 147 µg azoxystrobin/L, respectively), the chronic fish toxicity is clearly driven by the metabolite prothioconazole-desthio (contributing 98% of the toxicity), compared to azoxystrobin (contributing 2% of the toxicity). A chronic fish mixture toxicity risk assessment with

azoxystrobin and prothioconazole-desthio is not required, for the following three reasons:

- 1) The metabolite, prothioconazole-desthio, is primarily derived as a result of run-off exposure, for which it is not a current standard requirement to conduct a mixture risk assessment (see: FAQ Aquatic MixTox Tool, v1 – Version 1: January 2021, <https://zenodo.org/record/4593676>);
- 2) Comparison of the TUs shows that prothioconazole-desthio clearly drives the risk assessment (a risk assessment for this metabolite has been conducted and is demonstrated to be acceptable with mitigation) and
- 3) The chronic fish endpoints are not comparable. The fish early life-stage studies were not conducted on the same species (*O. mykiss* for prothioconazole-desthio and *P. promelas* for azoxystrobin) and the endpoints indicate a large difference in sensitivity, based on the worst-case NOEC values (0.00334 mg prothioconazole-desthio/L, for deformities, versus 0.147 mg azoxystrobin/L, for growth).

Considering these points, the relative toxicity of these substances has been compared. Clearly, the majority of chronic toxicity to fish is likely to be due to the presence of the prothioconazole-desthio, based on its higher toxicity when compared to that of azoxystrobin. Consequently, it is considered that the chronic risk assessment for prothioconazole-desthio is sufficient, without the need for the additional combined risk assessment, illustrated above, and that an acceptable risk can be demonstrated with appropriate FOCUS step 4 mitigation (10 m NSBZ + 10 m VFS or 20 m NSBZ +20 m VFS for certain FOCUS scenarios). Overall, it is concluded that a combined chronic fish aquatic risk assessment for the metabolite, prothioconazole-desthio, and the active substance, azoxystrobin, is not required.

**zRMS comments:**

We agree that following comparison of the toxic units for the metabolite prothioconazole-desthio and the active substance azoxystrobin relevant for the chronic fish risk assessment (based on NOEC values of 3.34 µg prothioconazole-desthio/L and 147 µg azoxystrobin/L, respectively), the chronic fish toxicity is clearly driven by the metabolite prothioconazole-desthio (contributing 98% of the toxicity), compared to azoxystrobin (contributing 2% of the toxicity).

### Aquatic invertebrates

For the active substance azoxystrobin, it should be noted that the acute and chronic aquatic invertebrate risk assessments are based on a RAC value of 3.3 µg/L, which is derived from an assessment of lower and higher tier data, including a mesocosm study, and the lower limit of a calculated HC<sub>5</sub> (EFSA Journal 2010; 8(4):1542).

To illustrate which active substance is the driver, in terms of aquatic invertebrate toxicity, Table 9.5-29, above, uses tier 1 laboratory data for azoxystrobin to enable a comparison with the corresponding lower tier data for prothioconazole and its metabolite, prothioconazole-desthio. It is important to note that for the purposes of this illustration, the difference in sensitivity between freshwater and marine species is lower than one order of magnitude (10). In addition, for the aquatic risk assessment (Tables 9.5-16 to 9.5-20), none of these lower tier data aquatic invertebrate endpoints for azoxystrobin have been used, only the overall EU-agreed RAC value of 3.3 µg/L, in line with EFSA Journal 2010; 8(4):1542.

The results in Table 9.5-29 demonstrate that, based on both the acute and chronic aquatic invertebrate endpoints, azoxystrobin is the clear driver of the aquatic risk assessment. This is most visibly illustrated when the most sensitive aquatic invertebrate data (*M. bahia*) are considered for the acute risk assessment, where azoxystrobin is shown to be the main acute toxicity driver when compared to both prothioconazole (contributing to 96% of the toxicity) and prothioconazole-desthio (contributing to 99% of the toxicity) data. The driver for the acute mixture toxicity was also evaluated considering the less conservative azoxystrobin data for *D. magna* showing that azoxystrobin lead to 85% of the toxicity units.

The chronic aquatic invertebrate toxicity driver is also shown to be azoxystrobin (TU 93%) using the *D. magna* NOEC value of 44 µg/L when compared to the prothioconazole NOEC value of 560 µg/L. When



comparing the chronic toxicity of azoxystrobin (NOEC value: 44 µg/L) with that of prothioconazole-desthio (NOEC value: 100 µg/L), the distinction is less clear (TU azoxystrobin 69% as compared to TU prothioconazole-desthio of 31%). However, the overall RAC value of 3.3 µg azoxystrobin/L, as noted above and used for the aquatic invertebrate risk assessment, is equivalent to a notional NOEC value of 33 µg/L, which leads to a higher driver for azoxystrobin (contributing to 75% of the toxicity). Nevertheless, a full comparison of chronic toxicity to aquatic invertebrates between azoxystrobin and the metabolite, prothioconazole-desthio, is not possible, since the respective aquatic invertebrate data sets are not directly comparable. Furthermore, as noted above, the highest  $PEC_{sw}$  values for prothioconazole-desthio occur primarily as a result of run-off exposure, for which there is no current standard requirement to conduct a mixture risk assessment (see: FAQ Aquatic MixTox Tool, v1 – Version 1: January 2021, <https://zenodo.org/record/4593676>).

Based on current guidance for mixture risk assessments (EFSA Journal 2013;11(7):3290, Sections 2.5 and 10.3), it is concluded that the separate acute and chronic aquatic invertebrate risk assessments for azoxystrobin, prothioconazole and the run-off metabolite, prothioconazole-desthio, are sufficient to assess the risk to these organisms and combined toxicity risk assessments are not required. A suitable risk mitigation measure (10-m NSBZ + 10-m VFS) is required to demonstrate an acceptable aquatic invertebrate risk assessment for certain FOCUS scenarios for use on cereals.

#### Combined toxicity risk assessment for acute fish

According to the EFSA Journal 2013;11(7):3290 guidance document, Section 10.3.8, for a mixture risk assessment based on calculated mixture toxicity, the ETR is calculated by dividing  $PEC_{mix}$  by the calculated mixture toxicity, assuming concentration addition (CA) (i.e.  $ECx_{mix-CA}$ ). The  $ETR_{mix-CA}$  is calculated according to Equation 18 (section 10.3.8):

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

Where  $PEC_{mix}$  is the total  $PEC_{sw}$  value for the mixture components in combination. An acceptable risk is demonstrated if  $ETR_{mix-CA} < 0.01$ .

Based on the discussion, above, the following conservative combined toxicity risk assessments are based on the acute fish endpoints for prothioconazole (1000 µg prothioconazole/L) and azoxystrobin (470 µg azoxystrobin/L) only.

**Table 9.5-39:** Acceptability of the risk ( $ETR_{mix-CA} < 0.01$ ) for the combined toxicity of prothioconazole and azoxystrobin for the critical organism group (acute fish), following use of CA3642 in cereals.

<b>Active substances</b>	Prothioconazole and azoxystrobin							
<b>Taxonomic Group</b>	Acute fish							
<b>ETR<sub>mix-CA</sub> Trigger</b>	0.01 ( $EC_{x_{mix-CA}} = 748$ µg product/L, based on acute fish $LC_{50}$ values of 1830 µg prothioconazole/L and 470 µg azoxystrobin/L)*							
<b>Crop</b>	<b>Spring cereals</b>				<b>Winter cereals</b>			
<b>Application Rate (g a.s./ha)</b>	<b>210 g prothioconazole/ha 210 g azoxystrobin/ha</b>				<b>210 g prothioconazole/ha 210 g azoxystrobin/ha</b>			
<b>No. applications</b>	<b>1x</b>		<b>2x</b>		<b>1x</b>		<b>2x</b>	
<b>FOCUS Scenario</b>	<b>PEC<sub>mix</sub> (µg a.s./L)</b>	<b>ETR<sub>mix-CA</sub><sup>1</sup></b>	<b>PEC<sub>mix</sub> (µg a.s./L)</b>	<b>ETR<sub>mix-CA</sub><sup>1</sup></b>	<b>PEC<sub>mix</sub> (µg a.s./L)</b>	<b>ETR<sub>mix-CA</sub><sup>1</sup></b>	<b>PEC<sub>mix</sub> (µg a.s./L)</b>	<b>ETR<sub>mix-CA</sub><sup>1</sup></b>
<b>Step 1</b>								
Worst case	N/A	-	118.6200	<b>0.16</b>	N/A	-	118.62000	<b>0.16</b>
<b>Step 2</b>								
N-EU‡	N/A	-	31.10000	<b>0.04</b>	N/A	-	31.10000	<b>0.04</b>
<b>Step 3</b>								
D3/Ditch	2.6610	0.0036	2.3270	0.0036	2.6590	0.0036	2.3260	0.0031
D4/Pond	0.5129	0.0007	0.9262	0.0014	0.4715	0.0006	1.0205	0.0014
D4/Stream	2.1760	0.0029	1.9434	0.0030	1.9663	0.0026	1.8747	0.0025
D5/Pond	0.1850	0.0002	0.3150	0.0005	0.1829	0.0002	0.3302	0.0004
D5/Stream	2.2390	0.0030	2.0130	0.0031	2.1290	0.0028	2.0360	0.0027
R1/Pond	-	-	-	-	0.1960	0.0003	0.4483	0.0006
R1/Stream	-	-	-	-	2.2745	0.0030	4.8971	0.0065
R3/Stream	-	-	-	-	3.1950	0.0043	5.5980	0.0075
R4/Stream	3.4483	0.0046	5.2517	0.0082	1.8906	0.0025	3.5902	0.0048

PEC<sub>mix</sub> = sum prothioconazole and azoxystrobin PEC<sub>sw</sub> values – worst case values used.

ETR<sub>mix</sub> values in **bold** >0.01 and denote an unacceptable risk, which require further risk mitigation measures. Appl.: application; No.: number.

<sup>1</sup>ETR<sub>mix-CA</sub> = PEC<sub>mix</sub>/EC<sub>x<sub>mix-CA</sub></sub>

\*Worst case prothioconazole endpoint from EFSA Scientific Report (2007), 106, 1-98 and *O. mykiss* azoxystrobin endpoint of 470 µg azoxystrobin/L as EU agreed acute fish endpoint (see aquatic table footnote a in EFSA Journal 2010: 8(4):1542 confirming this as only agreed acute fish endpoint for azoxystrobin). PEC<sub>sw</sub> values selected from the worst-case FOCUS Step-1 and 2 values. ‡FOCUS Steps 2 N-EU PEC<sub>sw</sub> values are relevant for all central zone member states.

For the intended uses of the product, CA3642, in winter and spring cereals, an acceptable risk from the active substances, prothioconazole and azoxystrobin in combination, is concluded for all C-EU relevant FOCUS Step-3 scenarios, without the need for risk-mitigation measures, for the critical organism group (based on the worse-case acute fish endpoints of 1000 µg prothioconazole/L and 470 µg azoxystrobin/L).

**Table 9.5-40** ~~31:~~ **Acceptability of the risk ( $ETR_{mix-CA} < 0.01$ ) for the combined toxicity of prothioconazole and azoxystrobin for the critical organism group (acute fish), following use of CA3642 in oilseed rape.**

<b>Active substances</b>	Prothioconazole and azoxystrobin					
<b>Taxonomic Group</b>	Acute fish					
<b><math>ETR_{mix-CA}</math> Trigger</b>	0.01 ( $EC_{xmix-CA} = 748 \mu\text{g product/L}$ , based on acute fish $LC_{50}$ values of $1830 \mu\text{g prothioconazole/L}$ and $470 \mu\text{g azoxystrobin/L}$ )					
<b>Crop</b>	<b>Spring OSR</b>			<b>Winter OSR</b>		
<b>Application Rate (g a.s./ha)</b>	<b>180 g prothioconazole/ha 180 g azoxystrobin/ha</b>			<b>180 g prothioconazole/ha 180 g azoxystrobin/ha</b>		
<b>No. applications</b>	<b>1x</b>			<b>Autumn applications</b>		<b>Spring applications</b>
<b>FOCUS Scenario</b>	<b><math>PEC_{mix}</math> (<math>\mu\text{g a.s./L}</math>)</b>	<b><math>ETR_{mix-CA}^1</math></b>	<b><math>PEC_{mix}</math> (<math>\mu\text{g a.s./L}</math>)</b>	<b><math>ETR_{mix-CA}^1</math></b>	<b><math>PEC_{mix}</math> (<math>\mu\text{g a.s./L}</math>)</b>	<b><math>ETR_{mix-CA}^1</math></b>
<b>Step 1</b>						
Worst case	60.6100	<b>0.08</b>	60.6100	<b>0.08</b>	60.6100	<b>0.09</b>
<b>Step 2</b>			<b>Step 2 (autumn)</b>			
N-EU‡	7.43000	<b>0.010</b>	11.98000	<b>0.02</b>		
			<b>Step 2 (spring)</b>			
			7.43000	<b>0.010</b>	7.43000	<b>0.012</b>
<b>Step 3</b>						
D3/Ditch	2.2820	0.0031	2.2890	0.0031	2.2730	0.0030
D4/Pond	0.4294	0.0006	1.1503	0.0015	0.3766	0.0005
D4/Stream	1.8677	0.0025	2.2484	0.0030	1.7706	0.0024
D5/Pond	0.1780	0.0002	0.5374	0.0007	0.1648	0.0002
D5/Stream	1.8147	0.0024	2.1270	0.0028	1.4949	0.0020
R1/Pond	0.1841	0.0002	0.0997	0.0001	0.1665	0.0002
R1/Stream	2.3164	0.0031	1.8574	0.0025	1.8532	0.0025
R3/Stream	-	-	3.8130	0.0051	2.9220	0.0039

$PEC_{mix}$  = sum prothioconazole and azoxystrobin  $PEC_{sw}$  values – worst case values used.

$ETR_{mix}$  values in **bold**  $> 0.01$  and denote an unacceptable risk, which require further risk mitigation measures. Appl.: application; No.: number.

$1 ETR_{mix-CA} = PEC_{mix}/EC_{xmix-CA}$

\*Worst case a.s. prothioconazole endpoint from EFSA Scientific Report (2007), 106, 1-98 i.e.fish  $LC_{50}$  values of  $1830 \mu\text{g prothioconazole/L}$  i.e.and *O. mykiss* azoxystrobin endpoint of  $470 \mu\text{g azoxystrobin/L}$  as EU agreed acute fish endpoint (see aquatic table footnote a in EFSA Journal 2010: 8(4):1542 confirming this as only agreed acute fish endpoint for azoxystrobin).  $PEC_{sw}$  values selected from the worst-case FOCUS Step-1 and 2 values. ‡FOCUS Steps 2 N-EU  $PEC_{sw}$  values are relevant for all central zone member states.

For the intended uses of CA3642 in winter and spring oilseed rape an acceptable risk from the combined toxicity of prothioconazole and azoxystrobin all C-EU relevant FOCUS Step-3 scenarios for the critical organism group, acute fish (based on the worse-case acute fish endpoints of  $1830 \mu\text{g prothioconazole/L}$  and  $470 \mu\text{g azoxystrobin/L}$ ).

**Table 9.5-41: Acceptability of the risk ( $ETR_{\text{mix-CA}} < 0.01$ ) for the combined toxicity of prothioconazole and azoxystrobin for the critical organism group (acute fish), following use of CA3642 in sunflower.**

<b>Active substances</b>	Prothioconazole and azoxystrobin	
<b>Taxonomic Group</b>	Acute fish	
<b><math>ETR_{\text{mix-CA}}</math> Trigger</b>	0.01 ( $EC_{\text{mix-CA}} = 748 \mu\text{g product/L}$ , based on acute fish $LC_{50}$ values of $1830 \mu\text{g prothioconazole/L}$ and $470 \mu\text{g azoxystrobin/L}$ )	
<b>Crop</b>	<b>Sunflower</b>	
<b>Application Rate (g a.s./ha)</b>	<b>180 g prothioconazole/ha 180 g azoxystrobin/ha</b>	
<b>No. applications</b>	<b>1x</b>	
<b>FOCUS Scenario</b>	<b><math>PEC_{\text{mix}}</math> (<math>\mu\text{g a.s./L}</math>)</b>	<b><math>ETR_{\text{mix-CA}}</math><sup>1</sup></b>
<b>Step 1</b>		
Worst case	60.61	<b>0.081</b>
<b>Step 2</b>		
N-EU <sup>†</sup>	8.96	<b>0.012</b>
<b>Step 3</b>		
D3/Ditch	1.888	0.003
D4/Pond	0.515	0.001
D4/Stream	1.620	0.002
R1/Pond	1.506	0.002
R1/Stream	2.742	0.004

$PEC_{\text{mix}}$  = sum prothioconazole and azoxystrobin  $PEC_{\text{sw}}$  values – worst case values used.

$ETR_{\text{mix}}$  values in **bold**  $> 0.01$  and denote an unacceptable risk, which require further risk mitigation measures. Appl.: application; No.: number.

<sup>1</sup>  $ETR_{\text{mix-CA}} = PEC_{\text{mix}}/EC_{\text{mix-CA}}$

\* Worst case a.s. prothioconazole endpoint from EFSA Scientific Report (2007), 106, 1-98 i.e. fish  $LC_{50}$  values of  $1830 \mu\text{g prothioconazole/L}$  and *O. mykiss* azoxystrobin endpoint of  $470 \mu\text{g azoxystrobin/L}$  as EU agreed acute fish endpoint (see aquatic table footnote a in EFSA Journal 2010: 8(4):1542 confirming this as only agreed acute fish endpoint for azoxystrobin).  $PEC_{\text{sw}}$  values selected from the worst-case FOCUS Step-1 and 2 values.

<sup>†</sup> FOCUS Steps 2 N-EU  $PEC_{\text{sw}}$  values are relevant for all central zone member states.

For the intended uses of CA3642 in sunflower an acceptable risk is concluded from the combined toxicity of prothioconazole and azoxystrobin all C-EU relevant FOCUS Step-3 scenarios for the critical organism group, acute fish (based on the worse-case acute fish endpoints of  $1830 \mu\text{g prothioconazole/L}$  and  $470 \mu\text{g azoxystrobin/L}$ ).

Risk assessment for the formulated product (spray drift exposure)

For completeness, the  $PEC_{\text{sw}}$  values for the formulated product were calculated using the FOCUS drift calculator for a single application. Multiple applications, drainflow, runoff and sediment concentrations are not relevant for the formulation, as it dissociates into its component substances on contact with soil or water.  $PEC_{\text{sw}}$  values are as shown in Table 8.9-57 of the B8 document.

**Table 9.5-42<sup>32</sup>: Acceptability of the risk (PEC/RAC <1) for CA3642 based on spray drift of intact formulation**

Group		Fish acute	Invertebrate acute	Algae
Test species		<i>O. mykiss</i>	<i>D. magna</i>	<i>S. costatum</i>
Endpoint		LC <sub>50</sub>	EC <sub>50</sub>	E <sub>r</sub> C <sub>50</sub>
(µg CA3642/L) – mean measured		2040	970	487
AF		100	100	10
RAC (µg a.s./L)		20.4	9.7	48.7
PEC <sub>sw</sub> (µg a.s./L) at 1 m		PEC/RAC		
Oilseed rape (winter and spring)	12.14	0.60	<b>1.25</b>	0.25
Cereals (winter and spring)	14.17	0.70	<b>1.46</b>	0.29
PEC <sub>sw</sub> (µg a.s./L) at 5 m		PEC/RAC		
Oilseed rape (winter and spring)	2.517	-	0.26	-
Cereals (winter and spring)	2.937	-	0.30	-

AF: assessment factor; PEC: predicted environmental concentration; RAC: regulatory acceptable concentration

An acceptable risk from spray drift exposure to CA3642 is concluded for all proposed uses with the utilisation of a 5 m no-spray buffer zone (NSBZ).

### 9.5.3 Overall conclusions

An acceptable risk is concluded for all aquatic organism groups for the intended uses of CA3642 in cereals and oilseed rape, for the active substance prothioconazole and its relevant metabolites (prothioconazole-desthio, prothioconazole-s-methyl and 1,2,4-triazole), as well as for azoxystrobin and its relevant metabolites (R234886, R401553, and R402173).

The critical areas of the aquatic risk assessment are for the active substance azoxystrobin and the metabolite prothioconazole-desthio. Their aquatic risk assessments are driven by the lowest RAC values of 3.3 µg/L (azoxystrobin EU-agreed mesocosm, tier 1 and HC<sub>5</sub> derived aquatic invertebrate endpoint) and 0.334 µg/L (prothioconazole-desthio chronic fish endpoint), respectively. Appropriate mitigation to enable an acceptable risk from each of these substances has been identified.

A detailed examination of the critical aquatic risk assessments (acute/chronic fish and aquatic invertebrates) considering combined exposure concluded that the assessments with the single active substances (or metabolite) sufficiently covered the chronic risk to fish and the acute and chronic risk to aquatic invertebrates. However, for the acute risk to fish, it was not demonstrated that that azoxystrobin was clearly driving the acute risk to fish (contributing to >90% of the toxicity), therefore, a risk assessment for the combined acute toxicity to fish has been investigated, using the EU agreed worse-case acute fish endpoints of 1830 µg prothioconazole/L and 470 µg azoxystrobin/L. The combined risk assessment for prothioconazole and azoxystrobin demonstrated an acceptable acute risk to fish, with no risk mitigation measures required for the FOCUSsw relevant scenarios in the Central EU zone.

In conclusion, acceptable aquatic risks, from prothioconazole, azoxystrobin and their metabolites, also taking into account combined toxicity can be demonstrated for all relevant FOCUS scenarios in the Central EU zone, following the intended use of CA3642, in cereals, ~~and~~ oilseed rape and sunflower, with the following risk mitigation measures:

FOCUS scenario	Spring cereals		Winter cereals	
	1 x application	2 x application	1 x application	2 x application
D3/Ditch	-	-	-	-
D4/Pond	-	-	-	-
D4/Stream	-	-	-	-
D5/Pond	-	-	-	-
D5/Stream	-	-	-	-
R1/Pond	-	-	-	-
R1/Stream	-	-	10-m NSBZ + 10-m VFS	20-m NSBZ + 20-m VFS
R3/Stream	-	-	10-m NSBZ + 10-m VFS	20-m NSBZ + 20-m VFS
R4/Stream	10-m NSBZ + 10-m VFS	20-m NSBZ + 20-m VFS	-	20-m NSBZ + 20-m VFS
FOCUS scenario	Mitigation required to conclude an acceptable aquatic risk, for the intended uses			
	Spring oilseed rape		Winter oilseed rape	
	Spring application		Spring application	Autumn application
D3/Ditch	-	-	-	-
D4/Pond	-	-	-	-
D4/Stream	-	-	-	-
D5/Pond	-	-	-	-
D5/Stream	-	-	-	-
R1/Pond	-	-	-	-
R1/Stream	10-m NSBZ + 10-m VFS		-	-
R3/Stream	-	-	10-m NSBZ + 10-m VFS	20-m NSBZ + 20-m VFS
R4/Stream	-	-	-	-
FOCUS scenario	Mitigation required to conclude an acceptable aquatic risk for the intended uses			
	Sunflower, 1 x application			
D3/Ditch	-	-	-	-
D4/Pond	-	-	-	-

D4/Stream	-
R1/Pond	-
R1/Stream	10-m NSBZ + 10-m VFS

Dashes (-) indicate no required mitigation. NSBZ: No-spray buffer zone (to mitigate drift); VFS: vegetated filter strip (to mitigate spray drift and runoff).

In addition, a 5-m NSBZ is required to mitigate the potential risk from spray drift exposure from CA3642.

Overall, relevant CA3642 aquatic mitigation should be as follows:

Single use on spring and winter cereals: 10-m NSBZ+ 10-m VFS  
Two-fold use on spring and winter cereals: 20-m NSBZ + 20-m VFS  
Use on spring oilseed rape: 10-m NSBZ + 10-m VFS  
Use on winter oilseed rape (spring application): 10-m NSBZ + 10-m VFS  
Use on winter oilseed rape (autumn application): 20-m NSBZ + 20-m VFS  
Use on sunflower: 10-m NSBZ + 10-m VFS

#### **zRMS comments:**

Based on the performed calculations for the worst-case scenario acceptable risk following application of CA3642 according to the Central Zone GAP may be concluded.

Single use on spring and winter cereals: 10-m NSBZ+ 10-m VFS  
Two-fold use on spring and winter cereals: 20-m NSBZ + 20-m VFS  
Use on spring oilseed rape: 10-m NSBZ + 10-m VFS  
Use on winter oilseed rape (spring application): 10-m NSBZ + 10-m VFS  
Use on winter oilseed rape (autumn application): 20-m NSBZ + 20-m VFS  
Use on sunflower: 10-m NSBZ + 10-m VFS

Concerned Member States must decide on applicability of indicated risk mitigation measures in their countries at the product authorisation.

Please note that additional aquatic risk assessment may be required by the concerned Member States that do not accept simulations performed according to FOCUS recommendations.

## **9.6 Effects on bees (KCP 10.3.1)**

The assessment for risks to bees is conducted 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 with the 2013 EFSA bee risk assessment guidance (EFSA Journal 2013;11(7):3295; updated July 2014), which is yet to be noted, although new data requirements are in place. In accordance with the ‘Outcome of pesticides peer review meeting on recurring issues in ecotoxicology; EFSA Supporting publication 2015:EN-924), the first-tier risk assessment to honey bees is conducted according to EFSA Journal 2013;11(7):3295, but for bumble bees and solitary bees currently it cannot be recommended to routinely perform a risk assessment. Nonetheless, data for the acute contact and oral effects of the product, CA3642, on bumble bees are available and, thus, for completeness, a risk assessment for this species is also presented below.

### **9.6.1 Toxicity data**

Studies on the toxicity to bees have been carried out with prothioconazole, azoxystrobin and the respective formulated products. Full details of these studies are provided in the respective EU DARs and related documents.

Effects on bees exposed to CA3642 were not evaluated as part of the EU assessment of prothioconazole and azoxystrobin. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

Species	Substance	Exposure System	Results	Reference
<i>Apis mellifera</i>	CA3642	Acute contact, 48 h	<b>LD<sub>50</sub> &gt;1464 µg CA3642/bee</b>	KCP 10.3.1.1/01
		Acute oral, 96-h	<b>LD<sub>50</sub> = 339.2 µg CA3642/bee</b>	
		Chronic oral, 10 d	<b>LDD<sub>50</sub> = 50.2 µg CA3642/bee/day</b>	KCP 10.3.1.2/01
<i>Apis mellifera</i>	CA3642	Larval toxicity, 22 d	<b>NOED = 80.1 µg CA3642/larva/dev. period</b> ED <sub>10</sub> = 92.6 µg CA3642/larva/dev. period	KCP 10.3.1.3/01
<i>Bombus terrestris</i>	CA3642	Acute contact, 48 h	<b>LD<sub>50</sub> &gt;1464 µg CA3642/bee</b>	KCP 10.3.1.1/02
		Acute oral, 96-h	<b>LD<sub>50</sub> = 989.7 µg CA3642/bee</b>	
<i>Apis mellifera</i>	CA3642	Semi-field tunnel, winter oilseed rape, 15-day interval between applications (one pre-flowering and one during flowering and during daily honey bee flight).	Target applications: 2 x 1.4 L CA3642/ha (2 x 210 g each a.s./ha (actual: 215.3 g prothioconazole/ha and 219.0 g azoxystrobin/ha (1414.4 mL product/ha) at the first application and 214.9 g prothioconazole/ha and 218.6 g azoxystrobin/ha (1411.6 mL product/ha) at the second application)  Overall, it can be concluded that CA3642 had no significant effect on overall honey bee colony strength (mean number of adult honey bees), overall amount of brood or the development of the food storage area	KCP 10.3.1.5/01
<b>Active substance</b>				
<i>Apis mellifera</i>	Prothioconazole	Acute oral, 48 h	LD <sub>50</sub> >71 µg a.s./bee	EFSA Sci. Report. 2007; 106, 1-98
		Acute contact, 48 h	LD <sub>50</sub> >200 µg a.s./bee	
<i>Apis mellifera</i>	Azoxystrobin	Acute oral, 48 h	LD <sub>50</sub> >25 µg a.s./bee	EFSA Journal 2010; 8(4):1542
		Acute contact, 48 h	LD <sub>50</sub> >200 µg a.s./bee	
		Chronic oral, 10 d	No data available	
		Larval toxicity, 22 d	No data available	

**zRMS comments:**

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

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

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

### 9.6.1.1 Justification for new endpoints

New studies have been provided with CA3642 – acute oral and contact toxicity studies on honey bee and bumblebee (KCP 10.3.1.1/01 and KCP 10.3.1.1/02), two chronic studies (one chronic oral test with adult honey bees, one chronic test with larval honey bees ((KCP 10.3.1.2/01, KCP 10.3.1.3/01)), and one higher-tier, semi-field tunnel test (KCP 10.3.1.5/01). The studies are submitted to satisfy new data requirements under Regulation (EU) No 284/2013. New data submitted with this application are listed in Appendix 1



and summarised 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 Services (SANCO/10329/2002 rev.2 (final), October 17, 2002) and the draft EFSA bee guidance (EFSA Journal 2013;11(7):3295; updated July 2014). In accordance with the ‘Outcome of pesticides peer review meeting on recurring issues in ecotoxicology (EFSA Supporting publication, 2015:EN-924), the first-tier risk assessment is carried out using the required endpoints according to the draft roadmap of the European Commission (EC, 2014), dated 16th May, 2014:

- Honey bee: acute oral and contact (adult), chronic adult and larval
- Bumble bee: acute oral and contact (adult)

To achieve a concise risk assessment, the risk-envelope approach is applied in the first instance. Here, the assessment for the use group cereals (maximum application rate of 2 x 1.4 L CA3642/ha) also covers the risk for bees from all other intended uses in the oilseed rape crop group and sunflower (see 9.1.2).

### 9.6.2.1 Hazard quotients for bees

First-tier risk assessment, according to SANCO/10329/2002

**Table 9.6-2: First-tier assessment of the acute oral and contact risk to adult honey bees and bumble bees due to the use of CA3642 in cereals (in accordance with SANCO/10329/2002) – CA3642**

<b>Intended use</b>		Cereals			
<b>Product</b>		CA3642			
<b>Application rate (g/ha)</b>		2 x 1.4 L CA3642/ha (14-day interval); BBCH 14-69 2 x 1540 g CA3642/ha (based on a product density of 1.1004 g/mL)			
Species	Type of exposure	LD <sub>50</sub> (µg/bee)	Single application rate (g/ha)	HQ*	Acceptable HQ:
<i>Apis mellifera</i>	Acute contact	>1464	1540	<1.05	≤50
	Acute oral	339.2		4.54	
<i>Bombus terrestris</i>	Acute contact	>1464		<1.05	
	Acute oral	989.7		1.56	

\*Hazard quotients for oral and contact exposure calculated in accordance with SANCO/10329/2002.

The hazard quotients (HQ) values are well below the trigger value of 50, therefore an acceptable acute oral and contact risk to honey bees and bumble bees, exposed to CA3642 is concluded at the first tier with a large margin of safety for the intended uses of CA3642 in cereals and via the risk envelope approach for oilseed rape and sunflower.

**Table 9.6-3: First-tier assessment of the acute contact and oral risk to adult honey bees due to the use of CA3642 in cereals (in accordance with SANCO/10329/2002) – prothioconazole and azoxystrobin**

<b>Intended use</b>		Cereals			
<b>Active substance</b>		Prothioconazole and azoxystrobin			
<b>Application rate (g/ha)</b>		2 x 210 g a.s./ha (14-day interval); BBCH 14-69			
Species	Type of exposure	LD <sub>50</sub> (µg/bee)	Single application rate (g/ha)	HQ*	Acceptable HQ:
<b>Prothioconazole</b>					
<i>Apis mellifera</i>	Acute contact	>200	210	<1.05	≤50

	Acute oral	>71		<2.96	
<b>Azoxystrobin</b>					
<i>Apis mellifera</i>	Acute contact	>200	210	<1.05	≤50
	Acute oral	>25		<8.40	

\*Hazard quotients for oral and contact exposure calculated in accordance with SANCO/10329/2002.

The hazard quotients (HQ) values are well below the trigger value of 50, therefore, an acceptable acute oral and contact risk to honey bees exposed to prothioconazole and azoxystrobin is concluded at the first-tier with a large margin of safety for the intended uses of CA3642 in cereals and via the risk envelope approach for oilseed rape and sunflower.

#### zRMS comments:

The acute risk assessment for bees presented in Table 9.6-2 and Table 9.6-3 is agreed by the zRMS. HQ<sub>oral</sub>, contact values for the active substances and the formulated product CA3642 are below the trigger of 50, indicating a low acute risk for bees.

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

Overall, acceptable risk to bees may be concluded from the intended uses of CA3642 in cereals and via the risk envelope approach for oilseed rape and minor crop uses.

#### Screening risk assessment, according to EFSA/2013/3295

**Table 9.6-4: Screening assessment of the acute contact risk to adult honey bees and bumble bees due to the use of CA3642 in cereals (in accordance with EFSA/2013/3295) – CA3642**

<b>Intended use</b> <b>Product</b> <b>Application rate (g/ha)</b>		Cereals CA3642 2 x 1.4 L CA3642/ha (14-day interval); BBCH 14-69 2 x 1540 g CA3642/ha (based on a product density of 1.1004 g/mL)			
<b>Species</b>	<b>Type of exposure</b>	<b>LD<sub>50</sub> (µg/bee)</b>	<b>Single application rate (g/ha)</b>	<b>HQ</b>	<b>HQ trigger value for DW spray</b>
<i>Apis mellifera</i>	Acute contact	>1464	1540	<1.05	≤42
<i>Bombus terrestris</i>		>1464		<1.05	≤7

An acceptable acute contact risk to honey bees and bumble bees from CA3642 is concluded at the screening step with a large margin of safety for the intended uses of CA3642 in cereals and via the risk envelope approach for oilseed rape and sunflower.

**Table 9.6-5: Screening assessment of the acute contact risk to adult honey bees due to the use of CA3642 in cereals (in accordance with EFSA/2013/3295) – prothioconazole and azoxystrobin**

<b>Intended use</b> <b>Active substance</b> <b>Application rate (g/ha)</b>		Cereals Prothioconazole and azoxystrobin 2 x 210 g a.s./ha (14-d interval); BBCH 14-69			
<b>Species</b>	<b>Type of exposure</b>	<b>LD<sub>50</sub> (µg/bee)</b>	<b>Single application rate (g/ha)</b>	<b>HQ</b>	<b>HQ trigger value for DW spray</b>
<b>Prothioconazole</b>					
<i>Apis mellifera</i>	Acute contact	>200	210	<1.05	≤42
<b>Azoxystrobin</b>					
<i>Apis mellifera</i>	Acute contact	>200	210	<1.05	≤42

An acceptable acute contact risk to honey bees from prothioconazole and azoxystrobin is concluded at the screening step with a large margin of safety for the intended uses of CA3642 in cereals and via the risk envelope approach for oilseed rape uses and sunflower.

**Table 9.6-6: Screening assessment of the oral acute and chronic risk to honey bees and bumble bees due to the use of CA3642 in cereals (in accordance with EFSA/2013/3295) – CA3642**

<b>Intended use</b>		Cereals				
<b>Product</b>		CA3642				
<b>Application rate (g/ha)</b>		2 x 1.4 L CA3642/ha (14-day interval); BBCH 14-69 2 x 1540 g CA3642/ha (based on a product density of 1.1004 g/mL)				
Species	Type of exposure	LD <sub>50</sub> (µg/bee)	Single application rate (g/ha)	EF x SV	ETR	HQ trigger value for DW spray
<i>Apis mellifera</i>	Acute oral	339.2	1540	7.6	0.03	<0.2
<i>Bombus terrestris</i>		989.7		11.2	0.02	<0.036
<i>Apis mellifera</i>	Chronic adult	50.2		7.6	<b>0.233</b>	<0.03
	Chronic larval	80.1		4.4	0.08	<0.2

ETR values in **bold** are above the trigger.

Acceptable acute oral risks to honey bees and bumble bees and acceptable chronic oral risks to honey bee larvae from CA3642 are concluded at the screening step with a large margin of safety for the intended uses of CA3642 in cereals and and via the risk envelope approach for oilseed rape uses and sunflower. However, the chronic oral risk to adult honey bees is not acceptable at the screening step, therefore a first-tier risk assessment is presented in Tables 9.6-8 and 9.6-9, below.

**Table 9.6-7: Screening assessment of the acute oral risk to honey bees due to the use of CA3642 in cereals (in accordance with EFSA/2013/3295) – prothioconazole and azoxystrobin**

<b>Intended use</b>		Cereals				
<b>Active substance</b>		Prothioconazole and azoxystrobin				
<b>Application rate (g/ha)</b>		2 x 210 g a.s./ha (14-d interval); BBCH 14-69				
Species	Type of exposure	LD <sub>50</sub> (µg/bee)	Single application rate (g/ha)	EF x SV	ETR	HQ trigger value for DW spray
<b>Prothioconazole</b>						
<i>Apis mellifera</i>	Acute oral	>200	210	7.6	<0.01	0.2
<b>Azoxystrobin</b>						
<i>Apis mellifera</i>	Acute oral	>25	210	7.6	<0.06	0.2

ETR values in **bold** are above the trigger.

Acceptable acute oral risks to adult honey bees from prothioconazole and azoxystrobin are concluded at the screening step with large margins of safety for the intended uses of CA3642 in cereals and via the risk envelope approach for oilseed rape and sunflower.

First-tier risk assessment of chronic risk , according to EFSA/2013/3295

**Table 9.6-8: First-tier assessment of the chronic oral risk to adult honey bees, due to the use of CA3642 in cereals (in accordance with EFSA/2013/3295) – CA3642**

<b>Intended use</b>		Cereals						
<b>Product</b>		CA3642						
<b>Application rate</b>		2 x 1.4 L CA3642/ha (14-day interval); BBCH 30-69 2 x 1540 g CA3642/ha (based on a product density of 1.1004 g/mL)						
Scenario	BBCH	LDD <sub>50</sub> (µg/bee)	EF	SV (DW spray)	TWA	ETR <sub>oral</sub>	Trigger	Acceptable risk?
Treated Crop	30-39	50.2	1.0	0.92	0.72	0.02	0.03	Yes
	40-69		1.0	0.92	0.72	0.02	0.03	Yes
Weeds	30-39	50.2	0.5	2.90	0.72	0.03	0.03	Yes
	40-69		0.3	2.90	0.72	0.02	0.03	Yes
Field Margin	30-39	50.2	0.0092	2.90	0.72	0.001	0.03	Yes
	40-69		0.0092	2.90	0.72	0.001	0.03	Yes
Adjacent Crop	30-39	50.2	0.0033	5.80	0.72	0.0004	0.03	Yes
	40-69		0.0033	5.80	0.72	0.0004	0.03	Yes
Next Crop	30-39	50.2	1.0	0.54	0.72	0.01	0.03	Yes
	40-69		1.0	0.54	0.72	0.01	0.03	Yes

ETR values in **bold** are above the trigger.

**Table 9.6-9: First-tier assessment of the chronic oral risk to adult honey bees, due to the use of CA3642 in oilseed rape (in accordance with EFSA/2013/3295) – CA3642**

<b>Intended use</b>		Oilseed rape						
<b>Product</b>		CA3642						
<b>Application rate</b>		1 x 1.2 L CA3642/ha (14-day interval); BBCH 14-69 1 x 1320 g CA3642/ha (based on a product density of 1.1004 g/mL)						
Scenario	BBCH	LDD <sub>50</sub> (µg/bee)	EF	SV (DW spray)	TWA	ETR <sub>oral</sub>	Trigger	Acceptable risk?
Treated Crop	10-29	50.2	1.00	5.8	0.72	<b>0.110</b>	0.03	<b>No</b>
	30-39		1.00	5.8	0.72	<b>0.110</b>	0.03	<b>No</b>
	40-69		1.00	5.8	0.72	<b>0.110</b>	0.03	<b>No</b>
Weeds	10-29	50.2	1.00	2.9	0.72	<b>0.055</b>	0.03	<b>No</b>
	30-39		0.30	2.9	0.72	0.016	0.03	Yes
	40-69		0.25	2.9	0.72	0.014	0.03	Yes
Field Margin	10-29	50.2	0.0092	2.9	0.72	0.001	0.03	Yes
	30-39		0.0092	2.9	0.72	0.001	0.03	Yes
	40-69		0.0092	2.9	0.72	0.001	0.03	Yes
Adjacent Crop	10-29	50.2	0.0033	5.8	0.72	0.0004	0.03	Yes
	30-39		0.0033	5.8	0.72	0.0004	0.03	Yes
	40-69		0.0033	5.8	0.72	0.0004	0.03	Yes
Next Crop	10-29	50.2	1.00	0.54	0.72	0.010	0.03	Yes
	30-39		1.00	0.54	0.72	0.010	0.03	Yes
	40-69		1.00	0.54	0.72	0.010	0.03	Yes

ETR values in **bold** are above the trigger.

**Table 9.6-10: First-tier assessment of the chronic oral risk to adult honey bees, due to the use of CA3642 in sunflower (in accordance with EFSA/2013/3295) – CA3642**

Intended use Product Application rate		Sunflower CA3642 1 x 1.2 L CA3642/ha (14-day interval); BBCH 16-64 1 x 1320 g CA3642/ha (based on a product density of 1.1004 g/mL)						
Scenario	BBCH	LDD <sub>50</sub> (µg/bee)	EF	SV (DW spray)	TWA	ETR <sub>oral</sub>	Trigger	Acceptable risk?
Treated Crop	10-29	50.2	1.0	5.8	0.72	<b>0.110</b>	0.03	No
	30-39		1.0	5.8	0.72	<b>0.110</b>	0.03	No
	40-69		1.0	5.8	0.72	<b>0.110</b>	0.03	No
Weeds	10-29	50.2	1	2.9	0.72	<b>0.055</b>	0.03	No
	30-39		0.5	2.9	0.72	0.027	0.03	Yes
	40-69		0.25	2.9	0.72	0.014	0.03	Yes
Field Margin	10-29	50.2	0.0092	2.9	0.72	0.0005	0.03	Yes
	30-39		0.0092	2.9	0.72	0.0005	0.03	Yes
	40-69		0.0092	2.9	0.72	0.0005	0.03	Yes
Adjacent Crop	10-29	50.2	0.0033	5.8	0.72	0.0004	0.03	Yes
	30-39		0.0033	5.8	0.72	0.0004	0.03	Yes
	40-69		0.0033	5.8	0.72	0.0004	0.03	Yes
Next Crop	10-29	50.2	1.0	0.54	0.72	0.010	0.03	Yes
	30-39		1.0	0.54	0.72	0.010	0.03	Yes
	40-69		1.0	0.54	0.72	0.010	0.03	Yes

ETR values in **bold** are above the trigger.

The results above demonstrate acceptable chronic oral risks to adult honey bees for uses in cereals, for all relevant scenarios and BBCH growth stages. For uses in oilseed rape and sunflower, an acceptable risk was demonstrated for all relevant scenarios and BBCH growth stages, except for all growth stages of the scenario “treated crop” and for BBCH 10-29 of scenario “weeds”. Therefore, further refinement is required. Oilseed rape and sunflower are likely to be attractive to bees when flowering and requires pollination to propagate<sup>4</sup>. However, since oilseed rape and sunflower only flower at growth stages of BBCH 60-69<sup>5</sup>, it is unlikely that bees will be at significant risk in the “treated crop” scenarios for BBCH 10-29 and 30-39. In these cases, ETR values will be much lower, as likely exposure will be minimal when oilseed rape and sunflower not flowering. Consequently, a significant unacceptable chronic risk to bees remains only in the “treated crop” and “weeds” scenarios for BBCH 40-69 and BBCH 10-29, respectively, and further risk assessment is required.

#### zRMS comments:

Please note that the evaluation has been performed in line with SANCO/10329/2002 rev 2 final, as according to conclusions of the Central Zone Steering Committee (CZSC), recommendations of EFSA (2013) should not be considered for the zonal evaluations until the guidance is noted at the EU level.

zRMS is aware that the new EFSA bee GD is not yet noted by the MSs, nonetheless several MS (partially) use it. This is in line with PRAS 133 (2015), stating that it is recommended to perform the first tier of the honeybee risk assessment following the EFSA (2013) Bee GD with available data.

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

Based on the results above acceptable chronic oral risks to adult honey bees for uses in cereals, for all relevant scenarios and BBCH growth stages. For uses in oilseed rape and sunflower, an acceptable risk was demonstrated for all relevant scenarios and BBCH growth stages, except for all growth stages of the scenario “treated crop” and for **BBCH 10-29 of scenario “weeds”**. Therefore, further refinement is required.

<sup>4</sup>Attractiveness of Agricultural Crops to Pollinating Bees for the Collection of Nectar and/or Pollen. 2017. USDA

<sup>5</sup>Growth stages of mono-and dicotyledonous plants. BBCH Monograph, 2001. 2<sup>nd</sup> Ed, Uwe Meier, Federal Biological Research Centre for Agriculture and Forestry

Risk assessment based on EFSA (2013) is provided above for informative purposes only and is not the basis for derivation of conclusion regarding the risk to bees at the zonal level.

In order to resolve the chronic risk for the Applicant submitted higher tier studies performed with formulation CA3642 applied as two foliar applications under semi-field conditions on winter oilseed rape, one pre flowering and one at flowering during daily honey bee flight (interval between applications: 15 days). Effects of two foliar applications of 1400 mL product/ha applied one pre-flowering and one during flowering and during daily honey bee flight) were evaluated against effects observed in honey bees treated with tap water (control) and a toxic-reference item (1200 g Insegar/ha, equivalent to 300 g fenoxycarb/ha).

Based on the results it can be concluded that CA3642 had no significant effect on overall honey bee colony strength (mean number of adult honey bees), overall amount of brood or the development of the food storage area.

#### Commenting period process:

It is noted that no specific guidance is proposed in EFSA bee guidance (2013) for the risk assessment via honeydew exposure.

Considering that prothioconazole and azoxystrobin caused no significant effects on honey bee colonies and brood development at proposed field application rate (direct exposure under realistic worst case conditions in a bee attractive crop oilseed rape), a fast conversion of prothioconazole to -desthio metabolite rapidly degrading in plant material, it is not expected that the residue levels of either active substance or relevant metabolites in plant sap ingested by aphids (and honeydew collected by honey bees) will lead to significant exposure or risk.

It is also noted that during the period when application on cereals is intended (BBCH 30-69, beginning of stem elongation- end of flowering), other plants are more attractive to honey bees are flowering (e.g. black locust, chestnuts, linden, clover). The semi-field study was carried out on a bee attractive crop (oilseed rape) and therefore the exposure of adult honey bees and honey bee brood will cover worst-case exposure.

Thus, the risk from the indirect exposure would be covered by the conclusions of the existing acute oral direct exposure risk assessment including the higher tier semi-field data.

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

The first-tier assessments demonstrate a chronic oral risk to adult honey bees. In accordance with EFSA/2013/3295, higher-tier data are required.

A honey bee semi-field study is available for the product, CA3642 (KCP 10.3.1.5/01). CA3642 was applied as two foliar applications under semi-field conditions on winter oilseed rape, one pre flowering and one at flowering during daily honey bee flight (interval between applications: 15 days). Effects of two foliar applications of 1400 mL product/ha (equivalent to nominal 210 g a.s./ha each of azoxystrobin and prothioconazole, respectively) were evaluated against effects observed in honey bees treated with tap water (control) and a toxic-reference item (1200 g Insegar/ha, equivalent to 300 g fenoxycarb/ha).

Immediately after the second application residues of prothioconazole in nectar were 0.191 mg/kg and residues of azoxystrobin were 0.606 mg/kg. Also residues of prothioconazole in pollen were found at 35.1 mg/kg and of azoxystrobin in pollen at 52.2 mg/kg. These residue data confirm exposure of the tunnel confined honey bees to both active substances in the product CA3642 during the study.

Chronic risk to honey bees is demonstrated at Tier 1 from the use of CA3642 in oilseed rape and sunflower. The honey bee semi-field study was performed in oilseed rape, which is, as well as Phacelia, one of the commonly used crops to perform OECD75 studies. Oilseed rape is considered a highly attractive model plant species by EFSA/2013/3295 (Appendix O), from which the results can be extrapolated to a range of crops. Further, oilseed rape requires bee pollination to propagate<sup>6</sup>. In selecting a suitable crop according to EFSA/2013/3295 the key issue is to ensure that it is attractive to honey bees (such as Phacelia or oilseed rape) and that the residues, and hence the exposure to honey bees, is environmentally relevant and at least as high as predicted in the exposure section. The application rate of CA3642 in the semi-field study is more worst-case (210 g a.s./ha each for azoxystrobin and prothioconazole) than the proposed use in sunflower (1

<sup>6</sup> Attractiveness of Agricultural Crops to Pollinating Bees for the Collection of Nectar and/or Pollen. 2017. USDA

application of 180 g a.s./ha each of azoxystrobin and prothioconazole), therefore the exposure is considered sufficiently covered. It is considered appropriate to use the results of the semi-field study in oilseed rape (representative bee attractive crop according to EFSA/2013/3295) for the refinement of risk to honey bees in sunflower.

Exposure to CA3642 did not lead to statistically significant adverse effects on mortality of honey bee worker bees. Transient effects on honey bee flight activity were only observed on the day of application. No biologically relevant CA3642 treatment related behavioural effects were observed. CA3642 had no statistically significant effects on honey bee colony strength (mean number of adult honey bees), mean number of brood cells containing food (nectar and pollen), mean compensation indices or mean termination rates in brood cells initially containing eggs or young larvae.

CA3642 lead to a significant transient reduction in the mean number of honey bee brood cells on two occasions (4DAA2, 15DAA2) but there was no statistically significant reduction seen on the last two assessment dates (21DAA2, 27DAA2). Overall brood cell number levels at the end of the study observation period were in line with the control therefore no biological adverse effect on honey brood cell number is concluded for CA3642.

A transient significant reduction of the honey bee brood index for cells initially containing old larvae was also observed at BFD+16. Nevertheless, the data from the colony condition assessments for 21DAA2 and 27DAA2 show that the total number of pupal and brood cells was similar for CA3642 and the control. No statistically significant effects of CA3642 were observed for the mean compensation index or termination rate of cells initially containing old larvae.

Overall, it can therefore be concluded that CA3642 had no significant effect on overall honey bee colony strength (mean number of adult honey bees), overall amount of brood or the development of the food storage area following a target application of 210 g a.s./ha of prothioconazole and azoxystrobin (actual: 215.3 g prothioconazole/ha and 219.0 g azoxystrobin/ha (1414.4 mL product/ha) at the first application and 214.9 g prothioconazole/ha and 218.5 g azoxystrobin/ha (1411.6 mL product/ha) at the second application) during bee flight. The two specific protection goals mentioned in EFSA (2014), i.e. no significant effects on forager mortality or honey bee colony strength, have been shown to be met for CA3642 when applied at 210 g a.s./ha each of prothioconazole and azoxystrobin once pre flowering and once at flowering during daily bee flight under semi-field conditions in winter oilseed rape.

As noted in the risk assessment section above the available honey bee laboratory data lead to acceptable honey bee risk assessment for cereals and most scenarios for oilseed rape and sunflower except for chronic oral risk to adult honey bees in the treated crop and BBCH 10-29 in the weeds scenario. The tunnel test performed in winter oilseed rape is considered sufficient to shown that significant effects on adult honey bee foragers or the honey bee colony is not expected following the proposed use of CA3642 in oilseed rape and sunflower.

### **9.6.3 Effects on bumble bees**

At the time of the present submission, the EFSA guidance for the risk assessment to bumble bees is neither formally accepted, nor are EU data requirements established as compulsory. Nonetheless, acute oral and contact toxicity to the bumblebee has been tested for CA3642. As adopted OECD guidelines for bumble bees exist, the risk assessment was conducted above, in Tables 9.6-2, 9.6-4 and 9.6-6. For full details of the acute toxicity study results, refer to A 2.3.1.1.1, KCP 10.3.1.1.1, (KCP 10.3.1.1/02).

### **9.6.4 Effects on solitary bees**

At the time of the present submission, the EFSA guidance for the risk assessment to solitary bees is neither formally accepted, nor are EU data requirements established as compulsory. No data have been provided.



## 9.6.5 Overall conclusions

Acceptable acute oral and contact risk to honey and bumble bees and the risk to larval honey bees from CA3642 and prothioconazole and azoxystrobin is concluded at the screening step, with a large margin of safety for the intended uses of CA3642 at a maximum application rate of 210 g of each a.s./ha in cereals (1.4 L product/ha) and 180 g of each a.s./ha (1.2 L product/ha) in oilseed rape and sunflower.

The first-tier chronic oral risk assessment for adult honey bees indicated acceptable risks in cereals for all scenarios and intended BBCH stages. For oilseed rape and sunflower, an acceptable chronic oral risk was demonstrated for all intended BBCH stages in field margin, adjacent crop, and next crop scenarios. However, the ETR<sub>oral</sub> values are above the trigger values for the “treated crop” scenario (all intended BBCH stages) and the “weeds” scenario (BBCH 10-29).

Based on a honey bee semi-field tunnel study in winter oilseed rape with CA3642 (KCP 10.3.1.5/01), it was concluded that CA3642 had no significant effect on overall honey bee colony strength (mean number of adult honey bees), overall amount of brood or the development of the food storage area following target application of 1.4 L product/ha during bee flight (actual application: 1.4144 L product/ha) at the first application and 1.4116 L product/ha) at the second application). The two specific protection goals mentioned in EFSA (2014) i.e. no significant effects on forager mortality or honey bee colony strength, have been shown to be met for CA3642 when applied at a nominal rate of 1.4 L product/ha, once pre flowering and once at flowering during daily bee flight, under semi-field conditions in winter oilseed rape. It is considered appropriate to extrapolate results of the semi-field study (1.4 L product/ha) to refine the chronic risk to honey bees for the use in sunflower (1.2 L product/ha). An acceptable risk to honey bees is concluded for the proposed uses of CA3642 in cereals, oilseed rape, and sunflower.

## 9.7 Effects on arthropods other than bees (KCP 10.3.2)

### 9.7.1 Toxicity data

Effects on non-target arthropods exposed to CA3642 were not evaluated as part of the EU assessments of prothioconazole and azoxystrobin. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

**Table 9.7-1: Endpoints and effect values relevant for the risk assessment for non-target arthropods**

Species	Substance	Exposure system	Results	Reference
<i>Typhlodromus pyri</i>	CA3642	Laboratory test, glass plates (2D)	LR <sub>50</sub> >15.5 L CA3642/ha ER <sub>50</sub> = 2.35 L CA3642/ha  Mortality NOER = 8.62 L CA3642/ha Reproduction NOER <1.48 L CA3642/ha	KCP 10.3.2.1/01
<i>Aphidius rhopalosiphi</i>		Laboratory test, glass plates (2D)	LR <sub>50</sub> = 2.9 L CA3642/ha ER <sub>50</sub> >10 L CA3642/ha  Mortality NOER = 0.256 L CA3642/ha Reproduction NOER = 4.0 L CA3642/ha	KCP 10.3.2.1/02

#### **zRMS comments:**

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

### 9.7.1.1 Justification for new endpoints

Studies with non-target arthropods are always conducted with a formulated product and no testing was carried out with unformulated technical material. It is not appropriate to rely on the data from the active substance representative formulations for the EU reviews for prothioconazole and azoxystrobin. Therefore, new studies are available for CA3642 for the risk assessment for non-target arthropods.

### 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 use group cereals also covers the risk for non-target arthropods from all other intended uses in the crop group oilseed rape and sunflower (see 9.1.2).

**Table 9.7-2: First-tier assessment of the in-field risk for non-target arthropods, due to the use of CA3642 in cereals**

<b>Intended use</b>	Cereals		
<b>Product</b>	CA3642		
<b>Application rate</b>	2 x 1.4 L CA3642/ha (14-d interval); BBCH 14-69		
<b>MAF</b>	1.7 (Appendix V of ESCORT 2) – foliar NTA MAF, as use is not pre-emergence		
<b>Test species</b>	<b>LR<sub>50</sub> (lab.) (L CA3642/ha)</b>	<b>PER<sub>in-field</sub> (L CA3642/ha)</b>	<b>HQ<sub>in-field</sub> (criterion: HQ ≤2)</b>
<b>First tier</b>			
<i>T. pyri</i>	>15.5	2.38	<0.15
<i>A. rhopalosiphi</i>	2.9		0.82
	<b>Reproduction ER<sub>50</sub> (lab.) (L CA3642/ha)</b>	<b>PER<sub>in-field</sub> (L CA3642/ha)</b>	<b>HQ<sub>in-field</sub> (criterion: HQ ≤2)</b>
<i>T. pyri</i>	2.35	2.38	1.01
<i>A. rhopalosiphi</i>	>10		0.238

Values in **bold** are above either the trigger value or the PER<sub>in-field</sub> value. MAF, multiple application factor; PER, predicted environmental rate; HQ, hazard quotient.

An acceptable in-field risk is concluded at the first-tier assessment, based on the glass-plate mortality and reproduction laboratory data, for *T. pyri* and *A. rhopalosiphi*, when considering application of 2 x 1.4 L CA3642/ha for the proposed use on cereals. The proposed use on oilseed rape and sunflower (maximum 1 x 1.2 L/ha) is covered based on the risk envelope approach.

#### zRMS comments:

Acceptable in-field and off-field risks were demonstrated for the indicator species *T. pyri* and *A. rhopalosiphi* at the first tier (HQ <2) using mortality and reproduction data.  
The proposed use on oilseed rape (maximum 1 x 1.2 L/ha) and minor crops uses covered based on the risk envelope approach.

## 9.7.2.2 Risk assessment for off-field exposure

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

**Table 9.7-3: First -tier assessment of the off-field risk for non-target arthropods due to the use of CA3642 in cereals**

<b>Intended use</b>	Cereals				
<b>Product/active substances</b>	CA3642/prothioconazole and azoxystrobin				
<b>Application rate</b>	2 x 1.4 L CA3642/ha (14-d interval); BBCH 14-69				
<b>MAF</b>	1.7 (Appendix V of ESCORT 2) – foliar NTA MAF, as use is not pre-emergence				
<b>vdf</b>	5 (first-tier studies) – <del>C EU requirements – May 2021 with update June 2022</del> 10 (Escort 2)				
<b>Test species First tier</b>	<b>LR<sub>50</sub> (lab.) (L CA3642/ha)</b>	<b>Drift (%)*</b>	<b>PER<sub>off-field</sub> (L CA3642/ha)</b>	<b>CF</b>	<b>HQ<sub>off-field</sub> (criterion: HQ ≤2)</b>
CA3642					
<i>T. pyri</i>	>15.5	2.38	0.1133	10	<0.0073 ≤0.00365
<i>A. rhopalosiphi</i>	2.9				0.039 0.0195
<b>Test species First tier</b>	<b>Reproduction ER<sub>50</sub> (lab.) (L CA3642/ha)</b>	<b>Drift (%)*</b>	<b>PER<sub>off-field</sub> (L CA3642/ha)</b>	<b>CF</b>	<b>HQ<sub>off-field</sub> (criterion: HQ ≤2)</b>
<i>T. pyri</i>	2.35	2.38	0.1133	10	0.048 0.024
<i>A. rhopalosiphi</i>	>10				<0.0113 ≤0.00565

Values in **bold** are above either the trigger value or the PER<sub>in-field</sub> value. \*Appendix VI of ESCORT 2 (drift rate at 1 m for two applications in field crops). MAF: multiple application factor; vdf: vegetation distribution factor; PER: predicted environmental rate; CF: correction factor; HQ: hazard quotient.

Based on the risk assessment for cereals presented above, an acceptable off-field risk can be concluded for the two standard indicator species, *T. pyri* and *A. rhopalosiphi* based on mortality and reproduction endpoints. The proposed use on oilseed rape and sunflower (maximum 1 x 1.2 L/ha) is covered based on the risk envelope approach.

### zRMS comments:

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

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

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

In line with Bullet Points: Ecotoxicology (CZSC November 2021) as long as adjustment to the guidance document has not been made, a VDF of 10 should be applied in core risk assessment. We are aware that VDF of 10 should be used until the update of the guidance document. However, despite these agreements, we constantly receive comments from several Central Zone Member States to present the off-field risk assessment performed with consideration of VDF of 5. Taking this into account, it was decided to accept such calculation to avoid these potential comments.

Nevertheless, calculations for both VDF values are presented in Table 9.7-3 and the concerned Member States may decide which calculation is relevant at the national level. Based on calculations performed with consideration of the Tier I laboratory data an acceptable off-field risk to non-target arthropods from the intended uses of CA3642 may be concluded with no need for risk mitigation measures.

### **9.7.2.3 Additional higher-tier risk assessment**

Not relevant.

### **9.7.2.4 Risk mitigation measures**

No risk mitigation is required for non-target arthropods is needed for the proposed use of CA3642.

### 9.7.3 Overall conclusions

The risk assessment was conducted according to 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.

The risk from the formulated product, CA3642, to non-target arthropods was assessed in first-tier assessments, from hazard quotients, between toxicity endpoints that were estimated from laboratory studies with CA3642 and crop-specific use patterns. The assessment was conducted for the worst-case application patterns of 2 x 1.4L product/ha (14-d interval), covering the risk for non-target arthropods from all other intended uses.

Acceptable in-field and off-field risks were demonstrated for the indicator species *T. pyri* and *A. rhopalosiphi* at the first tier (HQ <2) using mortality and reproduction data. The proposed use on oilseed rape and sunflower (maximum 1 x 1.2 L/ha) is covered based on the risk envelope approach.

## 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 prothioconazole, azoxystrobin and their relevant metabolites. Full details of these studies are provided in the respective EU DARs 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) exposed to CA3642, were not evaluated as part of the EU assessments of prothioconazole and azoxystrobin. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

**Table 9.8-1: Endpoints and effect values relevant for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) – prothioconazole**

Species	Substance	Exposure system	Results	Reference
Active substance				
<i>Folsomia candida</i>	Prothioconazole	Mixed into substrate, 28 d, chronic 10% peat content	NOEC ≥64 mg a.s./kg dw NOEC <sub>Corr</sub> ≥32 mg a.s./kg dw *	EFSA Sci. Report. 2007; 106, 1-98
<i>Eisenia fetida</i>	Prothioconazole 250 g/L EC	56 d, chronic 10 % peat	NOEC = 1.33 mg a.s./kg d.w. soil NOEC <sub>corr</sub> = 0.665 mg/kg dw	
<i>Hypoaspis aculeifer</i>	Prothioconazole	Mixed into substrate, 34 d, chronic, LUFA 2.1 soil	NOEC ≥100 mg a.s./kg dw NOEC <sub>Corr</sub> ≥50 mg a.s./kg dw *	
Metabolites				
<i>Eisenia fetida</i>	Prothioconazole-desthio	Mixed into substrate, 56 d, chronic, 10% peat content	NOEC = 1 mg/kg dw NOEC <sub>Corr</sub> = 0.5 mg/kg dw*	EFSA Sci. Report. 2007; 106, 1-98
<i>Folsomia candida</i>		Mixed into substrate, 28 d, chronic, 10% peat content	NOEC = 62.5 mg/kg dw NOEC <sub>Corr</sub> = 31.25 mg/kg dw*	
<i>Eisenia fetida</i>	Prothioconazole-S-methyl	Mixed into substrate, 56 d, chronic, 10% peat content	NOEC = 100 mg/kg dw NOEC <sub>Corr</sub> = 50 mg/kg dw*	EFSA Sci. Report. 2007; 106, 1-98
<i>Folsomia candida</i>		Mixed into substrate, 28 d, chronic, 10% peat content	NOEC ≥31.6 mg/kg dw NOEC <sub>Corr</sub> ≥15.8 mg/kg dw*	

Species	Substance	Exposure system	Results	Reference
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Values in **bold** used in the risk assessment. \*Corrected value derived by dividing the endpoint by a factor of 2, for substances with logPow >2, in accordance with the EPPO earthworm scheme 2002.

**Table 9.8-2: Endpoints and effect values relevant for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) – azoxystrobin**

Species	Substance	Exposure system	Results	Reference
Active substance				
<i>Eisenia fetida</i>	Azoxystrobin	Mixed into substrate, 14-d, acute 10% peat content	LC <sub>50</sub> =283 mg/kg dw LC <sub>50,Corr</sub> =141.5 mg/kg dw*	EFSA Journal 2010; 8(4):1542
<i>Hypoaspis aculeifer</i>		Mixed into substrate, 14-d, chronic 5% peat content	No data currently available <sup>1</sup>	-
Formulation				
<i>Eisenia fetida</i>	YF10537 (azoxystrobin 250 g/L SC)	Mixed into substrate 56-d, chronic 10% peat content	NOEC = 20 mg a.s./kg dw NOEC <sub>Corr</sub> = <b>10 mg a.s./kg dw*</b>	EFSA Journal 2010; 8(4):1542
<i>Folsomia candida</i>		Mixed into substrate 28-d, chronic	NOEC = 50 mg a.s./kg dw NOEC <sub>Corr</sub> = <b>25 mg a.s./kg dw*</b>	
Metabolites				
<i>Eisenia fetida</i>	R234886	Mixed into substrate 14 d, acute <sup>1</sup> 10 % peat content	LC <sub>50</sub> >1000 mg/kg dw LC <sub>50,Corr</sub> >500 mg/kg dw*	EFSA Journal 2010; 8(4):1542
		Mixed into substrate 56 d, chronic 10 % peat content	No data currently available <sup>1</sup>	-
<i>Folsomia candida</i>		Mixed into substrate 28 d, chronic 5 % peat content	No data currently available <sup>1</sup>	-
<i>Hypoaspis aculeifer</i>		Mixed into substrate 14 d, chronic 5 % peat content	No data currently available <sup>1</sup>	-
<i>Eisenia fetida</i>	R401553	Mixed into substrate 14 d, acute <sup>1</sup> 10 % peat content	LC <sub>50</sub> >1000 mg/kg dw NOEC <sub>Corr</sub> >500 mg/kg dw*	EFSA Journal 2010; 8(4):1542
		Mixed into substrate 56 d, chronic 10 % peat content	No data currently available <sup>1</sup>	-
<i>Folsomia candida</i>		Mixed into substrate 28 d, chronic 5 % peat content	No data currently available <sup>1</sup>	-
<i>Hypoaspis aculeifer</i>		Mixed into substrate 14 d, chronic 5 % peat content	No data currently available <sup>1</sup>	-
<i>Eisenia fetida</i>	R402173	Mixed into substrate 56 d, chronic 10 % peat content	LC <sub>50</sub> >1000 mg/kg dw NOEC <sub>Corr</sub> >500 mg/kg dw*	EFSA Journal 2010; 8(4):1542
<i>Folsomia candida</i>		Mixed into substrate 28 d, chronic 5 % peat content	No data currently available <sup>1</sup>	-
<i>Hypoaspis aculeifer</i>		Mixed into substrate 14 d, chronic 5 % peat content	No data currently available <sup>1</sup>	-

Values in **bold** used in the risk assessment. \*Corrected value derived by dividing the endpoint by a factor of 2, for substances with logPow >2, in accordance with the EPPO earthworm scheme 2002. <sup>1</sup>It is noted that the available data for azoxystrobin (EFSA Journal 2010; 8(4):1542) do not meet EU 283/2013 active substance data requirements. The current EU-agreed active substance endpoints should be used for the product registration and hence further consideration of the active substance data is not considered relevant here.

**Table 9.8-3: Endpoints and effect values relevant for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) – CA3642**

Species	Substance	Exposure system	Results	Reference
<b>Formulation</b>				
<i>Eisenia fetida</i>	CA3642	Mixed into substrate 56 d, chronic 10 % peat content	Mortality and Reproductive NOEC ≥14.0 mg CA3642/kg dry soil <b>NOEC<sub>corr</sub> ≥7.0 mg CA3642/kg dry soil*</b>  LC <sub>10/20/50</sub> >14.0 mg CA3642/kg dry soil LC <sub>10/20/50corr</sub> >7.0 mg CA3642/kg dry soil*  Corresponding to corrected values of 0.99 mg azoxystrobin/kg dry soil and 0.97 mg prothioconazole/kg dry soil	KCP 10.4.1.1/01
<i>Folsomia candida</i>		Mixed into substrate 28 d, chronic 5 % peat	LC <sub>50</sub> >612 mg CA3642/kg dry soil  Corresponding to >86.1 mg azoxystrobin/kg dry soil and >84.7 mg prothioconazole/kg dry soil  NOEC (reprod.) = 58.3 mg CA3642/kg dry soil <b>NOEC<sub>Corr</sub> = 29.2 mg CA3642/kg dry soil*</b>  Corresponding to corrected values of 4.10 mg azoxystrobin/kg dry soil and 4.03 mg prothioconazole/kg dry soil	KCP 10.4.2.1/01
<i>Hypoaspis aculeifer</i>		Mixed into substrate 14 d, chronic 5 % peat	LC <sub>50</sub> >177.16 mg CA3642/kg soil dw  EC <sub>50</sub> , EC <sub>20</sub> and EC <sub>10</sub> reproductive >177.16 mg CA3642/kg soil dw.  Overall NOEC ≥177.16 mg CA3642/kg soil dw <b>NOEC<sub>Corr</sub> = 88.6 mg CA3642/kg soil dw*</b> Corrected value of 88.6 mg CA3642/kg soil dw corresponds to 12.47mg azoxystrobin/kg soil dw and 12.26 mg prothioconazole/kg soil dw	KCP 10.4.2.1/02

Species	Substance	Exposure system	Results	Reference
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Values in **bold** used in the risk assessment. \*Corrected value derived by dividing the endpoint by a factor of 2, for substances with  $\log P_{ow} > 2$ , in accordance with the EPPO earthworm scheme 2002.

#### zRMS comments:

Data for earthworms for n and prothioconazole and azoxystrobin provided in Table 9.8-1 and 9.8-2 are in line with EU agreed endpoints reported in EFSA Journal 2010; 8(4):1542 and EFSA Scientific Report (2007) 106, respectively.

Studies on effects of the formulated product to earthworm and other soil macro-organism listed in Table 9.8-3 were evaluated by the zRMS and considered acceptable. The reported endpoints are confirmed. Summary of the performed studies together with zRMS evaluation may be found in Appendix 2.

### 9.8.1.1 Justification for new endpoints

New studies are available for CA3642 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. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

### 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).

PEC<sub>Soil</sub> values have been calculated for three uses (cereals, spring oilseed rape and winter oilseed rape and sunflower). The worst-case use is application in sunflower. ~~autumn application to winter oilseed rape, due to the earlier crop growth stage and lower crop interception.~~

#### 9.8.2.1 First-tier risk assessment

The relevant PEC<sub>soil</sub> values for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7, Tables 8.7-3 to 8.7-17.

Assessment of the risk from the intended uses of the product, CA3642, in cereals

**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 CA3642 in spring and winter cereals**

Cereals			
Intended use	Cereals		
Product/Active substance	CA3642, prothioconazole, azoxystrobin, and their metabolites		
Application rate	2 x 1.4 L CA3642/ha, corresponding to 2 x 210 g a.s./ha (BBCH 20-69)		
Species	NOEC <sub>Corr</sub> or LC <sub>50,Corr</sub> (mg/kg dw)	PEC <sub>Soil</sub> (mg/kg dw)*	TER <sub>LT</sub> (criterion TER ≥5)
CA3642			
<i>E. fetida</i>	7.0 1.96 sum of s.a	0.411 <sup>1</sup> 0.185***	17.03 10.6
<i>F. candida</i>	29.2 8.15 sum of s.a		71.0 44.05
<i>H. aculeifer</i>	88.6 24.80 sum of s.a		215.6 134.05
Prothioconazole			
<i>E.fetida</i>	0.665 corr	0.0578	11.5
<i>E.fetida</i>	0.97**		16.8
<i>F. candida</i>	32		553.6



<i>H. aculeifer</i>	50		865.1
Prothioconazole-desthio			
<i>E. fetida</i>	0.5	0.054 0.0525	9.25 9.5
<i>F. candida</i>	31.25		578.70 595.2
Prothioconazole-S-methyl			
<i>E. fetida</i>	50	0.0154	3246.8
<i>F. candida</i>	≥15.8		≤1026
Azoxystrobin			
<i>E. fetida</i>	10	0.1269 <sup>2</sup>	78.8
<i>F. candida</i>	25		197.0
R234886			
<i>E. fetida</i>	≥500	0.0353 <sup>3</sup>	≤14016
R401553			
<i>E. fetida</i>	≥500	0.0114 <sup>3</sup>	≤43860
R402173			
<i>E. fetida</i>	≥500	0.0178 <sup>3</sup>	≤28090

\*PEC<sub>soil</sub> values are worst-case for all intended uses of CA3642 in cereals. <sup>1</sup>PEC<sub>act</sub>: Only instantaneous PEC<sub>soil</sub> value for the formulation relevant, following a single application, since it will immediately separate into its components. <sup>2</sup>Worst-case PEC<sub>accumulation</sub> value for the parent. <sup>3</sup>Worst-case PEC<sub>accumulation</sub> value based on parent accumulation. TER values shown in **bold** fall below the relevant trigger,

<sup>4</sup>) Since no measured toxicity data are available, it was assumed that the metabolite is 10 x more toxic than the parent compounds prothioconazole (unrealistic worst-case approach)

\*\* Formulation study: NOEC<sub>corr</sub> ≥ 7.0 mg CA3642/kg dry soil (corresponding to corrected values of 0.99 mg azoxystrobin/kg dry soil and **0.97 mg prothioconazole/kg dry soil**)

\*\*\* PECs of sum of the a.s.kg dw

An acceptable risk is concluded at the first tier, for earthworms and other soil meso/macro fauna, for the intended uses of CA3642 in cereals, with consideration of the active substances prothioconazole and azoxystrobin, as well as their respective relevant soil metabolites prothioconazole-desthio, prothioconazole-s-methyl, R234886, R401553, and R402173. As the worst case PEC<sub>soil</sub> values are used from either the spring or winter applications to cereals, it is concluded that there should be no unacceptable risk to earthworms or other soil macro organisms from any of the proposed uses of CA3642 in cereals.

#### Assessment of the risk from the intended uses of CA3642 in oilseed rape

**Table 9.8-5: 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 CA3642 in spring and winter oilseed rape (based on worst-case autumn applications in oilseed rape).**

Intended use	Oilseed rape		
Product/Active substance	CA3642, prothioconazole, azoxystrobin, and their metabolites		
Application rate	1 x 1.2 L CA3642/ha, corresponding to 1 x 180 g a.s./ha (BBCH 14-69)		
Species	NOEC <sub>Corr</sub> or LC <sub>50,Corr</sub> (mg/kg dw)	PEC <sub>Soil</sub> (mg/kg dw)*	TER <sub>LT</sub> (criterion TER ≥5)
CA3642 – autumn applications			
<i>E. fetida</i>	7.0	1.056	6.6
<i>F. candida</i>	29.2		27.7
<i>H. aculeifer</i>	88.6		83.9
Prothioconazole			
<i>E.fetida</i>	0.665	0.1440*	4.61*
<i>E.fetida</i>	0.97		6.74**
<i>F. candida</i>	32		222.2
<i>H. aculeifer</i>	20		138.9
Prothioconazole-desthio			
<i>E. fetida</i>	0.5	0.0746	6.7

<i>F. candida</i>	31.25		418.9
Prothioconazole-S-methyl			
<i>E. fetida</i>	50	0.0219	2283.1
<i>F. candida</i>	≥15.8		<721.5
Azoxystrobin			
<i>E. fetida</i>	10	0.1661	60.2
<i>F. candida</i>	25		150.5
R234886			
<i>E. fetida</i>	≥500	0.0462 <sup>+</sup>	<10823
R401553			
<i>E. fetida</i>	≥500	0.0149 <sup>+</sup>	<3557
R402173			
<i>E. fetida</i>	≥500	0.0233 <sup>+</sup>	<21459

\*PEC<sub>Soil</sub> values are based on worst-case autumn applications for all intended uses of CA3642 in oilseed rape. TER values shown in **bold** fall below the relevant trigger. <sup>1</sup>Worst-case PEC<sub>accumulation</sub> value based on parent accumulation.

\*\* Formulation study: NOEC<sub>corr</sub> ≥7.0 mg CA3642/kg dry soil (corresponding to corrected values of 0.99 mg azoxystrobin/kg dry soil and 0.97 mg prothioconazole/kg dry soil)

An acceptable risk is concluded at the first tier for earthworms and other soil meso/macro- fauna for the intended uses of CA3642 in oilseed rape with consideration of the active substances ~~prothioconazole and azoxystrobin~~, as well as ~~their respective for relevant~~ soil metabolites prothioconazole-desthio, prothioconazole-s-methyl, R234886, R401553, and R402173. As the PEC<sub>Soil</sub> values from autumn applications to oilseed rape were worst-case, it is concluded that there should be no unacceptable risk from any of the proposed uses of CA3642 in spring oilseed rape.

#### Assessment of the risk from the intended uses of CA3642 in sunflower

**Table 9.8-6: 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 CA3642 in sunflower - covers linseed\*.**

Intended use	Sunflower		
Product/Active substance	CA3642, prothioconazole, azoxystrobin, and their metabolites		
Application rate	Sunflower 1 x 1.2 L CA3642/ha, corresponding to 1 x 180 g a.s./ha (BBCH 16-64), linseed 14-69 BBCH 1 x 180 g a.s./ha		
Species	NOEC <sub>Corr</sub> or LC <sub>50,Corr</sub> (mg/kg dw)	PEC <sub>Soil</sub> (mg/kg dw) <sup>1</sup>	TER <sub>LT</sub> (criterion TER ≥5)
CA3642			
<i>E. fetida</i>	≥ 7.0	1.4085	≥ 5.0
<i>F. candida</i>	29.2		20.7
<i>H. aculeifer</i>	88.6		62.9
Prothioconazole			
<i>E.fetida</i>	0.665	0.192	3.46
<i>E.fetida</i>	0.97*		5.05
<i>F. candida</i>	32		166.7
<i>H. aculeifer</i>	20		260.4
Prothioconazole-desthio			
<i>E. fetida</i>	0.5	0.0994	5.0
<i>F. candida</i>	31.25		314.4
Prothioconazole-S-methyl			
<i>E. fetida</i>	50	0.0292	1712.3
<i>F. candida</i>	≥15.8		<541.1
Azoxystrobin			
<i>E. fetida</i>	10	0.2215	45.1

<i>F. candida</i>	25		112.9
<b>R234886</b>			
<i>E. fetida</i>	≥500	0.0616 <sup>†</sup>	<8116.8
<b>R401553</b>			
<i>E. fetida</i>	≥500	0.0199 <sup>†</sup>	<25125.6
<b>R402173</b>			
<i>E. fetida</i>	≥500	0.0311 <sup>†</sup>	<25125.6

<sup>†</sup>Worst-case PEC<sub>accumulation</sub> value based on parent accumulation.

**\*\*Formulation study: NOEC<sub>corr</sub> ≥ 7.0 mg CA3642/kg dry soil (corresponding to corrected values of 0.99 mg azoxystrobin/kg dry soil and 0.97 mg prothioconazole/kg dry soil)**

An acceptable risk is concluded at the first tier for earthworms and other soil meso/macro- fauna for the intended uses of CA3642 in sunflower with consideration of the product CA3642 toxicity data and the data for the active substances prothioconazole and azoxystrobin, as well as their respective relevant soil metabolites prothioconazole-desthio, prothioconazole-s-methyl, R234886, R401553, and R402173.

#### **zRMS comments:**

The soil exposure provided in Table 9.8-4 and Table 9.5-5 for metabolites Prothioconazole-desthio, R401553 and R402173 was amended in line with PEC<sub>SOIL</sub> values agreed by the zRMS in area of Section 8.

It should be indicated, that no long-term toxicity data for other soil organisms (*H.aculeifer*) was available from the EU review of prothioconazole soil metabolites. In addition, no long-term toxicity data are available for *E. fetida*, *H.aculeifer* and *Folsomia candida* for technical azoxystrobin and its metabolites. For the risk assessment for *E.fetida* and *Folsomia candida* for azoxystrobin the Applicant used the endpoints from representative formulation YF10537 (azoxystrobin 250 g/L SC).

Additionally, chronic toxicity studies on earthworms, springtails (*Folsomia candida*) and predatory mites (*Hypoaspis aculeifer*) conducted with CA3642 have been performed to meet the data requirements set in the Annex to Reg. (EU) 284/2013. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

In the same time, it should be stressed out that studies on toxicity of CA3642 to *E.fetida*, *Folsomia candida* and *Hypoaspis aculeifer* covers effects of prothioconazole, prothioconazole and azoxystrobin metabolites' in the product and are considered sufficient until the new endpoints from renewal are available.

Based on the calculations provided in the Tables above an unacceptable the long- term risk for earthworms based on solo formulation Prothioconazole 250 g/L EC was identified in OSR and sunflower.

However, the risk for *E.fetida* for formulation CA3642 (expressed in units for formulation) indicated an acceptable risk and it considered sufficient to concluded safe use.

**Moreover, based on content of prothioconazole in formulation endpoint 7 mg CA3642 mg / Kg dry soil (=0.97 mg, see table 9.8-3), and considering this amount , the TER<sub>LT</sub> values are above 5 for Oilseed Rape (TER<sub>LT</sub>> 6.74) and Sunflower (TER<sub>LT</sub>> 5.05) concluding on acceptable risk assessment.**

Overall, all TER<sub>LT</sub> values for soil meso- and macrofauna (other than earthworms) are greater than the trigger of 5, indicating acceptable risk from exposure CA3642.

### **9.8.2.2 Higher-tier risk assessment**

Based on the results of the first-tier risk assessment, no further risk assessment is considered necessary.

### **9.8.3 Overall conclusions**

An acceptable risk from CA3642 to earthworms and other non-target soil meso- and macro-fauna is concluded at the first-tier assessment (TER values >5), for all intended uses in spring and winter cereals and oilseed rape and sunflower.

## 9.9 Effects on soil microbial activity (KCP 10.5)

### 9.9.1 Toxicity data

Studies on effects to soil microorganisms have been carried out with prothioconazole, azoxystrobin and their relevant metabolites. Full details of these studies are provided in the respective EU DARs and related documents.

Effects on soil microorganisms exposed to CA3642 were not evaluated as part of the EU assessments of prothioconazole and azoxystrobin. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

**Table 9.9-1: Endpoints and effect values relevant for the risk assessment for soil microorganisms**

Table 9.9-1. Endpoints and effect values relevant for the risk assessment for soil microorganisms				
Endpoint	Substance	Exposure system	Results	Reference
CA3642				
N-mineralisation	CA3642	28-day, aerobic soil type	<25% effects at 10.56 mg CA3642/kg soil dw	Hugill, E 2024 KCA 10.5/02
Prothioconazole and relevant metabolites				
N-mineralisation	Prothioconazole	28-day, aerobic soil type	<25% effects at 2.71 mg/kg soil dw* Application rate of 2.0 kg/ha	EFSA Sci. Report. 2007; 106, 1-98
	Prothioconazole-desthio		<25% effects at 1.37 mg/kg soil dw* Application rate of 1.0 kg/ha	
	Prothioconazole-S-methyl		<25% effects at 2.69 mg/kg soil dw* Application rate of 2.0 kg/ha	
Azoxystrobin and relevant metabolites				
N-mineralisation	Azoxystrobin 250 SC	28-day, aerobic soil type	<25% effects at 2.5 kg a.s./ha 3.3 mg a.s/kg dws**	Azoxystrobin Review Report for first inclusion; 7581/VI/97-Final; 1998# Tarry, A.R., Prevett, A., Mason, G., 1994, ICI5504/0960
	R234886	28-day, aerobic soil type	<25% effects at 10 mg/kg soil dw	
	R401553	28-day, aerobic soil type	<25% effects at 2.643 mg/kg soil dw	
	R402173	28-day, aerobic soil type	<25% effects at 4.131 mg/kg soil dw	

\*Endpoints in terms of mg/kg soil dw are derived from the applications rates listed in EFSA Conclusion 2007. #This endpoint is not listed in the latest azoxystrobin EFSA conclusion LoEP (2010), but is assumed to be sufficient as the EFSA conclusion concluded a low risk to the active substance and the DAR (Vol. 3, B.9.8.3; 2009) stated ‘the risk to soil microbial processes from the active substance is considered to be acceptable on the basis of the original data considered for Annex I listing.’

\*\*value based on information included in DAR

#### **zRMS comments:**

Soil microorganism toxicity data for azoxystrobin, prothioconazole and their relevant metabolites provided in Tables 9.9-1 above were confirmed by zRMS that they are in line with EU agreed endpoints reported in EFSA-Journal 2010; 8(4):1542, Azoxystrobin Review Report for first inclusion; 7581/VI/97-Final; 1998#d and EFSA Scientific Report (2007) 106, respectively.

### 9.9.1.1 Justification for new endpoints

Data for the product CA3642 is listed in Appendix 1 and summarised in Appendix 2 (see results of study KCP 10.5/02).

### 9.9.2 Risk assessment

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

The relevant  $PEC_{soil}$  values for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7, Tables 8.7-3 to 8.7-17, and have been used in the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) (see 9.1.2).

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the autumn treatment in sunflower ~~winter oilseed rape~~ (highest  $PEC_{soil}$  values) also covers the risk to the soil microorganisms from all other intended uses.

**Table 9.9-2: Assessment of the risk for effects on soil micro-organisms due to the use CA3642 on ~~oilseed rape (worst-case autumn applications to winter oilseed rape)~~ for sunflower as the worst case**

Intended use	Cereals, oilseed rape and sunflower		
Product	Max. conc. with effects $\leq 25\%$ (mg/kg dw)	Worst-case $PEC_{soil}$ (mg/kg dw)	Risk acceptable?
CA3642	10.56 (at 28-days)	1.4085	Yes
Active substance/metabolite	Max. conc. with effects $\leq 25\%$ (mg/kg dw)	Worst-case $PEC_{soil}$ (mg/kg dw)	Risk acceptable?
Prothioconazole	2.71 (at 28-days)	0.192 <del>0.1440</del>	Yes
Prothioconazole-desthio	1.37 (at 28-days)	0.0994 <del>0.0746</del>	Yes
Prothioconazole-S-methyl	2.69 (at 28-days)	0.0292 <del>0.0219</del>	Yes
Azoxystrobin	2.5 kg a.s./ha (at 28-days*) 3.3 mg a.s/kg dws	0.2215 <del>0.1661<sup>1</sup></del>	Yes
R234886	10 (at 28-days)	0.0616 <sup>1</sup> <del>0.0462<sup>1</sup></del>	Yes
R401553	2.643 (at 28-days)	(0.00199) 0.0746 <del>0.0149<sup>1</sup></del>	Yes
R402173	4.131 (at 28-days)	0.0311 <sup>1</sup> 0.0746 <del>0.0233<sup>1</sup></del>	Yes

<sup>1</sup>: Worst-case  $PEC_{accumulation}$  value.

\* Tested with formulation 250 SC

Comparison of the worst case  $PEC_{soil}$  for CA3642 with the maximum concentration tested for CA3642 clearly shows there is no risk to soil Nitrogen transformation microorganisms with a margin of safety (factor of 7.5). In addition, comparison of the relevant worst case  $PEC_{soil}$  values with the maximum concentrations tested with  $<25\%$  effects for soil Nitrogen transformation show acceptable risk assessment for prothioconazole and azoxystrobin, and relevant metabolites.

#### zRMS comments:

The risk assessment presented in Table 9.9-2 above is in general agreed by the zRMS with some minor correction of  $PEC_{soil}$  values agreed in the course of evaluation in area of Section 8.

The effects on the nitrogen transformations are acceptable (<25%) at concentration which is higher than the maximum relevant PECs for the maximum application rate of active substance prothiconazole and azoxystrobin and its metabolites.

According to EU Reg.284/2009 the study for formulation contains more than one active substance should be submitted. The study for formulation CA3642 has been performed and an acceptable risk is concluded.

In addition, the combination risk to soil microorganisms has been further assessed by combining the margins of safety (MoS) for the individual active substances in a way equivalent to the combi-TER approach. As the trigger values for each active substance are equal, the combined MoS value can be calculated according to:

$$\text{MoS}_{\text{combi}} = 1/((1/\text{MoS}_{\text{substance 1}})+(1/\text{MoS}_{\text{substance 2}}))$$

#### Assessment of the risk of soil microorganisms based on the combined MoS.

Intended use	Prothioconazole MoS	Azoxystrobin MoS	MoS <sub>combi</sub>
Sunflower-worst case	14.11	14.9	7.7

As the calculated MoS<sub>combi</sub> exceed the trigger of 1, an acceptable combination risk of soil microorganisms is concluded for all intended uses.

Overall, for microorganism acceptable risk from exposure of active substance and CA3642 is concluded

### 9.9.3 Overall conclusions

An acceptable risk can be concluded for soil microorganisms, for the active substance and relevant soil metabolites, for the intended uses of CA3642 assuming the worst-case PEC<sub>soil</sub> values from use in sunflower autumn application to winter oilseed rape. The risk-envelope approach confirms that all intended uses will lead to PEC<sub>soil</sub> values lower than the worst-case values calculated and, thus, no unacceptable risk to soil microorganisms from the proposed uses is expected.

### 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 CA3642 which were not evaluated as part of the EU assessments of prothioconazole and azoxystrobin. New data submitted with this application are listed in Appendix 1 summarised in Appendix 2.

**Table 9.10-1: Endpoints and effect values relevant for the risk assessment for non-target terrestrial plants**

Species		Substance	Exposure System	ER <sub>50</sub>	Reference
Cucumber	<i>Cucumis sativus</i> (d)	CA3642	21-d, Vegetative vigour	>2.8 L/ha	KCP 10.6.2/01
Oilseed rape	<i>Brassica napus</i> (d)				
Carrot	<i>Daucus carota</i> (d)				
Tomato	<i>Lycopersicon esculentum</i> (d)				
Beet	<i>Beta vulgaris</i> (d)				
Soybean	<i>Glycine max</i> (m)		21-d, Seedling emergence	>2.8 L/ha	KCP 10.6.2/02
Corn	<i>Zea mays</i> (m)				
Ryegrass	<i>Lolium perenne</i> (m)				
Common oat	<i>Avena sativa</i> (m)				
Onion	<i>Allium cepa</i> (m)				

Species	Substance	Exposure System	ER <sub>50</sub>	Reference
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m: monocotyledonous; d: dicotyledonous

#### **zRMS comments:**

Studies on toxicity of CA3642 to non-target terrestrial plants were evaluated by the zRMS and are considered acceptable. For details of evaluation, please refer to Appendix 2. The endpoints reported in Table 9.10-1 are confirmed to be correct.

### **9.10.1.1 Justification for new endpoints**

In accordance with Regulation (EU) No. 284/2013, studies on non-target terrestrial plants have been conducted with the formulated product (CA3642).

### **9.10.2 Risk assessment**

#### **9.10.2.1 Tier-1 risk assessment (based screening data)**

Not relevant.

#### **9.10.2.2 Tier-2 risk assessment (based on dose-response data)**

New studies on vegetative vigour and seedling emergence with CA3642 were conducted as rate-response tests, with a maximum exposure rate of 2.8 L CA3642/ha. The evaluation of the risk to non-target terrestrial plants 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).

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for use in the cereals group, also covers the risk to non-target terrestrial plants from all other intended uses.

Effects on non-target terrestrial plants are of concern in the off-field environment, where they might be exposed to spray drift. The potential risk of CA3642 to off-field non-target terrestrial plants was assessed by calculation of toxicity exposure ratios (TER), by dividing the application rate with the predicted environmental rate (PER).

The amount of spray drift reaching off-crop habitats is calculated using estimates derived by the BBA (2000) from the spray-drift predictions of Ganzelmeier & Rautmann (2000). Ground-directed application only is proposed for this fungicide; therefore, the field crop drift values are used for all crops, according to recommendations of the Guidance Document on Terrestrial Ecotoxicology (2002).

The resulting TER values are presented in the following table, using the endpoints from the vegetative-vigour and seedling emergence studies.

**Table 9.10-2: Assessment of the risk for non-target plants due to the use of CA3642 in cereals**

<b>Intended use</b>	Cereals covering all proposed uses			
<b>Product</b>	CA3642			
<b>Application rate</b>	Maximum 1.4 L/ha			
<b>MAF</b>	1			
<b>Study</b>	<b>ER<sub>50</sub></b> (L CA3642/ha)	<b>Drift rate*</b>	<b>PER<sub>off-field</sub></b> (L/ha)	<b>TER criterion:</b> TER ≥5

<b>Foliar</b>				
Vegetative vigour	>2.8	0.0238	0.033	>84.8
<b>Soil</b>				
Seedling emergence	>2.8	0.0238	0.033	>84.8

TER values shown in **bold** fall below the relevant trigger. PER: predicted environmental rate; TER: toxicity-to-exposure ratio.

\*Appendix VI of ESCORT 2 (drift rate at 1 m for two applications in field crops).

Based on the available non-target terrestrial plant toxicity data for CA3642, an acceptable off-field risk is demonstrated for the proposed worst-case use (maximum 1.4 L CA3642/ha) on cereals. The other intended uses of CA3642 in the oilseed rape crop and sunflower and minor crop uses ~~grouping~~ (maximum 1.2 L CA3642/ha) will also have an acceptable risk assessment to non-target terrestrial plants, using the risk envelope approach, without the need for mitigation measures.

#### **zRMS comments:**

The calculations of the risk assessment for non-target plants have been validated by zRMS.

Overall, an acceptable off-field risk is demonstrated for the proposed worst-case use (maximum 1.4 L CA3642/ha) on cereals. The other intended uses of CA3642 in the oilseed rape crop grouping (maximum 1.2 L CA3642/ha) and minor crop uses will also have an acceptable risk assessment to non-target terrestrial plants, using the risk envelope approach, without the need for mitigation measures.

### **9.10.2.3 Higher-tier risk assessment**

Based on the results of the first-tier risk assessment, no further non-target terrestrial plant risk assessment or studies are considered necessary.

### **9.10.2.4 Risk mitigation measures**

No risk mitigation measures for non-target terrestrial plants are needed for the proposed uses of CA3642.

## **9.10.3 Overall conclusions**

The risk assessment was conducted according to the Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002 rev.2 (final), October 17, 2002). Based on the results of the first-tier assessment, the risk to non-target terrestrial plants (seedling emergence and vegetative vigour), due to the proposed use of CA3642 is considered acceptable (all TER values >5), without the need for any mitigation measures.

## **9.11 Effects on other terrestrial organisms (flora and fauna) (KCP 10.7)**

Not relevant. No data submitted.

## **9.12 Monitoring data (KCP 10.8)**

Not relevant. No data submitted.

## **9.13 Classification and Labelling**

The proposed classification and labelling of CA3642, for environmental hazards, has been determined according to the ECHA guidance on the application of the CLP criteria, version 5.0 (July 2017).

Available aquatic data on the product, CA3642, are summarised below.

### **Available formulation data for CA3642 for classification to the aquatic environment**



96-hour LC<sub>50</sub>, fish CA3642 LC<sub>50</sub> >2.04 mg product/L (mm) (>0.57 sum mg a.s./L)  
48-hour EC<sub>50</sub>, crustacea CA3642 EC<sub>50</sub> = 0.97 mg product/L (mm) (0.27 sum mg a.s./L)  
72-hour EC<sub>50</sub>, algae *S. costatum* CA3642 E<sub>r</sub>C<sub>50</sub> = 0.487 mg product/L (0.136 sum mg a.s./L)  
NOEC = 0.068 mg product/L (mm)  
E<sub>r</sub>C<sub>10</sub> = 0.172 mg product/L (mm)

CA3642 contains two active substances.

Prothioconazole, which has a harmonised classification under Annex VI of Regulation (EC) No 1272/2008:

Classification categories for hazard to the aquatic environment	Acute category 1 Chronic category 1
Hazard Pictograms	GHS09
Signal words	Warning
Hazard Statements	H400 'Very toxic to aquatic life' H410 'Very toxic to aquatic life,' with long-lasting effects'

Azoxystrobin, which has a harmonised classification under Annex VI of Regulation (EC) No 1272/2008:

Classification categories for hazard to the aquatic environment	Acute category 1 Chronic category 1
Hazard Pictograms	GHS09
Signal words	Warning
Hazard Statements	H400 'Very toxic to aquatic life' H410 'Very toxic to aquatic life,' with long-lasting effects'

Based on Regulation (EC) 1272/2008, the following classification for CA3642 is proposed, which considers the above aquatic endpoints for CA3642 and the harmonised classification for prothioconazole and azoxystrobin:

#### Conclusion: short-term (acute) aquatic hazard

The available acute toxicity of CA3642 as a formulation can be used for classification of the mixture. CA3642 is classified for short-term (acute) hazard as Acute Category 1, based on the lowest E<sub>r</sub>C<sub>50</sub> value of 0.487 mg product/L for algae (i.e., ≤1 mg a.s./L)

#### Conclusion: long-term (chronic) aquatic hazard

Neither prothioconazole nor azoxystrobin are considered to be rapidly degradable due to formation of toxic relevant metabolites in the aquatic environment. Only long term (NOEC/EC<sub>10</sub>) values for algae are available for the formulation CA3642. As adequate chronic toxicity data for all aquatic organism groups are not available, the product should be classified as Chronic category 1 as EC<sub>50</sub> values are not all ≥1 mg product/L (Table 4.1.0(b)(iii), Guidance on the Application of the CLP Criteria). The proposed environmental classification and labelling of CA3642 according to the CLP Regulation (EC) No 1272/2008 is presented below.

**Table 9.13-1: Proposed environmental classification and labelling of CA3642 according to the CLP Regulation (EC) No 1272/2008**

Classification categories for hazard to the aquatic environment	Acute category 1 Chronic category 1
Hazard Pictograms	GHS09
Signal words	Warning
Hazard Statements	H400 'Very toxic to aquatic life' H410 'Very toxic to aquatic life,' with long-lasting effects'
Proposed precautionary statements	<del>P273</del> , P391, & P501.

#### **zRMS comments:**

The classification of the product have been validated by zRMS.  
**Finally, the label H410 is proposed.**

## Appendix 1 Lists of data considered in support of the evaluation

### List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study (Y/N)	Owner
KCP 10.2.1/01	██████	2022	CA3642 (Prothioconazole 150 g/L + Azoxystrobin 150 g/L SC) – Acute toxicity to rainbow trout ( <i>Oncorhynchus mykiss</i> ), in a static 96-hour test ██████ GLP Unpublished	Y	Nufarm
KCP 10.2.1/02	Dupont, A.	2022	CA3642 (Azoxystrobin 150 g/L + Prothioconazole 150 g/L) – Acute Toxicity to <i>Daphnia magna</i> in a 48-Hour Immobilization Test Report no. 20210196 IES, Ltd., Witterswil, Switzerland GLP Unpublished	N	Nufarm
KCP 10.2.1/03	Dupont, A.	2022	CA3642 (Azoxystrobin 150 g/L + Prothioconazole 150 g/L) - Effect on <i>Skeletonema sp.</i> in a 72-Hour Algal Growth Inhibition Test Report no. 20210197 IES, Ltd., Witterswil, Switzerland GLP Unpublished	N	Nufarm
KCP 10.3.1.1/01	Gimeno, I.	2022	CA3642 (Azoxystrobin 150 g/L + Prothioconazole 150 g/L SC): Acute Oral and Contact Toxicity to the Honey bee ( <i>Apis mellifera</i> L.), under Laboratory Conditions Report no. S21-04080 Eurofins Trialcamp S.L.U, Alcàsser (Valencia) Spain GLP Unpublished	N	Nufarm
KCP 10.3.1.1/02	Gimeno, I.	2022	CA3642 (Azoxystrobin 150 g/L + Prothioconazole 150 g/L SC): Acute oral and contact Toxicity to the Bumblebee <i>Bombus terrestris</i> L., under Laboratory Conditions Report no. : S21-04083 Eurofins Trialcamp S.L.U, Alcàsser (Valencia) Spain GLP Unpublished	N	Nufarm

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 10.3.1.2/01	Gimeno, I.	2022	CA3642 (Azoxystrobin 150 g/L + Prothioconazole 150 g/L SC): Honey Bee ( <i>Apis mellifera</i> L.) Chronic Oral Toxicity Test (10-Day Feeding), under Laboratory Conditions Report no. S21-04081 Eurofins Trialcamp S.L.U, Alc��sser (Valencia) Spain GLP Unpublished	N	Nufarm
KCP 10.3.1.3/01	Gimeno, I.	2022	CA3642 (Azoxystrobin 150 g/L + Prothioconazole 150 g/L SC): Honey Bee ( <i>Apis mellifera</i> L.) Larval Toxicity Test following Repeated Exposure under laboratory conditions Report no. S21-04082 Eurofins Trialcamp S.L.U, Alc��sser (Valencia) Spain GLP Unpublished	N	Nufarm
KCP 10.3.1.5/01	Bocksch	2022	A Semi-Field Study to Evaluate Potential Effects on the Honey Bee ( <i>Apis mellifera</i> L.) After Two Applications of CA3301 and CA3642 in Winter Oil Seed Rape in Germany 2022 Report no. S21-00461 Eurofins Agrosience Services Ecotox GmbH, Niefern-��schelbronn Germany GLP Unpublished	N	Nufarm
KCP 10.3.2.1/01	Cornement M.	2022	CA3642 - Toxicity to the Predatory Mite <i>Typhlodromus pyri</i> (Acari, Phytoseiidae), under Worst-Case Conditions in the Laboratory Report no. 20210200 IES, Ltd., Witterswil, Switzerland GLP Unpublished	N	Nufarm
KCP 10.3.2.1/02	Schmidt T.	2022	A Worst-Case Laboratory Test to Determine the Effects of CA3642 (Prothiconazole 150 g/L + Azoxystrobin 150 g/L SC) on the Parasitoid Wasp <i>Aphidius rhopalosiph</i> (Hymenoptera: Braconidae) Report no. 20210199 IES, Ltd., Witterswil, Switzerland GLP Unpublished	N	Nufarm
KCP 10.4.1.1/01	Schmidt T.	2022	CA3642 (Prothioconazole 150 g/L + Azoxystrobin 150 g/L SC) - Effects on Reproduction	N	Nufarm

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
			of <i>Eisenia fetida</i> (Annelida: Lumbricidae) in Artificial Soil Report no. 20210206 IES, Ltd., Witterswil, Switzerland GLP Unpublished		
KCP 10.4.2.1/01	Schmidt, T.	2022	CA3642 (Prothioconazole 150 g/L + Azoxystrobin 150 g/L SC) - Effects on Reproduction of <i>Folsomia candida</i> (Collembola: Isotomidae) in Artificial Soil Report no. 20210207 IES, Ltd., Witterswil, Switzerland GLP Unpublished	N	Nufarm
KCP 10.4.2.1/02	Parsons C.	2022	A3642 (Prothioconazole 150 g/L + Azoxystrobin 150 g/L SC): A Laboratory Study to Determine the Effects of Fresh Residues on the Predatory Soil Mite, <i>Hypoaspis aculeifer</i> , in an Artificial Soil Substrate Report no. NUF-22-03 Mambo-Tox, Southampton, UK GLP Unpublished	N	Nufarm
KCP 10.5/01	Hugill, E.	2023	CA3642 (Prothioconazole 150 g/L + Azoxystrobin 150 g/L SC): Nitrogen Transformation Test Report no. 3203658 Smithers ERS Limited, UK GLP Unpublished	N	Nufarm
KCP 10.6.2/01	Merkle M.	2022	CA3642 (Azoxystrobin 150 g/L + Prothioconazole 150 g/L SC): Effects on the Vegetative Vigour of Ten Non-Target Terrestrial Plant Species under Greenhouse Conditions Report no. S21-04085 Eurofins Trialcamp S.L.U, Alcàsser (Valencia) Spain GLP Unpublished	N	Nufarm
KCP 10.6.2/02	Merkle M.	2022	CA3642 (Azoxystrobin 150 g/L + Prothioconazole 150 g/L SC): Effects on the Seedling Emergence and Growth of Ten Non-Target Terrestrial Plant Species under Greenhouse Conditions Report no. S21-04084 SynTech Research, Chapelle de Guinchay, France	N	Nufarm

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		

**List of data submitted or referred to by the applicant and relied on, but already evaluated at EU peer review**

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
-	-	-	-	-	-

**List of data submitted by the applicant and not relied on**

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
-	-	-	-	-	-

**List of data relied on not submitted by the applicant but necessary for evaluation**

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
-	-	-	-	-	-

## Appendix 2 Detailed evaluation of the new studies

<b>A 2.1</b>	<b>KCP 10.1</b>	<b>Effects on birds and other terrestrial vertebrates</b>
<b>A 2.1.1</b>	<b>KCP 10.1.1</b>	<b>Effects on birds</b>
<b>A 2.1.1.1</b>	<b>KCP 10.1.1.1</b>	<b>Acute oral toxicity</b>
<b>A 2.1.1.2</b>	<b>KCP 10.1.1.2</b>	<b>Higher tier data on birds</b>
<b>A 2.1.2</b>	<b>KCP 10.1.2</b>	<b>Effects on terrestrial vertebrates other than birds</b>
<b>A 2.1.2.1</b>	<b>KCP 10.1.2.1</b>	<b>Acute oral toxicity to mammals</b>
<b>A 2.1.2.2</b>	<b>KCP 10.1.2.2</b>	<b>Higher tier data on mammals</b>
<b>A 2.1.3</b>	<b>KCP 10.1.3</b>	<b>Effects on other terrestrial vertebrate wildlife (reptiles and amphibians)</b>
<b>A 2.2</b>	<b>KCP 10.2</b>	<b>Effects on aquatic organisms</b>
<b>A 2.2.1</b>	<b>KCP 10.2.1</b>	<b>Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes</b>

### Study 1

Comments of zRMS:	<p>The study was conducted in line with OECD 203 with no minor deviations.</p> <p>Analytical verification of test concentrations confirmed that measured concentrations of azoxystrobin and prothioconazole were between 93-102% and 52-102% of nominal concentrations, respectively, in fresh and aged media throughout the test. Biological results are reported based on nominal and mean measured concentrations of CA3642 and on geometric mean measured concentrations of the sum of the active substances, azoxystrobin and prothioconazole</p> <p>All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>96-h LC<sub>50</sub> &gt;2.25 mg CA3642/L (nominal); &gt;2.04 mg CA3642/L (mean measured); equivalent to &gt;0.57 mg sum of a.s./L (geometric mean measured).</p>
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Reference:	KCP 10.2.1/01
Report	CA3642 (Prothioconazole 150 g/L + Azoxystrobin 150 g/L SC) – Acute toxicity to rainbow trout ( <i>Oncorhynchus mykiss</i> ), in a static 96-hour test [REDACTED] year 2023 [REDACTED]
Guideline(s):	OECD Guideline for the Testing of Chemicals, No. 203, Fish, Acute Toxicity Test, 2019. Commission Regulation (EC) No 440/2008 of May 2008, C.1: Acute Toxicity for Fish.
Deviations:	No
GLP:	Yes, conducted under GLP
Acceptability:	Yes (all validity criteria met in accordance with current guidance)
Duplication	No

(if vertebrate study)	
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## Executive summary

In a 96-hour acute toxicity study, juvenile rainbow trout (*Oncorhynchus mykiss*) were exposed to the product, CA3642, a suspension concentrate (SC) formulation (analysed active substances: 13.84% w/w prothioconazole and 14.07% w/w azoxystrobin) in a static system at the nominal concentrations of 0 (control) 0.20, 0.29, 0.44, 0.66, 1.0, 1.5, and 2.25 mg CA3642/L, corresponding to nominal 0, 0.056, 0.081, 0.12, 0.18, 0.28, 0.42 and 0.63 mg sum of active substances/L. The geometric mean measured values for the sum of the active substances were 0, 0.050, 0.074, 0.10, 0.17, 0.26, 0.39 and 0.57 mg sum of active substances/L. Analytical verification of test concentrations confirmed that measured concentrations of azoxystrobin and prothioconazole were between 93-102% and 52-102% of nominal concentrations, respectively, in fresh and aged media throughout the test. Biological results are reported based on nominal and mean measured concentrations of CA3642 and on geometric mean measured concentrations of the sum of the active substances, azoxystrobin and prothioconazole.

No mortalities were observed in the control nor product treatment groups. The 96-hour LC<sub>50</sub> value for rainbow trout (*Oncorhynchus mykiss*) was estimated to be >2.25 mg CA3642/L based on nominal concentrations (equivalent to >0.57 mg sum of a.s./L (geometric mean measured concentration) and corresponding to >2.04 mg CA3642/L (mean measured concentration)). The NOEC (mortality) value was determined to be 2.25 mg CA3642/L (nominal), equivalent to 0.57 mg sum of a.s./L (geometric mean measured concentration), the highest concentration tested.

This toxicity study is considered acceptable and valid. The study satisfies the guideline requirements and validity criteria provided in the OECD test guideline 203 (2019).

## Materials and methods

### Test material

Name:	CA3642 (prothioconazole 150 g/L + azoxystrobin 150 g/L SC)
Formulation type:	Suspension concentrate (SC)
Lot/batch no.:	A20026
Active substances:	a) azoxystrobin b) prothioconazole
Active substance content:	a) 14.07% (w/w), corresponding to 154.83 g/L b) 13.84% (w/w), corresponding to 152.23 g/L
Density:	1.1004 g/mL
Appearance:	Off-white, odourless suspension
Expiry date of lot/batch:	September 07, 2022
Storage conditions:	Store in the tightly closed original container, in a dry, cool and well-ventilated area, out of direct sunlight

### Test organism

Species:	Rainbow trout ( <i>Oncorhynchus mykiss</i> )
Strain/clone:	Not reported
Age at study initiation:	Juvenile
Wet weight at study initiation:	0.62 ± 0.09 g (Mean ± standard deviation)
Length at study initiation:	4.0 ± 0.37 cm (Mean ± standard deviation)
Source:	fish breeding farm Fischzucht Zofingen, Höfenstrasse 754, 4812 Mühlethal
Feeding during test:	No
Acclimation:	Acclimatised to the test water, at test conditions for 7 days prior to test

start. Fish were fed with approximately 3 g of a commercial fish diet.

### Test conditions

Hardness:	40-250 mg CaCO <sub>3</sub> /L (actual: 125 mg calcium carbonate/L)
Test temperature:	11 - 12 °C
pH:	6.9 - 7.2
Dissolved oxygen:	8.1 - 9.1 mg/L
Photoperiod:	16 hours light : 8 hours dark

One glass aquarium, containing 7 litres of test medium, was used per product treatment and control. Seven juvenile fish were placed at random in each glass aquarium, providing an initial loading of 0.62 g wet bodyweight/L. The study was conducted as a static test with no medium renewal for an exposure period of 96 hours. The fish were not fed throughout the exposure period nor during the 48 hours that immediately preceded it. Duplicate samples of test media of all test concentrations and the control were taken daily from the start until the end of the exposure (96 hours) and were analysed for azoxystrobin and prothioconazole by HPLC-MS/MS. Observations were made twice every 24-hour period (in the morning and the afternoon) for mortality and visible abnormalities until the end of the test. The test medium in each vessel was sampled daily to measure temperature, pH and dissolved oxygen concentrations.

The LC<sub>50</sub> value was empirically estimated from the raw data, due to the absence of mortality caused by the product.

## Results

### Analytical results

The HPLC-MS/MS analytical method for the determination of azoxystrobin and prothioconazole in test water was validated with regards to specificity, linearity, accuracy and precision. Specificity was demonstrated by the absence of a peak at the characteristic retention time for both active substances in the control sample. The analytical calibration was conducted over the range of 0.0006 – 3.5 mg CA3642/L ( $r > 0.99$ ). Accuracy was confirmed with recovery determined by fortification of both active substances at 0.00982 and 3.07 mg/L; all recoveries were within the range of 107-120% (i.e. within the guideline range of 70-120%). Precision was confirmed with two determinations made at each fortification level; the relative standard deviation was within the guideline limit of  $\leq 20\%$ . The study was conducted according to the current guideline SANTE/2020/12830, Rev.1.

A summary of the measured concentrations of azoxystrobin and prothioconazole in the test media is presented in the tables below.

**Table 10.2.1/01-01: Nominal and measured concentrations of azoxystrobin and prothioconazole in the test media at the start and end of the test**

Nominal concentration [mg/L]		Geometric mean measured azoxystrobin / prothioconazole concentrations and sum of a.s. concentrations (% of nominal) [mg/L]	Analytically measured azoxystrobin / prothioconazole concentrations [mg/L] (% of nominal)	
Product	Azoxystrobin / prothioconazole and sum of a.s. concentrations		Day 0	Day 4
0.20	0.028 / 0.028 <b>0.056</b>	0.027 / 0.023 <b>0.050 (89)</b>	0.027 / 0.027 (96 / 97)	0.026 / 0.020 (93 / 73)
0.29	0.041 / 0.040 <b>0.081</b>	0.040 / 0.034 <b>0.074 (91)</b>	0.041 / 0.040 (100 / 98)	0.038 / 0.030 (94 / 73)



0.44	0.062 / 0.061 <b>0.12</b>	0.060 / 0.044 <b>0.10 (84)</b>	0.060 / 0.061 (97 / 99)	0.060 / 0.032 (96 / 52)
0.66	0.093 / 0.091 <b>0.18</b>	0.090 / 0.080 <b>0.17 (92)</b>	0.093 / 0.091 (100 / 99)	0.087 / 0.070 (93 / 77)
1.0	0.14 / 0.14 <b>0.28</b>	0.14 / 0.123 <b>0.26 (93)</b>	0.14 / 0.14 (99 / 102)	0.13 / 0.11 (93 / 78)
1.5	0.21 / 0.21 <b>0.42</b>	0.21 / 0.182 <b>0.39 (93)</b>	0.22 / 0.21 (102 / 101)	0.20 / 0.16 (96 / 75)
2.25 (2.04)*	0.32 / 0.31 <b>0.63</b>	0.30 / 0.271 <b>0.57 (91)</b>	0.31 / 0.31 (97 / 99)	0.30 / 0.24 (94 / 76)

\*: The mean measured CA3642 concentration of 2.04 mg CA3642/L was back-calculated based on the sum of the geometric mean measured concentrations of active substances of 0.57 mg sum of a.s./L, taking into account the content of active substances in the product (14.07% w/w azoxystrobin + 13.84% w/w prothioconazole = 27.91% w/w sum of active substances).

The recoveries of prothioconazole ranged from 52 to 78 % of nominal values at test end, therefore, the mean measured concentrations of the active substances over the test period of 96 hours were calculated as the geometric means of the active substance concentrations measured at the start and at the end of the test. Subsequently, mean measured concentrations of total active substances were calculated as the sum of the geometric means of the measured values for the active substances azoxystrobin and prothioconazole.

Nominal concentrations for the sum of active substances, i.e. 0.056, 0.081, 0.12, 0.18, 0.28, 0.42 and 0.63 mg sum of active substances/L, relate to the product concentrations of 0.20, 0.29, 0.44, 0.66, 1.0, 1.5 and 2.25 mg CA3642/L, respectively. The geometric mean measured values for the sum of the active substances was 0.050, 0.074, 0.10, 0.17, 0.26, 0.39 and 0.57 mg sum of active substances/L.

### Biological results

A summary of the effects of CA3642 on fish mortality is presented in the table below.

**Table 10.2.1/01-02: Effect of CA3642 on mortality of juvenile rainbow trout (*Oncorhynchus mykiss*), after 96 hours of exposure**

Nominal concentration [mg CA3642/L]	Geometric mean measured sum of active substances concentration [mg/L]	Cumulative mortality (no. dead)	Cumulative mortality (%)	Visible abnormalities
Control		0	0	0
0.20	0.050	0	0	0
0.29	0.074	0	0	0
0.44	0.10	0	0	0
0.66	0.17	0	0	0
1.0	0.26	0	0	0
1.5	0.39	0	0	0
2.25	0.57	0	0	1 P 1 <sup>d</sup>

No.: number

Visible abnormalities: P: Abnormal skin pigmentation (darkened<sup>d</sup>)

The 96-hour acute LC<sub>50</sub> value was estimated to be >2.25 mg CA3642/L (equivalent to >0.57 mg sum of a.s./L geometric mean measured concentration, corresponding to >2.04 mg CA3642/L mean measured concentration), as no mortalities were observed at the highest concentration tested. The NOEC (mortality) value was determined to be 2.25 mg CA3642/L (nominal), equivalent to 0.57 mg sum of a.s./L (geometric mean measured concentration), corresponding to 2.04 mg CA3642/L mean measured concentration, the highest concentration tested.

In the highest tested concentration of 2.25 mg/L (>0.57 mg sum of a.s./L geometric mean measured, corresponding to mean measured >2.04 mg CA3642/L), one fish showed visible abnormalities. At the second exposure day, observed symptoms in this fish were abnormal swimming behaviour (hypoactivity, abnormal bottom distribution and under-reactive to stimulus) and abnormal skin pigmentation (darkened skin). This abnormal skin pigmentation (darkened skin) was observed until the

end of the exposure.

### *Validity*

Overall, the study satisfies the guideline requirements for acute toxicity testing in fish and all validity criteria were met in accordance with OECD test guideline 203 (2019):

- Mortality in the control group was  $\leq 10\%$  at test end (actual value: 0%).
- Dissolved oxygen concentration was  $\geq 60\%$  of air saturation value (ASV) in all test vessels throughout the exposure (actual value: 8.1 mg O<sub>2</sub>/L, which corresponds to  $\geq 60\%$  ASV).
- Analytical measurement of test concentrations was included.

### **Assessment and conclusion**

In a 96-hour acute toxicity study, juvenile rainbow trout (*Oncorhynchus mykiss*) were exposed to the product, CA3642, under static conditions. All analytical values were based on the nominal product concentrations and geometric mean measured sum of active substances (azoxystrobin and prothioconazole) concentrations.

*Oncorhynchus mykiss* (rainbow trout):

96-hour acute (static) LC<sub>50</sub> >2.25 mg CA3642/L (nominal); >2.04 mg CA3642/L (mean measured); equivalent to >0.57 mg sum of a.s./L (geometric mean measured).

96-hour acute (static) NOEC 2.25 mg CA3642/L (nominal); 2.04 mg CA3642/L (mean measured); equivalent to 0.57 mg sum of a.s./L (geometric mean measured).

This toxicity study is considered acceptable and valid.

## Study 2

Comments of zRMS:	<p>The study was conducted in line with OECD 202 with no minor deviations.</p> <p>During the test, the concentrations of azoxystrobin were in the range of 95 and 115% of nominal active substance concentration, while for prothioconazole, concentrations were in the range of 8 to 92% of nominal. The biological results were therefore based on nominal concentrations of CA3642, and on the sum of the geometric mean measured concentrations of the active substances, and on mean measured concentrations of CA3642, recalculated, based on measured concentrations of the active substances, considering the content of the active substances in CA3642.</p> <p>All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>48-h <math>EC_{50}</math> = 1.4 mg CA3642/L (based on nominal concentration); equivalent to 0.27 mg total a.s./L (based on geometric mean measured concentrations for the sum of the active substances); corresponding to 0.97 mg CA3642/L (based on mean measured concentrations, assuming total active substance content of 27.91% in CA3642).</p>
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Reference:	KCP 10.2.1/02
Report	<p>CA3642 (Azoxystrobin 150 g/L + Prothioconazole 150 g/L) – Acute Toxicity to <i>Daphnia magna</i> in a 48-Hour Immobilization Test</p> <p>Dupont, A., year 2023, Report No. 20210196</p>
Guideline(s):	OECD Guideline for Testing of Chemicals, No. 202, <i>Daphnia sp.</i> , Acute Immobilisation Test, 2004
Deviations:	No
GLP:	Yes, conducted under GLP
Acceptability:	Yes (all validity criteria met in accordance with current guidance)
Duplication (if vertebrate study)	No

## Executive summary

The 48-hour acute toxicity of the product, CA3642, a suspension concentrate (SC) formulation (analysed active substances: 13.84% w/w prothioconazole and 14.07% w/w azoxystrobin) to *Daphnia magna* was studied under static conditions in accordance with OECD guideline 202 (2004). Daphnids were exposed to nominal concentrations of 0 (control), 0.21, 0.47, 1.03, 2.27 and 5.0 mg CA3642/L, corresponding to 0, 0.0586, 0.131, 0.288, 0.633 and 1.396 mg sum of active substances/L for 48 hours. Immobilisation of daphnids was observed after 24 and 48 hours of exposure.

Analytical verification of test concentrations confirmed that initial measured concentrations of azoxystrobin and prothioconazole were between 93-105% and 86-99% of the nominal active substance values, respectively. During the test, the concentrations of azoxystrobin were in the range of 95 and 115% of nominal active substance concentration, while for prothioconazole, concentrations were in the range of 8 to 92% of nominal. The biological results were therefore based on nominal concentrations of CA3642, and on the sum of the geometric mean measured concentrations of the active substances, and on mean measured concentrations of CA3642, recalculated, based on measured concentrations of the active substances, considering the content of the active substances in CA3642.

The 48-hour EC<sub>50</sub> value for *Daphnia magna*, based on immobilisation, was calculated to be 1.4 mg CA3642/L (nominal concentration), equivalent to 0.27 mg total a.s./L, (geometric mean measured concentration for the sum of the active substances), corresponding to 0.97 mg CA3642/L (mean measured concentration, recalculated). The 48-hour NOEC (immobility) value was estimated to be <0.21 mg CA3642/L (nominal), equivalent to <0.037 mg total a.s./L (geometric mean measured concentration for the sum of the active substances), corresponding to <0.13 mg CA3642/L (mean measured concentration, recalculated).

This study is considered acceptable and valid. The study satisfies the guideline requirements and validity criteria provided in the OECD test guideline 202 (2004).

## Materials and methods

### Test material

Name:	CA3642 (prothioconazole 150 g/L + azoxystrobin 150 g/L SC)
Formulation type:	Suspension concentrate (SC)
Lot/batch no.:	A20026
Active substances:	a) azoxystrobin b) prothioconazole
Purity:	a) 14.07% (w/w), corresponding to 154.83 g/L b) 13.84% (w/w), corresponding to 152.23 g/L
Density:	1.1004 g/mL
Expiry date of lot/batch:	September 2022
Appearance:	Off-white, odourless suspension
Storage conditions:	Store the tightly closed original container in a dry, cool and well-ventilated area out of direct sunlight

### Reference item

Name:	Potassium dichromate
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### Test organism

Species:	<i>Daphnia magna</i>
Strain/clone:	Straus
Age at study initiation:	<24 hours old
Source:	Originally from <i>Daphnia</i> Collection of the University of Basel/Switzerland in 2015 and bred in IES Ltd Laboratories.

The cultivation of the parental daphnids was performed in reconstituted water of the quality identical to the water quality used in the test (in respect to pH, main ions, and total hardness). During breeding, daphnids were generally fed three times a week with an algal suspension of the green algae *Desmodesmus subspicatus*.

Feeding during test:	No
Acclimation:	Not applicable.

### Test conditions

Test medium:	Reconstituted test water (ISO Test water)
Hardness:	250 mg/L as CaCO <sub>3</sub>
Test temperature:	21°C (measured at 0 and 48 hours)

pH:	7.8 – 7.9 (measured at 0 and 48 hours)
Dissolved oxygen:	8.5 mg/L (measured at 0 and 48 hours)
Photoperiod:	Dark (except during maintenance / observation steps)
Light intensity:	Not applicable.

Test vessels were 100 mL glass beakers, each filled with 50 mL of test medium. For each treatment, 20 daphnids were randomly distributed into four replicates of five daphnids each. The volume of test medium provided for each daphnid was 10 mL (50 mL per replicate). The test vessels were loosely covered with glass sheets to reduce the loss of water by evaporation.

After 24 and 48 hours, immobilised daphnids were counted. All daphnids that were unable to swim for approximately 15 seconds after gentle agitation of the test vessel were considered to be immobilised.

At the start and end of the test, the pH values, dissolved oxygen concentrations and water temperature were determined in each treatment with surviving daphnids. Duplicate samples were taken from all test concentrations and the control at the start of the test, after 24 hours and at the end of the test (after 48 hours) and analysed for azoxystrobin and prothioconazole by HPLC-MS/MS.

Statistical analyses were performed using ToxRat Professional<sup>®</sup>. The 48-hour EC<sub>50</sub> values and the 95% confidence limits were calculated by Trimmed Spearman-Kärber Procedure using interpolation. The NOEC value was determined directly from the raw data.

## Results

### Analytical results

The HPLC-MS/MS analytical method for the determination of azoxystrobin and prothioconazole in test medium was validated with regards to specificity, linearity, accuracy and precision. Specificity was demonstrated by the absence of a peak at the characteristic retention time for both active substances in the control sample. The analytical calibration was performed over the range of 0.00014 to 7.54 mg CA3642/L ( $r > 99$ ). Accuracy was confirmed with recovery determined by fortification of untreated test medium with both active substances at 0.0205 and 6.04 mg/L. All recoveries were within the range of 79-101% (i.e. within the guideline range of 70-120%). Precision was confirmed with five determinations made at each fortification level; the relative standard deviation was within the guideline limit of  $\leq 20\%$ . The limit of quantification (LOQ) was 0.0205 mg CA3642/L (i.e. significantly below the biological EC<sub>50</sub> value of 1.4 mg CA3642/L). The limit of detection (LOD) was 0.02 µg a.s./L in the test medium. The study was conducted according to the current guideline SANTE/2020/12830, Rev.1.

The measured concentrations of azoxystrobin and prothioconazole during the 48-hour exposure period of the *Daphnia magna* toxicity study are summarised in the table below.

**Table 10.2.1/02-01: Nominal and measured concentrations of azoxystrobin and prothioconazole in the test media at each sampling point**

Nominal concentration [mg/L]		Analytically measured azoxystrobin / prothioconazole concentrations [mg/L]			Geometric mean measured azoxystrobin / prothioconazole concentrations and sum of a.s. concentrations [mg/L]	Mean measured CA3642 concentrations <sup>o</sup> [mg/L]
Product	Azoxystrobin / prothioconazole sum a.s.	Day 0	Day 1*	Day 2		
0.21	0.0295 / 0.0291 0.0586	0.0294 / 0.0254	0.0304 / 0.0236	0.0298 / 0.00225	0.0296 / 0.0076 <b>0.037</b>	0.13
0.47	0.0661 / 0.0650 0.131	0.0615 / 0.0558	0.0712 / 0.0489	0.0652 / 0.00606	0.0633 / 0.0184 <b>0.082</b>	0.29
1.03	0.145 / 0.143 0.288	0.152 / 0.136	0.166 / 0.131	0.151 / 0.0149	0.152 / 0.045 <b>0.20</b>	0.72

2.27	0.319 / 0.314 0.633	0.333 / 0.300	0.341 / 0.280	0.328 / 0.0415	0.331 / 0.112 <b>0.44</b>	1.58
5.0	0.704 / 0.692 1.396	0.700 / 0.686	0.754 / 0.609	0.670 / 0.218	0.685 / 0.387 <b>1.07</b>	3.83

\*: Analytical measurements from day 1 are not used for the calculation of the geometric mean values for a static test.

°: Recalculated from mean measured concentrations of active substances, taking into account the content of active substances in the test item (14.07% w/w azoxystrobin + 13.84% w/w prothioconazole = 27.91% w/w sum of active substances).

**Bold** values: sum of the a.s. concentrations, geometric mean measured.

**Table 10.2.1/02-02: Individual recoveries of azoxystrobin and prothioconazole in the test samples**

Nominal CA3642 concentration [mg/L]	Azoxystrobin (% of nominal)			Prothioconazole (% of nominal)		
	Day 0	Day 1	Day 2	Day 0	Day 1	Day 2
Control	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
0.21	100	103	101	88	81	8
0.47	93	108	99	86	75	9
1.03	105	115	104	95	92	10
2.27	104	107	103	96	89	13
5.0	99	107	95	99	88	31

n.a.: not applicable

The mean measured concentrations of the active substances over the test period of 48 hours were calculated as the geometric means of the active substance concentrations measured at the start and at the end of the test (after 48 hours). Subsequently, mean measured concentrations of total active substances were calculated as the sum of the geometric means of the measured values for the active substances azoxystrobin and prothioconazole, resulting in concentrations of 0.037, 0.082, 0.20, 0.44 and 1.07 mg sum of a.s./L for the nominal test item concentrations of 0.21, 0.47, 1.03, 2.27 and 5.0 mg CA3642/L, respectively.

#### *Biological results*

A summary of the effects of CA3642 on immobilisation of *Daphnia magna* after 48 hours of exposure is presented in the table below.

**Table 10.2.1/02-03: Effect of CA3642 on immobilisation of *Daphnia magna***

Nominal concentration (mg CA3642/L)	Mean measured sum of active substance concentration (mg/L)	Rep.	No. organisms at test start	No. immobilised daphnids		% immobilisation	
				24 hours	48 hours	24 hours	48 hours
Control	-	1	5	0	0	0	0
		2	5	0	0		
		3	5	0	0		
		4	5	0	0		
0.21	0.037	1	5	0	1	10	20
		2	5	0	0		
		3	5	1	1		
		4	5	1	2		
0.47	0.082	1	5	0	1	10	15
		2	5	0	0		
		3	5	1	1		
		4	5	1	1		
1.03	0.20	1	5	1	1	25	30
		2	5	1	1		
		3	5	2	3		
		4	5	1	1		
2.27	0.44	1	5	2	2	35	45
		2	5	3	4		
		3	5	1	2		
		4	5	1	1		
5.0	1.07	1	5	5	5	100	100
		2	5	5	5		
		3	5	5	5		
		4	5	5	5		

No.: number; Rep.: replicate

The 48-hour EC<sub>50</sub> value for *Daphnia magna* based on immobilisation was calculated to be 1.4 mg CA3642/L (95 % confidence limits (CLs): 1.0 - 1.9 mg CA3642/L) based on nominal concentrations, equivalent to 0.27 mg sum a.s./L (95% CLs: 0.20 to 0.38 mg sum of a.s./L) based on geometric mean measured concentrations for the sum of the active substances, corresponding to 0.97 mg CA3642/L, (based on mean measured concentrations, considering the content of the active substances in the product). The 48-hour NOEC (immobility) value was estimated to be <0.21 mg CA3642/L (nominal), equivalent to <0.037 mg total a.s./L (geometric mean measured concentration for the sum of the active substances), corresponding to <0.13 mg CA3642/L (mean measured concentration, recalculated).

#### Validity

Overall, the study satisfies the guideline requirements for acute toxicity testing in aquatic invertebrates and all validity criteria were met in accordance with OECD test guideline 202 (2004):

- Immobilisation in the control group was ≤10% (actual value: 0%).
- The dissolved oxygen concentration at the end of the test was ≥3 mg/L in all test vessels (actual value: ≥8.5 mg/L).

#### Assessment and conclusion

The 48-hour acute toxicity of the product, CA3642, to *Daphnia magna* was studied under static conditions in accordance with OECD guideline 202 (2004).

#### *Daphnia magna*:

48-hour (static) EC<sub>50</sub> (immobilisation) = 1.4 mg CA3642/L (based on nominal concentration); equivalent to 0.27 mg total a.s./L (based on geometric mean measured concentrations for the sum of the active substances); corresponding to 0.97 mg CA3642/L (based on mean measured concentrations, assuming total active substance content of 27.91% in CA3642).

This study is considered acceptable and valid.

### Study 3

Comments of zRMS:	<p>The study was conducted in line with OECD 202 with no minor deviations.</p> <p>Analytical verification of test concentrations confirmed that measured concentrations of fresh and 72-hour aged samples of azoxystrobin and prothioconazole in test media were between 73-120% and 5-111% of nominals, respectively. Therefore, the biological results for biomass in terms of growth rate and yield of the marine diatom <i>Skeletonema</i> sp., were based on nominal concentrations of CA3642, on the sum of the geometric mean measured concentrations of the active substances and on CA3642 mean measured concentrations, recalculated from the mean measured concentrations of the sum of the active substances, considering the total analysed active substance content of 27.91% in the product.</p> <p>All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>Based on nominal concentrations:  72-h <math>E_rC_{50}</math> (growth rate) = 0.680 mg CA3642/L  72-h <math>E_yC_{50}</math> (yield) = 0.361 mg CA3642/L</p> <p>Based on the geometric mean measured for the sum of the active substances:  72-h <math>ErC_{50}</math> (growth rate) = 0.136 mg total a.s./L  72-h <math>EyC_{50}</math> (yield) = 0.062 mg total a.s./L</p> <p>Based on mean measured CA3642 concentrations, recalculated from mean measured sum of active substance concentrations considering total a.s. content of 27.91% in CA3642:</p> <p>72-h <math>ErC_{50}</math> (growth rate) = 0.487 mg CA3642/L  72-h <math>EyC_{50}</math> (yield) = 0.222 mg CA3642/L</p>
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Reference:	KCP 10.2.1/03
Report	CA3642 (Azoxystrobin 150 g/L + Prothioconazole 150 g/L) - Effect on <i>Skeletonema</i> sp. in a 72-Hour Algal Growth Inhibition Test Dupont, A., year 2023, Report No. 20210197
Guideline(s):	<p>International Organization for Standardization, ISO 10253: Water quality – Marine algal growth inhibition test with <i>Skeletonema</i> sp. and <i>Phaeodactylum tricornutum</i>, November 2016.</p> <p>OECD Guidelines for the Testing of Chemicals, No. 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test, adopted 2006, corrected 2011.</p> <p>US EPA, Ecological Effects Test Guidelines, OCSPP 850.4500: Algal Toxicity, EPA 712-C-006, January 2012.</p>
Deviations:	No
GLP:	Yes, conducted under GLP
Acceptability:	Yes (all validity criteria met in accordance with current guidance)
Duplication (if vertebrate study)	No

### Executive summary

In a 72-hour toxicity study, cultures of *Skeletonema* sp. were exposed to the product, CA3642, a



suspension concentrate (SC) formulation (analysed active substances: 13.84% w/w prothioconazole and 14.07% w/w azoxystrobin) at nominal test item concentrations tested of 0 (control) 0.0041, 0.0123, 0.037, 0.11, 0.33 and 1.0 mg CA3642/L, corresponding to nominal azoxystrobin concentrations of 0, 0.000577 to 0.141 mg/L and nominal prothioconazole concentrations of 0, 0.000567 to 0.138 mg/L. The nominal sum of the active substances was 0.00114, 0.00343, 0.0103, 0.0307, 0.0921 and 0.279 mg/L.

Analytical verification of test concentrations confirmed that measured concentrations of fresh and 72-hour aged samples of azoxystrobin and prothioconazole in test media were between 73-120% and 5-111% of nominals, respectively. Therefore, the biological results for biomass in terms of growth rate and yield of the marine diatom *Skeletonema sp.*, were based on nominal concentrations of CA3642, on the sum of the geometric mean measured concentrations of the active substances and on CA3642 mean measured concentrations, recalculated from the mean measured concentrations of the sum of the active substances, considering the total analysed active substance content of 27.91% in the product.

The 72-hour  $E_rC_{50}$  (growth rate) value for the marine diatom *Skeletonema sp.* was determined to be 0.680 mg CA3642/L (nominal), equivalent to 0.136 mg total a.s./L (geometric mean measured), corresponding to 0.487 mg CA3642/L (mean measured, recalculated).

The 72-hour  $E_yC_{50}$  (yield) value was determined to be 0.361 mg CA3642/L (nominal), equivalent to 0.062 mg total a.s./L (geometric mean measured), corresponding to 0.222 mg CA3642/L (mean measured, recalculated).

The 72-hour NOEC value for the marine diatom *Skeletonema sp.* was determined to be 0.11 mg CA3642/L (nominal), based on growth rate and yield, respectively, equivalent to 0.019 mg total a.s./L (geometric mean measured), corresponding to 0.068 mg CA3642/L (mean measured, recalculated).

This study is considered acceptable and satisfies the guideline requirements for an algal growth inhibition study (ISO 10253:2016, OCSPP 850.4500, 2012 and OECD test guideline 201, 2011).

## Materials and methods

### Test material

Name:	CA3642 (prothioconazole 150 g/L + azoxystrobin 150 g/L SC)
Formulation type:	Suspension concentrate (SC)
Lot/batch no.:	A20026
Active substances:	a) azoxystrobin b) prothioconazole
Purity:	a) 14.07% (w/w), corresponding to 154.83 g/L b) 13.84% (w/w), corresponding to 152.23 g/L
Density:	1.1004 g/mL
Appearance:	Off-white, odourless suspension
Expiry date of lot/batch:	September 2022
Storage conditions:	Store the tightly closed original container in a dry, cool and well-ventilated area out of direct sunlight

### Reference item

Name:	Potassium dichromate
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### Test organism

Species:	<i>Skeletonema sp.</i>
Strain/clone:	Strain No. CCAP 1077/1C (formerly listed as <i>Skeletonema costatum</i> )
Source:	Culture Collection of Algae and Protozoa (CCAP, Scottish Marine Institute, Dunbeg, Oban, Argyll, PA37 1QA, Scotland / UK). The algae were cultivated at IES Ltd under standardized conditions according to the test guidelines. An inoculum culture was set up four days before the start of the exposure. The algae were cultivated under

the test conditions and were kept in the exponential growth phase until inoculation of the test solutions.

#### *Test conditions*

Test medium:	Reconstituted test water (MAA)
Test temperature:	19.5 – 19.6°C (daily measured)
pH:	8.0 – 8.6 (measured at 0 and 72 hours)
Photoperiod:	Continuously illuminated, reduced light intensity
Light intensity:	Approximately 4884 Lux

Test vessels comprised 125 mL glass Erlenmeyer flasks, each filled with 50 mL inoculated test medium, and loosely covered with a glass lid. A stock solution was prepared by weighing the appropriate amount of test item and adding to test water. Lower test concentrations were prepared by dilution of the appropriate solution with test water.

An initial nominal cell density of 5000 cells/mL was added to each test vessel, with three replicate vessels per treatment group and six replicates vessels for the control group. For the stability samples at 24 and 48 hours, a set of additional flasks containing the corresponding test medium (with algae) were incubated under conditions identical to the test. The test vessels were incubated in a temperature-controlled water bath at a temperature of 20°C for 72 hours. Gaseous exchange was ensured by the loosely covered flasks. To minimise the impact of differences in light intensity across the test area on algal growth, control and test flasks were re-positioned randomly in the test area each day.

The initial cell density was selected according to the recommendations of the OECD test guideline and measured using an electronic particle counter. Samples of test media were taken from each test vessel at 24, 48 and 72 hours and the cell densities determined by fluorescence measurement. Microscopic examinations of samples of cells from control and nominal concentration of 0.33 mg CA3642/L were also performed in order to identify the presence of any abnormal cells.

Statistical analysis was performed using ToxRat Professional (Version 3.3.0). The 72-hour EC<sub>10/20/50</sub> values for the inhibition of average growth rate and yield and their 95 % confidence intervals were calculated by 3-parametric normal Cumulative Distribution Function (CDF) with non-linear regression analysis. For the determination of the LOEC and NOEC values, the average growth rate and yield at the test concentrations were compared to the control values by Williams t-test.

## **Results**

### *Analytical results*

The HPLC-MS/MS analytical method for the determination of azoxystrobin and prothioconazole in test medium was validated with regards to specificity, linearity, accuracy and precision. Specificity was demonstrated by the absence of a peak at the characteristic retention time for both active substances in the control sample. The analytical calibration was performed over the range of 0.00014 to 1.5 mg CA3642/L ( $r > 99$ ). Accuracy was confirmed with recovery determined by fortification of untreated test medium with both active substances at 0.000445 and 1.20 mg/L. All recoveries were within the range of 77-104% (i.e. within the guideline range of 70-120%). Precision was confirmed with five determinations made at each fortification level; the relative standard deviation was within the guideline limit of  $\leq 20\%$ . The limit of quantification (LOQ) was 0.000445 mg CA3642/L (i.e. significantly below the biological E<sub>50</sub>C<sub>50</sub> value of 0.361 mg CA3642/L). The limit of detection (LOD) was 0.02 µg a.s./L in the test medium. The study was conducted according to the current guideline SANTE/2020/12830, Rev.1.

The measured concentrations of azoxystrobin and prothioconazole during the 72-hour exposure period of the algal growth inhibition study are summarised in the tables below.

**Table 10.2.1/03-01: Nominal and measured concentrations of azoxystrobin and prothioconazole at each sampling point**

Nominal concentration		Analytically measured azoxystrobin / prothioconazole concentrations [µg/L]				Geometric mean measured azoxystrobin / prothioconazole concentrations and sum of a.s. concentrations [µg/L]	Mean measured CA3642 concentrations* [mg/L]
Product [mg/L]	Azoxystrobin / prothioconazole sum of a.s. [µg/L]	Day 0	Day 1	Day 2	Day 3		
0.0041	0.577 / 0.567 <b>1.14</b>	0.472 / 0.53	0.556 / 0.195	0.601 / 0.0661	0.502 / 0.0307	0.546 / 0.118 <b>0.664</b>	0.00236
0.0123	1.73 / 1.70 <b>3.43</b>	1.81 / 1.52	1.66 / 0.354	1.43 / 0.168	1.44 / 0.095	1.57 / 0.283 <b>1.85</b>	0.00681
0.037	5.21 / 5.12 <b>10.33</b>	6.03 / 5.10	4.17 / 1.82	4.83 / 1.49	4.21 / 0.309	4.66 / 1.50 <b>6.17</b>	0.0222
0.11	15.5 / 15.2 <b>30.7</b>	15.6 / 15.1	12.8 / 6.76	14.5 / 1.96	13.6 / 3.25	13.9 / 4.52 <b>18.5</b>	0.0681
0.33	46.4 / 45.7 <b>92.1</b>	55.5 / 45.6	48.4 / 23.2	34.0 / 6.36	42.0 / 3.68	43.0 / 12.4 <b>55.4</b>	0.197
1.0	141 / 138 <b>279</b>	151 / 153	137 / 103	114 / 71.1	146 / 51.0	132 / 86.5 <b>219</b>	0.788

\*: Recalculated from mean measured concentrations of active substances, taking into account the content of active substances in the test item (14.07% w/w azoxystrobin + 13.84% w/w prothioconazole = 27.91% w/w sum of active substances).

**Bold** values: sum of the a.s. concentrations, geometric mean measured.

**Table 10.2.1/03-02: Individual recoveries of azoxystrobin and prothioconazole in the test samples**

Nominal CA3642 concentration [mg/L]	Azoxystrobin (% of nominal)				Prothioconazole (% of nominal)			
	Day 0	Day 1	Day 2	Day 3	Day 0	Day 1	Day 2	Day 3
Control	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
0.0041	82	96	104	87	93	34	12	5
0.0123	104	96	83	83	90	21	10	6
0.037	116	80	93	81	100	36	29	6
0.11	101	82	94*	88	99	44	13*	21
0.33	120	104	73*	90	100	51	14*	8
1.0	107	97	81*	104	111	74	51*	37

n.a.: not applicable

\* The mean recovery for a first taken sample was averaged with the recovery of a retain sample for this dose level.

The mean measured concentrations of the active substances over the test period of 72 hours were calculated as the geometric means of the a.s. concentrations measured at the start and at the end of the test. Subsequently, mean measured concentrations of total active substances were calculated as the sum of the geometric mean measured values for the active substances azoxystrobin and prothioconazole, resulting in concentrations of 0.00664, 0.00185, 0.00617, 0.0185, 0.0554 and 0.219 mg sum of a.s./L for the nominal test item concentrations of 0.0041, 0.0123, 0.037, 0.11, 0.33 and 1.0 mg CA3642/L, respectively. These values correspond to mean measured CA3642 concentrations of 0.00236, 0.00681, 0.0222, 0.0681, 0.197 and 0.788 mg CA3642/L, recalculated from mean measured sum of active substance concentrations, considering the analysed (GLP Certificate of Analysis) total active substance content of 27.91% in CA3642.

#### Biological results

A summary of the effects of CA3642 on biomass of *Skeletonema* sp. after 72 hours exposure is presented in the table below.

**Table 10.2.1/03-03: Effect of CA3642 on biomass (area under the growth curve) for *Skeletonema sp.* after 72 hours**

Nominal CA3642 concentration [mg/L]	Geometric mean measured sum of active substances concentration [mg/L]	Replicate	Biomass of algae* (relative fluorescence units x 10 <sup>4</sup> )		
			24 h	48 h	72 h
Control	-	1	5.51	22.7	68.4
		2	4.93	18.4	55.4
		3	5.25	23.5	66.4
		4	6.27	20.8	62.1
		5	5.72	19.7	56.1
		6	3.90	16.3	59.3
		<b>Mean</b>	<b>5.26</b>	<b>20.3</b>	<b>61.3</b>
		SD	0.81	2.68	5.33
0.0041	0.00066	1	4.69	20.2	62.1
		2	4.59	20.5	60.4
		3	5.60	18.6	65.0
		<b>Mean</b>	<b>4.96</b>	<b>19.7</b>	<b>62.5</b>
		SD	0.55	1.04	2.29
0.0123	0.0019	1	4.85	23.0	67.8
		2	4.64	22.9	59.3
		3	4.76	20.1	62.8
		<b>Mean</b>	<b>4.75</b>	<b>22.0</b>	<b>63.3</b>
		SD	0.10	1.67	4.24
0.037	0.0062	1	5.48	18.8	56.6
		2	4.81	17.8	55.9
		3	4.14	20.2	57.6
		<b>Mean</b>	<b>4.80</b>	<b>19.0</b>	<b>56.7</b>
		SD	0.67	1.20	0.85
0.11	0.019	1	4.65	18.3	55.2
		2	5.45	17.9	65.8
		3	4.05	18.7	52.4
		<b>Mean</b>	<b>4.72</b>	<b>18.3</b>	<b>57.8</b>
		SD	0.70	0.39	7.07
0.33	0.055	1	3.86	12.8	36.7
		2	3.78	10.9	32.8
		3	3.71	12.5	35.1
		<b>Mean</b>	<b>3.78</b>	<b>12.1</b>	<b>34.8</b>
		SD	0.08	1.02	1.93
1.0	0.219	1	0.83	1.57	2.90
		2	0.72	1.86	2.76
		3	0.72	1.97	2.86
		<b>Mean</b>	<b>0.76</b>	<b>1.80</b>	<b>2.84</b>
		SD	0.06	0.20	0.08

SD: Standard deviation

\*: The biomass of the algae was determined by fluorescence measurement (mean of duplicate measurements per replicate) and is given as relative fluorescence units (x 10<sup>4</sup>). At the start of the test, the initial cell density was 5000 algal cells/mL, corresponding to 0.89 x 10<sup>4</sup> relative fluorescence units.

A summary of the effects of CA3642 on the growth rate of *Skeletonema sp.* after 72 hours exposure is presented in the table below.

**Table 10.2.1/03-04: Effect of CA3642 on the cell density and growth rate of *Skeletonema sp.* after 72 hours exposure**

Nominal CA3642 concentration [mg/L]	Geometric mean measured sum of active substances concentration [mg/L]	Rep.	Cell density <sup>a</sup> (relative fluorescence units x 10 <sup>4</sup> ) 72 hours	0 -72 hours			
				Mean cell density <sup>a</sup> (relative fluorescence units x 10 <sup>4</sup> )	% inhibition of cell density <sup>b</sup>	Average growth rate $\mu$ [day <sup>-1</sup> ]	% inhibition of growth rate <sup>b</sup> [%]
Control	-	1	68.4	61.3	-	1.410	-
		2	55.4				
		3	66.4				
		4	62.1				
		5	56.1				
		6	59.3				
0.0041	0.00066	1	62.1	62.5	-2.0	1.417	-0.5
		2	60.4				
		3	65.0				
0.0123	0.0019	1	67.8	63.3	-3.3	1.421	-0.8
		2	59.3				
		3	62.8				
0.037	0.0062	1	56.6	56.7	7.5	1.385	1.8
		2	55.9				
		3	57.6				
0.11	0.019	1	55.2	57.8	5.7	1.390	1.4
		2	65.8				
		3	52.4				
0.33	0.055	1	36.7	34.8	43.2	1.222*	13.3
		2	32.8				
		3	35.1				
1.0	0.219	1	2.90	2.84	95.4	0.386*	72.6
		2	2.76				
		3	2.86				

Rep.: replicate

<sup>a</sup> At the start of the test, the initial cell density was 5000 algal cells/mL, corresponding to 0.89 x 10<sup>4</sup> relative fluorescence units.

<sup>b</sup> % inhibition relative to the control. Negative values indicate an increase relative to the control.

\*: Mean value statistically significantly lower than in the control (according to Williams t-test, one-sided smaller,  $\alpha = 0.05$ ).

After 72 hours, clear concentration-response relationship was observed for cell density and growth rate. Statistically significant inhibitory effects were observed on the growth rate ( $\mu$ ) of the algae at the two highest test item concentrations, compared to the control group.

The 72-hour  $E_rC_{50}$  (growth rate) value for the marine diatom *Skeletonema sp.* was determined to be 0.680 mg CA3642/L (with 95% confidence limits (CLs): 0.624-0.742 mg CA3642/L) (based on nominal concentrations); equivalent to 0.136 mg total a.s./L (with 95% CLs: 0.122-0.151 mg total a.s./L) (based on the geometric mean measured concentrations for the sum of the active substances), corresponding to 0.487 mg CA3642/L (based on mean measured concentrations, recalculated from the mean measured sum of active substance concentrations, considering total a.s. content of 27.91% in CA3642). The 72-hour NOEC (growth rate) value was determined to be 0.11 mg CA3642/L (nominal), equivalent to 0.019 mg total a.s./L (geometric mean measured), corresponding to 0.068 mg CA3642/L (mean measured, recalculated).

The shape and size of the algal cells were not obviously affected by the test item up to at least the test item concentration of 0.33 mg/L (nominal).

A summary of the effects of CA3642 on the yield of *Skeletonema sp.* after 72 hours of exposure is presented in the table below.

**Table 10.2.1/03-05: Effect of CA3642 on the yield of *Skeletonema sp.* after 72 hours**

Nominal CA3642 concentration [mg/L]	Geometric mean measured sum of active substances concentration [mg/L]	Replicate	Cell density <sup>a</sup> (relative fluorescence units x 10 <sup>4</sup> ) 72 hours	0 -72 hours	
				Yield (x10 <sup>4</sup> )	% inhibition of yield <sup>b</sup>
Control	--	1	68.4	60.4	-
		2	55.4		
		3	66.4		
		4	62.1		
		5	56.1		
		6	59.3		
0.0041	0.00066	1	62.1	61.6	-2.0
		2	60.4		
		3	65.0		
0.0123	0.0019	1	67.8	62.4	-3.3
		2	59.3		
		3	62.8		
0.037	0.0062	1	56.6	55.8	7.7
		2	55.9		
		3	57.6		
0.11	0.019	1	55.2	56.9	5.8
		2	65.8		
		3	52.4		
0.33	0.055	1	36.7	34.0*	43.8
		2	32.8		
		3	35.1		
1.0	0.219	1	2.90	1.9*	96.8
		2	2.76		
		3	2.86		

<sup>a</sup> At the start of the test, the initial cell density was 5000 algal cells/mL, corresponding to  $0.89 \times 10^4$  relative fluorescence units.

<sup>b</sup> % inhibition relative to the control. Negative values indicate an increase relative to the control.

\*: Mean value statistically significantly lower than in the control (according to Williams t-test, one-sided smaller,  $\alpha = 0.05$ ).

After 72 hours of exposure, a clear concentration-response relationship was observed for yield. Statistically significant inhibitory effects were observed on the yield (Y) of the algae at the two highest test item concentrations, compared to the control group.

The 72-hour  $E_yC_{50}$  (yield) value for the marine diatom *Skeletonema sp.* was determined to be 0.361

mg CA3642/L (with 95% CLs: 0.231-0.559 mg CA3642) (based on nominal concentrations); equivalent to 0.062 mg total a.s./L (with 95% CLs: 0.038-0.098 mg total a.s./L) (based on the geometric mean measured concentrations for the sum of the active substances), corresponding to 0.222 mg CA3642/L (based on mean measured concentrations, recalculated from mean measured sum of active substance concentrations, considering total a.s. content of 27.91% in CA3642). The 72-hour NOEC (yield) value was determined to be 0.11 mg CA3642/L (nominal), equivalent to 0.019 mg total a.s./L (geometric mean measured), corresponding to 0.068 mg CA3642/L (mean measured, recalculated).

### Validity

Overall, the study satisfies the guideline requirements for algal growth inhibition tests and all validity criteria were met in accordance with ISO, OECD and US EPA test guidelines:

- During the 72-hour test period, cell counts in the controls increased by a factor of at least 16 (actual value: biomass increased by a factor of 69 over 72 hours; see table 10.2.1/03-02).
- The mean coefficient of variation for section-by-section specific growth rates in the control cultures did not exceed 35% (actual value 0 – 72 hours: 24.0%, see table below).

Control replicate	Section-by-section specific growth rate*				Standard deviation	Coefficient of variation %
	0 - 24 hours	24 - 48 hours	48 - 72 hours	Mean		
1	1.823	1.418	1.101	1.447	0.362	25.0
2	1.713	1.317	1.102	1.377	0.310	22.5
3	1.775	1.498	1.039	1.437	0.372	25.9
4	1.952	1.199	1.094	1.415	0.468	33.1
5	1.860	1.237	1.048	1.382	0.425	30.8
6	1.478	1.433	1.289	1.400	0.099	7.1
Mean coefficient of variation of the section-by-section specific growth rate						24.0

\* calculated according to paragraph 48, OECD 201 (2011).

- The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures did not exceed 10% (actual value 0 – 72 hours: 2.0%, see table below).

Control Replicate	Average specific growth rate during whole test period*		Standard deviation	Mean coefficient of variation of the average specific growth rate during the whole test period (0 - 72 hours)
	0 - 72 hours	Mean		
1	1.447	1.410	0.029	2.0
2	1.377			
3	1.437			
4	1.415			
5	1.382			
6	1.400			

\* calculated according to paragraph 48, OECD 201 (2011).

As all validity criteria were met, the study is considered valid.

### Assessment and conclusion

In a 72-hour toxicity study, cultures of *Skeletonema sp.* were exposed to the product, CA3642, under static conditions, in accordance with the ISO 10253:2016, OCSPP 850.4500 (2012) and OECD test guideline 201 (2011).

*Skeletonema sp.*:

Based on nominal concentrations:

The 72-hour  $E_rC_{50}$  (growth rate) = 0.680 mg CA3642/L.

The 72-hour  $E_yC_{50}$  (yield) = 0.361 mg CA3642/L.

Based on the geometric mean measured for the sum of the active substances:

The 72-hour  $E_rC_{50}$  (growth rate) = 0.136 mg total a.s./L.

The 72-hour  $E_yC_{50}$  (yield) = 0.062 mg total a.s./L.

Based on mean measured CA3642 concentrations, recalculated from mean measured sum of active substance concentrations considering total a.s. content of 27.91% in CA3642:

The 72-hour  $E_rC_{50}$  (growth rate) = 0.487 mg CA3642/L.

The 72-hour  $E_yC_{50}$  (yield) = 0.222 mg CA3642/L.

The 72-hour NOEC = 0.11 mg CA3642/L (nominal), based on growth rate and yield, respectively, equivalent to 0.019 mg total a.s./L (geometric mean measured), corresponding to 0.068 mg CA3642/L (mean measured, recalculated).

This study is considered acceptable and valid.

**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**

### Study 1

Comments of zRMS:	<p>The study was conducted in line with OECD 213 and 214 with minor deviations. A topical application of 2 µL of the test solution(s) was applied onto the thorax. This is a higher volume than the guideline recommendation of 1 µL, due to the preparation of the required solutions (high concentrations). It has been validated with a non GLP pre-test and it is deemed unlikely to adversely affect the outcome of the study.</p> <p>All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>The contact LD50 (48 h) &gt;1464 µg product /bee The oral LD50 (96 h) = 339.19 µg product /bee</p>
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<b>Reference:</b>	KCP 10.3.1.1/01
<b>Report</b>	<p>CA3642 (Azoxystrobin 150 g/L + Prothioconazole 150 g/L SC): Acute Oral and Contact Toxicity to the Honey bee (<i>Apis mellifera</i> L.), under Laboratory Conditions</p> <p>Gimeno, I., 2022, report no. S21-04080</p>
<b>Guideline(s):</b>	Yes. OECD 213 and 214 (1998)
<b>Deviations:</b>	Yes. A topical application of 2 µL of the test solution(s) was applied onto the thorax. This is a higher volume than the guideline recommendation of 1 µL, due to the preparation of the required solutions (high concentrations). It has been validated with a non GLP pre-test and it is deemed unlikely to adversely affect the outcome of the study
<b>GLP:</b>	Yes, conducted under GLP/Officially recognised testing facilities
<b>Acceptability:</b>	Yes
<b>Duplication (if vertebrate study)</b>	No



## Executive summary

In a 96-hour laboratory study, the acute oral and contact toxicity of the product, CA3642 (analysed active substances: 14.07% w/w azoxystrobin and 13.84% w/w prothioconazole), to the European honey bee, *Apis mellifera*, was evaluated in dose-response tests, in accordance with OECD 213 and OECD 214 test guidelines.

In the acute oral test, adult worker honey bees were exposed to nominal test concentrations of 0.0 (untreated sucrose solution control), 87.84, 175.68, 351.36, 702.72, and 1405.44 µg product/bee, (equivalent to 0.0, 12.36, 24.72, 49.44, 98.88, and 197.75 µg azoxystrobin/bee and 0.0, 12.15, 24.30, 48.61, 97.21, and 194.43 µg prothioconazole/bee, respectively) dispersed in 50% aqueous sucrose solution, plus four nominal doses of a toxic reference (0.060, 0.090, 0.140, and 0.210 µg dimethoate/bee).

In the acute contact test, a topical solution was applied to the dorsal thorax of adult worker honey bees (2-µL droplets), with treatments of 0.0 (0.1% Triton X in deionised water) and 91.50, 183.00, 366.00, 732.00, and 1464.00 µg product/bee dispersed in 0.1% Triton-X solution (equivalent to 0.0, 12.87, 25.75, 51.50, 102.99, and 205.99 µg azoxystrobin/bee and 0.0, 12.66, 25.32, 50.63, 101.27, and 202.53 µg prothioconazole/bee, respectively), plus four doses of a toxic reference (0.080, 0.120, 0.180, and 0.270 µg dimethoate/bee).

The control and test-item treatment groups comprised five replicates of 10 bees each; the toxic-reference group comprised four replicates of 10 bees each. The acute oral test was extended to 96 hours because mortality in the test-item treatment groups increased by 10%, between 24 and 48 hours, while control mortality changed by ≤10%.

The 96-hour acute oral LD<sub>50</sub> value was calculated to be 339.19 µg product/bee (based on actual diet intake/consumption). The 96-h acute oral NOED and LOED values were 100.24 and 168.0 µg product/bee (based on actual consumption), respectively.

The 48-hour acute contact LD<sub>50</sub> value was estimated to be >1464.0 µg product/bee. The 48-hour acute contact NOED and LOED values were determined to be ≥1464.0 and >1464.0 µg product/bee, respectively.

For validation of the test system, the treatments with the toxic reference resulted in 24-hour LD<sub>50</sub> values of 0.11 and 0.17 µg dimethoate/bee, in the oral and contact tests, respectively. The control mortality was acceptable (0%) for both the acute oral and contact studies.

The study is considered acceptable and satisfies the guideline requirements for acute toxicity testing with honey bees (OECD 213 and 214 (1998) test guidelines).

## Materials and methods

### Test materials

#### *Test item*

Name:	CA3642 (Prothioconazole 150 g/L + Azoxystrobin 150 g/L SC)
Density:	1.1004 g/mL
Formulation type:	Suspension Concentrate (SC)
Batch no.:	A20026
Active substance (a.s.) 1:	Azoxystrobin 154.83 g/L or 14.07 w/w (analysed), 150 g/L (nominal)
Active substance (a.s.) 2:	Prothioconazole 152.23 g/L or 13.84 w/w (analysed), 150 g/L (nominal)
Appearance:	Off-white, odourless suspension
Expiry date of lot/batch:	September 2022
Storage conditions:	Keep at room temperature in a well-ventilated place

#### *Reference item*

Name:	BAS 152 65 I
Density:	1.062 g/mL (20°C)
Formulation type:	Emulsifiable concentrate (EC)
Batch no.:	10248664A
Active substance:	Dimethoate
a.s. content:	40.9% w/v or 409 g/L (analysed), 40.0% w/v (nominal)
Appearance:	Orange liquid
Expiry date of lot/batch:	16 Feb 2022
Storage conditions:	Between 5°C and 35°C, protected against moisture. Kept away from heat. Protected from direct sunlight. Protected against freezing.

#### *Adjuvant item*

Name:	Triton X-100
Density:	Not reported
Lot no.:	Not reported
Appearance:	Not reported

#### Test organism

Species:	European honey bee, <i>Apis mellifera</i> (Hymenoptera, Apidae)
Sub-species:	Spanish honey bee, <i>Apis mellifera iberiensis</i>
Age at study initiation:	Adult workers of similar age
Source:	Obtained from a commercial apiary (Eurofins Trialcamp S.L.U., Chella, València, Spain). The hive used in the test was adequately fed, healthy, disease-free, and queen-right. No chemical substances were used on the hive for at least one month prior to the test.
Feeding during test:	50% w/v sucrose solution
Acclimation:	Bees were randomly collected on the day before the experimental phase start and kept under test conditions

#### Test conditions

Test temperature:	25±2°C (actual: 25.0-25.4°C)
Relative humidity:	50-70% (actual: 56.4-59.0%)
Photoperiod:	Complete darkness, except during application and assessments

Test units (for both oral and contact tests) comprised well-ventilated, stainless-steel cages (base: 8.5 cm x 4.5 cm; height: 6.0 cm, approximately). The front side of the cages was equipped with a transparent pane to enable observation. The bottom of the cages consisted of perforated steel, which guaranteed sufficient air supply. The cages were lined with filter paper. Adult worker honey bees from the three colonies were randomly assigned to the treatment groups and were individually weighed before they were introduced to their test cages, one bee per cage.

Each test unit represented a replicate, with each test comprising one control group, five test-item treatment groups, and four reference-item treatment groups. The control and test-item treatment groups consisted of 50 test organisms (divided into 5 replicates, containing 10 bees each); the reference-item group contained 40 bees (4 replicates of 10 individuals).

#### Preparation of test solutions

The test and reference items were measured with a balance and calibrated micropipettes, respectively. The test-item solutions were prepared by mixing a defined amount of the test item with the required solvent. For the oral-toxicity test, the solvent was a 50% w/v aqueous sucrose solution. For the contact-toxicity test, a 0.1% Triton-X solution was used as a wetting agent. For the preparation of the test-item and toxic-reference treatments, the highest respective test-item treatments were used as stock solutions and diluted to produce the remaining treatments.

#### *Acute oral-toxicity test*

2-mL syringes were used as feeding devices; the tips of the syringes were cut off to provide the bees access to the feeding solutions. The feed volume was 200  $\mu\text{L}$ /replicate (corresponding to 20  $\mu\text{L}$ /bee); bees in one replicate shared the test solution and, thus, are assumed to have received similar doses (trophallaxis). The bees were starved 2 hours prior to treatment. The syringes of treated diet were provided for up to 6 hours, to ensure a sufficient intake. The feeders were then removed, and the bees were provided with a 50% w/v aqueous sucrose solution *ad libitum*. For dose verification, the amount of test solutions consumed was determined by weighing the feeders before and after feeding using a calibrated balance.

The nominal treatment doses were 0.0 (untreated diet), 87.84, 175.68, 351.36, 702.72, and 1405.44  $\mu\text{g}$  product/bee, (equivalent to 0.0, 12.36, 24.72, 49.44, 98.88, and 197.75  $\mu\text{g}$  azoxystrobin/bee and 0.0, 12.15, 24.30, 48.61, 97.21, and 194.43  $\mu\text{g}$  prothioconazole/bee, respectively). The toxic-reference treatment doses were 0.06, 0.09, 0.14, and 0.21  $\mu\text{g}$  dimethoate/bee.

Since the uptake of the treated sucrose solutions differed from the nominal doses, the results are based on measured consumption.

#### *Acute contact-toxicity test*

The test item treatment doses, obtained by dissolving test item in 0.1% Triton-X solution, were 0.0, 91.50, 183.00, 366.00, 732.00, and 1464.00  $\mu\text{g}$  product/bee, (equivalent to 0.0, 12.87, 25.75, 51.50, 102.99, and 205.99  $\mu\text{g}$  azoxystrobin/bee and 0.0, 12.66, 25.32, 50.63, 101.27, and 202.53  $\mu\text{g}$  prothioconazole/bee, respectively), plus four doses of a toxic reference (0.080, 0.120, 0.180, and 0.270  $\mu\text{g}$  dimethoate/bee). After being anaesthetised with  $\text{CO}_2$ , the test solutions were applied to the dorsal thorax of the bees (2- $\mu\text{L}$  droplets).

#### Assessments

The acute contact test lasted 48 hours, while the acute oral test was extended from 48 to 96 hours, since mortality increased by more than 10% after the first 24 hours in the test item group(s).

Mortality and behaviour were assessed by visual counting of dead honey bees per test unit, after 4, 24, and 48 h of exposure (plus at 72- and 96 hours for the oral test). Any behavioural abnormalities were observed. Mean mortality of the test-item and toxic-reference groups were corrected for mortality in the control group, using a modified Abbott's formula. Behaviour was quantitatively observed, according to the categories unaffected, affected (lacking coordination), apathetic (slow or no response to stimulation), cramping (abdominal or whole-body contractions), moribund (unable to walk, weak responses to stimuli), and vomiting. Behavioural data were not statistically analysed.

Mortality data were analysed with the statistical software R 3.3.0, and all statistical analyses were performed at 95% confidence level. The 24-h and 48-h  $\text{LD}_{50}$  values of the oral and contact tests could not be calculated, since mortality did not exceed 50% in any treatment group. Therefore, endpoints were empirically estimated. In the extended oral toxicity test the 72-hour and 96-hour  $\text{LD}_{50}$  values, and their corresponding 95% confidence limits (CLs) were calculated by probit analyses, using linear maximum-likelihood regressions. The 24-hour oral and contact  $\text{LD}_{50}$  values (including with 95% CLs) of the toxic reference item were both calculated by trimmed Spearman-Kärber procedures. The 24-, 48-, 72-, and 96-hour oral NOED values (for mortality) were determined by step-down Rao-Scott-Cochran-Armitage and step-down Cochran-Armitage test procedures. The 24-hour and 48-hour contact NOED values (for mortality) were determined by multiple sequentially rejective Fisher's tests, followed by Bonferroni-Holm corrections.

## **Results**

### *Biological results*

#### Mortality:

In the oral and contact tests, cumulative mortality of the test-item and toxic-reference treatment groups

were not corrected for control mortality, since there was zero mortality in the control groups.

*Acute oral toxicity:*

Mortality and behavioural abnormalities of honey bees exposed to CA3642 in the 96-hour acute oral toxicity test, are presented in table below.

**Table CP 10.3.1.1/01-01: The effects of exposure to CA3642 on the mortality and behaviour of *Apis mellifera*, in a 96-hour oral-toxicity test**

Treatment (µg product/bee)		Rep.	Mortality (no.)					Behaviour (no. affected bees/total bees)				
Nominal dose	Consumed dose		Time (hours)					Time (hours)				
			4	24	48	72	96	4	24	48	72	96
0.00 (control)	0.00	1	0	0	0	0	0	0/50	0/50	0/50	0/50	0/50
		2	0	0	0	0	0					
		3	0	0	0	0	0					
		4	0	0	0	0	0					
		5	0	0	0	0	0					
87.84	62.82	1	0	0	0	0	0	0/50	0/50	0/49	0/48	0/47
		2	0	0	0	1	1					
		3	0	0	0	0	1					
		4	0	0	1	1	1					
		5	0	0	0	0	0					
175.68	100.24	1	0	0	0	0	0	0/50	0/49	0/49	0/48	0/47
		2	0	0	0	0	0					
		3	0	0	0	0	0					
		4	0	0	0	1	2					
		5	0	1	1	1	1					
351.36	168.00	1	0	0	0	1	2	0/50	0/48	0/47	1/42	0/38
		2	0	0	1	2	2					
		3	0	1	1	2	2					
		4	0	1	1	3	4					
		5	0	0	0	1	2					
702.72	387.97	1	0	0	0	2	2	0/50	3/43	0/35	3/30	2/26
		2	0	2	5	6	7					
		3	0	2	3	5	5					
		4	0	3	4	5	6					
		5	0	3	3	5	6					
1405.44	454.70	1	0	5	9	9	10	0/50	8/44	1/29	3/24	2/19
		2	0	2	2	5	5					
		3	0	2	3	4	5					
		4	0	2	2	3	5					
		5	0	3	6	8	8					
0.06 µg dimethoate/bee		1	0	0	0	0	1	-	-	-	-	-
		2	0	0	0	0	0					
		3	0	0	0	0	0					
		4	0	0	0	0	0					
0.09 µg dimethoate/bee		1	0	0	0	0	0	-	-	-	-	-
		2	0	1	1	1	1					
		3	0	1	1	1	1					
		4	0	3	3	3	3					
0.14 µg dimethoate/bee		1	0	2	2	2	2	-	-	-	-	-
		2	0	0	0	1	2					
		3	0	2	4	4	4					
		4	0	1	1	1	1					
0.21 µg dimethoate/bee		1	0	6	6	7	7	-	-	-	-	-
		2	0	6	7	7	7					
		3	0	7	7	7	7					
		4	0	9	10	10	10					

Rep.: replicate (10 adult honey bees per replicate); no.: number.

The mean mortality and behavioural abnormalities, at 4-, 24- 48-, 72 and 96 hours after oral treatment of honey bees (*Apis mellifera*) with the product, CA3542, are reported in the table below.

**Table CP 10.3.1.1/01-02: Mean mortality of *Apis mellifera*, after 4, 24, 48, 72 and 96 hours of oral exposure to CA3642.**

Treatment (µg product/bee)			Mean cumulative mortality ± SD (%)					
ID	Nominal Dose	Consumed dose	4 hours	24 hours	48 hours	72 hours	96 hours	CV
C	0.00	0.00	0.00	0.00	0.00	0.00	0.00 ± 0.00	0.00
T1	87.84	62.82	0.00	0.00	2.00	4.00	6.00 ± 0.55	0.09
T2	175.68	100.24	0.00	2.00	2.00	4.00	6.00 ± 0.89	0.15
T3	351.36	168.00	0.00	4.00*	6.00*	18.00*	24.00 ± 0.89*	0.04
T4	702.72	387.97	0.00	20.00*	30.00*	46.00*	52.00 ± 1.92*	0.04
T5	1405.44	454.70	0.00	28.00*	44.00*	58.00*	66.00 ± 2.30*	0.03
R1	0.06 µg dimethoate/bee		0.00	0.00	0.00	0.00	2.50 ± 0.50	0.20
R2	0.09 µg dimethoate/bee		0.00	12.50	12.50	12.50	12.50 ± 1.26	0.10
R3	0.14 µg dimethoate/bee		0.00	12.50	17.50	20.00	22.50 ± 1.26	0.06
R4	0.21 µg dimethoate/bee		0.00	70.00	75.00	77.50	77.50 ± 1.50	0.02

\*Statistically significant differences in mortality, relative to the control group ( $p < 0.05$ , step-down Rao-Scott-Cochran-Armitage or step-down Cochran-Armitage tests). Mortality was not corrected, since there was zero mortality in the control group. SD: standard deviation; CV: coefficient of variation, at 96 h.

In the acute oral toxicity test, statistically significant increases in mortality compared to the control group, following exposure to CA3642 were observed between 24 and 96 hours, in the three-highest test-item treatment groups (T3, T4, and T5). After 96 hours of exposure, the actual consumed doses were 62.82, 100.24, 168.00, 387.97, and 454.70 µg product/bee, respectively, corresponding to a cumulative mean mortality of 0.00, 6.00, 6.00, 24.00, 52.00, and 66.00%, respectively.

The 96-hour acute oral LD<sub>50</sub> value was determined to be 339.01 µg product/bee (with 95% Confidence Limits (CLs) of 283.58 – 426.57 µg product/bee), equivalent to 47.73 µg azoxystrobin/bee (with 95% LCs of 39.90 – 60.02 µg a.s./bee) and 46.92 µg prothioconazole/bee (with 95% CLs of 39.23 – 59.01 µg a.s./bee) (based on mean measured consumption).

The 96-hour acute oral NOED value was determined to be 100.24 µg product/bee (equivalent to 14.10 µg azoxystrobin/bee and 13.87 µg prothioconazole/bee (based on mean measured consumption)).

The 24-hour oral LD<sub>50</sub> value (with 95% CLs) for the reference item was 0.11 (0.10 – 0.12) µg dimethoate/bee.

Behavioural abnormalities (i.e., bees lacking coordination) were observed between 24 and 96 hours. At 24 hours, there were 3 and 8 affected bees, with treatments T4 and T5, respectively; at 48 hours, there was only 1 affected bee (treatment T5); at 72 hours, there were 1, 3, and 3 affected bees, with treatments T3, T4, and T5 groups, respectively; and at 96 hours, there were 2 affected bees each in the T4 and T5 treatment groups.

#### *Acute contact toxicity:*

Mortality and behavioural abnormalities of honey bees exposed to CA3642 in the acute contact toxicity test, are presented in the tables below.

**Table CP 10.3.1.1/01-03: The effects of exposure to CA3642 on the mortality and behaviour of *Apis mellifera*, in a 48-hour acute contact toxicity test**

Treatment		Rep.	Mortality (no.)			Behaviour (no. affected bees/total bees)		
ID	Nominal dose (µg product/bee)		Time (hours)			Time (hours)		
			4	24	48	4	24	48
C	0.00 (control)	1	0	0	0	0/50	0/50	0/50
		2	0	0	0			
		3	0	0	0			
		4	0	0	0			
		5	0	0	0			
T1	91.50	1	0	0	0	0/50	0/50	0/50
		2	0	0	0			
		3	0	0	0			
		4	0	0	0			
		5	0	0	0			
T2	183.00	1	0	0	0	0/50	0/50	0/50
		2	0	0	0			
		3	0	0	0			
		4	0	0	0			
		5	0	0	0			
T3	366.00	1	0	0	0	0/50	0/49	0/48
		2	0	0	0			
		3	0	0	0			
		4	0	1	1			
		5	0	0	1			
T4	732.00	1	0	0	1	0/50	0/50	1/49
		2	0	0	1			
		3	0	0	0			
		4	0	0	0			
		5	0	0	0			
T5	1464.00	1	0	0	0	0/50	0/50	0/49
		2	0	0	0			
		3	0	0	0			
		4	0	0	0			
		5	0	0	1			
R1	0.08 µg dimethoate/bee	1	0	0	0	-	-	-
		2	0	0	0			
		3	0	1	1			
		4	0	1	1			
R2	0.12 µg dimethoate/bee	1	0	2	4	-	-	-
		2	0	5	7			
		3	0	2	8			
		4	0	3	6			
R3	0.18 µg dimethoate/bee	1	0	4	5	-	-	-
		2	0	8	8			
		3	0	7	7			
		4	0	6	7			
R4	0.27 µg dimethoate/bee	1	0	8	8	-	-	-
		2	1	8	8			
		3	0	4	4			
		4	3	7	7			

Rep.: replicate (10 adult honey bees per replicate); no.: number.

The mean mortality and behavioural abnormalities, at 4-, 24- and 48-hours after contact treatment of honey bees (*Apis mellifera*) are reported in the table below.

**Table CP 10.3.1.1/01-04: Mean mortality of *Apis mellifera*, after 4-, 24- and 48-hours of contact exposure to CA3642**

Application treatment		Mean cumulative mortality ± SD (%)			
ID	Nominal dose (µg product/bee)	4 h	24 h	48 h	CV
C	0.00	0.00	0.00	0.00 ± 0.00	0.00

<b>T1</b>	91.50	0.00	0.00	0.00 ± 0.00	0.00
<b>T2</b>	183.00	0.00	0.00	0.00 ± 0.00	0.00
<b>T3</b>	366.00	0.00	2.00	4.00 ± 0.55	0.14
<b>T4</b>	732.00	0.00	0.00	4.00 ± 0.55	0.14
<b>T5</b>	1464.00	0.00	0.00	2.00 ± 0.45	0.22
<b>R1</b>	0.08 µg dimethoate/bee	0.00	5.00	5.00 ± 0.58	0.12
<b>R2</b>	0.12 µg dimethoate/bee	0.00	30.00	62.50 ± 1.71	0.030
<b>R3</b>	0.18 µg dimethoate/bee	0.00	62.50	67.50 ± 1.26	0.02
<b>R4</b>	0.27 µg dimethoate/bee	10.00	67.50	67.50 ± 1.89	0.03

There were no significant differences in mortality, relative to the control group. Mortality was not corrected, since there was zero mortality in the control group. SD: standard deviation; CV: coefficient of variation, at 48 hours.

In the acute contact toxicity test, there were no statistically significant increases in mortality, compared to the control group, following 48-hours of exposure to CA3642. The cumulative mean mortality after 48 hours was 0.00, 0.00, 0.00, 4.00, 4.00, and 2.00%, at dose rates of 0.00, 91.50, 183.00, 366.00, 732.00, and 1464.00 µg product/bee, respectively.

The 48-hour acute contact LD<sub>50</sub> value was estimated to be >1464.0 µg product/bee, equivalent to >205.99 µg azoxystrobin/bee and >202.53 µg prothioconazole/bee, respectively, the highest dose tested (based on nominal doses).

The corresponding 48-hour acute contact NOED value was estimated to be ≥1464.00 µg product/bee equivalent to ≥205.99 µg azoxystrobin/bee and ≥202.53 µg prothioconazole/bee, respectively, the highest dose tested (based on nominal doses).

Only 1 bee was observed with behavioural abnormalities (apathy), in T4 treatment group (732 µg product/bee) after 48 hours.

The 24-hour contact LD<sub>50</sub> value (with 95% CLs) for the reference item was 0.17 (0.15 – 0.19) µg dimethoate/bee.

## Validity

All validity criteria were met, in accordance with the OECD 213 and 214 (1998) test guidelines:

- The mean mortality in the control was ≤10% at the end of both tests (actual values: 0.00% mortality in both oral and contact tests, respectively).
- 24-hour LD<sub>50</sub> value of the toxic reference was between 0.10-0.35 for the oral test (actual value: 0.11 µg dimethoate/bee) and 0.10-0.30 µg dimethoate/bee in the contact test (actual value: 0.17 µg dimethoate/bee).

## Conclusion

The acute oral and contact toxicity of CA3642 to honey bees (*Apis mellifera*) was evaluated in a GLP laboratory test, following OECD 213 and 214 (1998) test guidelines.

The 96-hour acute oral LD<sub>50</sub> value was determined to be 339.19 µg product/bee (with 95% Confidence Limits (CLs) of 283.58 – 426.57 µg product/bee), equivalent to 47.73 µg azoxystrobin/bee (with CLs of 39.90 – 60.02 µg a.s./bee) and 46.92 µg prothioconazole/bee (with CLs of 39.23 – 59.01 µg a.s./bee), respectively.

The corresponding 96-hour acute oral NOED value was determined to be 100.24 µg product/bee, equivalent to 14.10 µg azoxystrobin/bee and 13.87 µg prothioconazole/bee, respectively.

The 48-hour acute contact LD<sub>50</sub> value was estimated to be >1464.00 µg product/bee, equivalent to >205.99 µg azoxystrobin/bee and >202.53 µg prothioconazole/bee, respectively, the highest dose tested.

The corresponding 48-hour acute contact NOED value was estimated to be  $\geq 1464 \mu\text{g}$  product/bee, equivalent to  $\geq 205.99 \mu\text{g}$  azoxystrobin/bee and  $\geq 202.53 \mu\text{g}$  prothioconazole/bee, respectively, the highest dose tested (based on nominal doses)

This study is considered acceptable and valid.

## Study 2

Comments of zRMS:	<p>The study was conducted in line with OECD 246 and 247 (2017) with minor deviations:</p> <p>A topical application of <math>2 \mu\text{L}</math> of the test solution(s) was applied onto the thorax. This is a higher volume than the guideline recommendation of <math>1 \mu\text{L}</math>, due to the preparation of the required solutions (high concentrations). It has been validated with a non GLP pre-test and it is deemed unlikely to adversely affect the outcome of the study.</p> <p>Since analytical verification of the test concentrations confirm that individual measured concentrations of daily feeding solutions were between 94% and 108% of nominal values (i.e. within the recommended range of 80% to 120%), biological endpoints are expressed as nominal concentrations/doses.</p> <p>All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>The 96-h acute oral <math>\text{LD}_{50}</math> (mortality) = <math>989.70 \mu\text{g}</math> product/bumble bee, equivalent to <math>139.25 \mu\text{g}</math> azoxystrobin/bumble bee and <math>136.92 \mu\text{g}</math> prothioconazole/bumble bee, respectively, based on the actual intake/consumption of the feeding solutions (nominal).</p> <p>The 96-h acute oral NOED (mortality) = <math>348.67 \mu\text{g}</math> product/bumble bee, equivalent to <math>49.06 \mu\text{g}</math> azoxystrobin/bumble bee and <math>48.23 \mu\text{g}</math> prothioconazole/bumble bee, respectively, based on the actual intake/consumption of the feeding solutions (nominal).</p> <p>The 48-h acute contact <math>\text{LD}_{50}</math> (mortality) <math>&gt; 1464.0 \mu\text{g}</math> product/bumble bee, equivalent to <math>&gt; 205.99 \mu\text{g}</math> azoxystrobin/bumble bee and <math>&gt; 202.53 \mu\text{g}</math> prothioconazole/bumble bee, respectively (based on nominal concentrations).</p> <p>The 48-h acute contact NOED (mortality) <math>\geq 1464.0 \mu\text{g}</math> product/bumble bee, equivalent to <math>\geq 205.99 \mu\text{g}</math> azoxystrobin/bumble bee and <math>\geq 202.53 \mu\text{g}</math> prothioconazole/bumble bee, respectively (based on nominal concentrations).</p>
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Reference:	KCP 10.3.1.1/02
Report	<p>CA3642 (Azoxystrobin 150 g/L + Prothioconazole 150 g/L SC): Acute oral and contact Toxicity to the Bumblebee <i>Bombus terrestris</i> L., under Laboratory Conditions</p> <p>Gimeno, I., 2022, report no. S21-04083</p>
Guideline(s):	Yes. OECD 246 and 247 (2017)
Deviations:	Yes. A topical application of $2 \mu\text{L}$ of the test solution(s) was applied onto the thorax. This is a higher volume than the guideline recommendation of $1 \mu\text{L}$ , due to the preparation of the required solutions (high concentrations). It has been validated with a non GLP pre-test and it is deemed unlikely to adversely affect the outcome of the study
GLP:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability:	Yes
Duplication	No



(if vertebrate study)	
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## Executive summary

In a 48-hour laboratory study, the acute oral and contact toxicity of the product, CA3642 (analysed active substances: 14.07% w/w azoxystrobin and 13.84% w/w prothioconazole), to the buff-tailed bumble bee, *Bombus terrestris*, was evaluated in dose-response tests, in accordance with OECD 246 and OECD 247 test guidelines. Since analytical verification of the lowest and highest test concentrations confirmed that measured concentrations of daily feeding solutions were between 80% and 120% of nominals, biological endpoints are reported based on nominal concentrations.

In the acute oral test, adult worker bumble bees were exposed to nominal doses of 0.00 (untreated sucrose solution control), 87.84, 175.68, 351.36, 702.72, and 1405.44 µg product/bee (equivalent to 12.36, 24.72, 49.44, 98.88, and 197.75 µg azoxystrobin/bee and 12.15, 24.30, 48.61, 97.21, and 194.43 µg prothioconazole/bee, respectively), plus one nominal dose of a toxic reference (3.0 µg dimethoate/bee).

In the acute contact test, a topical solution was applied to the dorsal thorax of the bees (2-µL droplets), with treatments of 0.00 (0.1% Triton X in deionised water), 91.50, 183.00, 366.00, 732.00, and 1464.00 µg product/bee, (equivalent to 12.87, 25.75, 51.50, 102.99, and 205.99 µg azoxystrobin/bee and 12.66, 25.32, 50.63, 101.27, and 202.53 µg prothioconazole/bee, respectively), plus one dose of a toxic reference (10.0 µg dimethoate/bee).

The 96-hour acute oral LD<sub>50</sub> (mortality) value was determined to be 989.70 µg product/bumble bee, equivalent to 139.25 µg azoxystrobin/bumble bee and 136.92 µg prothioconazole/bumble bee, respectively, based on the actual intake/consumption of the feeding solutions (nominal). The 96-hour acute oral NOED (mortality) value was determined to be 348.67 µg product/bumble bee, equivalent to 49.06 µg azoxystrobin/bumble bee and 48.23 µg prothioconazole/bumble bee, respectively, based on the actual intake/consumption of the feeding solutions (nominal).

The 48-hour acute contact LD<sub>50</sub> (mortality) value was estimated to be >1464.0 µg product/bumble bee, equivalent to >205.99 µg azoxystrobin/bumble bee and >202.53 µg prothioconazole/bumble bee, respectively (based on nominal concentrations). The 48-hour acute contact NOED (mortality) value was estimated to be ≥1464.0 µg product/bumble bee, equivalent to ≥205.99 µg azoxystrobin/bumble bee and ≥202.53 µg prothioconazole/bumble bee, respectively (based on nominal concentrations).

For validation of the test system, treatment with the toxic reference resulted in 100.0% and 76.67% mortality, in the oral and contact tests, respectively.

The study is considered acceptable and satisfies the OECD 246 and 247 (2017) test guideline requirements.

## Materials and methods

### Test materials

#### Test item

Name:	CA3642 (Prothioconazole 150 g/L + Azoxystrobin 150 g/L SC)
Density:	1.1004 g/mL
Formulation type:	Suspension Concentrate (SC)
Batch no.:	A20026
Active substance (a.s.) 1:	Azoxystrobin 154.83 g/L or 14.075 w/w (analysed), 150 g/L (nominal)
Active substance (a.s.) 2:	Prothioconazole 152.23 g/L or 13.84% w/w (analysed), 150 g/L (nominal)
Appearance:	Off-white, odourless suspension
Expiry date of lot/batch:	September 2022
Storage conditions:	Keep at room temperature in a well-ventilated place

#### *Reference item*

Name:	BAS 152 65 I
Density:	1.062 g/mL (20°C)
Formulation type:	Emulsifiable concentrate (EC)
Batch no.:	10248664A
Active substance:	Dimethoate
a.s. content:	40.9% w/v (analysed) or 40.0% w/v (nominal)
Appearance:	Orange liquid
Expiry date of lot/batch:	16 Feb 2022
Storage conditions:	Between 5°C and 35°C, protected against moisture. Kept away from heat. Protected from direct sunlight. Protected against freezing.

#### *Adjuvant item*

Name:	Triton X-100
Density:	1.06 g/mL (nominal)
Lot no.:	9N014730
Appearance:	Clear, colourless, viscous liquid

#### Test organism

Species:	Buff-tailed bumble bee, <i>Bombus terrestris</i> L. (Hymenoptera, Apidae)
Age at study initiation:	Young, medium-sized, adult workers of similar age
Source:	Obtained from a commercial apiary (Biomip Biological Quality S.L., Campohermoso, Almería, Spain). The 13 hives used in the test were adequately fed, healthy, disease-free, and queen-right. No chemical substances were used on the hive for at least one month prior to the test.
Feeding during test:	50% w/v sucrose solution
Acclimation:	Bees were randomly collected on the day before the experimental phase start and kept under test conditions

#### Test conditions

Test temperature:	25 ± 2°C (actual: 24.99 – 25.38°C (oral and contact tests))
Relative humidity:	60 ± 20% (actual: 53.4 – 64.4.0% (oral test), 54.9 – 59.2% (contact test))
Photoperiod:	Complete darkness, except during application and assessments

Bumble bees were kept individually in Nicot® cages and fed via 1-mL syringes (with the tips cut off before use). The syringes were inserted into plastic sockets and kept in position with a piece of hose, which prevented the syringes from sticking into the Nicot® cages. These test units were slightly inclined to ensure that the sugar solutions flowed to the opening of the syringes. Individual cages were randomly placed next to each other.

Each test unit represented a replicate, with each test comprising one control group, five test-item treatment groups, and one toxic-reference treatment group. The treatment groups consisted of 35 (oral test) and 30 (contact test) replicates of 1 bee each. The control and reference item treatment groups were exclusively used for this study.

#### Preparation of test solutions

The test and reference items were measured with a balance and/or measured with calibrated micropipettes. The test-item solutions were prepared by mixing a defined amount of the test item with the required solvent. For the oral-toxicity test, the solvent used was a 50% w/v aqueous sucrose solution. For the contact-toxicity test, 0.1% Triton-X solution was used as the solvent and wetting agent.

For the preparation of the test-item and toxic-reference treatments, the highest respective test-item treatments were used as stock solutions and diluted to produce the remaining treatments. For the acute oral toxicity test, the highest test-item feeding solution was prepared by dissolving 0.7029 g test item in 20 mL

50% w/v aqueous sucrose solution. The lower test-item doses were prepared diluting 5.0 mL, 2.5 mL, 1.25 mL, and 625 µL of the highest test-item feeding solution in 10 mL 50% w/v aqueous sucrose solution. The concentrations of the diets were 0.00, 2.20, 4.39, 8278, 17.57, and 35.14 mg product/mL (corresponding to 0.00, 0.31, 0.62, 1.24, 2.47, and 4.94 mg azoxystrobin/mL and 0.00, 0.30, 0.61, 1.21, 2.43, and 4.86 mg prothioconazole/mL, respectively). The toxic-reference feeding solution was prepared by dissolving 0.92 µL BAS 125 65 I in 5 mL 50% w/v aqueous sucrose solution.

For the acute contact toxicity test, the highest test-item topical application solution was prepared by dissolving 7.32 g test item in 20 mL 0.1% Triton X solution. The lower test-item application solutions were prepared diluting 5.0 mL, 2.5 mL, 1.25 mL, and 625 µL of the highest test-item feeding solution in 10 mL 0.1% Triton X solution. The concentrations of the application solutions were 45.75, 91.50, 183.00, 366.00, and 732.00 mg product/mL (corresponding to 6.44, 12.87, 25.75, 51.50, and 102.99 mg azoxystrobin/mL and 6.33, 12.66, 25.32, 50.63, and 101.27 prothioconazole/mL, respectively). The toxic-reference application solution was prepared by dissolving 30.60 µL BAS 125 65 I in 5 mL 0.1% Triton X solution.

#### *Acute oral toxicity test:*

1-mL syringes were used as feeding devices; the tips of the syringes were cut off to provide the bees access to the feeding solutions. The feed volume was 40 µL/replicate (corresponding to 40 µL/bee). The bees were starved 2 hours prior to being offered the treated diet, to which they were provided for up to 4 hours, to ensure a sufficient intake. The treatment syringes were then removed and replaced with syringes filled with untreated 50% w/v aqueous sucrose solution, provided to the bees *ad libitum*. For dose verification, the amount of test solutions consumed was determined by weighing the feeders before and after feeding using a calibrated balance. Individuals that did not consume at least 80% of the mean consumption of the respective treatment group, within the four hours of exposure, were discarded from the test, for the calculation of the endpoints.

The nominal treatment doses were 0.00 (untreated diet), 87.84, 175.68, 351.36, 702.72, and 1405.44 µg product/bee (equivalent to 0.0, 12.36, 24.72, 49.44, 98.88, and 197.75 µg azoxystrobin/bee and 0.0, 12.15, 24.30, 48.61, 97.21, and 194.43 µg prothioconazole/bee, respectively). The toxic-reference treatment doses were 0.06, 0.09, 0.14, and 0.21 µg dimethoate/bee.

#### *Acute contact-toxicity test*

After being anesthetized with dry ice, 4 µL<sup>7</sup> of the corresponding test solution was applied individually to the dorsal side of the thorax of each bumble bee, using a calibrated micropipette. Treated bumble bees were returned to their individual test units and provided with aqueous 50% w/v sucrose solution *ad libitum*. All individuals recovered from the anesthetic sufficiently, with no adverse behaviour reported.

The nominal application concentrations were 0.00 (0.1% Triton X in deionised water), 91.50, 183.00, 366.00, 732.00, and 1464.00 µg product/bee (equivalent to 12.87, 25.75, 51.50, 102.99, and 205.99 µg azoxystrobin/bee and 12.66, 25.32, 50.63, 101.27, and 202.53 µg prothioconazole/bee, respectively), plus one concentration of a toxic reference (10.0 µg dimethoate/bee).

#### Assessments

Mortality and behaviour were assessed by visual counting of dead bumble bees per test unit, after 4, 24, and 48 hours of exposure (and after 72 and 96 hours in the oral test). Mean mortality of the test-item and toxic-reference groups were corrected for mortality in the control group, using a modified Abbott's formula. Behavioural abnormalities were evaluated at each observation interval for the test item group, according to the categories: unaffected, affected (bees were still upright and attempting to walk, but showing signs of reduced coordination or hyperactivity), and moribund (unable to walk, weak responses to stimuli).

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<sup>7</sup> The 4-µL droplet was applied, instead of a 2-µL droplet, because a higher volume ensures a more reliable dispersion of the application solution. Test facility experience has demonstrated that higher volumes are suitable and no adverse effects on the outcome of the study are to be expected.

Behavioural data were not statistically analysed and were not recorded for the toxic reference group.

Mortality data were analysed with the statistical software ToxRatPro Version 3.3.0, and all statistical analyses were performed at 95% confidence level. The 24-, 48-, 72-, and 96-hour NOED values for the oral test were determined by step-down Cochran-Armitage tests. The 24- and 48-hour NOED values for the contact test were determined by a multiple sequentially rejective Fisher's tests, followed by Bonferroni-Holm corrections. For the oral test, the 48-, 72-, and 96-hour LD<sub>50</sub> values, with corresponding 95% confidence limits (CLs), were determined by the trimmed Spearman-Kärber tests. However, the 24-h oral and contact LD<sub>50</sub> values could not be calculated since no test-item dose caused >50% mortality. Therefore, these values were empirically estimated from the results. Additionally, the normalised widths (NWs) of the 95% CLs were calculated for quantifying the reliability of the determined LD<sub>50</sub> values, when applicable. LD<sub>50</sub> values with NW <1 were considered acceptable.

The concentration of the test solutions was confirmed by analytical verification. Samples of the lowest and highest (T1 and T5 treatments) of the oral treated and contact solutions were taken directly after preparation.

## Results

### Analytical results

The HPLC -MS/MS method for the determination of prothioconazole and azoxystrobin in 50% aqueous sucrose solution and 0.1% Triton X solution was validated with regards to specificity, linearity, accuracy and precision in accordance with guideline SANTE/2020/12830 rev.1 24/02/2021. Specificity in 50% aqueous sucrose solution and 0.1% Triton X solution was demonstrated by the absence of significant interference above 30% of the LOQ in control and blank samples. For 50% aqueous sucrose solution, the linearity of the method was demonstrated using matrix-matched calibration standard. The analytical method was shown to be linear ( $r > 0.99$ ) over two ranges from 8.1 to 70 ng/mL and from 4 to 26 ng/mL for both prothioconazole and azoxystrobin. For 0.1% Triton X solution, the linearity of the method was demonstrated using solvent calibration standard. The analytical method was shown to be linear ( $r > 0.99$ ) over the range from 8.1 to 70 ng/mL for prothioconazole and from 4 to 30 ng/mL for azoxystrobin. Accuracy was confirmed with recovery of spiked samples at relevant concentrations of test item in 50% aqueous sucrose solution (200 (LOQ) and 46000 mg test item/L) and in 0.1% Triton X solution (2000 (LOQ) and 47700 mg test item/L). Mean recoveries for 50% aqueous sucrose solution were 89 and 103% at 200 and 46000 mg test item/L respectively (i.e., within 70-110%). Mean recoveries for 0.1% Triton X solution were 96 and 101% at 2000 and 47700 mg test item/L respectively (i.e., within 70-110%). Precision was confirmed. The relative standard deviation for 50% aqueous sucrose solution ( $n = 5$  for each fortification level) was 4% for both fortification level (i.e., within the guideline limit of  $\leq 20\%$ ). The relative standard deviation for 0.1% Triton X solution ( $n = 5$  for each fortification level) was 3% for both fortification level (i.e., within the guideline limit of  $\leq 20\%$ ). The limit of quantification (LOQ) for 50% aqueous sucrose solution was 200 mg test item/L (corresponding to 28.1 mg azoxystrobin/L and 27.7 mg prothioconazole/L). The limit of detection (LOD) was defined at 8 mg/L for both analytes. The limit of quantification (LOQ) for 0.1% Triton X solution was 2000 mg test item/L (corresponding to 281 mg azoxystrobin/L and 277 mg prothioconazole/L). The limit of detection (LOD) was defined at 80 mg/L for azoxystrobin and 81 mg/L for prothioconazole.

**Table CP 10.3.1.1/02-01: Nominal and measured concentrations of azoxystrobin and prothioconazole from sampled feeding solutions (lowest and highest concentrations)**

Sample solution	Active substance	Concentration (mg a.s./mL)		Recovery (% nominal)
		Nominal	Analysed	
50% w/v aqueous sucrose solution	Azoxystrobin	0.31	0.29	94
		4.94	4.89	99
	Prothioconazole	0.30	0.266	88
		4.86	4.63	95
0.1% Triton-X aqueous solution	Azoxystrobin	3.22	3.04	94
		51.50	52.00	101

	Prothioconazole	3.17	3.42	108
		50.63	51.40	102

Since analytical verification of the test concentrations confirm that individual measured concentrations of daily feeding solutions were between 94% and 108% of nominal values (i.e. within the recommended range of 80% to 120%), biological endpoints are expressed as nominal concentrations/doses.

### Biological results

#### *Mortality*

In the acute oral and contact toxicity tests, cumulative mortality of the test-item and toxic-reference treatment groups were not corrected for control mortality, since there was zero mortality in the control groups.

#### *Acute oral toxicity:*

A summary of the cumulative mortality of the bumble bee (*Bombus terrestris*), following exposure to CA3642, *via* the oral route, is presented in table below.

**Table CP 10.3.1.1/02-02: Mean mortality of *Bombus terrestris*, after 4, 24, 48, 72 and 96 hours of oral exposure to CA3642**

Treatment				Mean cumulative mortality ± SD (%)					
Test item	ID	Dose (µg product/bee)							
		Nominal	Measured intake	4 hours	24 hours	48 hours	72 hours	96 hours	CV (%)
CA3642	C	0.00	0.00	0.00	0.00	0.00	0.00	0.00 ± 0.00	-
	T1	87.84	83.37	0.00	0.00	0.00	0.00	2.86 ± 16.9	591.61
	T2	175.68	172.41	0.00	0.00	0.00	0.00	0.0 ± 0.0	-
	T3	351.36	348.67	0.00	0.00	0.00	0.00	2.94 ± 17.15	583.1
	T4	702.72	690.91	0.00	0.00	0.00	11.76*	14.71 ± 35.95*	244.45
	T5	1405.44	1348.18	0.00	30.30*	51.52*	72.73*	75.76 ± 43.52*	57.45
Dimethoate	R	3.00	2.85	70.00	100.00	100.00	100.00	100.00 ± 0.00	0.00

\*Statistically significant differences in mortality, relative to the control group (step-down Cochran-Armitage tests). SD: standard deviation; CV: coefficient of variation; Dash (-): indicates that the coefficient of variation could not be calculated.

In the 96-hour acute oral-toxicity test, statistically significant increases in mean cumulative mortality, relative to the control group, following exposure to CA3642, were observed between 24 and 96 hours, in the two highest test treatment groups (T4 and T5), (based on measured/consumed doses). The acute oral 96-hour LD<sub>50</sub> value was determined to be 989.70  $\mu$ g product/bumble bee (with 95% CLs of 858.08 – 1141.49  $\mu$ g product/bumble bee), equivalent to 139.25  $\mu$ g azoxystrobin and 136.92  $\mu$ g prothioconazole, respectively). The corresponding acute oral 96-hour NOED value was determined to be 348.67  $\mu$ g product/bumble bee (equivalent to 49.06  $\mu$ g azoxystrobin and 48.23  $\mu$ g prothioconazole, respectively). Acute oral toxicity endpoints are expressed as nominal doses (based on the actual intake/consumption of the feeding solutions).

Individuals with behavioural abnormalities (i.e., “affected” individuals) were recorded 72 hours after start of the test, in the three highest product treatment groups. At the end of the 96-hour test period, abnormalities in individuals were recorded in the two highest product treatment groups.

#### *Acute contact toxicity*

A summary of the cumulative mortality of the bumble bee (*Bombus terrestris*), following exposure to CA3642, *via* the contact route, is presented in table below.

**Table CP 10.3.1.1/02-03: Mean mortality of *Bombus terrestris*, after 48 hours of contact exposure to CA3642**

Treatment			Mean cumulative mortality ± SD (%)			
Test item	ID	Dose (µg product/bee)	4 hours	24 hours	48 hours	CV (%)
CA3642	C	0	0.0	0.0	0.0 ± 0.0	-
	T1	87.84	0.0	0.0	0.0 ± 0.0	-
	T2	175.68	0.0	0.0	0.0 ± 0.0	-
	T3	351.36	0.0	0.0	0.0 ± 0.0	-
	T4	702.72	0.0	0.0	0.0 ± 0.0	-
	T5	1405.44	0.0	3.33	3.33 ± 18.26	547.72
Dimethoate	R	10.0	3.33	50.0	76.67 ± 43.02	56.11

Statistical analysis: Multiple sequentially rejective Fisher's test, followed by Bonferroni-Holm corrections (no statistically significant differences in mortality identified). SD: standard deviation; CV: coefficient of variation at 48 hours; Dash (-): The coefficient of variation could not be calculated.

In the acute contact toxicity test, there were no statistically significant changes in mean cumulative mortality, compared to the control group, following 48 hours of exposure to CA3642. No individuals with symptoms of behavioural abnormalities were recorded. Since bumble bee mortality did not reach ≤50% at the end of the test in any treatment group, the 48-hour acute contact LD<sub>50</sub> value was estimated to be >1464.00 µg product/bumble bee, equivalent to >205.99 µg azoxystrobin/bee and >202.53 µg prothioconazole/bee, respectively. The corresponding 48-hour acute contact NOED value was estimated to be ≥1464.00 µg product/bumble bee, the highest dose tested.

Acute contact toxicity endpoints are based on nominal concentrations.

## Validity

All validity criteria were met, in accordance with the OECD 246 and 247 (2017) test guidelines:

- Mean mortality in the control group was ≤10% at the end of both tests (actual values: 0.00% mortality in both oral and contact tests, respectively).
- The toxic reference group mortality was >50% (actual values: 76.67% and 96.67% mortality in oral and contact tests, respectively).

## Conclusion

The acute oral and contact toxicity of CA3642 to bumble bees (*Bombus terrestris*) was evaluated in accordance with the OECD 246 and 247 (2017) test guidelines, under laboratory conditions, over 96 and 48 hours, respectively.

The 96-hour acute oral LD<sub>50</sub> (mortality) value: = 989.70 µg product/bumble bee, equivalent to 139.25 µg azoxystrobin/bumble bee and 136.92 µg prothioconazole/bumble bee, respectively, based on the actual intake/consumption of the feeding solutions (nominal).

The 96-hour acute oral NOED (mortality) value: = 348.67 µg product/bumble bee, equivalent to 49.06 µg azoxystrobin/bumble bee and 48.23 µg prothioconazole/bumble bee, respectively, based on the actual intake/consumption of the feeding solutions (nominal).

The 48-hour acute contact LD<sub>50</sub> (mortality) value: >1464.0 µg product/bumble bee, equivalent to >205.99 µg azoxystrobin/bumble bee and >202.53 µg prothioconazole/bumble bee, respectively (based on nominal concentrations). The 48-hour acute contact NOED (mortality) value: ≥1464.0 µg product/bumble bee, equivalent to ≥205.99 µg azoxystrobin/bumble bee and ≥202.53 µg prothioconazole/bumble bee, respectively (based on nominal concentrations).

This study is considered acceptable and valid.

### A 2.3.1.1.1 KCP 10.3.1.1.1 Acute oral toxicity to bees

See Section KCP 10.3.1.1, studies 1 and 2.

### A 2.3.1.1.2 KCP 10.3.1.1.2 Acute contact toxicity to bees

See Section KCP 10.3.1.1, studies 1 and 2.

### A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

#### Study 1

Comments of zRMS:	<p>The study was conducted in line with OECD 245 with no deviation.</p> <p>The concentrations of the active substances in the applied test item feeding solutions were within the required range of <math>\pm 20</math> % of the nominal concentrations.</p> <p>All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>LDD<sub>50</sub> = 50.21 µg CA3642/bee/day (equivalent to 7.06 µg azoxystrobin/bee/day and 6.95 µg prothioconazole/bee/day).</p> <p>NOEDD = 35.75 µg CA3642/bee/day (equivalent to 5.03 µg azoxystrobin/bee/day and 4.95 µg prothioconazole/bee/day).</p> <p>LC<sub>50</sub> = 3487.37 mg CA3642/kg diet (equivalent to 490.67 mg azoxystrobin/kg diet and 482.65 mg prothioconazole/kg diet).</p> <p>NOEC = 2291.67 mg CA3642/kg diet (equivalent to 322.44 mg azoxystrobin/kg diet and 317.17 mg prothioconazole/kg diet).</p>
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Reference:	KCP 10.3.1.2/01
Report	<p>CA3642 (Azoxystrobin 150 g/L + Prothioconazole 150 g/L SC): Honey Bee (<i>Apis mellifera</i> L.) Chronic Oral Toxicity Test (10-Day Feeding) under Laboratory Conditions</p> <p>Gimeno, I., 2022, report no. S21-04081</p>
Guideline(s):	Yes. OECD 245 (2017)
Deviations:	No
GLP:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability:	Yes
Duplication (if vertebrate study)	No

#### Executive summary

A 10-day chronic oral toxicity study of the product, CA3642 (analysed active substances: 14.07% w/w azoxystrobin and 13.84% w/w prothioconazole), on the adult European honey bee (*Apis mellifera*), was conducted as a dose-response test at nominally 0.00 (control; untreated diet), 165.77, 397.86, 954.86, 2291.67, and 5500.00 mg CA3643/kg diet, equivalent to 23.32, 55.98, 134.35, 322.44, and 773.85 mg azoxystrobin/kg diet and 22.94, 55.06, 132.15, 317.17, and 761.20 mg prothioconazole/kg diet, respectively. The reference item was provided at a concentration of 0.90 mg dimethoate/kg diet. The analysed active substance content was determined to be within  $\pm 20\%$  of the nominal value in the highest

and lowest concentrations in the feeding solutions, therefore the endpoints were calculated using the nominal concentrations and doses (but accounting for actual mean intake/consumption).

The 10-day  $LC_{10/20}$  values were both estimated to be >2291.67 mg CA3642/kg diet, corresponding to >322.44 mg azoxystrobin/kg diet and >317.17 mg prothioconazole/kg diet. The 10-day  $LC_{50}$ -value was determined to be 3487.37 mg CA3642/kg diet (with 95% Confidence Limits (CLs) of 3116.16 – 3902.80 mg CA3642/kg diet), corresponding to 490.67 mg azoxystrobin/kg diet (with 95% CLs of 438.44 – 549.12 mg a.s./kg diet) and 482.65 mg prothioconazole/kg diet (with 95% CLs of 431.28 – 540.15 mg a.s./kg diet) (based on nominal concentrations).

The 10-day  $LDD_{10/20}$  values were both estimated to be >35.75 µg CA3642/bee/day, corresponding to >5.03 µg azoxystrobin/bee/day and >4.95 µg prothioconazole/bee/day. The 10-day  $LDD_{50}$  value was calculated to be 50.21 µg CA3642/bee/day (with 95% Confidence Limits (CLs) of 45.95 – 54.88 µg CA3642/bee/day), corresponding to 7.06 µg azoxystrobin/bee/day (with 95% Confidence Limits (CLs) of 6.47 – 7.72 µg azoxystrobin/bee/day) and 6.95 µg prothioconazole/bee/day (with 95% Confidence Limits (CLs) of 6.36 – 7.60 µg prothioconazole/bee/day) (based on nominal concentrations).

The 10-day NOEC and NOEDD (mortality) values were determined to be 2291.67 mg CA3642/kg diet and 35.75 µg CA3642/bee/day, respectively; equivalent to 322.44 mg azoxystrobin/kg diet and 5.03 µg azoxystrobin/bee/day; and 317.17 mg prothioconazole/kg diet and 4.95 µg prothioconazole/bee/day, respectively (based on nominal concentrations).

This study satisfies the OECD 245 (2017) test-guideline requirements for a chronic oral honey bee study and is considered acceptable.

## Materials and methods

### Test materials

#### *Test item*

Name:	CA3642 (prothioconazole 150 g/L + azoxystrobin 150 g/L SC)
Density:	1.1004 g/mL
Formulation type:	Suspension Concentrate (SC)
Batch no.:	A20026
Active substance (a.s.) 1:	Azoxystrobin 154.83 g/L, corresponding to 14.07 w/w (analysed), 150 g/L (nominal)
Active substance (a.s.) 2:	Prothioconazole 152.23 g/L, corresponding to 13.84% w/w (analysed), 150 g/L (nominal)
Appearance:	Off-white, odourless suspension
Expiry date of lot/batch:	September 2022
Storage conditions:	Keep at room temperature in a well-ventilated place

#### *Reference item*

Name:	BAS 152 65 I
Active substance:	Dimethoate
Purity:	412 g dimethoate/L (analysed)
Batch no.:	10248664A
Appearance:	Orange liquid
Expiry date:	12 May 2022
Storage conditions:	Kept cool and away from heat, protected from moisture and direct sunlight.

### Test organism

Species:	European honey bee, subspecies <i>Apis mellifera iberiensis</i> (Hymenoptera, Apidae),
Age at study initiation:	<48-hour-old adult workers



Source:	Obtained from healthy beehives located in a commercial apiary near Eurofins Trialcamp facilities (Chella, València, Spain), where bees foraged on wildflowers
Feeding during test:	50% w/v sucrose solution <i>ad libitum</i> (with untreated controls or test concentrations)
Acclimation:	On day prior to start of study, under test conditions.

#### Test conditions

Test temperature:	33°C ± 2°C (actual: 32.4-32.8°C (acclimatisation period), 31.6-33.3°C (test period))
Relative humidity:	50-70% (actual: 52.4-70.1% (acclimatisation period), 42.7-69.8% (test period) – deviations in relative humidity (outside the range of 50-70%) were short-term (<2 hours))
Photoperiod:	Continuous darkness, except during feeding and assessments.

Two days before the beginning of the test, frames with capped cells were transferred from healthy beehives to a bioclimatic chamber. One day prior to the beginning of the test, the bees were randomly collected directly from the frames, introduced into the test units, and kept under test conditions until the beginning of the test. The test comprised seven treatment groups: one control, five test-item groups, and one reference-item group. Each treatment group consisted of 50 bees, divided into five replicates of 10 bees each. The bees were kept in cages made of stainless steel (base: 8.5 cm x 4.5 cm; height: 6.0 cm). The front side of the cages was equipped with a transparent pane to enable observation. The bottom of the cages consisted of perforated steel, which guaranteed sufficient air supply. The cages were lined with filter paper.

A fresh stock solution was prepared daily (the highest test-item treatment), by mixing 163.6 mg of CA3642 (148.7 µL of CA3642, based on a density of 1.1004 g/mL) in 25 mL of 50% w/v aqueous sucrose solution. The test-item feeding solutions were freshly prepared every day by mixing aliquots of the stock solution with 50% w/v aqueous sucrose solution. Individuals in the control group were fed with pure, untreated 50% w/v aqueous sucrose solution. Deionised water was used as the solvent for the preparation of the reference-item stock solution. A unique stock solution of the reference item was prepared on day 0 (D0) and it was stored in a refrigerator for use up to D9 (13.0 µL of BAS 152 65 I in 5 mL of deionised water).

The concentrations of feeding solutions were 0.00 (control; untreated diet) 197.27, 473.45, 1136.28, 2727.08, and 6545.00 mg CA3642/L diet, equivalent to 0.00, 165.77, 397.86, 954.86, 2291.67, and 5500.00 mg CA3642/kg diet, respectively (based on feeding solution density of 1.19 g/mL). These concentrations correspond to 27.76, 66.62, 159.88, 383.71, 920.90 mg azoxystrobin/L diet, equivalent to 23.33, 55.98, 134.35, 322.45, and 773.87 mg azoxystrobin/kg diet and 27.29, 65.50, 157.19, 377.27 and 905.44 mg prothioconazole/L diet, equivalent to 22.93, 55.04, 132.10, 317.03, and 760.87 mg prothioconazole/kg diet, respectively (based on feeding solution density of 1.19 g/mL). The reference item was provided at a concentration of 0.90 mg dimethoate/kg diet.

Daily feeding consumption was calculated by dividing the total daily consumption per replicate by the number of living bees at the beginning of the respective feeding interval. For each treatment group, the mean consumption of feeding solution per bee per day was calculated by averaging the replicate values, then correcting to account for daily values of evaporation.

Mortality was recorded daily, 24 hours after the first application and for the duration of the 10-day test. Mortality was corrected by adjusting cumulative mean mortality by the cumulative mean mortality of control group, using a modified Abbott's formula (1925).

Behavioural abnormalities were recorded as bees either affected (lacking coordination), apathetic (low or delayed reaction to stimulation), cramping (uncontrollable abdomen or bodily contractions), moribund (unable to walk or feeble movements), or vomiting. Behaviour was not assessed in the reference-item group.

Statistical calculations were made with the statistical program ToxRatPro Version 3.3.0. The NOEC value for mortality was determined with a multiple sequentially-rejective Fisher test, with Bonferroni-Holm

corrections ( $\alpha = 0.05$ ; one-sided greater), while the corresponding NOEDD for mortality value was determined by considering the consumption of feeding solutions. The  $LDD_{10/20}/LC_{10/20}$  values could not be statistically calculated, but were estimated, due to insufficient goodness of fit for concentration-response function. However, the empirically derived values for  $LDD_{10/20}/LC_{10/20}$  are not considered to be reliable due to the lack of goodness of fit and non-determined 95%-confidence limits. No statistically significant concentration-response was found ( $p(F) > 0.05$ ) at the intervals of interest. The  $LDD_{50}/LC_{50}$  values were statistically calculated by the trimmed Spearman-Kärber procedure.

Samples of each product concentration feeding solution and of control were taken on each application day (days 0 to 9), directly after preparation. Only samples of the highest and lowest product concentration feeding solutions (T5 and T1, respectively) were analysed.

## Results

### Analytical results

The HPLC -MS/MS method for the determination of prothioconazole and azoxystrobin in 50 % (w/v) aqueous sucrose solution was validated with regards to selectivity, linearity, accuracy and repeatability in accordance with guideline SANTE/2020/12830 rev.1. Selectivity was demonstrated by the absence of detected residues above LOD in the control (untreated) test portions used for recovery determinations. The linearity of the method was demonstrated using matrix-matched calibration standard. The analytical method was shown to be linear ( $r > 0.99$ ) over the range from 0.5 ng/mL to 6.5 ng/mL for azoxystrobin and prothioconazole (corresponding to fortification levels of 0.525 mg/kg to 6.83 mg/kg for both analytes and thus cover the range from no more than 30 % of the LOQ and at least + 20 % of the highest analyte concentration detected in any diluted sample). Accuracy was confirmed with recovery of spiked samples at relevant concentrations of test item in 50 % (w/v) aqueous sucrose solution: five fortifications of untreated control samples at the LOQ (15.0 mg test item/kg, corresponding to 2.11 mg azoxystrobin/kg and 2.08 mg prothioconazole/kg) and five fortifications at the level of 7194 mg test item/kg (corresponding to 1012 mg azoxystrobin/kg and 995 mg prothioconazole/kg). Mean recoveries were 90 and 97% for azoxystrobin at 15 and 7194 mg test item/kg, respectively (i.e., within 70-120%). Mean recoveries were 87% and 94% for prothioconazole at 15 and 7194 mg test item/kg, respectively (i.e., within 70-120%). Precision was confirmed. For azoxystrobin, the relative standard deviation ( $n = 5$  for each fortification level) was 2% and 1% at 15 and 7194 mg test item/kg, respectively (i.e., within the guideline limit of  $\leq 20\%$ ). For prothioconazole, the relative standard deviation ( $n = 5$  for each fortification level) was 3% and 2% at 15 and 7194 mg test item/kg, respectively (i.e., within the guideline limit of  $\leq 20\%$ ).

The limit of quantification (LOQ) of the analytical method for 50 % (w/v) aqueous sucrose solution was 15.0 mg test item/kg (corresponding to 2.11 mg azoxystrobin/kg and 2.08 mg prothioconazole/kg) with a limit of detection (LOD) set at 0.525 mg/kg for both analytes (defined as the lowest calibration standard, which is  $\leq 30$  % of the LOQ).

**Table CP 10.3.1.2/01-01. Recovery of azoxystrobin and prothioconazole from analysed samples**

Treatment ID	Time (days)	Azoxystrobin				Prothioconazole			
		Conc. (mg a.s./kg diet)		Recovery (% nominal)		Conc. (mg a.s./kg diet)		Recovery (% nominal)	
		Nominal	Analysed	Rep.	Mean $\pm$ SD	Nominal	Analysed	Rep.	Mean $\pm$ SD
T1	D0	23.33	20.5	88	96 $\pm$ 10	22.93	20.3	88	90 $\pm$ 4
	D1		25.3	108			21.8	95	
	D2		24	103			20.7	90	
	D3		<LOD <sup>1</sup>	-			<LOD <sup>1</sup>	-	
	D3*		<LOD <sup>1</sup>	-			<LOD <sup>1</sup>	-	
	D4		25.1	108			21.5	94	
	D5		19.7	84			20.0	87	
	D6		23.5	101			20.1	88	
	D7		20	86			19.7	86	
	D8		21.8	93			21.8	95	

	D9		22.2	95			20.4	89	
<b>T2</b>	D3	55.98	3.90 <sup>1</sup>	7	-	55.04	3.41 <sup>1</sup>	6	-
<b>T3</b>	D3	134.35	54.6 <sup>1</sup>	41	-	132.10	48.6 <sup>1</sup>	37	-
<b>T4</b>	D3	322.45	304.0	94	-	317.03	300.4	95	-
<b>T5</b>	D0	773.87	766.8	99	98 ± 2	760.87	724.8	95	95 ± 1
	D1		751.1	97			719.5	95	
	D2		761.6	98			719.5	95	
	D3		751.1	97			724.8	95	
	D4		764.2	99			730.0	96	
	D5		740.5	96			701.2	92	
	D6		769.4	99			724.8	95	
	D7		716.9	93			724.8	95	
	D8		785.2	101			740.5	97	
	D9		761.6	98			730.0	96	

\*Sample re-analysed. Conc.: concentration; LOD: Limit of detection = 0.525 mg azoxystrobin/kg diet and 0.525 mg prothioconazole/kg diet; Rep.: replicate; SD: standard deviation.

<sup>1</sup> Values not included in the calculation of Mean and RSD due to an error of the preparation of the 50 % (w/v) aqueous sucrose solution samples.

The mean-measured concentrations in analysed samples of azoxystrobin were 92 to 98% and of prothioconazole were 90 to 95% of nominal concentrations. These values are within ±20% of nominal concentrations, therefore endpoints are based on nominal concentrations.

## Biological results

### Feed consumption

A summary of the effect of CA3642 on food consumption, during the 10-day exposure, are presented in the tables below.

**Table CP 10.3.1.2/01-02: The effect of CA3642 on daily diet consumption, during the 10-day exposure**

Treatment Nominal mg CA3642/kg diet	Mean daily consumption (mg diet/bee) <sup>a</sup>										Mean consumption (mg diet/bee/day)
	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	
0.00	22.3	25.0	22.7	21.9	21.4	20.6	25.7	19.0	28.8	22.7	23.0
165.77	18.1	25.0	25.2	21.8	22.4	22.5	30.6	24.4	24.1	29.9	24.4
397.86	21.7	20.5	24.6	19.3	22.7	28.3	27.0	25.7	27.8	22.0	24.0
954.86	19.8	19.1	22.3	22.9	21.2	18.4	19.4	19.4	21.8	23.5	20.8
2291.67	17.6	18.0	19.6	13.7	14.1	16.4	14.2	10.6	17.4	14.2	15.6
5500.00	15.4	14.8	13.9	14.3	10.0	15.3	12.1	13.9	11.2	9.1	13.1
0.9 mg dimethoate/kg diet	20.6	16.5	13.8	11.4	19.3	16.7	19.9	12.1	16.6	n.s.	16.4

<sup>a</sup> Daily diet consumption was corrected for average daily loss via evaporation. Food consumption could not be analysed for treatment groups with no surviving (n.s.) individuals.

**Table CP 10.3.1.2/01-03: The effect of CA3642 on cumulative mean diet consumption, during the 10-day exposure**

Treatment Nominal mg CA3642/kg diet	Mean consumption (mg diet/bee/day)			Mean consumed dose (µg/bee)					
				CA3642		Azoxystrobin		Prothioconazole	
	Mean	SD	SE	Daily	Cuml	Daily	Cuml	Daily	Cuml
0.00	23.0	7.5	1.1	0.00	0.00	0.00	0.00	0.00	0.00
165.77	24.4	7.9	1.1	4.04	40.44	0.57	5.69	0.56	5.59
397.86	24.0	6.6	0.9	9.53	95.29	1.34	13.41	1.32	13.18
954.86	20.8	6.2	0.9	19.85	198.50	2.79	27.93	2.75	27.46
2291.67	15.6	6.8	1.0	35.75	357.46	5.03	50.30	4.95	49.45
5500.00	13.1	6.0	0.9	71.94	704.99	10.12	99.19	9.95	97.53
0.9 mg dimethoate/kg diet*	16.4	8.2	1.4	0.01	0.11	0.01	--	--	--

\*Values in this row are based on the amount of dimethoate consumed. SD: standard deviation; SE: standard error; Cuml: cumulative.

## Mortality

The effects of CA3642 on mortality, during the 10-days of exposure, are presented in tables below.

**Table CP 10.3.1.2/01-04: The effect of CA3642 on mortality and behaviour, after 10 days of exposure**

Treatment	Rep.	Cumulative mortality										Total mortality	
mg CA3642/kg diet		D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	No.	%
0	1	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0	0	0	0
	5	0	1	0	0	0	0	0	0	0	0	1	10
No. of surviving affected bees		-	-	-	-	-	-	-	-	-	-		
-165.77	1	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	1	0	0	1	10
	3	0	0	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0	0	0	0
	5	0	0	0	0	0	0	0	0	0	0	0	0
No. of surviving affected bees		-	-	-	-	-	-	-	-	-	-		
-397.86	1	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	1	1	0	1	0	0	3	30
	5	0	0	0	0	0	0	0	0	0	0	0	0
No. of surviving affected bees		-	-	-	-	1 <sup>a</sup>	-	2 <sup>a</sup>	1 <sup>a</sup>	-	-		
-954.86	1	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	1	0	0	1	10
	3	0	0	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0	0	0	0
	5	0	0	0	0	0	0	0	0	0	0	0	0
No. of surviving affected bees		-	-	-	-	-	-	1 <sup>a</sup>	-	-	-		
-2291.67	1	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	1	0	0	0	0	1	2	20
	4	1	0	0	0	0	0	0	0	0	0	1	10
	5	0	1	0	0	0	0	0	0	1	0	2	20
No. of surviving affected bees		-	-	-	-	-	-	-	-	-	-		
-5500.00	1	0	0	0	2	0	1	0	2	0	2	7	70
	2	0	0	1	0	1	1	0	1	4	2	10	100
	3	0	0	1	1	0	3	0	3	2	n.s.	10	100
	4	0	0	0	0	1	0	0	2	4	1	8	80
	5	0	0	1	0	0	0	3	3	1	2	10	100
No. of surviving affected bees		-	1 <sup>a</sup>	-	-	3 <sup>a</sup>	-	3 <sup>a</sup>	-	1 <sup>a</sup>	5 <sup>a</sup>		
0.90 mg dimethoate/kg diet	1	1	0	0	3	2	3	1	n.s.	n.s.	n.s.	10	100
	2	0	0	0	0	4	1	4	1	n.s.	n.s.	10	100
	3	0	0	0	0	0	3	2	4	1	n.s.	10	100
	4	0	0	4	0	2	4	n.s.	n.s.	n.s.	n.s.	10	100
	5	2	0	0	1	6	1	n.s.	n.s.	n.s.	n.s.	10	100

Rep.: replicate; No.: number; n.s.: no surviving individuals; a: affected (reduced coordination)

**Table CP 10.3.1.2/01-05: Summary of the effect of CA3642 on cumulative mortality, after 10 days of exposure**

Treatment	10-day cumulative mortality (%)		
mg CA3642/kg diet	Number	Mean ± SE	Corrected <sup>#</sup>
0.00	1	2.00 ± 2.00	0.00
165.77	1	2.00 ± 2.00	0.00
397.86	3	6.00 ± 6.00	4.08

954.86	1	2.00 ± 2.00	0.00
2291.67	5	10.00 ± 4.47	8.16
5500.00	45	90.00 ± 6.32*	89.80
0.9 mg dimethoate/kg diet	50	100.00 ± 0.00	100.00

\*Significant differences in mortality, relative to the control. #Corrected for control mortality, according to Abbott's formula (1925), modified by Schneider-Orelli (1947).

After 10 days of continuous exposure to CA3642, a statistically significant increase in mean mortality, relative to the control, was observed only with the highest treatment of 5500 mg CA3642/kg diet. Mean mortality in the control group was 2.00% and 100.00% in the reference-item group.

The chronic oral 10-day NOEC and NOEDD (mortality) values were determined to be 2291.67 mg CA3642/kg diet and 35.75 µg CA3642/bee/day, respectively; equivalent to 322.44 mg azoxystrobin/kg diet and 5.03 µg azoxystrobin/bee/day; and 317.17 mg prothioconazole/kg diet and 4.95 µg prothioconazole/bee/day, respectively (based on nominal concentrations).

In terms of dietary concentration, the 10-day LC<sub>10/20</sub> values were both estimated to be >2291.67 mg CA3642/kg diet, corresponding to >322.44 mg azoxystrobin/kg diet and >317.17 mg prothioconazole/kg diet. The 10-day LC<sub>50</sub>-value was determined to be 3487.37 mg CA3642/kg diet (with 95% Confidence Limits (CLs) of 3116.16 – 3902.80 mg CA3642/kg diet), corresponding to 490.67 mg azoxystrobin/kg diet (with 95% CLs of 438.44 – 549.12 mg a.s./kg diet) and 482.65 mg prothioconazole/kg diet (with 95% CLs of 431.28 – 540.15 mg a.s./kg diet) (based on nominal concentrations).

In terms of dose (related to the mean food consumption), the 10-day LDD<sub>10/20</sub> values were both estimated to be >35.75 µg CA3642/bee/day, corresponding to >5.03 µg azoxystrobin/bee/day and >4.95 µg prothioconazole/bee/day. The 10-day LDD<sub>50</sub> value was calculated to be 50.21 µg CA3642/bee/day (with 95% Confidence Limits (CLs) of 45.95 – 54.88 µg CA3642/bee/day), corresponding to 7.06 µg azoxystrobin/bee/day (with 95% Confidence Limits (CLs) of 6.47 – 7.72 µg azoxystrobin/bee/day) and 6.95 µg prothioconazole/bee/day (with 95% Confidence Limits (CLs) of 6.36 – 7.60 µg prothioconazole/bee/day) (based on nominal concentrations).

### Validity

All validity criteria were met, in accordance with the OECD 245 (2017) test guideline:

- Control group: mean mortality was ≤15%, at the end of the study (actual value: 2.0%).
- Reference-item group: mean mortality was ≥50% on day 8, at the end of the study (actual value: 100.0%).

### **Conclusion**

The toxicity of CA3642 to adult *Apis mellifera* during a 10-day laboratory test was studied, in accordance with the OECD 245 (2017) test guideline. Analytical verification confirmed that the mean-measured concentrations in analysed samples of azoxystrobin were 92 to 98% and of prothioconazole were 90 to 95% of nominal concentrations. These values are within ±20% of nominal concentrations, therefore endpoints are based on nominal concentrations.

The chronic 10-day NOEC (mortality) = 2291.67 mg CA3642/kg diet (equivalent to 322.44 mg azoxystrobin/kg diet and 317.17 mg prothioconazole/kg diet).

The chronic 10-day NOEDD (mortality) = 35.75 µg CA3642/bee/day (equivalent to 5.03 µg azoxystrobin/bee/day and 4.95 µg prothioconazole/bee/day).

The chronic 10-day LC<sub>50</sub> = 3487.37 mg CA3642/kg diet (equivalent to 490.67 mg azoxystrobin/kg diet and 482.65 mg prothioconazole/kg diet).

The chronic 10-day LDD<sub>50</sub> = 50.21 µg CA3642/bee/day (equivalent to 7.06 µg azoxystrobin/bee/day and 6.95 µg prothioconazole/bee/day).

This study is considered acceptable and valid.

### A 2.3.1.3 KCP 10.3.1.3 Effects on honey bee development and other honey bee life stages

#### Study 1

Comments of zRMS:	<p>The study was conducted in line with OECD 239 with no deviations.</p> <p>Analytical verification confirmed that mean measured concentrations of test solutions used to prepare the daily larval diets were between 88 and 96% of nominal concentration of azoxystrobin and between 86 and 97% of nominal concentrations of prothioconazole; biological endpoints are therefore reported based on nominal concentrations of CA3642.</p> <p>All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>The 22-day EC<sub>10</sub> (emergence) = 601.08 mg product/kg diet (equivalent to 84.57 mg azoxystrobin/kg diet and 83.15 mg prothioconazole/kg diet, respectively), based on nominal concentrations.</p> <p>The 22-day EC<sub>20</sub> (emergence) = 740.83 mg product/kg diet (equivalent to 104.24 mg azoxystrobin/kg diet and 102.49 mg prothioconazole/kg diet, respectively), based on nominal concentrations.</p> <p>The 22-day EC<sub>50</sub> (emergence) = 1105.07 mg product/kg diet (equivalent to 155.49 mg azoxystrobin/kg diet and 152.88 mg prothioconazole/kg diet, respectively), based on nominal concentrations.</p> <p>NOEC = 519.89 mg product/kg diet (equivalent to 73.15 mg azoxystrobin/kg diet and 71.92 mg prothioconazole/kg diet, respectively), based on nominal concentrations.</p> <p>The 22-day ED<sub>10</sub> (emergence) value was: = 92.56 µg product/larva/development period (equivalent to 13.02 µg azoxystrobin/larva/development period and 12.80 µg prothioconazole/larva/development period, respectively).</p> <p>The 22-day ED<sub>20</sub> (emergence) = 114.09 µg product/larva/development period (equivalent to 16.05 µg azoxystrobin/larva/development period and 15.78 µg prothioconazole/larva/development period, respectively).</p> <p>The 22-day ED<sub>50</sub> (emergence) = 170.18 µg product/larva/development period (equivalent to 23.94 µg azoxystrobin/larva/development period and 23.54 µg prothioconazole/larva/development period, respectively).</p> <p>NOED (D22) = 80.06 µg product/larva/development period (equivalent to 11.27 µg azoxystrobin/larva/development period and 11.08 µg prothioconazole/larva/development period).</p>
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Reference:	KCP 10.3.1.3/01
Report	<p>CA3642 (Azoxystrobin 150 g/L + Prothioconazole 150 g/L SC): Honey Bee (<i>Apis mellifera</i> L.) Larval Toxicity Test following Repeated Exposure under laboratory conditions</p> <p>Gimeno, I., 2022, report no. S21-04082</p>

Guideline(s):	Yes. Guidance Document on Honey bee ( <i>Apis mellifera</i> ) Larval Toxicity Test, Repeated Exposure, no. 239 (2016)
Deviations:	No
GLP:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability:	Yes
Duplication (if vertebrate study)	No

## Executive summary

In a repeated-exposure dose-response test, European honey bee (*Apis mellifera* L.) larvae were exposed to the product, CA3642 (analysed active substance content of 14.07% w/w azoxystrobin and 13.84% w/w prothioconazole), administered in the larval diet from Days 3 to 6 at nominal concentrations of 0.00 (control), 83.18, 207.96, 519.89, 1299.73, and 3249.32 mg product/kg diet (equivalent to 0.00, 11.70, 29.26, 73.15, 182.88, and 457.19 mg azoxystrobin/kg diet and 0.00, 11.51, 28.77, 71.92, 179.81, and 449.51 mg prothioconazole/kg diet, respectively). The test also included a toxic-reference treatment group of 48 mg dimethoate/kg diet, equivalent to a cumulative dose of 7.39 µg dimethoate/larva. The study was conducted in accordance with OECD test guidance document 239 (2016). Since analytical verification demonstrated that treatment solutions contained azoxystrobin and prothioconazole within  $\pm 20\%$  of nominal concentrations, endpoints were based on nominal concentrations.

The 22-day NOED (emergence) value was determined to be 80.06 µg product/larva/development period (equivalent to 11.27 µg azoxystrobin/larva/development period and 11.08 µg prothioconazole/larva/development period). The 22-day NOEC (emergence) value was determined to be 519.89 mg product/kg diet (equivalent to 73.15 mg azoxystrobin/kg diet and 71.92 mg prothioconazole/kg diet, respectively), based on nominal concentrations.

The 22-day ED<sub>10</sub> (emergence) value was determined to be 92.56 µg product/larva/development period (equivalent to 13.02 µg azoxystrobin/larva/development period and 12.80 µg prothioconazole/larva/development period, respectively). The 22-day ED<sub>20</sub> (emergence) value was determined to be 114.09 µg product/larva/development period (equivalent to 16.05 µg azoxystrobin/larva/development period and 15.78 µg prothioconazole/larva/development period, respectively). The 22-day ED<sub>50</sub> (emergence) value was determined to be 170.18 µg product/larva/development period (equivalent to 23.94 µg azoxystrobin/larva/development period and 23.54 µg prothioconazole/larva/development period, respectively).

The 22-day EC<sub>10</sub> (emergence) value was determined to be 601.08 mg product/kg diet (equivalent to 84.57 mg azoxystrobin/kg diet and 83.15 mg prothioconazole/kg diet, respectively), based on nominal concentrations. The 22-day EC<sub>20</sub> (emergence) value was determined to be 740.83 mg product/kg diet (equivalent to 104.24 mg azoxystrobin/kg diet and 102.49 mg prothioconazole/kg diet, respectively), based on nominal concentrations. The 22-day EC<sub>50</sub> (emergence) value was determined to be 1105.07 mg product/kg diet (equivalent to 155.49 mg azoxystrobin/kg diet and 152.88 mg prothioconazole/kg diet, respectively), based on nominal concentrations.

The study satisfies the OECD test guidance document no. 239 (2016) requirements for a repeated-exposure larval toxicity test with honey bees and is considered acceptable.

## Materials and methods

### Test materials

#### Test item

Name: CA3642 (prothioconazole 150 g/L + azoxystrobin 150 g/L SC)  
Density: 1.1004 g/mL  
Formulation type: Suspension Concentrate (SC)

Batch no.: A20026  
Active substance (a.s.) 1: Azoxystrobin  
154.83 g/L or 14.07 w/w (analysed), 150 g/L (nominal)  
Active substance (a.s.) 2: Prothioconazole  
152.23 g/L or 13.84% w/w (analysed), 150 g/L (nominal)  
Appearance: Off-white, odourless suspension  
Expiry date of lot/batch: September 2022  
Storage conditions: Keep at room temperature in a well-ventilated place

Reference item

Name: BAS 152 I  
Active substance: Dimethoate  
Purity: 98.2% w/w  
Batch no.: COD-002332  
Appearance: White to grey mass, solidified, crystalline flakes  
Expiry date: 23 Jan 2022  
Storage conditions: ≤25°C; dark and dry

Test organism

Species: European honey bee, *Apis mellifera* L. (Hymenoptera, Apidae),  
Subspecies: *A. mellifera iberiensis*  
Age at study initiation: <30-hours-old first instar larvae (L1)  
Source: Collected from three different beehives located in the commercial apiary of the test facility (Eurofins Trialcamp, Chella, València, Spain). The beehives were disease-free, adequately fed, healthy, and queen-right. No chemical substances were used on the hives within 4 weeks of the start of the test.  
Feeding during test: Yes, different diets according to development stage of larvae:  
Diet A (Day 1; 20 µL/larva): 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 2% weight of yeast extract, 12% weight of glucose and 12% weight of fructose  
Diet B (Day 3; 20 µL/larva): 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 3% weight of yeast extract, 15% weight of glucose and 15% weight of fructose  
Diet C (from Day 4 to Day 6; 30 µL/larva, 40 µL/larva and 50 µL/larva, respectively): 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 4% weight of yeast extract, 18% weight of glucose and 18% weight of fructose

Test conditions

Test temperature: 34-35°C (actual: 30.5 – 35.0°C)  
Relative humidity: 95% ± 5% (Day 1 - Day 8) (actual: 47.4\* – 97.8%)  
80% ± 5% (Day 8 - Day 15) (actual: 57.1\* – 97.5%)  
50 - 80% (Day 15 - 22) (actual: 52.9\* – 79.9%)

\*Minimum single value recorded due to opening of desiccators during feeding and assessments.

Photoperiod: Continuous darkness, except during feeding and assessments

Four days prior to grafting, to ensure the production of larvae, several queens were confined to their own colony, with an excluder cage containing a comb with empty cells. Three days prior to grafting, after a maximum 30 hours of caging, queens were released from their excluder cages. The combs containing the newly laid eggs were left in the excluder cages during the incubation stage, until hatching on Day 1. On Day 1, three of the combs (with the highest number of synchronised eggs) were selected and transferred to the laboratory, using an insulated container, to avoid variations in temperature. In the laboratory, the three combs were used for grafting.



The study was conducted as a dose-response test (from Day 1 to the final assessment on Day 22). It comprised of one control (C) and five product (T1-T5) treatment groups of 0.00 (control), 83.18, 207.96, 519.89, 1299.73, and 3249.32 mg product/kg diet, equivalent to 0.00, 11.70, 29.26, 73.15, 182.88, and 457.19 mg azoxystrobin/kg diet and 0.00, 11.51, 28.77, 71.92, 179.81, and 449.51 mg prothioconazole/kg diet. Based on the cumulative application volume of 140 µL/larva, the corresponding doses were 0.00, 12.81, 32.03, 80.06, 200.16, and 500.40 µg product/larva, equivalent to 0.00, 1.80, 4.51, 11.27, 28.16, and 70.41 µg azoxystrobin/larva and 0.00, 1.77, 4.43, 11.08, 27.69, and 69.22 µg prothioconazole/larva. One toxic-reference treatment (R) of 48.00 mg dimethoate/kg diet, equivalent to a cumulative dose of 7.39 µg dimethoate/larva, was also included in the study. Each treatment group consisted of 48 honey bee larvae, from three different hives. Each hive corresponded to one replicate; 16 larvae from each replicate were used.

#### *Larval diet and test units*

The larval diets were prepared from application solutions (which were made by diluting stock solutions of 3574.25 mg product/L or 52.8 mg dimethoate/L diet). Larvae were fed on Days 1, 3, and 4 to 6, with diets A, B, and C, respectively (see summary table, above).

Larvae were transferred into crystal polystyrene grafting cells (NICOTPLAST®), each with a diameter of 9 mm. Each cell was placed into a slot of a sterile 48-well cellular culture plate (Greiner Bio One®) and then placed into hermetically sealed Plexiglas desiccators, from Days 1 to 7. On Day 7, the well plates were transferred to another Plexiglas desiccator, to maintain a slightly lower relative humidity until Day 15. On Day 15, each plate was transferred into an emergence box (approximately 18 x 13 x 7 cm) in an incubator. Bees that emerged in the emergence box had access to aqueous sucrose solution *ad libitum*.

On Day 1, 20 µL of diet A was deposited into each grafting cell of the well plate and one larva was transferred into each cell. Larvae were grafted in excess, to allow replacement of unsuitable larvae, with individuals from the reserve plates, using larvae from the same hive, on Day 3. When all the plates were prepared, they were placed into the hermetically sealed Plexiglas desiccator.

#### *Assessments*

Larval mortality was assessed before feeding on Days 4, 5, and 6 and on Days 7 and 8, using a stereo microscope, to detect whether larvae were respiring (movement of spiracles). On Day 8, the presence of uneaten food was qualitatively recorded. Pupal mortality and emergence were assessed on Days 15 and 22, respectively.

For analytical verification, samples were taken of the stock solution, application solutions (the dilutions of the stock solution), the deionized water used for the application solutions, all treated diets, and the control diet from Day 3 to Day 6. Only samples of the application solutions used to prepare the highest and lowest diet concentrations from Day 3 to Day 6, and the control application solution taken on Day 6 were analysed.

#### *Statistical analyses*

The statistical software ToxRatPro® (v3.3.0) was used to analyse the data. A step-down Cochran-Armitage test ( $\alpha = 0.05$ , one-sided greater) was used to determine the NOEC/NOED and LOEC/LOED values. A probit analysis (using a maximum-likelihood regression) was used to calculate the EC<sub>x</sub>/ED<sub>x</sub> values and their 95% confidence limits (CLs). Additionally, the normalised widths (NWs) of the 95% CLs were calculated to reliably quantify the EC<sub>x</sub> values; values of NW <1 were considered as acceptable.

## **Results**

### Analytical results

The HPLC -MS/MS method for the determination of prothioconazole and azoxystrobin in deionised water was validated with regards to specificity, linearity, accuracy and precision in accordance with guideline SANTE/2020/12830 rev.1 24/02/2021. Specificity in deionised water was demonstrated by the absence of significant interference above 30% of the LOQ in control and blank samples. The linearity of the method was demonstrated using matrix-matched calibration standard. The analytical method was shown to be linear

( $r > 0.99$ ) over the range from 0.7 to 6.5 ng/mL for both prothioconazole and azoxystrobin (covering sample with test item concentration of 3.2 to 32.5 mg/L). Accuracy was confirmed with recovery of spiked samples at relevant concentrations of test item in deionised water (90 (LOQ) and 47000 mg test item/L). Mean recoveries were 97 and 94% for Azoxystrobin at 90 and 47000 mg test item/L respectively (i.e., within 70-110%). Mean recoveries were 94% and 95% for prothioconazole at 90 and 47000 mg test item/L respectively (i.e., within 70-110%). Precision was confirmed. For Azoxystrobin, the relative standard deviation ( $n = 5$  for each fortification level) was 4% and 3% at 90 and 47000 mg test item/L respectively (i.e., within the guideline limit of  $\leq 20\%$ ). For Prothioconazole, the relative standard deviation ( $n = 5$  for each fortification level) was 3% and 2% at 90 and 47000 mg test item/L respectively (i.e., within the guideline limit of  $\leq 20\%$ ).

The limit of quantification (LOQ) for deionised water was 90 mg test item/L (corresponding to 12.7 mg azoxystrobin/L and 12.5 mg prothioconazole/L). The limit of detection (LOD) was defined at 3.5 mg/L for both analytes.

**Table CP 10.3.1.3/01-01: Recovery of azoxystrobin and prothioconazole from analysed samples.**

Active substance	Concentration (mg a.s./L)		Recovery (% nominal)	
	Nominal	Analysed	Per sample	Mean
Azoxystrobin	0.00 (control)	<LOD	-	-
	128.74	118.0	92.0	88.0
		111.0	86.0	
		113.0	88.0	
		110.0	85.0	
	5029.09	4680.0	93.0	96.0
		5230.0	104.0	
		4800.0	95.0	
		4690.0	93.0	
Prothioconazole	0.00 (control)	<LOD	-	-
	126.58	112.0	88.0	86.0
		108.0	85.0	
		110.0	87.0	
		108.0	85.0	
	4944.64	4740.0	96.0	97.0
		5040.0	102.0	
		4730.0	96.0	
		4610.0	93.0	

LOD: limit of detection. Analysed concentration values were rounded to three (3) significant digits. LOD = 3.50mg/L. LOQ = 90.0 mg product/L (equivalent to 12.7 mg azoxystrobin/L and 12.5 mg prothioconazole/L).

Since the measured concentrations in all analysed samples (application solutions used to prepare the highest and lowest diet concentrations) were within the range of  $\pm 20\%$  of nominal concentrations (86–97%), biological endpoints are based on nominal concentrations.

### Biological results

#### *Larval mortality*

The cumulative effects of CA3642 on honey bee (*A. mellifera*) larval mortality, pupal mortality and adult emergence, are presented in tables below.

**Table CP 10.3.1.3/01-02: The effects of CA3642 on honey bee (*A. mellifera*) larval mortality and adult emergence from repeated exposure.**

Treatment			Rep.	Parameter (number)								
				L	Cumulative mortality (D4-D8: larvae; D8-D15: pupae; D22: emerged adults)							E
Test item	ID	mg product /kg diet		D1	D4	D5	D6	D7	D8	D15	D22	D22
CA3642	C	0.0	1	16	0	0	0	0	0	2	2	14
			2	16	1	1	1	1	1	2	3	13
			3	16	0	0	1	1	1	1	1	15
	T1	83.18	1	16	1	1	1	1	1	2	2	14
			2	16	0	0	0	0	0	1	1	15
			3	16	0	0	0	0	0	0	1	15
	T2	207.96	1	16	0	0	0	0	0	0	2	14
			2	16	0	0	0	0	0	0	0	16
			3	16	0	0	0	0	0	0	1	15
	T3	519.89	1	16	1	2	2	2	2	2	2	14
			2	16	0	3	3	3	3	4	4	12
			3	16	0	0	0	0	0	2	3	13
	T4	1299.73	1	14	1	5	8	8	9	12	12	4
			2	16	1	4	5	5	5	8	9	7
			3	15	1	4	5	8	9	10	10	6
	T5	3249.32	1	16	1	9	14	15	16	16	16	0
			2	16	1	12	14	15	16	16	16	0
			3	16	1	2	11	16	16	16	16	0
Dimethoate	R	48.0	1	16	5	12	16	16	16	16	16	0
			2	16	4	14	16	16	16	16	16	0
			3	16	8	12	15	16	16	16	16	0

ID: Treatment group code – T1-5 = product treatment groups, C = Control, R = Toxic reference; Rep.: replicate; L.: live larvae; E: emerged bees; D: Day.

**Table CP 10.3.1.3/01-03: Summary of the effects of CA3642 on honey bee (*A. mellifera*) larval and pupal mortality and adult emergence - mean cumulative mortality, from repeated exposure.**

Treatment			Mean cumulative mortality (%)						
Test item	ID	mg product /kg diet	D4	D5	D6	D7	D8	D15	D22
CA3642	C	0.0	2.1	2.1	4.2	4.2	4.2	10.4	12.5
	T1	83.18	2.1	2.1	2.1	2.1	2.1	6.3	8.3
	T2	207.96	0.0	0.0	0.0	0.0	0.0	0.0	6.3
	T3	519.89	2.1	10.4	10.4	10.4	10.4	16.7	18.8
	T4	1299.73	6.8	27.1	37.5	43.8	47.9	62.5	64.6*
	T5	3249.32	6.3	47.9	81.3	95.8	100.0	100.0	100.0*
Dimethoate	R	48.0 <sup>a</sup>	35.4	79.2	97.9	100.0	100.0	100.0	100.0

ID: Treatment group code – T1-5 = product treatment groups, C = Control, R = Toxic reference; D: Day

<sup>a</sup>For the reference item, the values indicate the amount of active substance (dimethoate)

\*Statistically significantly different compared to the control group (step-down Cochran-Armitage test;  $\alpha = 0.05$ ; one-side greater) at D22

**Table CP 10.3.1.3/01-04: Summary of the effects of CA3642 on honey bee (*A. mellifera*) larval and pupal mortality and adult emergence - mean corrected cumulative mortality, from repeated exposure.**

Treatment			Mean corrected cumulative mortality (%) <sup>#</sup>						
Test item	ID	mg product /kg diet	D4	D5	D6	D7	D8	D15	D22
CA3642	T1	83.18	0.0	0.0	-2.2	-2.2	-2.2	-4.7	-4.8
	T2	207.96	-2.1	-2.1	-4.3	-4.3	-4.3	-11.6	-7.1
	T3	519.89	0.0	8.5	6.5	6.5	6.5	7.0	7.1
	T4	1299.73	4.3	25.5	34.8	41.3	45.7	58.1	59.5*
	T5	3249.32	4.3	46.8	80.4	95.7	100.0	100.0	100.0*

ID: Treatment group code – T1-5 = product treatment groups, C = Control, R = Toxic reference; D: Day

\*Statistically significant differences, relative to the control (step-down Cochran-Armitage test ( $\alpha = 0.05$ ; one-side greater)) at D22. #Data were corrected for control mortality, using a modified Abbot's formula by Schneider-Orelli.

Statistically significant differences, compared to the control, on honey bee adult emergence were observed at the two highest product treatments of 1299.73 and 3249.32 mg product/kg diet (T4 and T5).

#### *Pupal mortality and adult emergence*

A summary of the effects of CA3642 on honey bee pupal mortality and adult emergence, following repeated exposure, is presented in the table below.

**Table CP 10.3.1.3/01-05: Summary of the effects of CA3642 on honey bee (*A. mellifera*) pupal mortality and adult emergence rate**

Treatment		Mean cumulative mortality (%)			Emergence (Mean %)
ID	Nominal Conc. (mg product /kg diet)	D8-D15 (Pupation)	D15-D22 (Pupation)	D8-D22 (Total)	D22
C	0.0	6.5	2.3	8.7	87.5
T1	83.18	4.3	2.2	6.4	91.7
T2	207.96	0.0	6.3	6.3	93.8
T3	519.89	7.0	2.5	9.3	81.3
T4	1299.73	28.0	5.6	32.0	35.4*
T5	3249.32	-	-	-	0.0*

ID: Treatment group code – T1-5 = product treatment groups, C = Control; D: Day; Conc.: concentration. \*Statistically significant differences, relative to the control group (step-down Cochran-Armitage test ( $\alpha = 0.05$ ; one-sided greater)).

Statistically significant differences in adult emergence, when compared to the control group, were observed in the two highest product treatment groups (T4 and T5).

The 22-day NOED value (emergence) was determined to be 80.06 µg product/larva/development period, equivalent to 11.27 µg azoxystrobin/larva/development period and 11.08 µg prothioconazole/larva/development period. The 22-day NOEC (emergence) value was determined to be 519.89 mg product/kg diet, equivalent to 73.15 mg azoxystrobin/kg diet and 71.92 mg prothioconazole/kg diet, respectively (based on nominal concentrations).

The 22-day ED<sub>10</sub> (emergence) value (with 95% Confidence Limits (CLs)) was determined to be 92.56 (70.52 – 110.77) µg product/larva/development period, equivalent to 13.02 (9.92 – 15.59) µg azoxystrobin/larva/development period and 12.80 (9.76 – 15.32) µg prothioconazole/larva/development period, respectively.

The 22-day ED<sub>20</sub> (emergence) value (with 95% CLs) was determined to be 114.09 (92.10 – 133.01) µg product/larva/development period, equivalent to 16.05 (12.96 – 18.71) µg azoxystrobin/larva/development period and 15.78 (12.74 – 18.40) µg prothioconazole/larva/development period, respectively.

The 22-day ED<sub>50</sub> (emergence) value (with 95% CLs) was determined to be 170.18 (147.35 – 196.61) µg product/larva/development period, equivalent to 23.94 (20.73 – 27.66) µg azoxystrobin/larva/development period and 23.54 (20.38 – 27.20) µg prothioconazole/larva/development period, respectively.

The corresponding 22-day EC<sub>10</sub> (emergence) value (with 95% CLs) was determined to be 601.08 (457.96 – 719.32) mg product/kg diet, equivalent to 84.57 (64.44 – 101.21) mg azoxystrobin/kg diet and 83.15 (63 – 99.51) mg prothioconazole/kg diet, respectively.

The 22-day EC<sub>20</sub> (emergence) value (with 95% CLs) was determined to be 740.83 (598.08 – 863.71) mg product/kg diet, equivalent to 104.24 (84.15 – 121.53) mg azoxystrobin/kg diet and 102.49 (82.74 – 119.49) mg prothioconazole/kg diet, respectively.

The 22-day EC<sub>50</sub> (emergence) value (with 95% CLs) was determined to be 1105.07 (956.82 – 1276.67) mg product/kg diet, equivalent to 155.49 (134.63 – 179.63) mg azoxystrobin/kg diet and 152.88 (132.37 – 176.62) mg prothioconazole/kg diet, respectively.

At the end of the test (Day 22), no emerged bees were observed to be visually or morphologically affected by treatment with CA3642.

### Validity

All validity criteria were met in accordance with the OECD test guidance document no. 239 (2016):

- Cumulative larval mortality in the control group was  $\leq 15\%$ , from Day 3 to Day 8, across all replicates (actual values: 0.0, 6.25, and 6.25% in replicates 1, 2, and 3, respectively).
- Emergence in the control group, on Day 22 was  $\geq 70\%$ , across all replicates (actual values: 87.5, 81.25, and 93.75% in replicates 1, 2, and 3, respectively).
- Cumulative larval mortality at Day 8 in the toxic-reference group was  $\geq 50\%$ , on day 8, across all replicates (actual values: 100% in all replicates).

### Conclusion

In a repeated exposure dose-response test, the toxicity of CA3642 to honey bee (*Apis mellifera* L.) larvae was tested under laboratory conditions, over a period of 22 days, in accordance with the OECD test guidance document no. 239 (2016). Analytical verification confirmed that mean measured concentrations of test solutions used to prepare the daily larval diets were between 88 and 96% of nominal concentration of azoxystrobin and between 86 and 97% of nominal concentrations of prothioconazole; biological endpoints are therefore reported based on nominal concentrations of CA3642.

The 22-day NOED (emergence) value was: = 80.06  $\mu\text{g}$  product/larva/development period (equivalent to 11.27  $\mu\text{g}$  azoxystrobin/larva/development period and 11.08  $\mu\text{g}$  prothioconazole/larva/development period).

The 22-day NOEC (emergence) value was: = 519.89 mg product/kg diet (equivalent to 73.15 mg azoxystrobin/kg diet and 71.92 mg prothioconazole/kg diet, respectively), based on nominal concentrations.

The 22-day ED<sub>10</sub> (emergence) value was: = 92.56  $\mu\text{g}$  product/larva/development period (equivalent to 13.02  $\mu\text{g}$  azoxystrobin/larva/development period and 12.80  $\mu\text{g}$  prothioconazole/larva/development period, respectively).

The 22-day ED<sub>20</sub> (emergence) value was: = 114.09  $\mu\text{g}$  product/larva/development period (equivalent to 16.05  $\mu\text{g}$  azoxystrobin/larva/development period and 15.78  $\mu\text{g}$  prothioconazole/larva/development period, respectively).

The 22-day ED<sub>50</sub> (emergence) value was: = 170.18  $\mu\text{g}$  product/larva/development period (equivalent to 23.94  $\mu\text{g}$  azoxystrobin/larva/development period and 23.54  $\mu\text{g}$  prothioconazole/larva/development period, respectively).

The 22-day EC<sub>10</sub> (emergence) value was: = 601.08 mg product/kg diet (equivalent to 84.57 mg azoxystrobin/kg diet and 83.15 mg prothioconazole/kg diet, respectively), based on nominal concentrations.

The 22-day EC<sub>20</sub> (emergence) value was: = 740.83 mg product/kg diet (equivalent to 104.24 mg azoxystrobin/kg diet and 102.49 mg prothioconazole/kg diet, respectively), based on nominal concentrations.

The 22-day EC<sub>50</sub> (emergence) value was: = 1105.07 mg product/kg diet (equivalent to 155.49 mg azoxystrobin/kg diet and 152.88 mg prothioconazole/kg diet, respectively), based on nominal concentrations.

This study is considered acceptable and valid.

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<b>A 2.3.1.4</b>	<b>KCP 10.3.1.4</b>	<b>Sub-lethal effects</b>
<b>A 2.3.1.5</b>	<b>KCP 10.3.1.5</b>	<b>Cage and tunnel tests</b>
<b>Study 1</b>		

<p>Comments of zRMS:</p>	<p>The study was conducted in line with OECD 75 (2007) and OEPP/EPPO 170(4) (2010) for semi-field honey bee study EU Guideline 7029/VI95 rev. 4 (1997) for residues study, with following deviation:</p> <ul style="list-style-type: none"> <li>○ Only 70 old larvae marked at BFD0, instead of 200 for one replicate tunnel – not expected to impact results across 4 replicates</li> <li>○ Hives were set up on 4DBA2 instead of 5DBA2 due to inclement weather conditions</li> <li>○ Colony strength outside recommended values of 6000-8000 per colony, At BFD0 the mean number of honey bees was 9701, 7670, 8450 and 8206 in each of these treatments respectively. The slightly larger numbers at BFD0 will not have an impact on the study.</li> </ul> <p>It should be noted that the crop area treated within the tunnels used in this study were also larger (82.72 m<sup>2</sup>) than the minimum specified in OECD 75 guideline (40 m<sup>2</sup>).</p> <p>CA3642 was applied as two foliar applications under semi-field conditions to oilseed rape, one at pre-flowering and one at flowering, during daily honey bee flight (interval between applications: 15 days). Applications were made at the target rate each of 210 g a.s./ha of prothioconazole and azoxystrobin (actual rate applied based on GLP Certificate of analysis): 215.3 g prothioconazole/ha and 219.0 g azoxystrobin/ha (1414.4 mL product/ha) at the first application and 214.9 g prothioconazole/ha and 218.6 g azoxystrobin/ha (1411.6 mL product/ha) at the second application.</p> <p>Residues of prothioconazole and azoxystrobin were detected in the nectar and pollen samples collected from the forager bees in the CA3642 treatment confirming that honey bees and their hives were exposed to the CA3642 treatment.</p> <p>CA3642 did not lead to statistically significant adverse effects on mortality of honey bee worker bees (adults, larvae or pupae). Transient effects on honey bee flight activity were only observed on the day of application. No biologically relevant CA3642 treatment related behavioural effects were observed. CA3642 had no statistically significant effects on honey bee colony strength (mean number of adult honey bees), mean number of cells containing food (nectar and pollen), mean compensation indices or mean termination rates in brood cells initially containing eggs or young larvae.</p> <p>CA3642 lead to a significant transient reduction in the mean number of honey bee pupal cells on 9DAA2 and of total brood cells on two occasions (4DAA2, 15DAA2) but there was no statistically significant reduction seen on the last two assessment dates (21DAA2, 27DAA2). Overall brood cell number levels at the end of the study observation period for CA3642 were in line with the control. Therefore, no biological adverse effect on honey brood cell number is concluded for CA3642.</p> <p>A transient significant reduction of the honey bee brood index for cells initially containing old larvae was observed at BFD+16. Nevertheless, the data from the colony condition assessments for 21DAA2 and 27DAA2 show that the total number of pupal and brood cells was similar for CA3642 and the control. No statistically significant effects of CA3642 were observed for the mean compensation index or termination rate of cells initially containing old larvae.</p> <p>Overall, it can be concluded that CA3642 had no significant effect on overall honey bee (<i>Apis mellifera</i>) colony strength (mean number of adult honey bees), overall amount of brood or the development of the food storage area following nominal target application of 1400 mL CA3642/ha (210 g a.s./ha each of prothioconazole and azoxystrobin) during honey bee flight.</p>
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Reference:	KCP 10.3.1.5/01
Report	A Semi-Field Study to Evaluate Potential Effects on the Honey Bee ( <i>Apis mellifera</i> L.) After Two Applications of CA3301 and CA3642 in Winter Oil Seed Rape in Germany 2022  Bocksch, S., 2023, report no. S21-00461
Guideline(s):	Yes. OECD 75 (2007) and OEPP/EPPO 170(4) (2010) for semi-field honey bee study EU Guideline 7029/VI95 rev. 4 (1997) for residues study
Deviations to bee guidelines:	Yes, but no significant impact on the study results: 1) Only 70 old larvae marked at BFD0, instead of 200 for one replicate tunnel – not expected to impact results across 4 replicates 2) Hives were set up on 4DBA2 instead of 5DBA2 due to inclement weather conditions 3) Colony strength outside recommended values of 6000-8000 per colony, At BFD0 the mean number of honey bees was 9701, 7670, 8450 and 8206 in each of these treatments respectively. The slightly larger numbers at BFD0 will not have an impact on the study. It should be noted that the crop area treated within the tunnels used in this study were also larger (82.72 m <sup>2</sup> ) than the minimum specified in OECD 75 guideline (40 m <sup>2</sup> ).
GLP:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability:	Yes
Duplication (if vertebrate study)	No

## Executive summary

In a semi-field brood study, the effects of the product, CA3642 (analysed a.s. content of 14.07% w/w azoxystrobin and 13.84% w/w prothioconazole), on the European honey bee (*Apis mellifera*) were investigated. Potential effects of two foliar applications, applied once pre-flowering and once at flowering, during daily bee flight, of 1400 mL product/ha (equivalent to nominal 210 g a.s./ha each of azoxystrobin and prothioconazole, respectively) were evaluated against those observed in honey bees treated with tap water (control) and a toxic-reference item (1200 g Insegar/ha, equivalent to 300 g fenoxycarb/ha).

The study comprised 19 tunnel tents (approximately 100 m<sup>2</sup>) each containing winter oilseed rape and one honey bee colony, five tents each for the control group and the product treatment group – four tents from each group for biological assessments and one tent each for residues sampling - and four tents for the reference item treatment group.

Colonies were introduced into the tunnel tents just before full bloom (BBCH 61), 4 days before the day of the second application (4DBA2), in the evening after daily bee flight, then relocated to a monitoring site, without flowering main crops nearby but with wildflower food source, 7 days after the second application (7DAA2), in the evening after daily bee flight.

After exposure to the product treatment in the tunnel tents, honey bee colonies were assessed for adult, larvae and pupae worker honey bee mortality, flight activity, behaviour, colony condition and brood development, throughout the 7-day exposure period in the tunnel. Assessment of all these parameters continued in the post-tunnel monitoring phase up to 27 days after the second application (27DAA2) excepting flight activity and behaviour.

For CA3642 residues of prothioconazole were detected in nectar at 0.191 mg/kg on 0DAA2 and decreased to < LOQ on 6DAA2 and residues of azoxystrobin were detected in nectar at 0.606 mg/kg on 0DAA2 and 0.758 mg/kg on 6DAA2. Residues of prothioconazole detected in pollen was 35.1 mg/kg on 0DAA2 and decreased to 0.0303 mg/kg on 6DAA2 and residues of azoxystrobin detected in pollen in the test item treatment (CA3642) replicate T2s was 52.2 mg/kg on 0DAA2 and decreased to 1.34 mg/kg on 6DAA2. No residues of prothioconazole or azoxystrobin were found in nectar or pollen from the bees in the control treatment. These residue data confirm exposure of the confined honey bees to both active substances in the product CA3642 during the study.



CA3642 did not lead to statistically significant adverse effects on mortality of honey bee worker bees. Transient effects on honey bee flight activity were only observed on the day of application. No biologically relevant CA3642 treatment related behavioural effects were observed. CA3642 had no statistically significant effects on honey bee colony strength (mean number of adult honey bees), mean number of cells containing food (nectar and pollen), mean compensation indices or mean termination rates in brood cells initially containing eggs or young larvae.

CA3642 lead to a significant transient reduction in the mean number of honey bee pupal cells on 9DAA2 and of total brood cells on two occasions (4DAA2, 15DAA2) but there was no statistically significant reduction seen on the last two assessment dates (21DAA2, 27DAA2). Overall brood cell number levels at the end of the study observation period for T2 (CA3642) were in line with the control. Therefore, no biological adverse effect on honey brood cell number is concluded for T2 (CA3642).

A transient significant reduction of the honey bee brood index for cells initially containing old larvae was observed at BFD+16. Nevertheless, the data from the colony condition assessments for 21DAA2 and 27DAA2 show that the total number of pupal and brood cells was similar for CA3642 and the control. No statistically significant effects of CA3642 were observed for the mean compensation index or termination rate of cells initially containing old larvae.

Overall, it can be concluded that the product, CA3642, had no significant effect on overall honey bee colony strength (mean number of adult honey bees), overall amount of brood or the development of the food storage area following application of 1400 mL CA3642/ha (target application rate: 210 g prothioconazole/ha actual: 215.3 g prothioconazole/ha and 219.0 g azoxystrobin/ha (1414.4 mL product/ha) at the first application and 214.9 g prothioconazole/ha and 218.6 g azoxystrobin/ha (1411.6 mL product/ha) at the second application).

The study satisfies the OECD 75 (2007) and OEPP/EPPO 170(4) (2010) test-guideline requirements for a semi-field brood study with *Apis mellifera* and is considered acceptable. The study also satisfies the requirements of EU (1997) 7029/VI/95 regarding the design, preparation and realisation of residue trials.

## Materials and methods

### Test materials

#### *Test item*

Name:	CA3642 (azoxystrobin 150 g/L + prothioconazole 150 g/L SC)
Density:	1.1004 g/cm <sup>3</sup>
Active substances:	Azoxystrobin and prothioconazole
Formulation type:	Soluble concentrate (SC)
Source and lot/batch no.:	A20026
Active substance 1 content:	Azoxystrobin 150 g/L nominal (154.83 g/L, 14.07 % w/w - analysed)
Active substance 2 content:	Prothioconazole 150 g/L nominal (152.23 g/L, 13.84 % w/w – analysed)
Appearance:	Off-white liquid
Expiry date of lot/batch:	07 September 2022
Storage conditions:	Cool (1°C -10°C), dark, dry

#### *Reference item*

Name:	Insegar
Active substance:	Fenoxycarb
Active substance content:	25.0% w/w nominal; 24.9% w/w analysed

#### *Test crop*

Name:	Winter Oilseed Rape ( <i>Brassica napus</i> )
Age:	BBCH 57 at application 1; BBCH 61 at time of tunnel set up and BBCH 63 (full flowering) at application 2
Location of test crop:	Near Pforzheim, Baden-Württemberg, Germany

### Test organism

Species:	Western honey bee, ( <i>Apis mellifera</i> L.), Hymenoptera, Apidae
Source:	Colonies supplied from Eurofins Agrosience Services' apiary; queen-right and free from symptoms of nosemosis, varroaosis or other honey bee diseases.

The trial was performed in a field of winter oilseed rape (*Brassica napus*), located near Pforzheim, Baden-Württemberg, Germany. Shortly prior to the onset of flowering (BBCH 61), 14 tunnel tents were set up to accommodate five control replicates (four biological replicates Ca-Cd and one residue sampling replicate Cs), five product treatment replicates (four biological replicates Ta-Td and one residue sampling replicate Ts) and four reference item replicates (Ra-Rd, no residue sampling replicate for the reference item), on honey bee colony per tent. Twenty-five hives were assessed at the first colony assessment for selection of the 12 biological hives and 2 residue sampling hives. They were ranked according to their colony strength (number of bees) and then the 3 strongest were distributed over each treatment group, followed by the second strongest 3 and so on. The number of brood cells and nectar cells were then as evenly distributed over the treatment groups as possible.

Each of the tunnel tents covered an area of 100 m<sup>2</sup> (20 m length x 5 m width) and had a height of approximately 3.5 m at the tunnel centre. Each tunnel frame was covered with light plastic gauze (mesh size: 1.5 mm). Before the start of the trial, paths (0.6 m) were created in the centre along the tunnel length and across the tunnel at both ends by removing the plants and smoothing the ground. These paths were covered with water-permeable linen sheets to enable the counting of dead honey bees during mortality assessments. The treated area crop (i.e. tunnel area minus path area) was 82.72 m<sup>2</sup>.

Honey bee colonies were relocated from the field site to the monitoring site (located near Oeschelbronn, 39 km from the field site) on 7DAA2 in the evening after daily honey bee flight and monitored further until 27DAA2. This site provided sufficient food sources on which the honey bees could forage (e.g. wild flowers). There were no intensive agriculture and no flowering main crops which might have been attractive to the honey bees in the near surroundings (3 km radius).

Two applications were made, one before flowering (BBCH 57) and the second at full flowering (BBCH 63), with honeybees actively foraging on the crop. The target application rate of the product, CA3642, was 1400 mL product/ha, equivalent to nominally 210 g a.s./ha each prothioconazole and azoxystrobin, Actual application rate 215.3 g prothioconazole/ha and 219.0 g azoxystrobin/ha (1414.4 mL product/ha) at the first application and 214.9 g prothioconazole/ha and 218.5 g azoxystrobin/ha (1411.6 mL product/ha) at the second application. Tap water was applied in the control group and the reference item was applied at a target rate of 1200 g Insegar/ha (equivalent to 300 g fenoxycarb/ha). The target spray volume was 300 L/ha in all treatment groups and were within the tolerance limits of  $\pm 10\%$ . The application was carried out with a calibrated portable boom sprayer, simulating a commercial application.

The following conditions for the second application were met:

- Winter oilseed rape in full bloom (BBCH 63)
- $\geq 5$  bees/m<sup>2</sup> honey bees were actively foraging shortly before the applications in control (C), product (T), and reference-item (R) treatment groups (mean values: 10.3 in C, 10.5 in T and 7.8 in R)

The following conditions for the first and second applications were met:

- Wind speed outside the tunnel tents did not exceed 2.0 m/s during all applications (max. 1.6 m/s for C and R, 1.9 m/s for T)
- Air temperature did not exceed 30°C (max. 24.3°C)
- The accepted spray tolerance of  $\pm 10\%$  per treatment group was met in all treatment groups (deviations ranged from -2.10 % to +3.95 %)
- There was no rainfall recorded on the day of the first application, 15DBA2, (and up to 3DBA2) nor on the day of second application, 0DAA2 (and up to 7DAA2).

Thirteen days before the second application (13DBA), a colony pre-assessment was conducted, to select

the most suitable honey bee colonies for the study, in which colonies for biological assessments contained between 5135 and 8905 honey bees per colony; the colonies for biological assessments (replicates a-d) contained 3-9 combs containing brood and 8-10 combs containing food. The mean number of honey bees was considered comparable with 7459 honey bees in the control group (C), 6858 honey bees in test item treatment group for CA3642 (T) and 7069 honey bees in the reference item treatment group (R). Hives intended for residue sampling contained 6-7 combs containing brood and 10 combs containing food. At brood fixing day 0 (BFD0), one day before the second application, the colonies contained between 5850 and 12220 honey bees. At BFD0 the mean number of honey bees was 9701 in the control, 8450 in CA3642 treatment and 8206 in the reference item treatment.

Before the start of mortality assessments, dead honey bee traps were fixed in front of the hives to record the number of dead honey bees which were carried out of the hives. The bottoms of the hives were equipped with a retractable bottom drawer to count the number of dead honey bees on the bottom of the hives without opening the hives.

One honey bee colony was placed in each tunnel, shortly before winter oilseed rape reached full bloom (BBCH 61, 4DBA2), in the evening after honey bee flight. During the exposure period in the tunnels, the honey bees were supplied with water from a water feeder or water bucket in biological and sampling replicates, respectively. The water feeders and buckets were removed from the tunnel during the application.

The honey bee colonies were relocated from the tunnels to the monitoring location after the last assessment of mortality, foraging activity and behaviour in the tunnels (in the evening after daily honey bee flight on 7DAA2), and monitored further until 27DAA2.

**Table CP 10.3.1.5/01-01. Critical dates of the test in the year 2022**

Activity	Timing	Date
Sampling of spray solution samples in all replicates of product test treatment T (S1)	0DBA1	28 Mar 2022
First spray application in treatment T (A1) at BBCH 57	15DBA2	28 Mar 2022
Colony pre-assessment	13DBA2	30 Mar 2022
Installation of the bee colonies in the tunnels (BBCH 61; evening after bee flight)	4DBA2	08 Apr 2022
Start of daily assessments of mortality, flight and behaviour inside tunnels	3DBA2	09 Apr 2022
1 <sup>st</sup> colony assessment (CCA1), photographic brood assessment (BFD0)	1DBA2/ BFD 0	11 Apr 2022
Sampling of spray solution in all replicates	0DBA2	12 Apr 2022
Second spray application during daily honey bee flight at BBCH 63 (C, T, R)	0DAA2	
Sampling of forager bees for analysis and nectar and pollen preparation (Cs, Ts,)		
Multiple assessments of mortality, flight and behaviour		
Multiple assessments of flight and behaviour, assessment of mortality	1DAA2	13 Apr 2022
2 <sup>nd</sup> colony assessment (CCA2), photographic brood assessment (BFD5)	4DAA2/ BFD5	16 Apr 2022
Sampling (S3) of forager bees for analysis and nectar and pollen preparation (Cs, T2s)	6DAA2	18 Apr 2022
Last assessments of mortality, flight and behaviour at the field site	7DAA2	19 Apr 2022
Relocation of the colonies to the monitoring site (evening after bee flight)		
Start of daily assessments of mortality and behaviour at the monitoring site	8DAA2	20 Apr 2022
3 <sup>rd</sup> colony assessment (CCA3), photographic brood assessment (BFD10)	9DAA2/ BFD10	21 Apr 2022
4 <sup>th</sup> colony assessment (CCA4), photographic brood assessment (BFD16)	15DAA2/ BFD16	27 Apr 2022
5 <sup>th</sup> colony assessment (CCA5), photographic brood assessment (BFD22)	21DAA2/ BFD22	03 May 2022
Last assessment of mortality and behaviour at the monitoring site	27DAA2	09 May 2022
6 <sup>th</sup> colony assessment (CCA6)		

DBA: days before application; DAA: days after application; BFD: brood area fixing day; CCA: condition of the colonies assessment

The data evaluated:

- Mortality (mean number of dead honey bees): recorded by counting the total number of dead honey bees on the linen sheets, in the dead bee traps and the bottom drawer inside the hive. Dead honey bees were differentiated into adult worker honey bees, pupae and larvae during each assessment and the exact number was recorded (dead male bees were also recorded, but excluded from the mortality evaluation). After each assessment, all dead honey bees found were removed.
- Flight activity (mean number of forager honey bees/m<sup>2</sup>/10-15 sec): the number of honey bees that were both foraging and flying over the crop within three areas of 1 m<sup>2</sup> were counted for 10-15 seconds. Assessment areas were chosen randomly prior to each assessment.
- Behaviour of the honey bees: during the assessments of mortality and flight activity, the behaviour of the honey bees in the crop and around the hive was observed according to the following categories:
  - o intensive cleaning
  - o trembling
  - o cramping
  - o locomotion problems
  - o inactivity
  - o hanging on the crop
  - o filtering (i.e. guard honey bees attacking and/or preventing returning honey bees from entering the hive)
  - o clustering of large numbers of honey bees at the hive entrance
  - o flying without landing
- Condition of the honey bee colonies: Number of honey bees (colony strength), presence of a healthy queen, development of the bee brood (area containing cells with eggs, larvae and capped cells, and food storage area (area with pollen, nectar and honey)
- Development of the honey bee brood (photographic assessments): assessed in individually marked brood cells at the assessment before application (Brood Area Fixing Day = BFD). Several brood combs were removed and cells containing eggs, young larvae and old larvae, where possible 200 cells for each parameter, were identified and monitored for brood development. During each brood stage, the fixed brood areas were photographed and used for analysis.
- Brood index as an indicator for the honey bee brood development (derived from values generated by analysis of the photographic assessment results)
  - o An assessed value (1-5) was assigned to all cells containing the expected brood stage at the respective day. For all cells that did not contain the expected brood stage, “0” was used for calculation on this date and all following assessments. The values of individual cells per assessment day were summed up and divided by the number of observed cells to obtain the average brood index
- Compensation index as an indicator for recovery the honey bee colony (derived from values generated by analysis of the photographic assessment results)
  - o An assessed value (1-5) was assigned to all cells containing the expected brood stage at the respective day. For all cells that did not contain the expected brood stage the actual observed cell content was used for calculation. “0” was used for calculation if cells were empty or filled with nectar or pollen. The values of all individual cells per assessment day were summed up and divided by the number of observed cells to obtain the compensation index
- Brood termination rate (%) of the initially marked honey bee eggs, young larvae and old larvae (derived from values generated by analysis of the photographic assessment results)
  - o Gives the number of the marked cells where a termination of the honey bee brood development (i.e. no successful development, the honey bee brood did not reach the expected brood stage at one of the assessment days, or food was stored in the cell) was recorded, expressed as percentage of the sum of all marked cells
- Results of spray solution residue analysis: samples were taken directly after preparation and mixing, shortly before each application and frozen within 6 hours of sampling. Analysis and quantification of active substance residues in spray solutions was performed in the analytical laboratories of Eurofins Agroscience Services EcoChem GmbH
- Results of residues analyses in pollen and nectar collected from forager honey bees: At least 75

forager honey bees were prepared for the nectar samples and at least 30 forager honey bees were prepared for the pollen samples. All samples were frozen within 2 hours of preparation. Analysis and quantification of active substance residues in nectar and pollen was performed in the analytical laboratories of Eurofins Agrosience Services EcoChem GmbH

The following meteorological data were recorded:

- During the application, the climatic conditions (temperature, humidity, wind speed) were measured using portable equipment at the trial site
- Daily precipitation was measured using a rain gauge (GLP record) located in a control group tunnel (Ca) during the exposure phase, or next to the colonies during the monitoring phase. The collected rain was recorded in the morning during the mortality assessments and the rain gauge was emptied thereafter
- Temperature and humidity were measured hourly using a calibrated data logger (GLP record) located in a control group tunnel (Ca) during the exposure phase, or next to the colonies during the monitoring phase
- Weather data for the pre- and post-exposure periods at the monitoring site were obtained from the LTZ (“Landwirtschaftliches Technologie Zentrum” of the German Government) weather station in Bauschlott located 5.9 – 14.2 km from the monitoring sites (non-GLP).

Various statistical analyses were conducted using SAS® (SAS Institute Inc., Version 9.4). A significance level of 0.05 was chosen for all comparisons. Data of the product treatment group (T) and the control group (C) were checked for normality using Shapiro-Wilks test. If the distribution of the data fitted the normal distribution very well (Shapiro-Wilks test,  $p \geq 0.2$ ) then Bartlett’s test was used to check for homoscedasticity of data, in the other cases, Levene’s test was used. If logarithmic transformation of data solved problems with normality or homoscedasticity, then the data were transformed for analysis to enable use of tests with higher statistical power. If normality and homoscedasticity were proven, Dunnett’s t-test was used for analysis of the data from the product treatments. If normality was met but homoscedasticity was disturbed, the Bonferroni-Holms corrected t-test (same as Welch test) was used for analysis. If data were not normal, the Bonferroni-Holms corrected U-test was used.

Data of the reference item treatment group (R) and corresponding data of the control were tested for normality using Shapiro-Wilks Test and for homoscedasticity using the Folded F-Test. Transformed data (logarithmic transformation as described above) were used for analysis if these data allowed the use of tests with higher power. The Student’s t-Test (pooled) was used for reference item data meeting normal distribution and homoscedasticity. In case of no homoscedasticity but proven normality Satterthwaite’s t-test was used. In case of no normal distribution of data, the Mann-Whitney Exact Test was used.

## Results

### Biological results

#### *Mortality*

A summary of the effects of CA3642 on honey bee mortality is presented in the tables below.

**Table CP 10.3.1.5/01-02. Summary of the effects of CA3642 on mean mortality of adult worker honey bees (*Apis mellifera*)**

Assessment timing	Application rate		
	Control (tap water)	T (nominal 1400 mL CA3642/ha)	R (300 g fenoxycarb/ha)
	Daily mean mortality <sup>1</sup> (dead worker bees/colony) ± SD		
Mean 3DBA2 – 0 DBA2	36.1 ± 14.2	26.2 ± 8.5	26.5 ± 6.2
Mean sum 0DAA2	27.3 ± 22.0	16.3 ± 8.1	17.5 ± 8.5
Mean 0DAA2 – 7 DAA2	38.1 ± 24.3	31.0 ± 27.8	23.3 ± 11.5
Mean 8DBA2 – 27DAA2	13.0 ± 2.0	12.9 ± 2.8	16.7 ± 3.5

DBA: days before application; DAA: days after application; SD: standard deviation; <sup>1</sup>Mortality: number of dead honey bee

worker adult/day found on linen sheets, in dead bee traps and bottom drawers

Throughout the study (before and following exposure), adult worker honey bee mortality was comparable between all treatments and no statistically significant differences were observed, indicating no adverse effects following exposure to CA3642.

**Overall, there was no statistically significant effects of CA3642 on daily adult worker honey bee mortality.**

**Table CP 10.3.1.5/01-03. Summary of the effects of CA3642 on mean mortality of worker honey bee (*Apis mellifera*) larvae and pupae**

Assessment timing	Application rate		
	Control (tap water)	T (nominal 1400 mL CA3642/ha)	R (300 g fenoxycarb/ha)
	Daily mean mortality <sup>1</sup> (dead larvae + dead pupae/colony) ± SD		
Mean 3DBA2 – 0 DBA2	0.3 ± 0.4	0.8 ± 0.6	0.3 ± 0.4
Mean sum 0DAA2	0.3 ± 0.5	0.0 ± 0.0	0.3 ± 0.5
Mean 0DAA2 – 7 DAA2	0.2 ± 0.1	0.2 ± 0.2	0.2 ± 0.2
Mean 8DBA2 – 27DAA2	0.7 ± 0.4	0.3 ± 0.1	106.9* ± 43.6

DBA: days before application; DAA: days after application; SD: standard deviation; <sup>1</sup>Mortality: number of dead honey bee worker larvae and pupae/day found on linen sheets, in dead bee traps and bottom drawers; \*Statistically significantly different compared to the control group.

Daily mortalities of honey bee worker larvae and pupae were comparable between the control group, (C), and the product treatment group (T) during the post-exposure phase at the monitoring site (8DAA2 to 27DAA2). In contrast, a statistically significant effect on honey bee larval and pupal mortality in the reference item group was observed for the exposure period from 8DAA2 to 27DAA2, confirming sensitivity of the test design.

**Overall, there were no statistically significant effects from CA3642 on honey bee worker larvae and pupae mortality. Sensitivity of the test design was confirmed, as the reference item treatment group, R, displayed the expected significant increase in larvae and pupae mortality over the expected timeframe.**

#### Flight activity

A summary of the effects of CA3642 on honey bee flight activity is presented in the table below.

**Table CP 10.3.1.5/01-04. Summary of the effects of CA3642 on mean honey bee (*Apis mellifera*) flight intensity**

Assessment timing	Application rate		
	Control (tap water)	T (nominal 1400 mL CA3642/ha)	R (300 g fenoxycarb/ha)
	Honey bee flight activity (mean ± SD number of forager bees/m <sup>2</sup> /10-15 seconds)		
Mean 3DBA2 – 0 DBA2	5.0 ± 0.8	4.9 ± 2.2	3.7 ± 1.0
Mean sum 0DAA2	17.8 ± 1.1	9.4* ± 3.3	6.7** ± 3.3
Mean sum 1DAA2	19.9 ± 1.9	17.1 ± 6.6	10.1** ± 5.2
Mean 0DAA2 – 7DAA2	13.0 ± 1.9	9.8 ± 2.6	6.7** ± 2.2

DBA: days before application; DAA: days after application; SD: standard deviation.

\*Statistically significantly different compared to the control group (Dunnett's t-test, left-sided, p≤0.05)

\*\*Statistically significantly different compared to the control group (pooled t-test, left-sided, p≤0.05)

On the day of application (0DAA2), after application, the mean sum of honey bee flight activity was statistically significantly reduced for the CA3642 and reference item treatment groups (T) and (R), compared to the control group (C). However, on the day after application (1DAA2), the mean honey bee flight activity was assessed three times (morning, noon and afternoon) and was observed to be comparable between the product treatment and control groups, while that in the reference item group remained statistically significantly reduced.

**Overall, a slight transient reduction in honey bee flight activity (forager bees/m<sup>2</sup> per 10-15 seconds) was observed in CA3642 treatment group on the day of application (0DAA2), but no statistically significant effect was observed for the remainder of the exposure period.**

#### Behaviour

A summary of the effects of CA3642 on honey bee behaviour is presented in the table below.

**Table CP 10.3.1.5/01-05. Summary of the effects of CA3642 on honey bee (*Apis mellifera*) behaviour**

Application rate	Assessment timing	Abnormal honey bee behaviour								
		LP	IA	CR	TR	IC	HA	Filt	FwL	Clu
Control (tap water)	Sum 3DBA2 – 0 DBA2	2	0	0	0	0	0	0	0	0
	Sum 0DAA2 – 7DAA2	2	2	6	0	0	0	0	0	0
	Sum 8DAA2 – 27DAA2	10	7	6	0	0	0	0	0	0
T (nominal 1400 mL CA3642/ha)	Sum 3DBA2 – 0 DBA2	0	0	3	0	0	0	0	0	0
	Sum 0DAA2 – 7DAA2	30	2	9	0	3	0	0	0	0
	Sum 8DAA2 – 27DAA2	19	70	10	0	0	0	0	0	0
R (300 g fenoxycarb/ha)	Sum 3DBA2 – 0 DBA2	0	0	1	0	1	0	0	0	0
	Sum 0DAA2 – 7DAA2	15	22	4	2	10 + many	2	0	0	0
	Sum 8DAA2 – 27DAA2	23	30	7	1	0	0	0	0	0

DBA: days before application; DAA: days after application;

LP: locomotion problems; IA: inactive bees; CR: cramping; TR: trembling; IC: intensive cleaning; HA: hanging on crop; Filt: Filtering (aggression) at hive entrance; FwL: flying without landing; Clu: clustering at hive entrance

Over the entire observation period from 3DBA2 to 27DAA2, honey bees displaying unusual behaviours such as locomotion problems, inactivity, cramping, intensive cleaning and trembling were observed across all treatment groups (C, T, R). Compared to the control group, honey bees of the CA3642 treatment group (T) showed slightly elevated numbers of honey bees displaying locomotion problems during exposure in the tunnels, but absolute numbers were still low (30 individuals) considering the total number of honey bees in the colony. After exposure, on two days (17DAA2 and 26DAA2) inactive bees were observed in the CA3642 treatment group at the monitoring site. Nevertheless, the frequency and magnitude of the observed behaviour are not considered to be treatment related, but rather, are probably due to unknown external reasons.

**Some unusual honey bee behaviour was displayed in all treatment groups (including the control group) at low levels. There is an indication for slightly elevated numbers of honey bees with locomotion problems following exposure to CA3642 treatment in the tunnel, but overall no biologically relevant product treatment effect on behaviour was observed during the assessments.**

#### Condition of the honey bee colonies

##### Honey bee colony strength – mean number of honey bees

The following table summarises the mean total number of honey bees at each assessment point.

**Table CP 10.3.1.5/01-06. The effect of CA3642 on mean honey bee colony strength - total number of adult honey bee (*Apis mellifera*) for each assessment point**

Assessment timing	Application rate		
	Control (tap water)	T (nominal 1400 mL CA3642/ha)	R (300 g fenoxycarb/ha)
	Mean total number of honey bees/colony ± SD		
Mean 13DBA2 (for hive selection)	7459 ± 1622	6858 ± 723	7069 ± 1307
Mean BFD0/1DBA2	9701 ± 1118	8450 ± 1751	8206 ± 2906
Mean BFD5/4DAA2	10563 ± 804	9588 ± 2503	9035 ± 2158
Mean BFD10/9DAA2	12724 ± 1066	13211 ± 3010	12773 ± 2625
Mean BFD16/15DAA2	14138 ± 2367	13861 ± 3064	14511 ± 1990
Mean BFD22/21DAA2	15714 ± 3503	15405 ± 2894	13699 ± 3599

<b>Mean CCA6/27DAA2</b>	12951 ± 2772	13033 ± 439	10481 ± 4033
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DBA: days before application; DAA: days after application; SD: standard deviation.

Over the course of the observation period, the colony strength increased in all treatment groups reaching maxima of 15714 (± 3503) in the control group (C), 15405 (± 2894) in the CA3642 treatment group (T) and 13699 (± 3599) in the reference item treatment group (R) on 21DAA2/BFD22.

After these maxima of mean number of honey bees, numbers declined in all treatment groups, including the control. At the end of the observation period (27DAA2), mean honey bee numbers were 12951 (± 2772) in the control group (C) 13033 (± 439) in the CA3642 treatment group (T) and 10481 (± 4033) in the reference item treatment group.

No statistically significant differences in honey bee colony strength (number of adult bees) were observed for any of the treatment groups.

**Overall, there were no statistically significant effects on honey bee colony strength (mean number of adult bees per colony), following exposure to CA3642.**

#### *Number of Brood Cells*

The following table summarises the mean number of honey bee brood cells (number of egg, larval and pupal cells/colony) at each assessment point.

**Table CP 10.3.1.5/01-07. Summary of the effects of CA3642 on mean number of honey bee (*Apis mellifera*) brood cells per colony for each assessment point**

Assessment timing	Application rate		
	Control (tap water)	T (nominal 1400 mL CA3642/ha)	R (300 g fenoxycarb/ha)
	Mean number of brood cells/colony (all stages <sup>1</sup> ) ± SD		
<b>13DBA2</b>	17179 ± 169	15050 ± 4703	14250 ± 5249
<b>1DBA2 / BFD0</b>	22679 ± 3297	18700 ± 3856	19250 ± 4793
<b>4DAA2 / BFD5</b>	23500 ± 1510	20300* ± 2075	20279 ± 6763
<b>9DAA2 / BFD10</b>	26500 ± 931	23500 ± 1291	22150 ± 6525
<b>15DAA2 / BFD16</b>	32850 ± 2259	27850* ± 1370	25400** ± 6112
<b>21DAA2 / BFD22</b>	33650 ± 3057	35100 ± 1949	30650 ± 8985
<b>27DAA2</b>	33109 ± 3618	31935 ± 1979	27555 ± 8911

<sup>1</sup>Mean number of cells containing eggs, larvae and pupae/colony.

DBA: days before application; DAA: days after application; BFD: Brood Fixing Day; SD: standard deviation.

\* Statistically significantly different compared to the control group (Dunnett's t-test, left-sided, p≤0.05)

\*\*Statistically significantly different compared to the control group (left-sided pooled t-test p≤0.05)

All brood stages were present during the entire observation period in all treatment groups.

The mean number of brood cells was slightly reduced in the CA3642 treatment compared to the control at BFD+5 (4DAA2) and BFD+16 (15DAA2), and in (R) at BFD16 (15DAA2) (p ≤ 0.05, left-sided Dunnett's test (T2) or pooled t-Test (R)). The slight transient effects at 4DAA2 and 15DAA2 for the CA3642 treatment are more likely to be related to the differences in initial colony size between (T) (CA3642) and control hives rather than any treatment related effect (brood cells: 13DBA2 C 17179 SD ±169 and T (CA3642) 15050 ± 4703).

Over the course of the observation period, the number of brood cells increased in all treatment groups reaching maxima of 33650 (± 3057) in the control group (C), 39050 (± 8888) 35100 (± 1949) in the test item treatment group (T) (CA3642) and 30650 (± 8985) in the reference item treatment group (R) on 21DAA2 / BFD+22. Between 21DAA2 / BFD+22 and the last assessment on 27DAA2, the number of brood cells decreased in all test item and reference item treatment groups and reached 33109 (± 3618) in the control group (C), 31935 (± 1979) in the test item treatment group (T) (CA3642) and 27555 (± 8911) in the reference item treatment group (R).



Honey bee colonies in the control (C) and CA3642 treatment (T) groups showed normal colony growth over time with an increase from around 15000 honey bee brood cells at 13DBAA2 to around 35000 honey bee brood cells at 27DAA2.

A slight, transient effect on the mean number of honey bee brood cells cannot be excluded for CA3642. However, the difference between the control group and the CA3642 treatment was only statistically significant on 4DAA2 and 15DAA2, but not statistically significant on 21DAA2 and 27DAA2. Overall, for the CA3642 treatment group, honey bee brood cell number at the end of the observation period was in line with the control group. Therefore, no biological adverse effect on honey bee brood cell number is concluded for CA3642.

#### *Number of food cells*

The following table summarises the mean number of cells containing food per colony (i.e. containing pollen and nectar) at each assessment point.

**Table CP 10.3.1.5/01-08. Summary of the effects of CA3642 on mean number of food cells for each assessment point**

Assessment timing	Application rate		
	Control (tap water)	T (nominal 1400 mL CA3642/ha)	R (300 g fenoxycarb/ha)
	Mean number of food cells/colony (all stages <sup>1</sup> ) ± SD		
<b>13DBA2</b>	26250 ± 7102	27850 ± 7195	30100 ± 6728
<b>1DBA2 / BFD0</b>	20700 ± 4678	22400 ± 5062	24100 ± 5461
<b>4DAA2 / BFD5</b>	17650 ± 3601	17750 ± 4136	19000 ± 5463
<b>9DAA2 / BFD10</b>	17600 ± 5210	20200 ± 6802	22450 ± 6690
<b>15DAA2 / BFD16</b>	15050 ± 4905	19200 ± 4880	19850 ± 5825
<b>21DAA2 / BFD22</b>	19150 ± 7819	21950 ± 957	20800 ± 5411
<b>27DAA2</b>	26950 ± 11020	25000 ± 4808	27300 ± 4113

<sup>1</sup>Mean number of cells containing nectar and pollen/colony.

DBA: days before application; DAA: days after application; BFD: Brood Fixing Day; SD: standard deviation.

There were no statistically significant adverse effects on the number of food cells in the CA3642 treatment group (T), compared to the control group, on any of the assessment dates.

Despite some variability between replicates and over the time-course of the trial, due to variable weather conditions, both prior to the trial period as well as during the trial, both nectar and pollen were generally sufficiently available to all colonies to sustain colony development and survival.

**Overall, there were no statistically significant effects CA3642 on the mean number of food cells containing nectar and pollen, compared to the control group. Therefore, it can be concluded that there were no adverse effects of CA3642 on honey bee food collection and/or storage.**

#### *Development of the honey bee brood*

##### Development in cells initially containing eggs

The mean brood indices of cells initially containing eggs from the start (BFD0) to the end (BFD22) of the brood cycle are summarised in the following table.

**Table CP 10.3.1.5/01-09. Summary of brood and compensation indices for honey bee (*Apis mellifera*) brood cells initially containing eggs**

Treatment		Brood index / Compensation index at x days after brood area fixing day (BFD) for marked cells initially containing eggs					Termination Rate BFD22 [%]
		0	+5	+10	+16	+22	
C	mean	1.00 / 1.00	2.78 / 2.79	3.31 / 3.48	3.28 / 3.60	4.10 / 4.60	18.03
	SD	0.00 / 0.00	0.12 / 0.11	0.91 / 0.63	0.91 / 0.43	1.14 / 0.42	22.68
T	mean	1.00 / 1.00	2.75 / 2.75	3.53 / 3.60	3.52 / 3.73	4.40 / 4.76	12.08
	SD	0.00 / 0.00	0.20 / 0.20	0.47 / 0.43	0.47 / 0.30	0.60 / 0.23	11.95
R	mean	1.00 / 1.00	2.40* / 2.42*	3.00 / 3.23	2.45 / 2.95*	2.99 / 4.05*	40.29
	SD	0.00 / 0.00	0.37 / 0.35	0.67 / 0.49	0.51 / 0.48	0.73 / 0.34	14.60

Treatments: C (Control), T (CA3642), R (toxic reference); BFD: brood area fixing day; SD: standard deviation; \*Statistically significantly lower (brood index and compensation index) than the control group (one-sided pooled t-Test;  $p \leq 0.05$ )

The mean compensation indices of honey bee brood cells initially containing eggs were 1.00 in all treatment groups at the first assessment (BFD0) and were 4.60 in the control group (C), 4.76 in the test item treatment group (T) (CA3642) and 4.05 in the reference item treatment group (R) at the end of the brood cycle (BFD+22). There was no statistically significant difference in the mean compensation index between (T) (CA3642) and the control. Compensation indices of cells initially containing eggs of the reference item group (R) were significantly lower than those of the control group (C) on BFD+5, BFD+16 and BFD+22.

The mean termination rates in cells initially containing eggs were 18.03% in the control group (C), 12.08% in the test item treatment group (T) (CA3642) and 40.29% in the reference item treatment group (R) at the end of the brood cycle (BFD+22). No statistically significant difference between the treatment group (T) (CA3642) and the control group (C), regarding the brood termination rate, was detected.

**Overall, no statistically significant adverse effects of CA3642 were observed on the compensation indices or mean termination rate for worker honey bee development, in cells initially containing eggs at all time points. Significantly decreased brood and compensation indices in the reference item compared to the control group confirm the sensitivity of the test system.**

#### Development in cells initially containing young honey bee larvae

The mean brood indices of cells initially containing young larvae from the start (BFD0) to the end (BFD22) of the brood cycle are summarised in the following table.

**Table CP 10.3.1.5/01-10. Summary of brood and compensation indices for cells initially containing young honey bee (*Apis mellifera*) larvae**

Treatment		Brood index / Compensation index at x days after brood area fixing day (BFD) for marked cells initially containing young larvae					Termination Rate BFD22 [%]
		0	+5	+10	+16	+22	
C	mean	2.00 / 2.00	3.74 / 3.79	3.70 / 3.79	4.23 / 4.43	4.49 / 2.73	10.17
	SD	0.00 / 0.00	0.15 / 0.13	0.15 / 0.10	0.17 / 0.14	0.14 / 0.38	2.83
T	mean	2.00 / 2.00	3.44 / 3.55	3.48 / 3.70	3.98 / 4.32	4.29 / 2.76	14.17
	SD	0.00 / 0.00	0.33 / 0.23	0.37 / 0.19	0.28 / 0.25	0.46 / 1.02	9.27
R	mean	2.00 / 2.00	3.66 / 3.73	3.64 / 4.30	4.07 / 4.30	4.20 / 3.01	16.05
	SD	0.00 / 0.00	0.37 / 0.26	0.37 / 0.31	0.37 / 0.31	0.43 / 0.35	8.60

Treatments: C (Control), T (CA3642), R (toxic reference); BFD: brood area fixing day; SD: standard deviation

The mean honey bee brood indices of cells initially containing young larvae were 2.00 in all treatment groups at the first assessment (BFD0) and were 4.49 in the control group (C), 4.29 in the test item treatment group (T) (CA3642) and 4.20 in the reference item treatment group (R) at the end of the brood cycle (BFD+22).

The mean compensation indices of cells initially containing young larvae were 2.00 in all treatment groups at the first assessment (BFD0) and were 2.73 in the control group (C), 2.76 in the test item treatment group (T) (CA3642) and 3.01 in the reference item treatment group (R) at the end of the brood cycle (BFD+22). The mean termination rates in cells initially containing young larvae were 10.17% in the control group (C), 14.17% in the test item treatment group (T) (CA3642) and 16.05% in the reference item treatment group (R) at the end of the brood cycle (BFD+22).

**Overall, no statistically significant adverse effects of CA3642 were observed on the worker honey bee development in cells initially containing young larvae at all time points.**

#### Development in cells initially containing old honey bee larvae

The mean brood indices of cells initially containing old larvae from the start (BFD0) to the end (BFD16) of the brood cycle are summarised in the following table. Emergence of adult worker honey bee from cells initially containing old larvae is expected to occur by BFD+16.

**Table CP 10.3.1.5/01-11. Summary of brood and compensation indices for cells initially containing old honey bee (*Apis mellifera*) larvae**

Treatment		Brood index / Compensation index at x days after brood area fixing day (BFD) for marked cells initially containing old larvae				Termination Rate BFD16 [%]
		0	+5	+10	+16	
C	mean	3.00 / 3.00	3.91 / 3.93	3.90 / 3.92	4.87 / 4.92	2.58
	SD	0.00 / 0.00	0.06 / 0.05	0.06 / 0.04	0.07 / 0.05	1.41
T	mean	3.00 / 3.00	3.17 / 3.35	3.16 / 3.55	3.66* / 4.44	26.76
	SD	0.00 / 0.00	1.00 / 0.75	1.01 / 0.39	1.19 / 0.48	23.85
R	mean	3.00 / 3.00	3.83 / 3.86	2.71 / 2.85	3.19* / 3.85*	36.24
	SD	0.00 / 0.00	0.14 / 0.09	1.17 / 1.15	1.29 / 1.05	25.67

Treatments: C (Control), T (CA3642), R (toxic reference; BFD: brood area fixing day; SD: standard deviation; \*Statistically significantly lower (brood and compensation indices) than the control group (one-sided Dunnett's test, Satterthwaite t-Test or Mann Whitney Exact test, as applicable,  $p \leq 0.05$ )

The mean compensation indices of cells initially containing old larvae were 3.00 in all treatment groups at the first assessment (BFD0) and were 4.92 in the control group (C), 4.44 in the test item treatment group (T) (CA3642) and 3.85 in the reference item treatment group (R) at the end of the relevant period (BFD+16). The compensation index of cells initially containing old larvae of the reference item treatment group (R) was significantly lower than the one of the control group (C) on BFD+16.

The mean brood termination rates in cells initially containing old larvae were 2.58% in the control group (C), 26.76% in the test item treatment group (T) (CA3642) and 36.24% in the reference item treatment group (R) at the end of the relevant period (BFD+16/15 DAA2). Although the brood termination rate for the test item treatment group was elevated compared to the control, no statistical significances between the test item treatment groups with regard to the brood termination rate of old larvae was detected. The data from the colony condition assessments for 21DAA2 and 27DAA2 show that the total number of pupal and brood cells was at similar levels in the control and treatment (CA3642) groups, which allows the conclusion that the reduction of pupal cells was only transient. Treatment group (T) (CA3642) had a slightly lower number of brood cells than the control group (C) from the first colony assessment up until BFD16.

**A transient effect of CA3642 on the development in cells initially containing old larvae was observed for the brood index at BFD+16. Since the total number of brood and pupal cells was at similar levels on the subsequent colony assessments 21DAA2 and 27DAA2 this difference has no overall biological effect on the brood development. No statistically significant effects of CA3642 were observed on mean compensation index or termination rate of cells initially containing old larvae.**

#### *Analytical results*

##### Residue analysis of spray solution samples

The HPLC -MS/MS method for the determination of prothioconazole and azoxystrobin in pollen, nectar and spray solution was validated with regards to specificity, linearity, accuracy and precision in accordance with guideline SANTE/2020/12830 rev.1 24/02/2021. Specificity was demonstrated by the absence of significant interference above 30% of the LOQ in reagent blanks and control samples. The linearity of the method was demonstrated using solvent calibration standards for spray solutions and matrix-matched calibration standard for pollen and nectar matrices.

The analytical method was shown to be linear for prothioconazole ( $r > 0.995$ ) over the following ranges:

- Pollen: 0.1 – 9.0 ng/mL (n = 9), corresponding to 0.003 – 0.27 mg/kg
- Nectar: 0.03 – 3.0 ng/mL (n = 8), corresponding to 0.003 – 0.3 mg/kg
- Spray sol.: 0.04 – 3.0 ng/mL (n = 8), corresponding to 20 – 1500 mg/L

The analytical method was shown to be linear for azoxystrobin ( $r > 0.995$ ) over the following ranges:

- Pollen: 0.1 – 9.0 ng/mL (n = 9), corresponding to 0.003 – 0.27 mg/kg
- Nectar: 0.03 – 3.0 ng/mL (n = 8), corresponding to 0.003 – 0.3 mg/kg
- Spray sol.: 0.04 – 3.0 ng/mL (n = 8), corresponding to 20 – 1500 mg/L

Analytical results confirmed that spray solutions of CA3642 (T), ranged between 540 and 568 mg prothioconazole/L (equivalent to 77% to 81% of the target concentration (700 mg/L)), for the first application, and from 552 to 582 mg prothioconazole/L (equivalent to 79% to 83% of the target concentration), for the second application.

Similarly, analytical results confirmed azoxystrobin ranged between 535 and 563 mg azoxystrobin/L (equivalent to 76% to 80% if the target concentration (700 mg/L)), for the first application, and from 552 to 566 mg azoxystrobin/L (equivalent to 79% to 81% of the target concentration), for the second application.

#### Residue analysis of pollen and nectar samples taken from forager honey bees

In nectar, mean recoveries were 97%, 100% and 100% for prothioconazole and 108%, 106% and 107% for azoxystrobin at 0.01, 0.1 and 1 mg/kg respectively (i.e., within 70-110%). For prothioconazole, the relative standard deviations (n = 5 for each fortification level) were 5%, 2% and 4% at 0.01, 0.1 and 1 mg/kg respectively (i.e., within the guideline limit of  $\leq 20\%$ ). For azoxystrobin, the relative standard deviations (n = 5 for each fortification level) were 2%, 2% and 3% at 0.01, 0.1 and 1 mg/kg respectively (i.e., within the guideline limit of  $\leq 20\%$ ).

In pollen, mean recoveries were 92%, 91% and 93% for prothioconazole and 88%, 95% and 98% for azoxystrobin at 0.01, 1 and 100 mg/kg respectively (i.e., within 70-110%). For prothioconazole, the relative standard deviations (n = 5 for each fortification level) were 6%, 9% and 12% at 0.01, 0.1 and 1 mg/kg respectively (i.e., within the guideline limit of  $\leq 20\%$ ). For azoxystrobin, the relative standard deviation (n = 5 for each fortification level) were 7%, 6% and 7% at 0.01, 0.1 and 1 mg/kg respectively (i.e., within the guideline limit of  $\leq 20\%$ ).

In spray solutions, mean recoveries were 100% and 92% for prothioconazole at 66666 and 907715 mg/L respectively (i.e., within 70-110%) and 102% for azoxystrobin at 66666 and 908282 mg/L (i.e., within 70-110%). For prothioconazole, the relative standard deviation (n = 5 for each fortification level) was 3% at 66666 and 907715 mg/L (i.e., within the guideline limit of  $\leq 20\%$ ). For azoxystrobin, the relative standard deviation (n = 5 for each fortification level) was 3% and 2% at 66666 and 908282 mg/L respectively (i.e., within the guideline limit of  $\leq 20\%$ ).

The limit of quantification (LOQ) for pollen and nectar was 0.01 mg/kg for both analytes. The limit of detection (LOD) was defined at 0.003 mg/kg (30% of the LOQ) for both analytes. The limit of quantification (LOQ) for spray solution was 67 mg/L for both analytes.

No residues of prothioconazole or azoxystrobin above the LOD (0.003 mg/kg for both compounds) were detected in any of the nectar and pollen samples taken from forager honey bees in the control.

Residues in nectar samples taken from forager honey bees in the CA3642 treatment were from 0.191 mg prothioconazole/kg (0DAA2), decreasing to <LOD (6DAA2) and 0.606 mg azoxystrobin/kg (0DAA2) decreasing to 0.758 mg azoxystrobin/kg (6DAA2).

Residues in pollen samples taken from forager honey bees in the CA3642 treatment were from 35.1 mg

prothioconazole/kg (0DAA2), decreasing to 0.0303 mg prothioconazole/kg (6DAA2) and 52.2 mg azoxystrobin/kg (0DAA2) decreasing to 1.34 mg azoxystrobin/kg (6DAA2).

### Validity

The study is considered valid because the following validity criteria were fulfilled:

- Effect of the reference item: brood termination rate in cells initially containing eggs was significantly increased above control levels and a significantly increased mortality of worker honey bee larvae and pupae of the reference item treatment group was observed in the relevant time frame;
- Control brood termination rate: the mean brood termination rate averaged over the four biological replicates of the control group was not higher than 50% on any assessment date for all brood stages.

### Conclusion

CA3642 was applied as two foliar applications under semi-field conditions to oilseed rape, one at pre-flowering and one at flowering, during daily honey bee flight (interval between applications: 15 days). Applications were made at the target rate each of 210 g a.s./ha of prothioconazole and azoxystrobin (actual rate applied based on GLP Certificate of analysis): 215.3 g prothioconazole/ha and 219.0 g azoxystrobin/ha (1414.4 mL product/ha) at the first application and 214.9 g prothioconazole/ha and 218.6 g azoxystrobin/ha (1411.6 mL product/ha) at the second application.

Residues of prothioconazole and azoxystrobin were detected in the nectar and pollen samples collected from the forager bees in the CA3642 treatment confirming that honey bees and their hives were exposed to the CA3642 treatment.

CA3642 did not lead to statistically significant adverse effects on mortality of honey bee worker bees (adults, larvae or pupae). Transient effects on honey bee flight activity were only observed on the day of application. No biologically relevant CA3642 treatment related behavioural effects were observed. CA3642 had no statistically significant effects on honey bee colony strength (mean number of adult honey bees), mean number of cells containing food (nectar and pollen), mean compensation indices or mean termination rates in brood cells initially containing eggs or young larvae.

CA3642 lead to a significant transient reduction in the mean number of honey bee pupal cells on 9DAA2 and of total brood cells on two occasions (4DAA2, 15DAA2) but there was no statistically significant reduction seen on the last two assessment dates (21DAA2, 27DAA2). Overall brood cell number levels at the end of the study observation period for CA3642 were in line with the control. Therefore, no biological adverse effect on honey brood cell number is concluded for CA3642.

A transient significant reduction of the honey bee brood index for cells initially containing old larvae was observed at BFD+16. Nevertheless, the data from the colony condition assessments for 21DAA2 and 27DAA2 show that the total number of pupal and brood cells was similar for CA3642 and the control. No statistically significant effects of CA3642 were observed for the mean compensation index or termination rate of cells initially containing old larvae.

**Overall, it can be concluded that CA3642 had no significant effect on overall honey bee (*Apis mellifera*) colony strength (mean number of adult honey bees), overall amount of brood or the development of the food storage area following nominal target application of 1400 mL CA3642/ha (210 g a.s./ha each of prothioconazole and azoxystrobin) during honey bee flight.**

This study is considered reliable and acceptable.

## A 2.3.1.6 KCP 10.3.1.6 Field tests with honeybees

### A 2.3.1.7 Effects on non-target arthropods other than bees

#### Study 1

Comments of zRMS:	<p>The study was conducted in line with the respective guideline by Blümel S. et al. (2000) with the following deviations.</p> <ul style="list-style-type: none"> <li>○ The mortality in the reference item treatment was slightly below the 50 % mortality validity criteria on Day 7 but was above on Day 14. The mean mortality of protonymphs exposed to the reference item was 48.8 % (47.4 % corrected) after 7 days of exposure and 52.5 % (51.3 % corrected) after 14 days of exposure.</li> <li>○ Time to insert the mites in the test arena was slightly above 90 minutes (96 minutes) for the highest test item treatment at 15.5 L Test item/ha.</li> <li>○ The number of individuals inserted in the control replicates 2 and 4 were 21 instead of 20.</li> <li>○ The measured temperature ranged from 21.2 to 22.7 °C with a mean of 21.9 °C. Due to an issue with the climate chamber used, the temperature was slightly below the required range (<math>25 \pm 2</math> °C). This slightly lower temperature is not considered to have had a significant effect on the performance of the organisms as the control <i>T. pyri</i> met their mortality and reproductive validity criteria.</li> </ul> <p>The deviations are not considered to have had a significant impact on the outcome of the study.</p> <p>All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>The 7-day LR<sub>50</sub> (mortality) &gt;15.5 L product/ha, equivalent to &gt;2360 g prothioconazole/ha and &gt;2400 g azoxystrobin/ha, respectively, the highest application rate tested.</p> <p>The 7-day NOER (mortality) = 8.62 L product/ha, equivalent to 1312 g prothioconazole and 1335 g azoxystrobin, respectively.</p> <p>The 14-day ER<sub>50</sub> (reproduction) = 2.35 (with 95% Confidence Limit (CL): 0.28 - n.d.) L product/ha, equivalent to 358 (95% CL: 43.1 – n.d.) g prothioconazole/ha and 364 (95% CL: 43.8 – n.d.) g azoxystrobin/ha, respectively.</p> <p>The 14-day NOER (reproduction) &lt;1.48 L product/ha, equivalent to &lt;225 g prothioconazole/ha and &lt; 229 g azoxystrobin/ha, respectively.</p>
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Reference:	KCP 10.3.2.1/01
Report	CA3642 - Toxicity to the Predatory Mite <i>Typhlodromus pyri</i> (Acari, Phytoseiidae), under Worst-Case Conditions in the Laboratory  Cornement, M., year 2023, Report No. 20210200
Guideline(s):	Blümel S. et al. (2000) in "Guidelines to evaluate side-effects of plant protection products to non-target arthropods"
Deviations:	<p>Yes.</p> <ol style="list-style-type: none"> <li>1. The mortality in the reference item treatment was slightly below the 50 % mortality validity criteria on Day 7 but was above on Day 14. The mean mortality of protonymphs exposed to the reference item was 48.8 % (47.4 % corrected) after 7 days of exposure and 52.5 % (51.3 % corrected) after 14 days of exposure.</li> <li>2. Time to insert the mites in the test arena was slightly above 90 minutes (96 minutes) for the</li> </ol>

	highest test item treatment at 15.5 L Test item/ha. 3. The number of individuals inserted in the control replicates 2 and 4 were 21 instead of 20. 4. The measured temperature ranged from 21.2 to 22.7 °C with a mean of 21.9 °C. Due to an issue with the climate chamber used, the temperature was slightly below the required range ( $25 \pm 2$ °C). This slightly lower temperature is not considered to have had a significant effect on the performance of the organisms as the control <i>T. pyri</i> met their mortality and reproductive validity criteria. The deviations are not considered to have had a significant impact on the outcome of the study.
GLP:	Yes, conducted under GLP.
Acceptability:	Yes (all validity criteria met in accordance with current guidance)
Duplication (if vertebrate study)	No.

## Executive summary

A laboratory dose-response study of the predatory mite, *Typhlodromus pyri*, exposed to dried residues of the product, CA3642, a suspension concentrate (SC) formulation (analysed active substances: 13.84% w/w prothioconazole and 14.07% w/w azoxystrobin), was conducted on glass plates in accordance with Blümel *S. et al.* (2000). There were seven treatment groups of 0 (water control), 1.48, 2.66, 4.79, 8.62 and 15.5 L product/ha, equivalent to 225, 405, 729, 1312 and 2360 g prothioconazole/ha and to 229, 412, 742, 1335 and 2400 g azoxystrobin/ha (based on the content of active substances in CA3642 of 152.23 g/L of prothioconazole and 154.83 g/L of azoxystrobin), and a toxic reference (Perfekthion (37.4% w/w dimethoate)).

Each treatment consisted of four replicates containing 20 protonymphs each. Adult mortality was assessed after 3, 7 and 14 days' exposure and reproduction (number of eggs per surviving female) from Day 7 to 14.

The 7-day LR<sub>50</sub> value (mortality) was estimated to be >15.5 L product/ha, equivalent to >2360 g prothioconazole/ha and >2400 g azoxystrobin/ha, respectively, the highest application rate tested. The 7-day NOER value (mortality) was determined to be 8.62 L product/ha, equivalent to 1312 g prothioconazole and 1335 g azoxystrobin, respectively.

The 14-day ER<sub>50</sub> value (reproduction) was determined to be 2.35 (with 95% Confidence Limit (CL): 0.283 - n.d.) L product/ha, equivalent to 358 (95% CL: 43.1 - n.d.) g prothioconazole/ha and 364 (95% CL: 43.8 - n.d.) g azoxystrobin/ha, respectively. The 14-day NOER value (reproduction) was estimated to be <1.48 L product/ha, equivalent to <225 g prothioconazole/ha and <229 g azoxystrobin/ha, respectively.

For validation of the test system, treatment with Perfekthion (20 mL/ha), as the reference item, resulted in a mortality slightly below the 50% mortality validity criteria on Day 7 (i.e. 47.4 %) but above on Day 14 (i.e. 51.3 %).

Overall, the study satisfies the IOBC Blümel *et al.* (2000) test-guideline requirements for chronic toxicity to *Typhlodromus pyri* and is considered acceptable.

## Materials and methods

### Test material

Name:	CA3642 (azoxystrobin 150 g/L + prothioconazole 150 g/L SC)
Formulation type:	SC (suspension concentrate)
Source and Lot/batch no.:	Nufarm - A20026
Active substances:	a. Azoxystrobin b. Prothioconazole
Active substance content:	a. 14.07% w/w corresponding to 154.83 g/L b. 13.84% w/w corresponding to 152.23 g/L
Density:	1.1004 g/mL

Appearance: Off-white, odourless suspension  
Expiry date of lot/batch: September 07, 2022  
Storage conditions: Store in the closed, original container in a cool, well-ventilated area.

#### Reference item

Name: Perfekthion  
Density: 1.04 – 1.10 g/cm<sup>3</sup>  
Active substance: Dimethoate  
Batch no.: 0001122611  
Active substance content: 37.4% w/w, corresponding to 400 g/L, based on a density of 1.07 g/cm<sup>3</sup>  
Appearance: Blue liquid  
Expiry date: January 31, 2026

#### Test organism

Species: *Typhlodromus pyri* Scheuten (Acari, Phytoseiidae)  
Age at study initiation: <24-hours-old protonymphs  
Source: Katz Biotech AG, Birkenpfuhlheide 10, 15837 Baruth, Germany  
Feeding during test: Yes. Apple pollen (*Malus vulgaris*) on days 0, 3, 7, 10, and 13 after application.

#### Test conditions

Test temperature: 25 ± 2 °C (actual: 21.2 to 22.7 °C, mean of 21.9 °C\*)  
Relative humidity: 60-90% (actual: 61.6 to 84.9%, mean of 75.8%)  
Light intensity: 466 and 688 Lux (measured at test unit level)

\* Due to an issue with the climate chamber used, the temperature was below the required range (25 ± 2 °C). This slightly lower temperature is not considered to have had any impact on the study as the control validity criteria were met.

The test arena consisted of two glass cover slides (approximately 24 × 50 mm), fixed together in longitude by a small strip of an adhesive tape. The gap between the two slides was sufficiently close to prevent mites from escaping, but sufficiently large to ensure availability of drinking water for the mites by capillary forces. The mites were confined to an area of approximately 10-13 cm<sup>2</sup> within the test arena by a barrier of non-drying glue gel. Test arenas were placed on a layer of a wet black filter paper, positioned on top of a water-soaked piece of foam on the bottom of the test unit. The test units were placed in plastic petri dishes (approximately 9-cm diameter, 1.6-cm height) partly filled with water. Holes in the bottom of the test units allowed water supply of the test arena via the water-soaked foam and the filter paper inside the test container. Plastic lids with fine gauze protected the test systems from outside disturbances during the exposure period.

A reproduction phase (assessment of fecundity) followed the exposure phase for an additional week, during which female mites were assessed for fecundity. The test units of the mortality phase were also used for the subsequent reproduction phase assessing the adults, which had developed from the exposed protonymphs.

Prior to application, a stock solution was made, by mixing 93.06 mL product in 1200 mL of ultrapure water. The application rates used in the study were 1.48, 2.66, 4.79, 8.62, and 15.5 L product/ha, equivalent to 225, 405, 729, 1312, and 2360 g prothioconazole/ha and 229, 412, 742, 1335, and 2400 g azoxystrobin/ha (accounting for the nominal product a.s. content of 152.23 g/L of prothioconazole and 154.83 g/L of azoxystrobin). The product concentrations in the application solutions were based on the spray volume of 200 L/ha. The study also included a control, where only ultrapure water was applied. The reference item treatment was applied at a rate of 20 mL Perfekthion/ha (equivalent to 8 g dimethoate/ha, when accounting for the nominal reference-item purity of 400 g a.s./L), which was expected to result in a mean mortality ranging between 50 and 100% after 7 days of exposure. The stock solution for the reference item was made by mixing 20.16 mg Perfekthion in 500 mL of ultrapure water.

After application and after the spray deposits on the glass plates had dried, 20 or 21 protonymphs per each of 4 replicates were transferred onto the surface, using a fine peaked brush (start of test). The transfer of the mites from the holding vessels to all test units was completed within 90 minutes after the application



except for the highest product treatment, i.e. 15.5 L product/ha, where it was completed after 96 minutes (Deviation 2). Cumulative mortality was assessed 7 days after treatment (DAT) and cumulative reproduction per female was assessed from 7 to 14 DAT.

Test organisms were considered dead when motionless, even after touching with a fine hairbrush. Mortality was calculated as the sum of dead and escaped mites (sum of unaccounted mites: not found on the test arena, stuck in the glue barrier or drowned in the water supply). Mortality for treatment groups was corrected for dead animals in the control, using the Abbott and Schneider-Orelli formula.

The reproduction phase was carried out with the control and product treatment groups only. The sex of the protonymphs at test initiation could not be determined, since protonymphs were sexually immature. Sexing of the mites was performed at the adult stage, when the females differed from the males by size and colour.

For the assessment of reproduction, the sex ratio of each replicated was adjusted to a minimum of 1 male: 5 females 7 DAT (i.e. the start of the reproduction phase). The number of larvae and eggs was recorded on each assessment day and were removed afterwards. The number of eggs per female during the reproduction period was calculated for each replicate separately. The result for each treatment group was the mean cumulative number of eggs per female.

The differences in mortality between the control and the product treatment groups was tested using Fisher's Exact Binomial Test ( $\alpha = 0.05$ , one sided greater). As the product treatments induced mortality  $<15\%$ , the NOER, LOER and LR<sub>50</sub> values were determined directly from the raw data. No LR<sub>10</sub> and LR<sub>20</sub> values were determined.

The differences in reproduction between the control and the test item treatments was tested using Multiple sequentially-rejective Welsh-t-test after Bonferroni-Holm ( $\alpha = 0.05$ , one-sided smaller). The ER<sub>x</sub> values were calculated using a non-linear 4-parametric Weibull CDF. However, to increase the statistical power, the lowest treatment was excluded from the statistical analysis.

Statistical analysis was performed using ToxRat Professional, Version 3.2.1.

## Results and discussions

### Biological results

A summary of the effects of CA3642 on adult mortality following 7 days of exposure, is presented in the table below.

**Table 10.3.2/01-01: The effect of CA3642 on adult *Typhlodromus pyri* cumulative mortality, after 7 days of exposure**

Application rate			Rep.	N° introduced	7 DAT			
L product/ha	g azoxystrobin/ha	g prothioconazole/ha			N° dead <sup>1</sup>	Mortality (%)	Mean mortality $\pm$ SD (%)	Corrected mortality <sup>2</sup> (%)
0 (control)	0	0	1	20	0	0.0	2.50 $\pm$ 5.00	-
			2	21	0	0.0		
			3	20	2	10.0		
			4	21	0	0.0		
1.48	229	225	1	20	1	5.0	5.00 $\pm$ 4.08	2.56
			2	20	2	10.0		
			3	20	1	5.0		
			4	20	0	0.0		
2.66	412	405	1	20	2	10.0	10.0 $\pm$ 0.0	7.69
			2	20	2	10.0		
			3	20	2	10.0		
			4	20	2	10.0		

Application rate			Rep.	N° introduc ed	7 DAT			
L product/h a	g azoxystro bin/ha	g prothiocona zole/ha			N° dead <sup>1</sup>	Mortality (%)	Mean mortality ± SD (%)	Corrected mortality <sup>2</sup> (%)
4.79	742	729	1	20	2	10.0	12.5 ± 8.66	10.3
			2	20	1	5.0		
			3	20	5	25.0		
			4	20	2	10.0		
8.62	1335	1312	1	20	4	20.0	12.5 ± 9.57	10.3
			2	20	0	0.0		
			3	20	2	10.0		
			4	20	4	20.0		
15.5	2400	2360	1	20	3	15.0	13.8 ± 6.29	11.5*
			2	20	3	15.0		
			3	20	4	20.0		
			4	20	1	5.0		
20 mL Perfekthion/ha			1	20	10	50.0	48.8 ± 12.5	47.4
			2	20	7	35.0		
			3	20	13	65.0		
			4	20	9	45.0		

Rep.: Replicate

DAT: days after treatment

SD: Standard Deviation

<sup>1</sup>: Includes escapees, not found and dead mites

<sup>2</sup>: Mortality corrected with control mortality by using the formula of Abbott with improvements by Schneider-Orelli. A negative value corresponds to a treatment mortality lower than the control mortality.

\*: Statistically significantly different to control mortality (Fisher's exact binomial test with Bonferroni correction ( $\alpha = 0.05$ , one-sided greater)).

After seven days of exposure, statistically significant effects on mean mortality of *Typhlodromus pyri* were observed at the highest product treatment group, when compared to the control group.

The 7-day LR<sub>50</sub> value (mortality) was estimated to be >15.5 L product/ha, equivalent to >2360 g prothioconazole/ha and >2400 g azoxystrobin/ha, respectively, the highest application rate tested. The 7-day NOER value (mortality) was determined to be 8.62 L product/ha, equivalent to 1312 g prothioconazole and 1335 g azoxystrobin, respectively.

### Reproduction

A summary of the effects of CA3642 on reproduction of *Typhlodromus pyri*, after 14 days of exposure, is presented in the table below.

**Table 10.3.2/01-02. Summary of the effects of CA3642 on *Typhlodromus pyri* cumulative reproduction (fecundity), between 7 and 14 days after exposure**

Application rate			Rep.	Cumulative reproduction (eggs (+ larvae)/female)		% Reduction in reproduction (compared to control) <sup>1</sup>
L product/ha	g azoxystrobin/ha	g prothioconazole/ha		Reproduction	CMR (Treatment mean ± SD)	
0 (control)	0	0	1	7.01	6.87 ± 0.570	100
			2	6.62		
			3	6.26		
			4	7.59		
1.48	229	225	1	2.61	3.42 ± 0.608	49.8*
			2	4.07		
			3	3.43		
			4	3.56		
	412	405	1	5.39	4.72 ± 0.467	68.7*

2.66			2	4.36		
			3	4.65		
			4	4.47		
4.79	742	729	1	4.18	3.65 ± 1.10	53.1*
			2	3.24		
			3	4.84		
			4	2.32		
8.62	1335	1312	1	5.45	3.51 ± 2.05	51.1*
			2	4.96		
			3	1.11		
			4	2.50		
15.5	2400	2360	1	3.52	3.06 ± 1.38	44.6*
			2	3.31		
			3	1.10		
			4	4.32		

CMR: Cumulative treatment mean reproduction.

<sup>1</sup>: % reduction in reproduction obtained by  $(R_t/R_c) * 100 \%$ , where:  $R_t$  and  $R_c$  are the absolute reproduction values observed in the treatment and control groups, respectively.

Rep., replicate; SD, standard deviation.

\*: Statistically significantly different to control reproduction (multiple sequentially-rejective Welsh-t-test after Bonferroni-Holm ( $\alpha = 0.05$ , one-sided greater))

After 14 days of exposure, statistically significant effects on mean egg production of *Typhlodromus pyri* were observed at all product treatment groups, when compared to the control group. The 14-day  $ER_{50}$  value was determined to be 2.35 (95% CL: 0.283 – n.d.) L product/ha, equivalent to 358 (95% CL: 43.1 – n.d.) g prothioconazole/ha and 364 (95% CL: 43.8 – n.d.) g azoxystrobin/ha, respectively.

The 14-day NOER value for reproduction was estimated to be <1.48 L product/ha, equivalent to <225 g prothioconazole and <229 g azoxystrobin, respectively.

### Validity

All validity criteria were met in accordance with IOBC Blümel *et al.* (2000) test guideline:

- Mean mortality in the control was  $\leq 20\%$ , after 7 days' exposure (actual value: 2.50%).
- Mean corrected mortality in the reference-item group was between 50-100%, after 7 days' exposure (actual value: 48.8 % (47.4% corrected) after 7 days of exposure and 52.5 % (51.3% corrected) after 14 days of exposure).
- Cumulative mean number of eggs per female in the control was  $\geq 4$  (actual value: 6.87).

The control validity criteria for both mortality and reproduction were met. The reference item mortality validity criterion was not quite met on Day 7 but was met by Day 14. The mortality was only just under 50% at day 7 and the study did show effects at the higher concentration (mortality) and on reproduction.

### Conclusion

In a glass plate laboratory test, the effects of dried residues of CA3642 on glass plates, on the predatory mite, *Typhlodromus pyri*, were assessed in accordance with IOBC Blümel *et al.* (2000) test guidelines.

The 7-day  $LR_{50}$  value (mortality): >15.5 L product/ha, equivalent to >2360 g prothioconazole/ha and >2400 g azoxystrobin/ha, respectively.

The 7-day NOER value (mortality): 8.62 L product/ha, equivalent to 1312 g prothioconazole and 1335 g azoxystrobin, respectively.

The 14-day  $ER_{50}$  value (reproduction): 2.35 L product/ha, equivalent to 358 g prothioconazole/ha and 364 g azoxystrobin/ha, respectively.

The 14-day NOER value (reproduction): <1.48L product/ha, equivalent to <225 g prothioconazole/ha and <229 g azoxystrobin/ha, respectively.

This study is considered acceptable and valid.

## Study 2

Comments of zRMS:	<p>The study was conducted in line with the respective guideline according to Yes. IOBC, Mead-Briggs et al. (2000) with no deviations.</p> <p>All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>The 48-h LR<sub>50</sub> (mortality) = 2.895 g product/ha (equivalent to 440.71 g prothioconazole/ha and 448.23 g azoxystrobin/ha, respectively), based on nominal concentrations.</p> <p>The 48-h NOER (mortality) = 0.256 L product/ha (equivalent to 38.97 g prothioconazole/ha and 39.64 g azoxystrobin/ha, respectively), based on nominal concentrations.</p> <p>The 48-h ER<sub>50</sub> (reproduction) &gt;10.0 L product/ha, (equivalent to &gt;1522.3 g prothioconazole/ha and &gt;1548.3 g azoxystrobin/ha, respectively), based on nominal concentrations.</p> <p>The 48-h NOER (reproduction) = 4.0 g product/ha (equivalent to 608.92 g prothioconazole/ha and 619.32 g azoxystrobin/ha, respectively), based on nominal concentrations.</p>
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Reference:	KCP 10.3.2.1/02
Report	<p>A Worst-Case Laboratory Test to Determine the Effects of CA3642 (Prothioconazole 150 g/L + Azoxystrobin 150 g/L SC) on the Parasitoid Wasp <i>Aphidius rhopalosiphi</i> (Hymenoptera: Braconidae)</p> <p>Schmidt, T., 2022, report no. 20210199</p>
Guideline(s):	Yes. IOBC, Mead-Briggs et al. (2000) in “Guidelines to evaluate side-effects of plant protection products to non-target arthropods”
Deviations:	No
GLP:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability:	Yes
Duplication (if vertebrate study)	No

## Executive summary

The effects of the product, CA3642 (azoxystrobin active substance content (a.s.) of 14.07% (w/w) or 154.83 g/L and prothioconazole a.s. content of 13.84% (w/w) or 152.23 g/L) on the survival and reproduction of the parasitoid wasp, *Aphidius rhopalosiphi* De Stefani-Perez (Hymenoptera: Braconidae) were assessed in a glass plate laboratory dose response test, in accordance with IOBC (2000) Guidelines.

CA3642 was evaluated at five nominal application rates of 0.256, 0.64, 1.6, 4.0, and 10 L product/ha, (equivalent to 38.97, 97.43, 243.57, 608.92, and 1522.3 g prothioconazole/ha and 39.64, 99.09, 247.73, 619.32, and 1548.3 g azoxystrobin/ha, respectively), compared to a control (purified water) and a toxic reference treatment of 0.3 g Perfekthion/ha (equivalent to 0.12 g dimethoate/ha). Treatments were applied to glass plates. Each treatment group comprised four replicates, with ten wasps per replicate ( $\leq 48$  hours old and at least five females per replicate). The health and mortality of the wasps was assessed 2-, 24- and 48 hours after test initiation. After 48 hours of exposure, the surviving female wasps from the control and treatment groups were removed from the exposure units and transferred to fecundity assessment units,

where the parasitic capacity per female was evaluated.

The 48-hour LR<sub>50</sub> value (mortality) (with 95% Confidence Intervals (CLs)) was determined to be 2.895 (with 95% CLs of 1.471 to 5.278) L product/ha (equivalent to 440.71 (with 95% CLs of 223.93 to 803.47) g prothioconazole/ha and 448.23 (with 95% CLs of 227.76 to 817.19) g azoxystrobin/ha, respectively). The 48-hour NOER value for mortality was determined to be 0.256 L product/ha (equivalent to 38.97 g prothioconazole/ha and 39.64 g azoxystrobin/ha, respectively).

The 48-hour ER<sub>50</sub> value for reproduction was estimated to be >10.000 L product/ha (equivalent to >1522.3 g prothioconazole/ha and >1548.3 g azoxystrobin/ha, respectively). The 48-hour NOER value for reproduction was determined to be 4.0 L product/ha (equivalent to 608.92 g prothioconazole/ha and 619.32 g azoxystrobin/ha, respectively). No behavioural abnormalities of the treated organisms were observed during the test.

For validation of the test system, treatment with the toxic reference item, Perfekthion (0.12 g dimethoate/ha), resulted in 100% mortality.

The study satisfies the IOBC Mead-Briggs *et al.* (2000) test guideline requirements to evaluate side-effects of plant protection products to non-target arthropods and is considered acceptable.

## Materials and methods

### Test materials

#### *Test item*

Name:	CA3642 (Prothioconazole 150 g/L + Azoxystrobin 150 g/L SC)
Active substance (a.s.):	Azoxystrobin and prothioconazole
Formulation type:	Suspension Concentrate (SC)
Source and lot/batch no.:	A20026
Active substance content:	Azoxystrobin: 14.07% w/w, corresponding to 154.83 g/L (analysed) Prothioconazole: 13.84% w/w, corresponding to 152.23 g/L (analysed)
Appearance:	Off-white, odorless suspension
Expiry date of lot/batch:	September 2022
Storage conditions:	Stored in the closed, original container in a dry, cool, well-ventilated area, away of direct sunlight.

#### *Reference item*

Name:	Perfekthion
Density:	1.04 – 1.10 g/cm <sup>3</sup>
Active substance:	Dimethoate
Batch no.:	0001122611
Active substance content:	37.4% w/w or 400 g/L (based on a density of 1.07 g/cm <sup>3</sup> )
Expiry date:	31 January 2026

### Test organism

Species:	<i>Aphidius rhopalosiphi</i> De Stefani-Perez (Hymenoptera: Braconidae)
Age at study initiation:	Adults ≤48 h
Sex:	Mixed, with at least 5 wasps per replicate
Source:	Pupal wasps, obtained from Katz Biotech AG, An der Birkenpfuhlheide 10, 15837 Baruth, Germany.
Feeding during test:	Exposure phase: 1:3 v/v solution of honey-in-water

### Test conditions

Test temperature:	20 ± 2°C (measured: 20.5-22.5°C, mean of 21.3°C*)
Relative humidity:	60-90% (measured: 47.9-67.7%, mean of 63.1%*)
Photoperiod:	16 h light:8 h dark

Light intensity: 400-3000 Lux (measured 769-1296 Lux) (exposure phase); 40000-20000 Lux (measured 4320-6770 Lux) (fecundity phase)

\*There were short-term deviations from the target temperature range of  $20 \pm 2^\circ\text{C}$  and consistent deviations from the target relative humidity of 60-90%. However, these deviations in temperature and relative humidity did not impact the outcome of the study or its results, as shown by the performance of the test organisms in the control group.

### *Exposure phase*

The test arenas consisted of two glass plates (each  $12 \times 12 \text{ cm}^2$ ), held apart by a stainless-steel square frame ( $9.7 \times 9.7 \text{ cm}^2$ , 2-cm tall). Before assembling, the treatments were sprayed onto the surface of the glass plates and allowed to dry. Each metal frame had four holes (0.7-cm diameter) on each side, to provide ventilation, which were covered by a fine-gauge mesh to prevent wasps from escaping, except for one hole, located on the bottom of the cage. This hole was uncovered for the introduction of the parasitoids and was later connected to a water bath positioned below the test units by a cotton wick. To avoid the build-up of product vapour within the test units, air was drawn through plastic tubing from one of the mesh-covered holes, with a small pump. Parasitoids were provided honey-water solution via a solution-soaked cotton wick, placed in the bottom ventilation hole, which was moistened daily to ensure adequate food supply.

Prior to application, a stock solution was made of the highest application rate, by mixing 25 mL of CA3642, in 500 mL of ultrapure water. The lower application rates were prepared by diluting aliquots of the stock solution with ultrapure water, based on a geometric order with a factor of 2.5). The application rates, 0.256, 0.64, 1.6, 4.0, and 10.0 L product /ha, (equivalent to 38.97, 97.43, 243.57, 608.92, and 1522.3 g prothioconazole/ha and 39.64, 99.09, 247.73, 619.32, and 1548.3 g azoxystrobin/ha, respectively), plus a water control and reference item treatment, were sprayed onto the glass plates of each replicate with a laboratory sprayer (Spray Lab, Schachtner, Germany), calibrated to deliver a target volume of 200 L/ha.

Once test arenas were dried, ten  $\leq 48$ -hour old adult wasps per replicate (with at least five females per replicate), were randomly transferred from the emergence boxes to the test units with an aspirator. After approximately 2, 24, and 48 hours of exposure, the condition of the wasps in the treated test units was assessed. The mortality of wasps was calculated for each treatment as number of moribund and dead wasps (wasps not seen were counted as dead) combined relative to the number of wasps at test start. Mortality in the treatments was corrected by the mortality of the control group, using the formula of Abbott with improvements by Schneider-Orelli.

### *Fecundity phase*

The fecundity test units comprised untreated pots of aphid-infested barley seedlings (*Hordeum vulgare*), which were covered with cylinders (9-cm diameter, 20-cm high), made of clear acryl acetate for observation. Before the start of the reproduction phase, a minimum of 15 pots of 10-20 barley seedlings, infested with  $>100$  adult and nymphal cereal aphids (*Rhopalosiphum padi* L.) per treatment, were prepared. The soil was covered with quartz sand to allow better observation of the wasps.

Following the exposure phase, 15 surviving females were transferred individually from the exposure test units to the fecundity test units and were allowed to parasitize aphids for 24 hours. Note that only 12 surviving females could be used in the treatment with 4.0 L product /ha, due to higher mortality in this treatment group plus the escape of all test organisms from one of the replicate units. Only 13 surviving females could be used in the treatment with 10 L product /ha, due to higher mortality in this treatment group. These deviations from the required number of 15, as mentioned in the guideline, did not impact the outcome of the study since the number of remaining replicates was still sufficient for statistical analysis.

### *Mortality assessments*

The number of mummies (i.e., parasitized aphids) developing 13 days after start of the exposure of the adults (equivalent to 11 days after start of the 24-hour parasitisation phase and 10 days after termination of the 24-hour parasitisation phase) was recorded for each individual pot (i.e., for each individual replicate). The mean number of mummies produced per individual female ( $\pm$  standard deviation) was calculated for each treatment and any reduction in reproduction, when compared to the control was calculated as follows:

$$R\% = (1 - R_t/R_c) \times 100$$

R% = Reduction in reproduction rate related to control

R<sub>c</sub> = Cumulative mean number of mummies per female in the control group

R<sub>t</sub> = Cumulative mean number of mummies per female in the treated group

### Statistical analyses

The statistical analyses were performed using ToxRat Professional. A Williams' t-test was used to assess statistically significant increases in mortality, relative to the control group (one-sided smaller,  $\alpha = 0.05$ ), and to determine the NOER and LOER values for mortality. The LR<sub>10</sub>, LR<sub>20</sub>, and LR<sub>50</sub> values (and their 95% confidence intervals) were calculated using a non-linear, 3-parametric, normal cumulative-distribution-function regression.

A Williams' t-test was also used to assess statistically significant reductions in reproductive output, relative to the control group (one-sided smaller,  $\alpha = 0.05$ ), and to determine the NOER and LOER values for reproduction. The ER<sub>10</sub>, ER<sub>20</sub>, and ER<sub>50</sub> values (and their 95% confidence intervals) were calculated using a non-linear 2-parametric normal cumulative-distribution-function regression.

## Results

### Biological results

#### Mortality

A summary of the mean values for the adult mortality data, recorded during the 48-hour exposure phase, is presented in the Table CP 10.3.2/02-01, below.

**Table CP 10.3.2/02-01. Mean mortality of *Aphidius rhopalosiphi*, after 48 hours of exposure to CA3642**

Treatment Rate (L product /ha)	Rep.	No. of wasps introduced	Number Dead	48 hours	
				Mean (%) ± SD	Corrected mean mortality (%)
0.0	1	11	1	4.8 ± 5.5	n/a
	2	10	1		
	3	10	0		
	4	10	0		
0.256	1	10	1	10.0 ± 14.1	5.5
	2	10	0		
	3	10	1		
	4	10	0		
0.64	1	10	3	37.5 ± 9.6*	34.4
	2	10	3		
	3	10	4		
	4	10	3		
1.6	1	10	3	37.5 ± 17.1*	34.4
	2	10	5		
	3	10	1		
	4	10	3		
4.0	1	10	4	70.0 ± 24.5*	58.0
	2 <sup>#</sup>	10	0		
	3	10	5		
	4	10	5		
10.0	1	10	5	70.0 ± 18.3*	68.5
	2	10	6		
	3	10	6		
	4	10	4		
0.12 g dimethoate/ha	1	10	10	100.0 ± 0.0	100.0
	2	10	10		
	3	10	10		
	4	10	9		

\*Statistically significant increases in mortality, relative to the control, (one-sided Williams' t-test ( $\alpha = 0.05$ )). Mortality was

corrected by the mortality in the control group, using Abbott's formula. #Replicate 2 (in red) was excluded from the analysis, since all 10 test organisms escaped their test unit. Rep.: replicate; No.: number; SD: standard deviation; n/a: not applicable.

After 48 hours of exposure, no statistically significant effects on mortality of the test organisms were observed at the lowest application rate (0.256 L product/ha). Statistically significant increases in mortality, compared with the control group, were observed at application rates 0.64, 1.6, 4.0 and 10.0 L product/ha. Mean mortality in the control and reference-item treatment groups, after 48 hours of exposure, was 4.8% and 100%, respectively. Mean mortality in the test-item treatments ranged from 10.0 to 70.0% and followed a rate-response relationship.

The 48-hour LR<sub>50</sub> value (with 95% Confidence Intervals (CLs)) was determined to be 2.895 (with 95% CLs of 1.471 to 5.278) L product/ha (equivalent to 440.71 (with 95% CLs of 223.93 to 803.47) g prothioconazole/ha and 448.23 (with 95% CLs of 227.76 to 817.19) g azoxystrobin/ha, respectively).

The 48-hour NOER value for mortality was determined to be 0.256 L product/ha (equivalent to 38.97 g prothioconazole/ha and 39.64 g azoxystrobin/ha, respectively).

### Reproduction

A summary of the effects of CA3642 on the reproduction capacity of *Aphidius rhopalosiphi* after 48 hours of exposure, is presented in the Table CP 10.3.2/02-02 below.

**Table CP 10.3.2/02-02. The effects on reproduction capacity of *Aphidius rhopalosiphi* after 48 hours of exposure to CA3642**

Replicate	Reproductive output (number of aphid mummies/female)					
	Treatment application rate (L product/ha)					
	0.0	0.256	0.64	1.6	4.0	10.0
1	57	56	6	66	34	11
2	23	69	41	44	11	37
3	26	68	31	46	20	0
4	52	67	40	86	42	50
5	48	76	49	56	13	0
6	0	43	61	66	60	23
7	44	54	25	41	34	32
8	44	60	46	75	26	17
9	40	59	64	58	28	2
10	46	35	27	38	31	21
11	24	27	43	81	15	20
12	13	52	14	49	10	33
13	48	2	37	68	n.s.	29
14	14	34	11	37	n.s.	n.s.
15	46	69	49	3	n.s.	n.s.
<b>Treatment mean ± SD</b>	35 ± 17	51.4 ± 20	36.3 ± 17.3	54.3 ± 21	27 ± 14.7	21.2 ± 15.2*
<b>% Reduction in reproduction compared to the control</b>	0.0	-46.86	-3.62	-55.05	22.86	39.6

\*Statistically significant reductions in reproduction, relative to the control (one-sided Williams' t-test ( $\alpha = 0.05$ )). Negative and positive values indicate an increase and decrease in reproductive output, respectively. n.s., no surviving females available for the assessment; SD, standard deviation;

A statistically significant decrease in reproduction capacity, compared to the control group, was observed in the highest product application rate, only (10.0 L product/ha). Mean mummy production after 24 hours of parasitisation in the control was 35 mummies per female. Mean mummy production of females in the test-item treatments was between 21.2 and 54.3 mummies per female and followed a rate-response relationship.

The 48-hour ER<sub>50</sub> value for reproduction was estimated to be >10.000 L product/ha (equivalent to >1522.3 g prothioconazole/ha and >1548.3 g azoxystrobin/ha, respectively). The 48-hour NOER and value for



reproduction was determined to be 4.0 L product/ha (equivalent to 608.92 g prothioconazole/ha and 619.32 g azoxystrobin/ha, respectively). No behaviour abnormalities of the treated organisms were observed during the test.

## Validity

All validity criteria were met in accordance with IOBC Mead-Briggs et al. (2000) test guideline:

- Mean mortality in the control was  $\leq 13\%$ , after 48 h of exposure (actual value: 4.8%).
- Mean mortality in the reference-item group was between  $\geq 50\%$  after 48 h of exposure (actual value: 100%)
- Mean mummy production per female in the control group was  $\geq 5$  (actual mean value: 35.0 mummies per female).
- No more than two wasps in the control fail to produce aphid mummies (actual value: one female wasp failed).

## Conclusion

In a laboratory study, the parasitoid wasp, *Aphidius rhopalosiphi* was exposed to dried residues of CA3642 on treated glass plates, in accordance with the IOBC Mead-Briggs et al. (2000) test guideline.

The 48-hour LR<sub>50</sub> value (mortality) = 2.895 g product/ha (equivalent to 440.71 g prothioconazole/ha and 448.23 g azoxystrobin/ha, respectively), based on nominal concentrations.

The 48-hour NOER value (mortality) = 0.256 L product/ha (equivalent to 38.97 g prothioconazole/ha and 39.64 g azoxystrobin/ha, respectively), based on nominal concentrations.

The 48-hour ER<sub>50</sub> value (reproduction) >10.0 L product/ha, (equivalent to >1522.3 g prothioconazole/ha and >1548.3 g azoxystrobin/ha, respectively), based on nominal concentrations.

The 48-hour NOER value (reproduction) = 4.0 g product/ha (equivalent to 608.92 g prothioconazole/ha and 619.32 g azoxystrobin/ha, respectively), based on nominal concentrations.

This study is considered acceptable.

## A 2.4 KCP 10.4 Effects on non-target soil meso- and macrofauna

### A 2.4.1 KCP 10.4.1 Earthworms

#### A 2.4.1.1 KCP 10.4.1.1 Earthworms - sub-lethal effects

## Study 1

Comments of zRMS:	<p>The study was conducted in line with OECD 222 with the following deviations.</p> <ul style="list-style-type: none"> <li>○ Temperature was between 22.0 and 23.0 °C for an average of 3 hours a day and one time at 23.1 °C and therefore above the upper limit of 22 °. Temperature was one time at 17.5 °C and therefore below the lower limit of 18 °C. These deviations were caused by the unintentionally incomplete temperature control of the climate chamber and had no impact on the study as shown by the performance of the control and the validity criteria that were met.</li> <li>○ At test termination the water content of the soil was in a range of 29.49 to 38.41 % corresponding to 55.7 and 72.6 % of the maximum water holding capacity in the control and in the test item treatments respectively. For the treatments with 0.229, 1.33, 2.40, 4.32, 7.78, and 14.0 mg product/kg dry soil, the MWHC exceeded the target range of 60 %. However, this deviation did not have a significant impact on the study since significantly more than 30 juveniles per treatment replicate hatched and thus validity criteria were met</li> </ul> <p>All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>The 56-day NOEC (reproduction) = 14.0 mg product/kg dry soil, equivalent to 1.94 mg prothioconazole/kg dry soil and 1.97 mg azoxystrobin/kg dry soil, respectively, based on nominal concentrations.</p> <p>The 56-day EC10/20/50 (reproduction) &gt;14.0 mg product/kg dry soil, equivalent to &gt;1.94 mg prothioconazole/kg dry soil and &gt;1.97 mg azoxystrobin/kg dry soil, respectively, based on nominal concentrations.</p>
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Reference:	KCP 10.4.1.1/01
Report	CA3642 (Prothioconazole 150 g/L + Azoxystrobin 150 g/L SC) - Effects on Reproduction of <i>Eisenia fetida</i> (Annelida: Lumbricidae) in Artificial Soil  Schmidt, T., year 2023, Report No. 20210206
Guideline(s):	OECD Guideline for the testing of chemicals No. 222: Earthworm Reproduction Test ( <i>Eisenia fetida</i> / <i>Eisenia andrei</i> ) (Adopted: 29 <sup>th</sup> July, 2016)
Deviations:	<p>Yes.</p> <p>1. Temperature was between 22.0 and 23.0 °C for an average of 3 hours a day and one time at 23.1 °C and therefore above the upper limit of 22 °. Temperature was one time at 17.5 °C and therefore below the lower limit of 18 °C. These deviations were caused by the unintentionally incomplete temperature control of the climate chamber and had no impact on the study as shown by the performance of the control and the validity criteria that were met.</p> <p>2. At test termination the water content of the soil was in a range of 29.49 to 38.41 % corresponding to 55.7 and 72.6 % of the maximum water holding capacity in the control and in the test item treatments respectively. For the treatments with 0.229, 1.33, 2.40, 4.32, 7.78, and 14.0 mg product/kg dry soil, the MWHC exceeded the target range of 60 %. However, this deviation did not have a significant impact on the study since significantly more than 30 juveniles per treatment replicate hatched and thus validity criteria were met.</p>
GLP:	Yes, conducted under GLP.
Acceptability:	Yes (all validity criteria met in accordance with current guidance)
Duplication (if vertebrate study)	No.

## Executive summary

In a 56-day chronic toxicity study, earthworms (*Eisenia fetida*) were exposed to the product, CA3642, a suspension concentrate (SC) formulation (analysed active substances: 13.84% w/w prothioconazole and 14.07% w/w azoxystrobin), in accordance with the OECD guideline N° 222 (2016). Nine treatment groups of, nominally, 0 (water control), 0.229, 0.412, 0.741, 1.33, 2.4, 4.32, 7.78 and 14.0 mg product/kg dry soil, equivalent to 0, 0.06, 0.10, 0.19, 0.34, 0.61, 1.09 and 1.97 mg azoxystrobin/kg dry soil and 0.03, 0.06, 0.10, 0.18, 0.33, 0.60, 1.08 and 1.94 mg prothioconazole/kg dry soil (accounting for the measured a.s. content of 154.83 g azoxystrobin/L and 152.23 g prothioconazole/L), 8 replicates for the control group and 4 replicates for each product treatment group, ten adult earthworms per replicate. After 28 days' exposure, adult worms were removed from their test units, to assess body weight change and mortality.

The 28-day NOEC value for earthworm (based on body weight change) was estimated to be  $\geq 14.0$  mg product/kg dry soil, equivalent to  $\geq 1.97$  mg azoxystrobin/kg dry soil and  $\geq 1.94$  mg prothioconazole/kg dry soil, respectively. The 56-day NOEC value for reproduction was estimated to be  $\geq 14.0$  mg product/kg dry soil, equivalent to  $\geq 1.97$  mg azoxystrobin/kg dry soil and  $\geq 1.94$  mg prothioconazole/kg dry soil, respectively.

The 56-day  $EC_{10/20/50}$  values for earthworm (*Eisenia fetida*), based on reproductive output, were each estimated to be  $>14.0$  mg product/kg soil dry weight, equivalent to  $>1.97$  mg azoxystrobin/kg dry soil and  $>1.94$  mg prothioconazole/kg dry soil, respectively.

The results of the most recent test with the reference substance, carbendazim, confirm the sensitivity of the test organism (i.e. significant effects on earthworm reproduction within the range of 1 and 5 mg carbendazim/kg dry soil).

Overall, the study satisfies the OECD 222 (2016) test-guideline requirements for chronic toxicity to earthworms and is considered acceptable.

## Materials and methods

### Test material

Name:	CA3642 (prothioconazole 150 g/L + azoxystrobin 150 g/L SC)
Formulation type:	SC (suspension concentrate)
Source and Lot/batch no.:	Nufarm - A20026
Active substances:	a) prothioconazole b) azoxystrobin
Active substance content:	a) 13.84% (w/w), corresponding to 152.23 g/L b) 14.07% (w/w), corresponding to 154.83 g/L
Density:	1.1004 g/mL
Appearance:	Off-white odourless suspension
Expiry date of lot/batch:	07 September 2022 (before recertification) 11 October 2024 (after recertification)
Storage conditions:	Store at environmental temperature

### Reference item

Name:	Carbendazim
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### Test organism

Species:	<i>Eisenia fetida</i> (Annelida: Lumbricidae)
Age at study initiation:	Synchronized <i>E. fetida</i> adults with an age of 11-12 months and a clitellum
Weight at study initiation:	Between 300 and 600 mg (wet mass)
Source:	From a stock culture maintained at IES Ltd
Feeding during test:	Yes. One day after test initiation and once a week during the first four

weeks of the study, earthworms were fed with approximately 6 g of horse manure. A further amount of approximately 6 g of food moistened with 12 mL ultrapure water was administered to each test container when adults were removed from the test units on day 28. No further feeding took place during the remaining four weeks of the test.

Acclimation: Two days in the artificial soil used for the test

#### Test medium

Soil type: Artificial soil  
7.0 kg sphagnum peat (air dried and finely ground, with no visible plant remains) corresponding to 9.98% of the substrate.  
14.0 kg kaolin clay (kaolinite content >30%) corresponding to 19.96% of the substrate.  
49.0 kg fine quartz-sand corresponding to 69.85% of the substrate.  
150 g calcium carbonate (CaCO<sub>3</sub>, pulverised, analytical grade, corresponding to 0.21% of substrate) to obtain a pH of 6.0 ± 0.5.

pH: 5.9 to 6.1 at test initiation and from 6.5 to 6.8 at test termination

Water-holding capacity: 52.90% (MWHC)

#### Test conditions

Test temperature: 20 ± 2 °C (actual: 17.5 - 23.1 °C, mean temperature of 20.9 °C<sup>1</sup>)  
Photoperiod: 16 hours light and 8 hours dark  
Light intensity: 400-800 Lux (actual: 583-642 Lux)

<sup>1</sup> Temperature was one time at 17.5 °C and therefore below the lower limit of 18 °C. These deviations were caused by the unintentionally incomplete temperature control of the climate chamber and had no impact on the study as shown by the performance of the control and the validity criteria that were met.

Test units comprised plastic vessels (17 × 12 × 11.8 cm<sup>3</sup>) that were covered with lids that enabled exchange of air and minimized evaporation from artificial soil. Each container was filled with 510 g of dry artificial soil (equivalent to 647 g wet soil). The artificial soil was prepared with a laboratory mixer. The maximum water-holding capacity (MWHC) was determined to be 52.90%. The final water content was 570.40 mL purified water corresponding to 26.78% water content and 50.63% of MWHC.

A stock solution was prepared by homogeneously mixing 119.35 mg product (target 119.26 mg) in 200 mL of ultrapure water, the highest test concentration, which was then prepared further into a dilution series of stock solutions. The treated artificial soil substrate needed for the replicates of each treatment was prepared as one batch. On application day for each treatment, 2200 g of soil was wetted with 450 mL of ultrapure water and 50 mL of the application solutions and thoroughly mixed.

The study consisted of nine treatments: one water control and eight product concentrations of 0.229, 0.412, 0.741, 1.33, 2.4, 4.32, 7.78 and 14.0 mg product/kg dry soil (spacing factor: 1.8), equivalent to 0, 0.03, 0.06, 0.10, 0.19, 0.34, 0.61, 1.09 and 1.97 mg azoxystrobin/kg dry soil and 0.03, 0.06, 0.10, 0.18, 0.33, 0.60, 1.08 and 1.94 mg prothioconazole/kg dry soil, respectively (based on the measured a.s. content of 152.23 g prothioconazole/L and 154.83 g azoxystrobin/L). For validation of the test system, treatment with carbendazim was assessed separately.

On the day of application, groups of ten earthworms were washed with ultrapure water, weighed individually and transferred to each test unit, by placing them onto the soil surface. After introducing the earthworms, each test unit was weighed, to monitor the water content throughout the test. Test units were positioned randomly in a temperature-controlled room and these positions were re-randomized weekly. There were 8 control replicates (test units) and 4 product treatment replicates, consisting of 10, randomly assigned adult earthworms per replicate.

After four weeks of exposure to the treated artificial soil substrate, *E. fetida* adults were counted and any unusual behaviour and symptoms were observed. Adults were removed from the test units, washed with ultrapure water, dried and subsequently weighed. The mean earthworm adult body weight of replicates at test start and at day 28 was determined and the weight change was calculated. Adult mortality was also

assessed on day 28. Any earthworm not found was recorded as dead. The soil was then incubated for an additional four weeks under the same test conditions.

After the additional 56 days, test containers were placed in a 45°C bath for 20 minutes. Most of the juveniles appeared on the soil surface and were removed and counted. After a second heating period of the test container for approximately 7 minutes, additional earthworms were sampled from the soil surface and the whole soil was thoroughly examined for juveniles potentially overlooked after the first heating period. The reproductive output of the earthworms in the test item treatments was determined and compared to the control group for both the number of juveniles and number of filled cocoons.

Differences between the control and the test item treatments were tested using the multiple Fisher test after Bonferroni-Holm for earthworm mortality (one-sided greater,  $\alpha = 0.05$ ), ANOVA for earthworm body weight at day 0 ( $\alpha = 0.05$ , two-sided), the Dunnett t-test for earthworm body weight, body weight change at day 28 and reproduction at day 56 ( $\alpha = 0.05$ , one-sided smaller).

NOEC values were determined based on the outcome of the statistics.  $LC_x$  and  $EC_x$  values could not be calculated due to lack of effects up to and including the highest test concentration of 14.0 mg/kg dry soil and were determined directly from the raw data.

The statistical software ToxRat Professional was used.

## Results and discussions

### *Biological results*

A summary of the effects of CA3642 on adult mortality of *Eisenia fetida*, after 28 days of exposure, is presented in the table below.

**Table 10.4.1.1/01-01. The effects of CA3642 on adult *Eisenia fetida* mortality, after 28 days of exposure**

Nominal concentration			Rep.	Number of earthworms			Mean Mortality (%)
				Live		Dead	
mg product/kg soil d.w.	mg azoxystrobin/kg soil d.w.	mg prothioconazole/kg soil d.w.		Day 0	Day 28		
0 (control)	0	0	1	10	10	0	0.0
			2	10	10		
			3	10	10		
			4	10	10		
			5	10	10		
			6	10	10		
			7a	10	10		
			8	10	10		
0.229	0.03	0.03	1	10	10	0	0.0
			2	10	10		
			3	10	10		
			4	10	10		
0.412	0.06	0.06	1	10	10	1	2.5
			2	10	10		
			3	10	9		
			4	10	10		
0.741	0.10	0.10	1	10	10	0	0.0
			2	10	10		
			3	10	10		
			4	10	10		
1.33	0.19	0.18	1	10	10	0	0.0
			2	10	10		
			3	10	10		
			4	10	10		
2.40	0.34	0.33	1	10	10	0	0.0
			2	10	10		
			3	10	10		
			4	10	10		
4.32	0.61	0.60	1	10	10	0	0.0
			2	10	10		
			3	10	10		
			4	10	10		
7.78	1.09	1.08	1	10	10	1	2.5
			2	10	10		
			3	10	9		
			4	10	10		
14.0	1.97	1.94	1	10	10	0	0.0
			2	10	10		
			3	10	10		
			4	10	10		

a: In replicate 7 of the control one adult earthworm was not removed at Day 28 but at Day 56

There were no statistically significant differences in adult mortality in any product treatment groups when compared to the control group. The LC<sub>50</sub> value for adult earthworm mortality was estimated to be >14.0 mg product/kg dry soil, equivalent to >1.97 mg azoxystrobin/kg dry soil and >1.94 mg prothioconazole/kg dry soil, respectively. The NOEC value for mortality was determined to be 14.0 mg product/kg dry soil, equivalent to 1.97 mg azoxystrobin/kg dry soil and 1.94 mg prothioconazole/kg dry soil, respectively.

A summary of the effect of CA3642 on adult body weight of *Eisenia fetida*, after 28 days of exposure, is presented in the table below.

**Table 10.4.1.1/01-02: The effect of CA3642 on adult *Eisenia fetida* body weight, after 28 days of exposure**

Nominal concentration			Rep.	Mean replicate bodyweight (mg)		Mean values	
						Body weight $\pm$ SD (mg)	Change in bodyweight $\pm$ SD (%)
mg product/kg soil d.w.	mg azoxystrobin/kg soil d.w.	mg prothioconazole/kg soil d.w.		Day 0	Day 28	Day 28	
0 (control)	0	0	1	471	455	454 $\pm$ 20.4	1.2 $\pm$ 7.4
			2	471	458		
			3	444	435		
			4	443	496		
			5	491	467		
			6	434	446		
			7a	468	444		
			8	383	432		
0.229	0.03	0.03	1	506	472	456 $\pm$ 38.1	-0.6 $\pm$ 9.2
			2	444	442		
			3	444	410		
			4	444	499		
0.412	0.06	0.06	1	465	460	449 $\pm$ 33.4	3.6 $\pm$ 7.8
			2	456	453		
			3	398	402		
			4	418	481		
0.741	0.10	0.10	1	523	492	462 $\pm$ 33.2	1.3 $\pm$ 6.4
			2	431	430		
			3	483	489		
			4	399	437		
1.33	0.19	0.18	1	477	412	462 $\pm$ 35.0	4.1 $\pm$ 15
			2	477	488		
			3	459	485		
			4	379	463		
2.40	0.34	0.33	1	504	458	431 $\pm$ 39.5	-5.1 $\pm$ 8.9
			2	444	375		
			3	443	460		
			4	430	431		
4.32	0.61	0.60	1	472	460	472 $\pm$ 28.7	4.9 $\pm$ 11
			2	458	462		
			3	451	451		
			4	423	514		
7.78	1.09	1.08	1	426	416	455 $\pm$ 34.3	3.8 $\pm$ 4.6
			2	424	437		
			3	458	487		
			4	444	479		
14.0	1.97	1.94	1	474	486	470 $\pm$ 39.6	8.4 $\pm$ 9.9
			2	401	411		
			3	473	499		
			4	393	483		

Rep.: Replicate

SD: Standard Deviation

a: In replicate 7 of the control one adult earthworm was not removed at Day 28 but at Day 56

After 28 days of exposure to CA3642, there were no statistically significant differences in adult earthworm (*Eisenia fetida*) body weight changes in the product treatment groups compared to the control group, up to and including the highest test concentration of 14.0 mg product/ha.

The 28-day NOEC value for adult body weight change was determined to be 14.0 mg product/kg dry soil,

equivalent to 1.97 mg azoxystrobin/kg dry soil and 1.94 mg prothioconazole/kg dry soil, respectively.

A summary of the effect of CA3642 on the number juvenile earthworms (reproductive output) of *Eisenia fetida*, after 56 days of exposure, is presented in the table below.

**Table 10.4.1.1/01-03. The effect of CA3642 on reproductive output (number of juvenile earthworms) of *Eisenia fetida*, after 56 days of exposure**

Nominal concentration			Rep.	N° of juveniles	Mean ± SD	CV (%)	% ctrl
mg product/kg soil d.w.	mg azoxystrobin/kg soil d.w.	mg prothioconazole/kg soil d.w.					
0 (ctrl)	0	0	1	60	75.1 ± 14.4	19.1	-
			2	86			
			3	85			
			4	81			
			5	78			
			6	50			
			7a	95			
			8	86			
0.229	0.03	0.03	1	97	79.3 ± 14.0	17.6	105
			2	65			
			3	83			
			4	72			
0.412	0.06	0.06	1	90	91.3 ± 27.8	30.5	121
			2	89			
			3	127			
			4	59			
0.741	0.10	0.10	1	89	75.5 ± 14.2	18.8	100
			2	60			
			3	86			
			4	67			
1.33	0.19	0.18	1	90	67.5 ± 18.0	26.6	90
			2	67			
			3	67			
			4	46			
2.40	0.34	0.33	1	67	66.0 ± 6.83	10.4	88
			2	63			
			3	75			
			4	59			
4.32	0.61	0.60	1	98	68.0 ± 22.0	32.4	90
			2	50			
			3	71			
			4	53			
7.78	1.09	1.08	1	98	67.3 ± 20.6	30.7	89
			2	57			
			3	54			
			4	60			
14.0	1.97	1.94	1	71	73.0 ± 15.9	21.8	97
			2	61			
			3	96			
			4	64			

Rep.: Replicate; ctrl.: control

SD: Standard Deviation

CV: Coefficient of Variation

a: In replicate 7 of the control one adult earthworm was not removed at Day 28 but at Day 56. This replicate was excluded from the calculations and statistics since this individual might have continued reproducing between Day 28 and Day 56.



After 56 days of exposure, there were no statistically significant differences in the mean number of juvenile earthworms in the product treatment groups compared to the control group, up to and including the highest test concentration of 14.0 mg product/ha.

The 56-day EC<sub>10/20/50</sub> values for reproduction were all estimated to be >14.0 mg product/kg dry soil, equivalent to >1.97 mg azoxystrobin/kg dry soil and >1.94 mg prothioconazole/kg dry soil, respectively. The 56-day NOEC value for earthworm reproduction was determined to be 14.0 mg product/kg dry soil, equivalent to 1.97 mg azoxystrobin/kg dry soil and 1.94 mg prothioconazole/kg dry soil, respectively.

### Validity

The following validity criteria from OECD test guideline 222 (2016) were met in the control group:

- Each replicate produced  $\geq 30$  juveniles by the end of the test (actual values: 50-86 juveniles per replicate in the control).
- The coefficient of variation (reproduction) was  $\leq 30\%$  (actual value: 19.1% for the control).
- Adult mortality over the initial four weeks of the test was  $\leq 10\%$  (actual value: 0.0% in the control).

### Conclusion

The 56-day chronic toxicity of CA3642 to the earthworm species *Eisenia fetida*, was assessed in artificial soil, in accordance with OECD test guideline 222 (2016).

The 56-day NOEC (reproduction) value: 14.0 mg product/kg dry soil, equivalent to 1.94 mg prothioconazole/kg dry soil and 1.97 mg azoxystrobin/kg dry soil, respectively, based on nominal concentrations.

The 56-day EC<sub>10/20/50</sub> (reproduction) values: >14.0 mg product/kg dry soil, equivalent to >1.94 mg prothioconazole/kg dry soil and >1.97 mg azoxystrobin/kg dry soil, respectively, based on nominal concentrations.

The study is considered acceptable and valid.

#### 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

### Study 1

Comments of zRMS:	<p>The study was conducted in line with OECD 232 with no deviations.</p> <p>All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>The 28-d NOEC and LOEC values (mortality) were determined to be <math>\geq 612</math> and <math>&gt;612</math> mg product/kg soil dw, respectively, (equivalent to <math>&gt;84.7</math> mg prothioconazole/kg soil dw and <math>&gt;86.1</math> mg azoxystrobin, respectively).</p> <p>The 28-d NOEC and LOEC (reproduction) = 58.3 mg product/kg soil dw (equivalent to 8.1 mg prothioconazole/kg soil dw and 8.2 mg azoxystrobin) and 105 mg product/kg soil dw (equivalent to 14.5 mg prothioconazole/kg soil dw and 14.8 mg azoxystrobin/kg soil dw), respectively.</p> <p>The 28-d EC<sub>10</sub>, (reproduction) = 83.9 mg product/kg soil dw (equivalent to 11.6 mg prothioconazole/kg soil dw and 11.8 mg azoxystrobin/kg soil dw, respectively).</p>
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	<p>The 28-day EC<sub>20</sub> (reproduction) = 121 mg product/kg soil dw (equivalent to 16.7 mg prothioconazole/kg soil dw and 17 mg azoxystrobin/kg soil dw, respectively).</p> <p>The 28-day EC<sub>50</sub> value (reproduction) = 241 mg product/kg soil dw (equivalent to 33.4 mg prothioconazole/kg soil dw and 33.9 mg azoxystrobin/kg soil dw, respectively).</p>
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Reference:	KCP 10.4.2.1/01
Report	CA3642 (Prothioconazole 150 g/L + Azoxystrobin 150 g/L SC) - Effects on Reproduction of <i>Folsomia candida</i> (Collembola: Isotomidae) in Artificial Soil  Schmidt, D., 2022, report no. 20210207
Guideline(s):	Yes. OECD 232 (2016)
Deviations:	No
GLP:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability:	Yes
Duplication (if vertebrate study)	No

## Executive summary

In a 28-day chronic toxicity study, springtails (*Folsomia candida*) were exposed to the product, CA3642 (analysed active substances: 14.07% w/w azoxystrobin and 13.84% w/w prothioconazole), at nominal 0 (control), 10.0, 18.0, 32.4, 58.3, 105, 189, 340, and 612 mg product/kg soil dry weight (dw), (corresponding to 0, 1.4, 2.5, 4.6, 8.2, 14.8, 26.6, 47.8, and 86.1 mg azoxystrobin/kg soil dw and 0, 1.4, 2.5, 4.5, 8.1, 14.5, 26.2, 47.1, and 84.7 mg prothioconazole/kg soil dw, respectively), in artificial soil with 5% peat, according to OECD test guideline 232 (2016). The toxic reference item, boric acid, was tested in a separate study to validate the test system.

The control group comprised eight replicates and the test-item treatment groups comprised four replicates each, with ten female adult collembola per replicate. After 28 days' exposure, adult and juvenile mites were removed from their test units, to assess adult mortality and reproduction (fecundity).

The 28-day NOEC and LOEC values, based on mortality, were estimated to be  $\geq 612$  and  $>612$  mg product/kg soil dw, respectively. The corresponding 28-day LC<sub>10</sub>, LC<sub>20</sub>, and LC<sub>50</sub> values, based on mortality, were all estimated to be  $>612$  mg product/kg soil dw.

The 28-day NOEC and LOEC values, based on reproduction, were determined to be 58.3 and 105 mg product/kg soil dw, respectively. The corresponding 28-day EC<sub>10</sub>, EC<sub>20</sub>, and EC<sub>50</sub> values, based on reproduction, were determined to be 83.9, 121, and 241 mg product/kg soil dw, respectively.

For validation of the test system, treatment with boric acid, the reference item, resulted in  $>50\%$  reduction in reproduction at 100 mg a.s./kg soil dw.

The study satisfies the OECD 232 (2016) test-guideline requirements for a chronic toxicity study with *Folsomia candida* and is considered acceptable.

## Materials and methods

### Test materials

#### Test item

Name: CA3642 (Prothioconazole 150 g/L + Azoxystrobin 150 g/L SC)  
Active substances (a.s.): Azoxystrobin and prothioconazole

Formulation type:	Suspension Concentrate (SC)
Source and lot/batch no.:	A20026
a.s. content:	Azoxystrobin: 14.07% w/w, corresponding to 154.83 g/L Prothioconazole: 13.84% w/w, corresponding to 152.23 g/L
Appearance:	Off-white, odorless suspension
Expiry date of lot/batch:	September 2022
Storage conditions:	Stored in the closed, original container in a dry, cool, well-ventilated area, away of direct sunlight.

#### Reference item

Name:	Boric acid, was assessed separately
Formulation type:	Not Reported
Density:	Not Reported
Batch no.:	Not Reported
Purity:	Not Reported
Expiry date:	Not Reported

#### Test organism

Species:	<i>Folsomia candida</i>
Age at study initiation:	9-to-12-day old juveniles (synchronised cohort)
Sex:	Female
Source:	Laboratory culture at IES Ltd.
Feeding during test:	2-10 mg of granulated dried baker's yeast was added to each test unit, at beginning of test (Day 0) and on Day 14

#### Test medium:

Soil type:	Artificial soil (according to OECD test guideline 232 4.99% sphagnum peat 19.96% kg kaolin clay (kaolinite content >30 %), 74.85% industrial quartz sand (air dried, particle size between 50 and 200 microns), 0.2% CaCO <sub>3</sub> , to obtain optimal pH of 6.0 ± 0.5.
pH:	6.35-7.29
Maximum Water-holding capacity:	49.3% of soil dry weight

#### Test conditions

Test temperature:	20 ± °C (actual: 19.9 – 21.9°C (mean value of 20.8°C))
pH:	6.0 ± 0.5 (actual 5.4 – 5.6 at test start; 5.6 – 5.7 at test end)
Photoperiod:	16 hours' light:8 hours' dark
Light intensity:	400 – 800 lux (actual: 453 - 565 lux)

Test units consisted of 100-mL glass containers (8.5-cm high x 4-cm diameter), covered with lids that enabled air exchange and minimised evaporation of water from the artificial soil. Test units were filled with 30 g (dry weight) of artificial soil, with a depth of 2-4 cm within each test unit. During the study, the test units were placed in a climate-controlled chamber.

For each treatment, 180 g of fresh soil with a remaining moisture of 0.99 % (corresponding to 178.22 g dry soil with 0.0% moisture) was moistened with 45 mL ultrapure water in two steps:

- 20 mL were added two days before application by pre-wetting the soil substrate, to obtain the half of the MWHC,
- during application, the remaining 25 mL [ultrapure water, either without test item (control) or with test item] was mixed into the pre-wetted soil.

Therefore, the whole water content of the soil substrate was 46.78 g water resulting in 26.25 % moisture

content (regarding the amount of dry soil) and corresponding to 53.25 % of the MWHC (water content at 100% MWHC: 49.3 %).

A stock solution was prepared by suspending 436.66 mg product in 100 mL ultrapure water and the lower test item concentrations were prepared from a dilution series of the highest concentration under intense stirring. A volume of 25 mL of each test concentration was mixed with the pre-wetted soil batch for approximately 5 minutes with a hand mixer.

Ten Collembola were introduced to each test unit containing the treated artificial soil. Four replicates were tested per nominal concentration of 10.0, 18.0, 32.4, 58.3, 105, 189, 340, and 612 mg product/kg soil dw, (equivalent to 1.4, 2.5, 4.6, 8.2, 14.8, 26.6, 47.8, and 86.1 mg azoxystrobin/kg soil dw and 1.4, 2.5, 4.5, 8.1, 14.5, 26.2, 47.1, and 84.7 mg prothioconazole/kg soil dw, respectively) and eight replicates for the ultrapure water controls were tested.

28 days after the applications (the test termination), the test organisms were extracted from the test soil by a controlled temperature-gradient extractor (by the MacFadyen principles). The soil from each replicate was placed in an extraction funnel of the extractor and an increasing temperature regime (from 25 to 40 °C) was set up for 48 hours (the heat was applied at the top of the funnel). Test organisms migrated away from the warming soil above and fell from the bottom of the funnel into a small empty container. Afterwards, the number of dried adult and juvenile *F. candida* in the extraction containers was counted using a binocular microscope. *Folsomia candida* adults that were not recovered were noted as dead. The mortality in the test-item treatments was corrected by the control mortality, using Abbott's formula, with modifications by Schneider-Orelli.

Statistical analyses were performed using ToxRat Professional® (version 3.3.0, ToxRat Solutions GmbH). For mortality, a Chi<sup>2</sup> 2x2 table test, with Bonferroni corrections (one-sided greater,  $\alpha = 0.05$ ), was performed to test the differences between test-item concentrations and the control. The NOEC and LOEC values for mortality were determined according to the results of the statistics. The LC<sub>10/20/50</sub> values were determined directly from the raw data.

For reproduction, a William's multiple-sequential t-test (one-sided smaller,  $\alpha = 0.05$ ) was performed to test the differences between test-item concentrations and control. The NOEC and LOEC values for reproduction were determined according to the results of the statistics. The EC<sub>10/20/50</sub> values for reproduction were determined using a 3-parametric normal cumulative distribution function (CFD).

## Results

### Biological results

#### *Mortality*

The effects of CA3642 on adult mortality, after 28 days of exposure, are presented in the table below.

**Table CP 10.4.2.1/01-01. The effect of CA3642 on adult *F. candida* mortality, after 28 days of exposure.**

Nominal concentration (mg product/kg soil dw)	Rep.	Live juveniles (No.)	Live adults (No.)	Dead adults (No.)	Mean mortality (%)	
		D0	D28	D28	Mean ± SD	Corrected
0.0	1	10	10	0	4.3 ± 5.3	0.0
	2	10	10	0		
	3	10	10	0		
	4	10	9	1		
	5	10	9	1		
	6	10	10	0		
	7	10	9	1		
	8 <sup>#</sup>	-	-	-		

10.0	1 <sup>#</sup>	-	-	-	0.0 ± 0.0	-4.5 <sup>n.s.</sup>
	2	10	10	0		
	3	10	10	0		
	4	10	10	0		
18.0	1	10	9	1	2.5 ± 8.2	-1.9 <sup>n.s.</sup>
	2	10	10	0		
	3	11	11	0		
	4	10	10	0		
32.4	1	10	9	1	2.5 ± 5.0	-1.9 <sup>n.s.</sup>
	2	10	10	0		
	3	10	10	0		
	4	10	10	0		
58.3	1	10	9	1	2.5 ± 5.0	-1.9 <sup>n.s.</sup>
	2	10	10	0		
	3	10	10	0		
	4	10	10	0		
105	1	10	10	0	5.0 ± 5.8	0.7 <sup>n.s.</sup>
	2	10	10	0		
	3	10	9	1		
	4	10	9	1		
189	1	10	9	1	5.0 ± 5.8	0.7 <sup>n.s.</sup>
	2	10	9	1		
	3	10	10	0		
	4	10	10	0		
340	1	10	9	1	6.7 ± 5.8	2.5 <sup>n.s.</sup>
	2 <sup>#</sup>	-	-	-		
	3	10	10	0		
	4	10	9	1		
612	1	10	10	0	0.0 ± 0.0	-4.5 <sup>n.s.</sup>
	2	10	10	0		
	3	10	10	0		
	4	10	10	0		

<sup>n.s.</sup> Not statistically significant (Chi<sup>2</sup> 2x2 table test (one-sided greater,  $\alpha = 0.05$ ), followed by Bonferroni corrections). Positive and negative corrected mortality values indicate lower and higher mortality than the control group, respectively. <sup>#</sup>Replicate excluded, due to technical problems during the extraction of adults and juveniles, when using the MacFayden device. C: control group; Rep.: replicate; No.: number; D0-D28: days 0-28; SD: standard deviation.

There were no statistically significant differences in mean adult mortality in any product treatment group, up to and including 612 mg product/kg soil dw (the highest concentration tested), when compared to the control group. The 28-day NOEC value for mortality was estimated to be  $\geq 612$  mg product/kg soil dw (equivalent to  $\geq 84.7$  mg prothioconazole/kg soil dw and  $\geq 86.1$  mg azoxystrobin, respectively). The corresponding 28-day LOEC value for mortality was estimated to be  $> 612$  mg product/kg soil dw, (equivalent to  $> 84.7$  mg prothioconazole/kg soil dw and  $> 86.1$  mg azoxystrobin/kg soil dw, respectively).

Since there was no concentration-response pattern and mortality was not  $\geq 10\%$  in any of the product treatment groups, the LC<sub>10/20/50</sub> values were all estimated to be  $> 612$  mg product/kg soil dw (equivalent to  $> 84.7$  mg prothioconazole/kg soil dw and  $> 86.1$  mg azoxystrobin/kg soil dw, respectively), the highest concentration tested.

### Reproduction

The effects of CA3642 on reproduction, after 28 days of exposure, are presented in the table below.

**Table CP 10.4.2.1/01-02. The effect of CA3642 on reproductive output (number of juvenile springtails) of *F. candida*, after 28 days of exposure.**

Nominal concentration (mg product/kg soil dw)	Rep.	Adults per replicate	No. of juveniles on D28 (juveniles)			
			No. juveniles per replicate	Mean $\pm$ SD (No.)	CV (%)	Reduction (% control)
0.0	1	10	1163	977 $\pm$ 172	18	0.0

	2	10	785			
	3	10	800			
	4	9	1028			
	5	9	978			
	6	10	1219			
	7	9	864			
	8 <sup>#</sup>	-	-			
	1 <sup>#</sup>	-	-			
10.0	2	10	1130	1130 ± 31	2.7	-16 <sup>n.s.</sup>
	3	10	1099			
	4	10	1160			
18.0	1	9	1102	1138 ± 77	6.8	-16 <sup>n.s.</sup>
	2	10	1171			
	3	11	1227			
	4	10	1051			
32.4	1	9	919	1093 ± 142	13.0	-12 <sup>n.s.</sup>
	2	10	1108			
	3	10	1266			
	4	10	1077			
58.3	1	9	864	1092 ± 182	17	-12 <sup>n.s.</sup>
	2	10	1192			
	3	10	1033			
	4	10	1277			
105	1	10	877	779 ± 126	16	20*
	2	10	869			
	3	9	762			
	4	9	606			
189	1	9	726	793 ± 140	18	19*
	2	9	715			
	3	10	729			
	4	10	1003			
340	1	9	321	316 ± 24	7.7	68*
	2 <sup>#</sup>	-	-			
	3	10	289			
	4	9	337			
612	1	10	203	133 ± 66.3	50	86*
	2	10	123			
	3	10	158			
	4	10	46			

\*Statistically significant decreases in reproduction, relative to the control (William's multiple-sequential t-test (one-sided smaller,  $\alpha = 0.05$ )); n.s.: not significant; Positive and negative reductions in reproduction indicate lower and higher reproductive output than the control group, respectively. <sup>#</sup>Replicate excluded, due to technical problems during the extraction of adults and juveniles, when using the MacFayden device. Rep.: replicate; No.: number; D0-D28: days 0-28; SD: standard deviation; CV: coefficient of variation.

There were no statistically significant differences in reproductive output in any test-item treatment group up to and including 58.3 mg product/kg soil dw, when compared to the control group. The 28-day NOEC value for reproduction was determined to be 58.3 mg product/kg soil dw (equivalent to 8.1 mg prothioconazole/kg soil dw and 8.2 mg azoxystrobin, respectively). The corresponding 28-day LOEC value for reproduction was determined to be 105 mg product/kg soil dw, (equivalent to 14.5 mg prothioconazole/kg soil dw and 14.8 mg azoxystrobin/kg soil dw, respectively).

The 28-day EC<sub>10</sub>, EC<sub>20</sub> and EC<sub>50</sub> values (with 95% confidence intervals) for reproduction were determined to be 83.9 (57.3 – 117), 121 (89.1 – 155), and 241 (203 – 275) mg product/kg soil dw, respectively (equivalent to 11.6 (7.9 – 16.2) mg prothioconazole/kg soil dw and 11.8 (8.1 – 16.5) mg azoxystrobin/kg soil dw; 16.7 (12.3 – 21.5) mg prothioconazole/kg soil dw and 17 (12.5 – 21.8) mg azoxystrobin/kg soil dw; 33.4 (28.1 – 38.1) mg prothioconazole/kg soil dw and 33.9 (28.6 – 38.7) mg azoxystrobin/kg soil dw, respectively).

## Validity

All validity criteria were met, in accordance with OECD 232 (2016) test guideline:

- The mean adult mortality in the control group was <20% (actual value: 4.3%)
- The mean number of juveniles per replicate in the control group was >100 (actual value: 976.7)
- The coefficient of variation calculated for the number of juveniles in the control group was <30% (actual value: 17.6%)

## Conclusion

The 28-day chronic toxicity of CA3642 to *Folsomia candida* was studied in artificial soil with 5% peat, in accordance with the OECD 232 (2016) test guideline.

The 28-day NOEC and LOEC values (mortality) were determined to be  $\geq 612$  and  $>612$  mg product/kg soil dw, respectively, (equivalent to  $\geq 84.7$  and  $>84.7$  mg prothioconazole/kg soil dw and  $\geq 86.1$  and  $>86.1$  mg azoxystrobin, respectively).

The 28-day LC<sub>10</sub>, LC<sub>20</sub>, and LC<sub>50</sub> values (mortality) were all estimated to be  $>612$  mg product/kg soil dw, respectively (equivalent to  $>84.7$  mg prothioconazole/kg soil dw and  $>86.1$  mg azoxystrobin, respectively).

The 28-day NOEC and LOEC values (reproduction) were determined to be 58.3 mg product/kg soil dw (equivalent to 8.1 mg prothioconazole/kg soil dw and 8.2 mg azoxystrobin) and 105 mg product/kg soil dw (equivalent to 14.5 mg prothioconazole/kg soil dw and 14.8 mg azoxystrobin/kg soil dw), respectively.

The 28-day EC<sub>10</sub> value (reproduction) was determined to be 83.9, mg product/kg soil dw (equivalent to 11.6 mg prothioconazole/kg soil dw and 11.8 mg azoxystrobin/kg soil dw, respectively).

The 28-day EC<sub>20</sub> value (reproduction) was determined to be 121 mg product/kg soil dw (equivalent to 16.7 mg prothioconazole/kg soil dw and 17 mg azoxystrobin/kg soil dw, respectively).

The 28-day EC<sub>50</sub> value (reproduction) was determined to be 241 mg product/kg soil dw (equivalent to 33.4 mg prothioconazole/kg soil dw and 33.9 mg azoxystrobin/kg soil dw, respectively).

This study is considered valid and acceptable.

## Study 2

Comments of zRMS:	<p>The study was conducted in line with OECD 226 with no deviations.</p> <p>All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>The 14-day NOEC (mortality) = 177.16 mg product/kg soil dw (equivalent to 24.52 mg prothioconazole/kg soil dw and 24.93 mg azoxystrobin/kg soil dw), the highest concentration tested.</p> <p>The 14-day EC<sub>50</sub> (reproduction) &gt;177.16 mg product/kg soil dw (equivalent to &gt;24.52 mg prothioconazole/kg soil dw and &gt;24.93 mg azoxystrobin/kg soil dw, respectively).</p> <p>The 14-day NOEC (reproduction)= 177.16 mg product/kg soil dw (equivalent to 24.52 mg prothioconazole/kg soil dw and 24.93 mg azoxystrobin/kg soil dw, respectively), the highest concentration tested.</p>
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Reference:	KCP 10.4.2.1/02
Report	CA3642 (Prothioconazole 150 g/L + Azoxystrobin 150 g/L SC): A Laboratory Study to Determine the Effects of Fresh Residues on the Predatory Soil Mite, <i>Hypoaspis aculeifer</i> , in an Artificial Soil Substrate  Parsons, C. 2022, Report No. NUF-22-03
Guideline(s):	OECD test guideline 226 (2016)
Deviations:	No.
GLP:	Yes, conducted under GLP/Officially recognised testing facilities.
Acceptability:	Yes
Duplication (if vertebrate study)	No

## Executive summary

In a 14-day laboratory test study, the predatory soil mite, *Hypoaspis aculeifer*, was exposed to the product, CA3642, a suspension concentrate (SC) formulation (analysed active substances: 13.84% w/w prothioconazole and 14.07% w/w azoxystrobin). The mites were exposed to eight treatment concentrations, incorporated into an artificial soil substrate (containing 5% w/w peat), of 177.16, 98.42, 54.68, 30.38, 16.88, 9.38, 5.21 and 2.89 mg product/kg soil dry weight (dw) (equivalent to 24.52, 13.62, 7.57, 4.20, 2.34, 1.30, 0.72 and 0.40 mg prothioconazole/kg soil dw and 24.93, 13.85, 7.69, 4.27, 2.38, 1.32, 0.73 and 0.41 mg azoxystrobin/kg soil dw). Treatments were compared to an untreated water control. A toxic reference, boric acid (99.99% w/w purity), was assessed in a separate bioassay.

The control group comprised eight replicates and the product treatment groups comprised four replicates each, with ten adult mites per replicate. Adult female mites were introduced into replicate test units containing treated soils and assessment of adult mortality and reproductive output (fecundity) was conducted after 14 days.

The 14-day LC<sub>50</sub> value (mortality) was estimated to be >177.16 mg product/kg soil dw. The corresponding 14-day NOEC value (mortality) was determined to be 177.16 mg product/kg soil dw, the highest concentration tested.

The 14-day EC<sub>10/20/50</sub> values (reproduction) were all estimated to be >177.16 mg product/kg soil dw. The corresponding 14-day NOEC value (reproduction) was determined to be 177.16 mg product/kg soil dw, the highest concentration tested.

The treatment concentration 177.16 mg product/kg soil dw is equivalent to 24.52 mg prothioconazole/kg soil dw and 24.93 mg azoxystrobin/kg soil dw.

This study is considered acceptable and satisfies the guideline requirements for a reproduction toxicity study with soil mites (OECD test guideline 226, 2016).

## Materials and methods

### Test material

Name:	CA3642
Active substances:	Prothioconazole and azoxystrobin
Formulation type:	Suspension concentrate (SC)
Lot/batch no.:	A20026
Active substance content:	azoxystrobin: 150 g/L (nominal), 154.83 g/L (measured), 14.07% w/w (analysed) prothioconazole: 150 g/L (nominal), 152.23 g/L (measured), 13.84% w/w (analysed)
Expiry date of lot/batch:	September 2022
Appearance:	Off-white liquid



Storage conditions: Ambient laboratory conditions

*Reference item*

Name: Boric acid  
Formulation type: Technical  
Batch no.: A0429088  
Purity: 99.99% w/w  
Expiry date: Not reported

*Test organism*

Species: *Hypoaspis aculeifer* (predatory soil mites)  
Age at study initiation: 31 and 30 days after start of egg laying  
Sex: Adult females  
Source: Synchronised culture from in-house source at Bias Labs Ltd., Fife, UK.  
Feeding during test: Yes. Cheese mites and juvenile springtails were added to the soil surface at test start; cheese mites were replenished *ad libitum* (at least twice a week).  
Acclimation: Controlled-environment cabinet, 19.5-20.0 °C, 16h photoperiod of 500-800 Lux.

*Test medium:*

Soil type: Artificial soil  
5% sphagnum peat  
20% kg kaolin clay (kaolinite content >30%),  
74.76% w/w sand, air-dried, predominantly fine sand with >50% particle size 0.05-0.2mm.  
0.24% w/w calcium carbonate (CaCO<sub>3</sub>), to obtain pH of 6.0 ± 0.5.  
pH: 6.26-6.48 (test initiation), 6.09-6.12 (test termination)  
Water-holding capacity: 50% ± 10%

*Test conditions*

Test temperature: 19.4-20.9°C.  
Photoperiod: 16 hours' light: 8 hours' dark  
Light intensity: 500-800 lux

Stock solutions and dilutions of the product were made shortly before they were required, which were then mixed evenly into the pre-moistened soil. The test consisted of an untreated water control and eight treatment concentrations of CA3642 of 177.16, 98.42, 54.68, 30.38, 16.88, 9.38, 5.21 and 2.89 mg product/kg soil dw (equivalent to 24.93, 13.85, 7.69, 4.27, 2.38, 1.32, 0.73 and 0.41 mg azoxystrobin/kg soil dw and 24.52, 13.62, 7.57, 4.20, 2.34, 1.30, 0.72 and 0.40 mg prothioconazole/kg soil dw, respectively, based on the measured active substance content).

The test arenas were 60 mL capacity glass jars (5.5-cm tall x 5.2-cm outer diameter, 4.4-cm inner diameter), with screw-top lids. An 8-mm diameter hole was made in the lid for ventilation, which was covered with fine nylon netting (80-µm<sup>2</sup> aperture mesh). Eight replicate arenas for the control treatment group and four replicate arenas for each test-item treatment concentration were used, with 10 adult female mites per replicate.

Assessments of both surviving adults and F<sub>1</sub> progeny in each test arena was carried out 14 days after treatment (DAT). Mites were removed from the test units using light and heat extraction. The numbers of extracted juveniles and adults were counted and recorded using a binocular microscope.

*Analysis of the data*

Statistical analyses were performed following the recommendations given in OECD Guidance Document 54 (2006), using validated computer software (ToxRat Professional, ToxRat® Solutions GmbH, 2018).

As there was no concentration response, the mortality data was not suitable for regression analysis to calculate the *median lethal concentration* (LC<sub>50</sub>). Therefore, the LC<sub>50</sub> value was visually derived from the data. The mortality data for the test-item treatments were subject to qualitative trend analysis by contrasts (monotonicity of concentration/response). Since there was no significant linear trend, the test-item treatments were compared to the control by chi<sup>2</sup> 2x2 table test with Bonferroni correction ( $\alpha = 0.05$ , one-sided, >control).

The reproduction data were not suitable for regression analysis to determine X% effect (EC<sub>x</sub>) for key points on the response curve (i.e. EC<sub>50</sub>, EC<sub>20</sub> and EC<sub>10</sub>). Therefore, the EC<sub>50</sub>, EC<sub>20</sub> and EC<sub>10</sub> values were visually derived from the data. The data from the reproduction assessments in the individual treatments were checked for normality (Shapiro-Wilk test,  $\alpha = 0.01$ ) and for homogeneity of variance (Levene's test,  $\alpha = 0.01$ ). Trend analysis by contrasts (monotonicity of concentration/response) showed a significant linear trend, therefore the test-item treatments were compared to the control using Williams' multiple sequential t-test procedure ( $\alpha = 0.05$ , one-sided, <control).

## Results

### Mortality

The observations of female adult mortality were carried out 14 DAT, detailed in the table below.

**Table CP 10.4.2.1/02-01. The effects of CA3642 on adult *Hypoaspis aculeifer* mean mortality, after 14 days' exposure.**

Nominal concentration (mg product/kg soil dw)	Rep. no.	Day 0	Day 14		
		Number Live adults	Number Live adults	Mortality per replicate [%]	Mortality per treatment [%]
0.0 (Control)	1	10	10	0	3
	2	10	9	10	
	3	10	10	0	
	4	10	9	10	
	5	10	10	0	
	6	10	10	0	
	7	10	10	0	
	8	10	10	0	
177.16	1	10	10	0	8
	2	10	9	10	
	3	10	9	10	
	4	10	9	10	
98.42	1	10	10	0	5
	2	10	10	0	
	3	10	10	0	
	4	10	8	20	
54.68	1	10	9	10	10
	2	10	9	10	
	3	10	8	20	
	4	10	10	0	
30.38	1	10	10	0	8
	2	10	10	0	
	3	10	9	10	
	4	10	8	20	
16.88	1	10	10	0	10
	2	10	9	10	
	3	10	7	30	
	4	10	10	0	
9.38	1	10	8	20	15*
	2	10	8	20	
	3	10	9	10	
	4	10	9	10	
5.21	1	10	9	10	13
	2	10	8	20	
	3	10	10	0	
	4	10	8	20	

2.89	1	10	9	10	13
	2	10	8	20	
	3	10	10	0	
	4	10	8	20	

Rep. no.: Replicate number. Mortality in the test item treatments was compared to the control using a  $\chi^2$  2x2 table test with Bonferroni correction ( $\alpha = 0.05$ , one-sided, >control). \*Indicates a statistically significant difference (this was observed in the 9.38 mg product/kg soil dw treatment only and was considered to be an anomaly).

There were no statistically significant differences in mite mortality between any treatment group, compared to the control group, except for the 9.38 mg product/kg soil dw treatment group ( $\chi^2$  2x2 table test with Bonferroni correction;  $\alpha = 0.05$ , one-sided, >control). However, this was considered to be an anomaly, since the five higher treatment groups were not significantly different to the control group and there was no clear rate response. The 14-day  $LC_{50}$  value (mortality) was estimated to be >177.16 mg product/kg soil dw. The corresponding 14-day NOEC value (mortality) was determined to be 177.16 mg product/kg soil dw, the highest concentration tested. (The treatment concentration 177.16 mg product/kg soil dw is equivalent to 24.52 mg prothioconazole/kg soil dw and 24.93 mg azoxystrobin/kg soil dw).

### Reproductive output

Observations on reproductive output of *Hypoaspis aculeifer* were carried out 14 days after treatment incorporation in the control and product treatment groups, detailed in the tables below.

**Table CP 10.4.2.1/02-02. The effects of CA3642 on reproductive output (number of juvenile mites after 14 days' exposure) of *Hypoaspis aculeifer***

Nominal Concentration (mg product/kg soil dw)	Rep. No.	No. juveniles		Reproductive output	
		Per rep.	Mean <sup>a</sup>	CV%	% Reduction relative to the control
0.0 (Control)	1	261	276.0	7.3	-
	2	251			
	3	281			
	4	289			
	5	283			
	6	253			
	7	279			
	8	311			
177.16	1	280	281.3	9.9	-2
	2	260			
	3	264			
	4	321			
98.42	1	297	283.5	6.3	-3
	2	264			
	3	273			
	4	300			
54.68	1	244	279.5	9.1	-1
	2	284			
	3	304			
	4	286			
30.38	1	301	269.3	16.9	2
	2	243			
	3	219			
	4	314			
16.88	1	286	280.5	4.4	-2
	2	262			
	3	289			
	4	285			
9.38	1	248	253.5	15.7	8
	2	201			
	3	294			
	4	271			
5.21	1	277	252.0	14.2	9
	2	215			
	3	288			

	4	228			
2.89	1	256	241.8	6.4	12
	2	237			
	3	252			
	4	222			

<sup>a</sup>The test item treatments were compared to the control using Williams' multiple sequential t-test procedure ( $\alpha = 0.05$ , one-sided, <control). There were no statistically significant differences for the mean number of juveniles per replicate.

<sup>b</sup>A negative value indicates an increase in reproduction and a positive value a decrease, relative to the control mean.

**Table CP 10.4.2.1/02-03. Summary of effects of CA3642 on reproductive output (number of juvenile mites after 14 days' exposure) of *Hypoaspis aculeifer***

Nominal Concentration (mg product/kg soil dw)	14 days after soil incorporation		
	Mean No. of juveniles	R%	CV%
0.0 (Control)	276	-	7.3
177.16	281	-2	9.9
98.42	284	-3	6.3
54.68	280	-1	9.1
30.38	269	2	16.9
16.88	281	-2	4.4
9.38	254	8	15.7
5.21	252	9	14.2
2.89	242	12	6.4

No.: Number; R%: reduction in reproductive output relative to the control; CV%: coefficient of variation

There were no statistically significant differences in reproduction between the control group and any of the product treatment groups. The 14-day  $EC_{10/20/50}$  values, based on reproduction, were all estimated to be >177.16 mg product/kg soil dw. The corresponding 14-day NOEC value, based on reproduction, was determined to be 177.16 mg product/kg soil dw, the highest concentration tested.

Test concentration 177.16 mg product/kg soil dw is equivalent to 24.52 mg prothioconazole/kg soil dw and 24.93 mg azoxystrobin/kg soil dw, based on the measured content of each active substance in the formulated product.

### Validity

All the validity criteria were met, in accordance with OECD test guideline 226 (OECD, 2016):

- Mean mortality in the control treatment group was <20 % (actual value: 3%)
- Mean number of the juveniles in the control treatment group should be at least 50 (actual value: 276)
- The coefficient of variation calculated for the number of juveniles in the control was <30% (actual value: 7.3%)
- The reduction of fecundity in the toxic reference, relative to the control was in the range between 100 and 500 mg boric acid/kg soil dw (actual  $EC_{50}$  value: 334 mg boric acid/kg soil dw)

### Conclusion

The 14-day toxicity of CA3642 to predatory soil mites (*Hypoaspis aculeifer*) was studied in artificial soil with 5% peat, according to OECD test guideline 226 (2016).

The 14-day  $LC_{50}$  value (mortality): >177.16 mg product/kg soil dw (equivalent to >24.52 mg prothioconazole/kg soil dw and >24.93 mg azoxystrobin/kg soil dw, respectively).

The 14-day NOEC value (mortality): = 177.16 mg product/kg soil dw (equivalent to 24.52 mg prothioconazole/kg soil dw and 24.93 mg azoxystrobin/kg soil dw), the highest concentration

tested.

The 14-day EC<sub>50</sub> value (reproduction): >177.16 mg product/kg soil dw (equivalent to >24.52 mg prothioconazole/kg soil dw and >24.93 mg azoxystrobin/kg soil dw, respectively).

The 14-day NOEC value (reproduction): = 177.16 mg product/kg soil dw (equivalent to 24.52 mg prothioconazole/kg soil dw and 24.93 mg azoxystrobin/kg soil dw, respectively), the highest concentration tested.

This study is considered acceptable and valid.

#### A 2.4.2.2 KCP 10.4.2.2 Higher tier testing

#### A 2.5 KCP 10.5 Effects on soil nitrogen transformation

Comments of zRMS:	<p>The study was conducted in line with OECD 216 with no deviations.</p> <p>All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>The formulated product CA3642 at rates up to <u>10.56 mg CA3642/kg soil</u> has no significant effect on soil nitrogen transformation micro-organisms.</p>
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Reference:	KCP 10.5/01
Report	<p>CA3642 (Prothioconazole 150 g/L + Azoxystrobin 150 g/L SC): Nitrogen Transformation Test</p> <p>Hugill, E., 2024 (Experimental phase concluded, study report to be submitted)</p>
Guideline(s):	Yes. OECD 216 (2000)
GLP:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability:	Yes, validity criteria met

### Executive summary

The objective of this study was to determine the effect of the test substance, CA3642 (Prothioconazole 150 g/L + Azoxystrobin 150 g/L SC) at rates of 1.056 and 10.56 mg CA3642/kg dry soil on soil N transformation micro-organisms by determination of inhibition of nitrogen transformation rate. The effect of the test substance on soil micro-organisms was determined by investigation of nitrate formation in treated soil amended with an organic substrate (alfalfa/lucerne). An untreated control (water) was also included in the study to enable comparison of nitrate formation in treated soils.

Statistical analysis was conducted on each intermediate time interval. The results showed no statistically significant adverse effects at the day 7 – 14 and 14 – 28 time intervals indicating that CA3642 had no impact on nitrogen transformation of soil microorganisms when applied at 1.056 and 10.56 mg CA3642/kg dry soil.

Soil nitrate formation rates for the 0-7, day 7-14 and 14-28 day periods showed that formation rates in the CA3642 treated soils were less than ±25% different compared to rates in the control soil. It can therefore be concluded that CA3642 at rates up to 10.56 mg CA3642/kg soil has no significant effect on soil nitrogen transformation micro-organisms.

### Materials and methods

#### Test item

Name: CA3642 (Prothioconazole 150 g/L + Azoxystrobin 150 g/L SC)  
Density: 1.1044 g/mL  
Formulation type: Suspension Concentrate (SC)  
Batch no.: FRAI008392

Active substance (a.s.) 1:	Azoxystrobin 150.86 g/L or 13.66% w/w (analysed), 150 g/L (nominal)
Active substance (a.s.) 2:	Prothioconazole 146.89 g/L or 13.30% w/w (analysed), 150 g/L (nominal)
Appearance:	Off-white, odourless suspension
Expiry date of lot/batch:	16 May 2025
Storage conditions on receipt:	Room temperature (15 - 25°C), protect from sunlight
<i>Test soil</i>	
Soil type:	Silty sand
Batch no.:	Lufa 23/006
Particle size distribution:	2.5% (630-2000 µm); 29.5% (200-630 µm); 25.6% (63-200 µm); 19.8% (20-63 µm); 11.2% (6-20 µm); 4.5% (2-6 µm); 7.0% (<2 µm) Organic carbon: 0.66 % ± 0.05
Total nitrogen content:	0.08 % ± 0.01
Source:	Lufa Speyer (Germany)
Moisture content:	35.2±2.7% of the maximum water holding capacity throughout the test
Acclimation:	14 days under test environmental conditions for the definitive test prior to starting the test

#### *Test conditions*

The temperature was recorded throughout the test using a min/max thermometer. The temperature range during the test was maintained within 19.2 and 20.7°C and therefore, was maintained within the range specified in the protocol ( $20 \pm 2^\circ\text{C}$ ). The moisture content of the test substrate was adjusted gravimetrically by the addition of RO water, as required, to every replicate every 7 days, prior to sampling. The soil moisture content was determined on bulk control and test preparations on day 0 and on individual vessels at the end of the test. The soil moisture ranged between 40.59% and 50.13% throughout the test. The pH of the soil was determined at the start and end of the test. pH ranged between 6.08 and 6.21 throughout the test.

The test soil was amended with powdered alfalfa (also known as lucerne) and treated by incorporation of the test substance at 1.056 and 10.56 mg CA3642/kg dry soil. An untreated control was also included in the study with water only incorporated. The units for the nitrogen transformation test comprised 1000 mL amber glass jars, each filled with a final weight of approximately 200 g dry weight of soil. Three replicates were tested per treatment. On days 7, 14 and 28, sub-samples of soil of approximately 8 g of soil wet weight (7 g soil dry weight) were removed and extracted with 0.1 M potassium chloride (KCl). The extracts were centrifuged and filtered, then analysed by spectrophotometry to determine concentrations of nitrate.

Statistical analysis was performed using the CETIS program v 1.8.6.8 on all intermediate time intervals (i.e. day 0 -7, day 7 – 14 and day 14 -28). The nitrate formation rates were assessed using either a Williams Multiple Comparison test (day 0 – 7 Nitrate formation rates) or Dunnett Multiple Comparison test (day 7 – 14 and 14 - 28 Nitrate formation rates) based on whether the data was monotonic or non-monotonic (both appropriate parametric tests for multiple continuous data), respectively. A Bartlett Equality of Variance, Shapiro Wilk W Normality and Grubbs test were performed alongside the multiple comparison analysis to confirm the distribution of each data set and assess for outliers. The data confirmed that the data distribution for each time interval was parametric (showed equal variance and normal distribution) and that there were no statistical outliers.

#### *Protocol Deviations*

The following protocol deviation was noted during the test:

- A 20 g soil sample was used for moisture content determination at day 0 instead of 10 g in error. This occurred as it is standard practice to use a 20 g soil weight for this and it had no impact on the outcome of the study, as the larger quantity was removed from the treatment excess soil was treated at day 0.
- The protocol states that 'test will be terminated after 28 days, unless the difference between treated and untreated soils is equal to or greater than 25%'. The definitive test was continued until day 36 while the results were discussed with the study monitor and sponsor and therefore, a protocol deviation occurred. This study extension was not considered to have an impact on the test results, as the study was terminated before any further analysis was performed so this had no effects on the nitrate end points.

### **Interim results**

#### *Biological results*

A summary effects of CA3642 on soil nitrate concentrations in each replicate at different time-points exposure is presented in the table below.

**Table KCP 10.5/02-1. Effects of the product CA3642 on soil nitrate concentrations**

Nominal concentration (mg product/kg soil dw)	Replicate number	Nitrate concentration (mg NO <sub>3</sub> /kg soil dw)			
		Day 0	Day 7	Day 14	Day 28
<b>Control (water)</b>	1	22.48	11.84	19.76	35.86 (1A-1)*, 36.26 (1A-2)*
	2	22.48	12.50	19.27	36.61
	3	21.78	13.07	20.55	37.84
	<b>Mean (CV)</b>	<b>22.25 (1.82)</b>	<b>12.47 (4.94)</b>	<b>19.86 (3.25)</b>	<b>36.64 (2.73)</b>
<b>1.056</b>	1	26.93	14.70	20.64	35.64
	2	26.27	14.65	21.47	36.26
	3	25.65	14.61	20.77	37.27
	<b>Mean (CV)</b>	<b>26.28 (2.44)</b>	<b>14.65 (0.31)</b>	<b>20.96 (2.13)</b>	<b>36.39 (2.26)</b>
<b>10.56</b>	1	26.00	14.96	21.96	39.51
	2	27.10	16.54	21.47	36.43
	3	27.41	13.73	23.23	37.93
	<b>Mean (CV)</b>	<b>26.84 (2.76)</b>	<b>15.08 (9.34)</b>	<b>22.22 (4.09)</b>	<b>37.96 (4.06)</b>

CV = Coefficient of Variation, dw = dry weight

\* Vessel Control 1A broke during moisture check at day 21. Most of the soil was retrieved, and identified in two sub-replicates: clean soil (1A-1) and potentially contaminated soil (1A-2)

The variation in nitrate concentrations between the control replicates was less than  $\pm 15\%$  on days 0, 7, 14 and 28. Therefore, the validity criterion of the OECD Guideline 216 was fully met for the duration of the test.

A summary of the effects of CA3642 on mean nitrate formation rates during 28 days exposure is presented in the table below.

**Table KCP 10.5/02-2. Summary of the effects of the product CA3642 on soil nitrate formation rates**

Nominal concentration (mg CA3642/kg soil dw)	Mean Nitrate formation rate (mg NO <sub>3</sub> /kg soil dw/day)	Mean deviation in nitrate formation rate relative to control (%)
<b>Days 0 - 7</b>		
<b>Control</b>	-1.40	NA
<b>1.056</b>	-1.66	-18.57 <sup>b*</sup>
<b>10.56</b>	-1.68	-20.00 <sup>b*</sup>
<b>Days 7 - 14</b>		
<b>Control</b>	1.06	NA
<b>1.056</b>	0.901	15.00
<b>10.56</b>	1.02	3.77
<b>Days 14 – 28</b>		
<b>Control</b>	1.21	NA
<b>1.056</b>	1.10	9.09
<b>10.56</b>	1.12	7.44

NA Not applicable

<sup>b</sup> Any negative percentage difference values of the test treatments compared to the control indicates a positive effect and therefore, this is not considered to suggest an adverse effect on the nitrogen transformation of soil.

\* Results identified to be statistically significant compared to the control.

The mean nitrate formation rates of the treated soils at 1.056 and 10.56 mg CA3642/kg soil dw test concentrations were lower than  $\pm 25\%$  compared to the control for all inter-periods (i.e. 0-7, 7-14 and 14-28 day).



The results showed no statistically significant adverse effects at the day 7 – 14 and 14 – 28 time intervals indicating that CA3642 had no impact on nitrogen transformation of soil microorganisms when applied at 1.056 and 10.56 mg CA3642/kg dry soil at these timepoints. A statistically significant adverse effect was noted at the day 0 - 7 time interval, however, as the difference was less than 25% and the rates were negative (nitrate levels lower than day 0) this was not considered to indicate a biological adverse effect.

### Validity

The validity criterion of the OECD test guideline 216 (2000) was met since the coefficient of variation in nitrate concentrations between replicate control samples was lower than 15% at all timepoints (Table KCP 10.5/02-1).

### Conclusion

Nitrate formation rates for the 0-7, day 7-14 and 14-28 day periods showed that the formation rates in the CA3642 treated soils were less than  $\pm 25\%$  different compared to rates in the control soil. It can therefore be concluded that CA3642 at rates up to 10.56 mg CA3642/kg soil has no significant effect on soil nitrogen transformation micro-organisms.

## 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

#### Study 1

Comments of zRMS:

The study was conducted in line with OECD 227 with no deviations.

The analytical recoveries were all within  $\pm 20\%$  of nominal concentrations ( 104% and 103% for azoxystrobin and prothioconazole, respectively), therefore, results and endpoints are based on nominal concentrations.

All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment:

ER<sub>50</sub>, survival, plant length and shoot dry weight > 2.8 L product/ha

Visual phytotoxicity:

Symptoms of phytotoxicity were observed in all the dicotyledonous species, with exception of *Daucus carota* (carrot), while no phytotoxicity symptoms were observed in the monocotyledonous species. The most sensitive species, with respect to phytotoxicity, was *C. sativus*. For this species

NOER and LOER values for phytotoxicity (based on nominal rates of CA3642)

Endpoint (L CA3642/ha)	<i>Cucumis sativus</i>	<i>Brassica napus</i>	<i>Daucus carota</i>	<i>L. esculentum</i>	<i>Beta vulgaris</i>	<i>Glycine max</i>	<i>Zea mays</i>	<i>Lolium perenne</i>	<i>Avena sativa</i>	<i>Allium cepa</i>
LOER	0.357	2.8	>2.8	2.8	2.8	2.8	>2.8	>2.8	>2.8	>2.8
NOER	0.128	1.0	$\geq 2.8$	1.0	1.0	1.0	$\geq 2.8$	$\geq 2.8$	$\geq 2.8$	$\geq 2.8$

**The phytotoxic effects of CA3642 on 10 plant species, after 21 days of exposure.**

Reference:	KCP 10.6.2/01
Report	CA3642 (Azoxystrobin 150 g/L + Prothioconazole 150 g/L SC): Effects on the Vegetative Vigour of Ten Non-Target Terrestrial Plant Species under Greenhouse Conditions  Huerta, F., 2023, report no. S21-04085
Guideline(s):	Yes. OECD 227 (2006)
Deviations:	No
GLP:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability:	Yes
Duplication (if vertebrate study)	No

## Executive summary

In a laboratory rate-response test, the effects of the product, CA3642, a suspension concentrate (SC) formulation (analysed active substances: 13.84% w/w prothioconazole and 14.07% w/w azoxystrobin), were assessed on the vegetative vigour of ten selected non-target plant species. The plants were exposed to six application rates of 0.0 (tap water control), 0.046, 0.128, 0.357, 1.00, and 2.80 L product/ha (equivalent to 0.00, 7.00, 19.49, 54.35, 152.23 and 426.24 g prothioconazole/ha and 0.00, 7.12, 19.82, 55.27, 154.83 and 433.52 g azoxystrobin/ha, respectively, based on measured active substance content). Since analytical verification of test concentrations confirmed that measured concentrations of all treatments were with  $\pm 20\%$  of nominal concentrations, biological endpoints are reported based on nominal application rates of CA3642.

Six dicotyledonous and 4 monocotyledonous species, cultivated in artificial soil, were recorded for plant survival, phytotoxicity and growth stage, 7, 14 and 21 days after treatment, and measure for shoot height and shoot dry weight, 21 days after treatment.

The 21-day LR<sub>50</sub> value (mortality) was estimated to be >2.8 L product/ha, equivalent to >426.24 g prothioconazole and >433.52 g azoxystrobin, respectively (based on nominal concentrations). The corresponding NOER (mortality) value for all species was estimated to be  $\geq 2.80$  L product/ha, equivalent to  $\geq 426.24$  g prothioconazole and  $\geq 433.52$  g azoxystrobin, respectively (based on nominal concentrations).

The 21-day ER<sub>50</sub> values (mean shoot height and mean shoot dry weight) were estimated to be >2.800 L product/ha, equivalent to >426.24 g prothioconazole and >433.52 g azoxystrobin, respectively, (based on nominal concentrations). The corresponding 21-day NOER value (mean shoot height), based on decreased shoot height of *Lycopersicon Esculentum* and *Glycine max*, was determined to be 0.357L product/ha, equivalent to 54.35 g prothioconazole/ha and 55.27 g azoxystrobin/ha, respectively (based on nominal concentrations).

The 21-day NOER value (mean shoot dry weight) based on decreased shoot dry weight of *C. sativus*, was determined to be 0.128 L product/ha, equivalent to 19.49 g prothioconazole/ha and 19.82 g azoxystrobin/ha, respectively, (based on nominal concentrations).

This study satisfies the OECD 227 (2006) test-guideline requirements for a vegetative-vigour study with non-target terrestrial plants and is considered acceptable.

## Materials and methods

### Test materials

#### Test item

Name: CA3642 (Prothioconazole 150 g/L + Azoxystrobin 150 g/L SC)

Density:	1.1004 g/mL
Formulation type:	Suspension Concentrate (SC)
Batch no.:	A20026
Active substance (a.s.) 1:	Azoxystrobin 154.83 g/L or 14.07 w/w (analysed), 150 g/L (nominal)
Active substance (a.s.) 2:	Prothioconazole 152.23 g/L or 13.84% w/w (analysed), 150 g/L (nominal)
Appearance:	Off-white, odourless suspension
Expiry date of lot/batch:	September 2022
Storage conditions:	Keep at room temperature in a well-ventilated place

#### Test organisms

Species/source:	Cucumber ( <i>Cucumis sativus</i> )/Batlle Oilseed rape ( <i>Brassica napus</i> )/KWS Carrot ( <i>Daucus carota</i> )/Sakata Tomato ( <i>Lycopersicon esculentum</i> )/Monsanto Holland BV Beet ( <i>Beta vulgaris</i> )/Strube Soybean ( <i>Glycine max</i> )/Saatbau Corn ( <i>Zea mays</i> )/Tozer Seeds Ryegrass ( <i>Lolium perenne</i> )/Herbiseed Common oat ( <i>Avena sativa</i> )/Intersemillas Onion ( <i>Allium cepa</i> )/Batlle
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#### Test medium:

Soil type:	Artificial soil (sandy loam) Soil texture: 4.00% silt, 14.72% clay, and 81.28% sand
pH:	7.26
Conductivity:	0.561 mS/cm

#### Test conditions

Test temperature:	12°C - 32°C (actual: 17.9°C – 33.7°C)
Relative humidity:	45%-95% (actual: 40.0% – 89.0%)
Photoperiod:	16 hours' light: 8 hours' dark
Light intensity:	>300 µE/m <sup>2</sup> /s (actual: 946- 978 µE/m <sup>2</sup> /s)

Untreated seeds of ten higher terrestrial plant species were sown in sandy loam soil, in 1.5-L, non-porous, plastic plant pots (15.1-cm diameter), which were filled with the test soil. Four seeds were sown per replicate pot (5 replicate pots per treatment) for *Lolium perenne*, *Avena sativa* and *Allium cepa*; two seeds were sown per replicate pot (10 replicate pots per treatment) for the remaining test species. ;

The study was conducted as a rate-response test, with six treatment groups: a tap-water control group and five test-item treatment groups of 0.0, 0.046, 0.128, 0.357, 1.00, and 2.80 L product/ha, equivalent to 0.00, 7, 19.49, 54.35, 152.23, and 426.24 g prothioconazole/ha and 0.00, 7.12, 19.82, 55.27, 154.83, and 433.52 g azoxystrobin/ha, respectively.

The product treatment solutions were prepared as close as practically possible to the time of spray application. Tap water was used as a solvent and the highest treatment solution served as the stock solution, with all lower treatment rates diluted in water. Treatments were applied by spraying the soil surface in the appropriate pots with the corresponding spray mixture at a spray volume of 200 L/ha (with an accuracy of ±10% per ha) from the control (tap water) followed by treatments from the lowest to the highest application rate.

The replicate pots were then arranged, according to treatment group, randomly in a glasshouse and were repositioned on the first and second assessment days (7 and 14 days after treatment). The plant pots were watered from beneath with a controlled and regularly replenished water supply. Temperature and relative humidity were monitored continuously, and light conditions were automatically regulated.

Survival/mortality, phytotoxicity, and growth stage (BBCH stage) were assessed 7, 14, and 21 days after treatment application. Phytotoxicity was assessed visually, by observing morphology, specifically chlorosis, necrosis, and other characteristics. Shoot height was measured on day 21, by measuring the length of the above-ground vegetation of each surviving plant, from the soil surface to the apical tip (oilseed rape, carrot, sugar beet, maize, ryegrass, oat, and onion) or highest aerial part (cucumber, tomato, and soybean). Shoot dry weight was recorded on day 21, by clipping each plant at its soil surface, drying it at 60°C, and weighing the dried plant.

Analytical verification of the stock product spray solution was conducted during the analytical phase of the study. Two samples (each 10 mL), one for the analytical rate verification (A) and one retain sample (R) were taken from the stock solution. Specimens were put in flasks, sealed tightly, labelled correspondingly, and placed into dry ice.

#### *Data analysis*

Data were statistically evaluated with the software ToxRat Solutions (ToxRat® Professional Version 3.3.0.). Data for mortality, shoot height, and shoot dry weight were statistically evaluated, and significant differences, relative to the control, were determined using  $\alpha = 0.05$  for all tests. Phytotoxicity and BBCH growth stages were qualitatively tabulated for each species and were not analysed statistically.

Quantal data were analysed with Fisher's exact binomial tests, with Bonferroni corrections, while metric data were analysed for normality (Shapiro-Wilk's test) and homoscedasticity (Levene's test), before performing the appropriate statistical tests. For normally distributed data, with equal variances, data were analysed with either a Williams' or Dunnett's test. When data were not normally distributed or did not have equal variances, either a step-down-Jonckheere Terpstra test ( $\alpha=0.05$ ) or multiple sequentially-rejective U-test was used. The data were then corrected with Bonferroni-Holm tests.  $LR_x$  and  $ER_x$  values were calculated using probit analyses, using linear maximum-likelihood regressions.

## **Results**

### Analytical results

The HPLC -MS/MS method for the determination of prothioconazole and azoxystrobin in spray solution (tap water) was validated with regards to specificity, linearity, accuracy and precision in accordance with guideline SANTE/2020/12830 rev.1 24/02/2021.

Specificity in tap water was demonstrated by the absence of significant interference above 30% of the LOQ in control and blank samples. The linearity of the method was demonstrated using solvent calibration standards. The analytical method was shown to be linear ( $r > 0.99$ ) over the range from 10 to 100 ng/mL for both prothioconazole and azoxystrobin. Accuracy was confirmed with recovery of spiked samples at relevant concentrations of test item in tap water (1560 (LOQ) and 20350 mg test item/L). Mean recoveries were 89 and 103% for Azoxystrobin at 1560 and 20350 mg test item/L respectively (i.e., within 70-110%). Mean recoveries were 99% and 101% for prothioconazole at 1560 and 23500 mg test item/L respectively (i.e., within 70-110%). Precision was confirmed. For Azoxystrobin, the relative standard deviation ( $n = 5$  for each fortification level) was 17% and 1% at 1560 and 23500 mg test item/L respectively (i.e., within the guideline limit of  $\leq 20\%$ ). For Prothioconazole, the relative standard deviation ( $n = 5$  for each fortification level) was 4% and 2% at 1560 and 23500 mg test item/L respectively (i.e., within the guideline limit of  $\leq 20\%$ ).

The limit of quantification (LOQ) for tap water was 1560 mg test item/L (corresponding to 219 mg azoxystrobin/L and 216 mg prothioconazole/L). The limit of detection (LOD) was defined at 40 mg/L for both analytes.

**Table CP 10.6.2/01-01: Summary of the analytical results in the spray solutions.**

Substance analysed	Concentration (mg a.s./L)		Recovery (% nominal)
	Nominal	Analysed	
Azoxystrobin	2168	2264	104
Prothioconazole	2132	2194	103

The analytical recoveries were all within  $\pm 20\%$  of nominal concentrations, therefore, results and endpoints are based on nominal concentrations.

### Biological results

#### *Survival and mortality*

The effects of CA3642 on survival and mortality, after 21 days of exposure, are presented in the table below.

**Table CP 10.6.2/01-02: The effect of CA3642 on the mortality of 10 plant species, after 21 days of exposure.**

Treatment		<i>C. sativus</i>			<i>B. napus</i>		
ID	L product/ha	No.	Mean ± SD (%)	CV (%)	No.	Mean ± SD (%)	CV (%)
C	0.0	0	0.0 ± 0.0	-	0	0.0 ± 0.0	-
T1	0.046	0	0.0 ± 0.0	-	0	0.0 ± 0.0	-
T2	0.128	0	0.0 ± 0.0	-	0	0.0 ± 0.0	-
T3	0.357	0	0.0 ± 0.0	-	0	0.0 ± 0.0	-
T4	1.0	0	0.0 ± 0.0	-	0	0.0 ± 0.0	-
T5	2.8	0	0.0 ± 0.0	-	0	0.0 ± 0.0	-
Treatment		<i>D. carota</i>			<i>L. esculentum</i>		
ID	L product/ha	No.	Mean ± SD (%)	CV (%)	No.	Mean ± SD (%)	CV (%)
C	0.0	-1	5.0 ± 15.8	316.2	0	0.0 ± 0.0	-
T1	0.046	0	0.0 ± 0.0	-	0	0.0 ± 0.0	-
T2	0.128	-2	10.0 ± 21.1	210.8	0	0.0 ± 0.0	-
T3	0.357	0	0.0 ± 0.0	-	0	0.0 ± 0.0	-
T4	1.0	0	0.0 ± 0.0	-	0	0.0 ± 0.0	-
T5	2.8	0	0.0 ± 0.0	-	0	0.0 ± 0.0	-
Treatment		<i>B. vulgaris</i>			<i>G. max</i>		
ID	L product/ha	No.	Mean ± SD (%)	CV (%)	No.	Mean ± SD (%)	CV (%)
C	0.0	0	0.0 ± 0.0	-	0	0.0 ± 0.0	-
T1	0.046	0	0.0 ± 0.0	-	0	0.0 ± 0.0	-
T2	0.128	0	0.0 ± 0.0	-	0	0.0 ± 0.0	-
T3	0.357	0	0.0 ± 0.0	-	0	0.0 ± 0.0	-
T4	1.0	0	0.0 ± 0.0	-	0	0.0 ± 0.0	-
T5	2.8	0	0.0 ± 0.0	-	0	0.0 ± 0.0	-
Treatment		<i>Z. mays</i>			<i>L. perenne</i>		
ID	L product/ha	No.	Mean ± SD (%)	CV (%)	No.	Mean ± SD (%)	CV (%)
C	0.0	0	0.0 ± 0.0	-	0	0.0 ± 0.0	-
T1	0.046	0	0.0 ± 0.0	-	0	0.0 ± 0.0	-
T2	0.128	0	0.0 ± 0.0	-	0	0.0 ± 0.0	-
T3	0.357	0	0.0 ± 0.0	-	0	0.0 ± 0.0	-
T4	1.0	0	0.0 ± 0.0	-	0	0.0 ± 0.0	-
T5	2.8	0	0.0 ± 0.0	-	0	0.0 ± 0.0	-
Treatment		<i>A. sativa</i>			<i>A. cepa</i>		
ID	L product/ha	No.	Mean ± SD (%)	CV (%)	No.	Mean ± SD (%)	CV (%)
C	0.0	0	0.0 ± 0.0	-	0	0.0 ± 0.0	-
T1	0.046	0	0.0 ± 0.0	-	0	0.0 ± 0.0	-
T2	0.128	0	0.0 ± 0.0	-	0	0.0 ± 0.0	-
T3	0.357	0	0.0 ± 0.0	-	0	0.0 ± 0.0	-
T4	1.0	0	0.0 ± 0.0	-	0	0.0 ± 0.0	-
T5	2.8	0	0.0 ± 0.0	-	0	0.0 ± 0.0	-

No.: number; SD: standard deviation; CV: coefficient of variation.

No statistically significant differences in plant mortality were recorded for any species and product treatment tested, when compared to the control group. The 21-day LR<sub>50</sub> value (mortality) was estimated to be >2.8 L product/ha, equivalent to >426.24 g prothioconazole and >433.52 g azoxystrobin, respectively (based on nominal concentrations). The corresponding NOER (mortality) value for all species was estimated to be ≥2.80 L product/ha, equivalent to ≥426.24 g prothioconazole and ≥433.52 g azoxystrobin, respectively, the highest rate tested, (based on nominal concentrations).

#### *Shoot height and shoot dry weight*

A summary of the effects of CA3642 on shoot height and dry shoot weight, after 21 days of exposure, is presented in tables below.

**Table CP 10.6.2/01-03: The effects of CA3642 on shoot height of 10 plant species, after 21 days of exposure.**

Treatment		<i>Cucumis sativus</i>		<i>Glycine max</i>	
ID	L product/ha	Mean ± SD (cm)	Inhibition (% control)	Mean ± SD (cm)	Inhibition (% control)
C	0.0	141.5 ± 10.91	0.00	77.68 ± 7.47	0.00
T1	0.046	134.55 ± 7.32	4.91	69.85 ± 3.32*	10.07
T2	0.128	143.95 ± 11.58	-1.73	74.3 ± 2.14	4.35
T3	0.357	145.55 ± 9.63	-2.86	76.6 ± 5.68	1.38
T4	1.0	139.25 ± 11.04	1.59	69.9 ± 3.9*	10.01
T5	2.8	141 ± 11.67	0.35	72.6 ± 5.19*	6.53
Treatment		<i>Brassica napus</i>		<i>Zea mays</i>	
ID	L product/ha	Mean ± SD (cm)	Inhibition (% control)	Mean ± SD (cm)	Inhibition (% control)
C	0.0	48.05 ± 1.88	0.00	138.7 ± 3.41	0.00
T1	0.046	45.95 ± 2.91	4.37	131.05 ± 7.29*	5.52
T2	0.128	44.75 ± 2.28*	6.87	134.65 ± 3.44	2.92
T3	0.357	46.75 ± 4.34	2.71	137.15 ± 2.75	1.12
T4	1.0	46.2 ± 2.42	3.85	134.9 ± 5.57	2.74
T5	2.8	45.2 ± 2.87	5.93	136.15 ± 5.27	1.84
Treatment		<i>Daucus carota (carrot)</i>		<i>Avena sativa</i>	
ID	L product/ha	Mean ± SD (cm)	Inhibition (% control)	Mean ± SD (cm)	Inhibition (% control)
C	0.0	41.1 ± 2.46	0.00	55.45 ± 4.96	0.00
T1	0.046	41.85 ± 3.4	-1.82	50.1 ± 4.16	9.65
T2	0.128	40.95 ± 4.65	0.36	56.5 ± 5.07	-1.89
T3	0.357	40.2 ± 4.83	2.19	56.75 ± 5.33	-2.34
T4	1.0	38.75 ± 2.9	5.72	50.9 ± 2.49	8.21
T5	2.8	41.8 ± 3.63	-1.70	58.25 ± 5.18	-5.05
Treatment		<i>Lycopersicon esculentum</i>		<i>Lolium perenne</i>	
ID	L product/ha	Mean ± SD (cm)	Inhibition (% control)	Mean ± SD (cm)	Inhibition (% control)
C	0.0	58.4 ± 3.13	0.00	32.8 ± 0.54	0.00
T1	0.046	55.25 ± 2.19	5.39	28.9 ± 3.26*	11.89
T2	0.128	57.5 ± 2.75	1.54	33.65 ± 2.23	-2.59
T3	0.357	58.6 ± 3.14	-0.34	33.8 ± 1.95	-3.05
T4	1.0	49.6 ± 6.67*	15.07	29.5 ± 3.61	10.06
T5	2.8	50.15 ± 4.2*	14.13	34.4 ± 1.06	-4.88
Treatment		<i>Beta vulgaris</i>		<i>Allium cepa</i>	
ID	L product/ha	Mean ± SD (cm)	Inhibition (% control)	Mean ± SD (cm)	Inhibition (% control)
C	0.0	36.4 ± 1.91	0.00	51.35 ± 0.98	0.00
T1	0.046	35.85 ± 1.25	1.51	53.75 ± 3.32	-4.67
T2	0.128	37.25 ± 1.72	-2.34	49.05 ± 3.9	4.48
T3	0.357	37.75 ± 2.66	-3.71	51.5 ± 2.78	-0.29
T4	1.0	35.35 ± 3.07	2.88	53.25 ± 2.9	-3.70
T5	2.8	37.45 ± 2.9	-2.88	52.05 ± 1.68	-1.36

Negative inhibition values indicate a increase in shoot height, relative to the control group.

\*: Statistically significant differences, relative to the control group (P < 0.05).

C: control; Tn: test-item treatment code; SD: standard deviation.

Statistically significant differences in mean shoot height of *L. esculentum* and *G. max*, relative to the control group, were observed at the two highest product treatment rates. For *B. napus*, *Z. mays*, and *L. perenne*, statistically significant effects on shoot height were observed at either of the two lowest treatment rates, while for the higher treatment rates there was no clear dose response. These discrepancies are considered likely attributed to biological variability and, consequently, NOER values for these species could not be determined. Conversely, the NOER value for mean shoot height of *L. esculentum* (tomato) and *G. max* (soybean) was determined to be 0.357 L product/ha (equivalent to 54.35 g prothioconazole/ha and 55.27 g



azoxystrobin/ha, respectively). For the remaining test species, the NOEC value for mean shoot height was estimated to be  $\geq 2.8$  L product/ha (equivalent to  $\geq 426.24$  g prothioconazole/ha and  $\geq 433.52$  g azoxystrobin/ha, respectively).

The ER<sub>10</sub> value (mean shoot height) for *L. esculentum* was determined to be 0.921 L product/ha [with 95% Confidence Limits (CLs) of 0.425 – 1.681 L product/ha], equivalent to 140.20 g prothioconazole/ha (with 95% CLs of 64.70 – 255.90 g prothioconazole/ha and 142.60 g azoxystrobin/ha [with 95% CLs of 65.80 – 260.27 g azoxystrobin/ha, respectively. For the remaining species, the ER<sub>10</sub>, ER<sub>20</sub>, ER<sub>25</sub>, and ER<sub>50</sub> values were all estimated to be  $>2.80$  L product/ha (equivalent to  $>426.24$  g prothioconazole/ha and  $>433.52$  g azoxystrobin/ha, respectively).

**Table CP 10.6.2/01-04: The effects of CA3642 on shoot dry weight of 10 plant species, after 21 days of exposure.**

Treatment		<i>Cucumis sativus</i>		<i>Glycine max</i>	
ID	L product/ha	Mean $\pm$ SD (g)	Inhibition (% control)	Mean $\pm$ SD (g)	Inhibition (% control)
C	0.0	22.52 $\pm$ 1.13	0.00	18.56 $\pm$ 2.54	0.00
T1	0.046	20.78 $\pm$ 1.1	7.74	17.71 $\pm$ 2.73	4.60
T2	0.128	23.44 $\pm$ 1.24	-4.05	20.53 $\pm$ 2.17	-10.64
T3	0.357	20.98 $\pm$ 1.59*	6.87	18.81 $\pm$ 3.07	-1.36
T4	1.0	21.3 $\pm$ 0.75*	5.43	19.11 $\pm$ 1.96	-2.99
T5	2.8	20.03 $\pm$ 1.02*	11.06	17.62 $\pm$ 3.24	5.04
Treatment		<i>Brassica napus</i>		<i>Zea mays</i>	
ID	L product/ha	Mean $\pm$ SD (g)	Inhibition (% control)	Mean $\pm$ SD (g)	Inhibition (% control)
C	0.0	14.06 $\pm$ 2.45	0.00	22.45 $\pm$ 1.44	0.00
T1	0.046	14.11 $\pm$ 2.18	-0.36	20.86 $\pm$ 2.77	7.06
T2	0.128	13.32 $\pm$ 3.33	5.29	22.7 $\pm$ 2.02	-1.14
T3	0.357	11.81 $\pm$ 2.12	15.99	21.94 $\pm$ 2.7	2.27
T4	1.0	11.84 $\pm$ 2.54	15.84	21.29 $\pm$ 2.67	5.17
T5	2.8	13.91 $\pm$ 1.5	1.06	22.05 $\pm$ 2.27	1.80
Treatment		<i>Daucus carota</i>		<i>Avena sativa</i>	
ID	L product/ha	Mean $\pm$ SD (g)	Inhibition (% control)	Mean $\pm$ SD (g)	Inhibition (% control)
C	0.0	4.06 $\pm$ 0.7	0.00	2.81 $\pm$ 0.2	0.00
T1	0.046	4.1 $\pm$ 0.58	-0.92	2.7 $\pm$ 0.22	3.81
T2	0.128	4.35 $\pm$ 1.26	-7.25	2.67 $\pm$ 0.37	4.95
T3	0.357	3.66 $\pm$ 0.89	9.90	3.28 $\pm$ 0.38	-16.85
T4	1.0	3.84 $\pm$ 0.55	5.51	3.02 $\pm$ 0.27	-7.28
T5	2.8	4.04 $\pm$ 0.91	0.42	2.92 $\pm$ 0.56	-4.05
Treatment		<i>Lycopersicon esculentum</i>		<i>Lolium perenne</i>	
ID	L product/ha	Mean $\pm$ SD (g)	Inhibition (% control)	Mean $\pm$ SD (g)	Inhibition (% control)
C	0.0	14.69 $\pm$ 1.38	0.00	1.09 $\pm$ 0.22	0.00
T1	0.046	13.96 $\pm$ 1.17	4.98	1.12 $\pm$ 0.27	-2.98
T2	0.128	13.98 $\pm$ 1.29	4.87	1.09 $\pm$ 0.21	-0.08
T3	0.357	15.52 $\pm$ 1.81	-5.64	1.34 $\pm$ 0.22	-23.28
T4	1.0	10.66 $\pm$ 3.34*	27.47	1.08 $\pm$ 0.24	0.72
T5	2.8	10.65 $\pm$ 2.22*	27.51	1.21 $\pm$ 0.14	-11.36
Treatment		<i>Beta vulgaris</i>		<i>Allium cepa</i>	
ID	L product/ha	Mean $\pm$ SD (g)	Inhibition (% control)	Mean $\pm$ SD (g)	Inhibition (% control)
C	0.0	10.79 $\pm$ 0.94	0.00	1.77 $\pm$ 0.16	0.00
T1	0.046	11.01 $\pm$ 1.25	-2.06	1.66 $\pm$ 0.26	6.55
T2	0.128	9.63 $\pm$ 2.16	10.74	1.64 $\pm$ 0.38	7.55
T3	0.357	10.24 $\pm$ 1.35	5.08	1.73 $\pm$ 0.32	2.59
T4	1.0	9.85 $\pm$ 1.49	8.70	1.77 $\pm$ 0.17	0.15

C; control; Tn: test-item treatment code; SD: standard deviation.

**Table CP 10.6.2/01-06: The phytotoxic effects of CA3642 on 10 plant species, after 21 days of exposure.**

[illegible]

T2	0.128	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T3	0.357	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T4	1.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T5	2.8	0	10*	10*	0	0	0	0	0	0	0	0	0	0	0
Treatment		<i>Beta vulgaris</i>							<i>Allium cepa</i>						
C	0.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T1	0.046	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T2	0.128	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T3	0.357	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T4	1.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T5	2.8	0	10*	0	10*	0	0	0	0	0	0	0	0	0	0

\*Statistically significant differences, relative to the control group ( $P < 0.05$ ).

C: control; T<sub>n</sub>: test-item treatment code.

Symptoms of phytotoxicity were observed in all the dicotyledonous species, with exception of *Daucus carota* (carrot), while no phytotoxicity symptoms were observed in the monocotyledonous species. The most sensitive species, with respect to phytotoxicity, was *C. sativus*.

### Validity

All validity criteria were met in accordance with the OECD 227 (2006) test guideline:

- No visible phytotoxic effects in the control group (actual result: no control plant species exhibited symptoms of phytotoxicity).
- Mean survival of seedlings for the duration of the study was  $\geq 90\%$  in the control group (actual values: 95% to 100%)
- Seedling emergence was  $\geq 70\%$  (actual values: 91% to 100%)

For each given species, environmental conditions were identical.

### Conclusion

A 21-day laboratory rate-response test was conducted to assess the effects of CA3642 on the vegetative vigour of ten non-target terrestrial plant species, according to OECD test guidelines 227 (2006).

The 21-day LR<sub>50</sub> (mortality) value for all species was:  $>2.800$  L product/ha (equivalent to  $>426.24$  g prothioconazole/ha and  $>433.52$  g azoxystrobin/ha, respectively), based on nominal concentrations.

The 21-day NOER (mortality) value for all species was:  $\geq 2.800$  L product/ha (equivalent to  $\geq 426.24$  g prothioconazole/ha and  $\geq 433.52$  g azoxystrobin/ha, respectively), based on nominal concentrations.

The 21-day ER<sub>50</sub> (mean shoot height and mean shoot dry weight) value for all species was:  $>2.800$  L product/ha (equivalent to  $>426.24$  g prothioconazole/ha and  $>433.52$  g azoxystrobin/ha, respectively), based on nominal concentrations.

The 21-day NOEC (mean shoot height) value for *L. esculentum* (tomato) and *G. max* (soybean) was:  $= 0.357$  L product/ha (equivalent to  $54.35$  g prothioconazole/ha and  $55.27$  g azoxystrobin/ha, respectively) based on nominal concentrations.

The 21-day NOEC (mean shoot dry weight) value for *C. sativus* was:  $= 0.128$  L product/ha, (equivalent to  $19.49$  g prothioconazole/ha and  $19.82$  g azoxystrobin/ha, respectively) based on nominal concentrations.

This study is considered acceptable and valid.

## Study 2

Comments of zRMS:	<p>The study was conducted in line with OECD 208 with no deviation.</p> <p>The analytical recoveries were all within <math>\pm 20\%</math> of nominal concentrations (101 and 10, therefore, results and endpoints are based on nominal concentrations or nominal rates.</p> <p>All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>The 21-d ER<sub>50</sub> values, for seedling emergence, shoot height, and shoot dry weight, of all tested species, were all estimated to be <math>&gt;2.80</math> L product/ha, corresponding to <math>&gt;426.24</math> g prothioconazole/ha and <math>&gt;433.52</math> g azoxystrobin/ha, respectively (based on nominal concentrations)</p> <p><u>Visual phytotoxicity:</u></p> <p>No statistically significant phytotoxic effects, relative to the control, were observed for all tested plant species. Consequently, the NOER value was estimated to be <math>\geq 2.80</math> L CA3642/ha, equivalent to <math>\geq 426.24</math> g prothioconazole/ha and <math>\geq 433.52</math> g azoxystrobin/ha, respectively. The ER<sub>10</sub>, ER<sub>20</sub>, ER<sub>25</sub>, and ER<sub>50</sub> values for mean shoot dry weight were estimated to be <math>&gt;2.80</math> L product/ha, equivalent to <math>&gt;426.24</math> g prothioconazole/ha and <math>&gt;433.52</math> g azoxystrobin/ha, respectively, for all the species tested.</p>
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Reference:	KCP 10.6.2/02
Report	<p>CA3642 (Azoxystrobin 150 g/L + Prothioconazole 150 g/L SC): Effects on the Seedling Emergence and Growth of Ten Non-Target Terrestrial Plant Species under Greenhouse Conditions</p> <p>Huerta, F., 2023, report no. S21-04084</p>
Guideline(s):	Yes. OECD 208 (2006)
Deviations:	No
GLP:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability:	Yes
Duplication (if vertebrate study)	No

## Executive summary

In a laboratory rate-response test, the effects of the product, CA3642, a suspension concentrate (SC) formulation (analysed active substances: 13.84% w/w prothioconazole and 14.07% w/w azoxystrobin), were assessed on the vegetative vigour of ten selected non-target plant species. The plants were exposed to six application treatments of 0.0 (tap water control), 0.046, 0.128, 0.357, 1.00, and 2.80 L product/ha, corresponding to 0.00, 7, 19.49, 54.35, 152.23, and 426.24 g prothioconazole/ha and 0.00, 7.12, 19.82, 55.27, 154.83, and 433.52 g azoxystrobin/ha, respectively. Since analytical verification of test concentrations confirmed that measured concentrations of all treatments were with  $\pm 20\%$  of nominal concentrations, biological endpoints are reported based on nominal application rates of CA3642.

The 21-day LR<sub>50</sub> (mortality) values for all tested species, were estimated to be  $>2.80$  L product/ha, equivalent to  $>426.24$  g prothioconazole/ha and  $>433.52$  g azoxystrobin/ha, respectively.

The NOER values, for seedling emergence, mortality, shoot height, and shoot dry weight, for all tested species, were all estimated to be  $\geq 2.80$  L product/ha, equivalent to  $\geq 426.24$  g prothioconazole/ha and  $\geq 433.52$  g azoxystrobin/ha, respectively (based on nominal concentrations and GLP analysed active substance content of the formulation).

The 21-day ER<sub>50</sub> values, for seedling emergence, shoot height, and shoot dry weight, of all tested species, were all estimated to be >2.80 L product/ha, corresponding to >426.24 g prothioconazole/ha and >433.52 g azoxystrobin/ha, respectively (based on nominal concentrations and GLP analysed active substance content of the formulation).

This study satisfies the OECD 208 (2006) test-guideline requirements for a study on seedling emergence with non-target terrestrial plants and is considered acceptable.

## Materials and methods

### Test materials

#### Test item

Name:	CA3642 (Prothioconazole 150 g/L + Azoxystrobin 150 g/L SC)
Density:	1.1004 g/mL
Formulation type:	Suspension Concentrate (SC)
Batch no.:	A20026
Active substance (a.s.) 1:	Azoxystrobin 154.83 g/L or 14.07 w/w (analysed), 150 g/L (nominal)
Active substance (a.s.) 2:	Prothioconazole 152.23 g/L or 13.84% w/w (analysed), 150 g/L (nominal)
Appearance:	Off-white, odourless suspension
Expiry date of lot/batch:	September 2022
Storage conditions:	Keep at room temperature in a well-ventilated place

#### Test organisms

Species/source:	Cucumber ( <i>Cucumis sativus</i> )/Batlle Oilseed rape ( <i>Brassica napus</i> )/KWS Carrot ( <i>Daucus carota</i> )/Sakata Tomato ( <i>Lycopersicon esculentum</i> )/Monsanto Holland BV Beet ( <i>Beta vulgaris</i> )/Strube Soybean ( <i>Glycine max</i> )/Saatbau Corn ( <i>Zea mays</i> )/Tozer Seeds Ryegrass ( <i>Lolium perenne</i> )/Herbiseed Common oat ( <i>Avena sativa</i> )/Intersemillas Onion ( <i>Allium cepa</i> )/Batlle
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#### Test medium:

Soil type:	Artificial soil (sandy loam) Soil texture: 4.00% silt, 14.72% clay, 81.28% sand, and 1.26% organic carbon
pH:	7.26
Conductivity:	0.561 mS/cm

#### Test conditions

Test temperature:	12.0°C-32.0°C (actual: 17.9°C – 35.1°C)
Relative humidity:	45% - 95% (actual: 38.0% – 92.0%)
Photoperiod:	16 hours' light:8 hours' dark
Light intensity:	≥300 µE/m <sup>2</sup> /s (actual: 545 - 943 µE/m <sup>2</sup> /s)

Untreated seeds of ten higher terrestrial plant species were sown in sandy loam soil, in 1.5-L, non-porous, plastic plant pots (15.1-cm diameter), which were filled with the test soil. Four seeds were sown per replicate pot (5 replicate pots per treatment) for *Lolium perenne*, *Avena sativa* and *Allium cepa*; two seeds were sown per replicate pot (10 replicate pots per treatment) for the remaining test species..

The study was conducted as a rate-response test, with six treatment groups: a tap water control group and

five test-item treatment groups of 0.046, 0.128, 0.357, 1.00, and 2.80 L product/ha, corresponding to 7, 19.49, 54.35, 152.23, and 426.24 g prothioconazole/ha and 7.12, 19.82, 55.27, 154.83, and 433.52 g azoxystrobin/ha, , respectively.

The product treatment solutions were prepared as close as practically possible to the time of spray application. Tap water was used as a solvent and the highest treatment solution served as the stock solution, with all lower treatment rates diluted in water. Treatments were applied by spraying the soil surface in the appropriate pots with the corresponding spray mixture at a spray volume of 200 L/ha (with an accuracy of  $\pm 10\%$  per ha) from the control (tap water) followed by treatments from the lowest to the highest application rate.

The replicate pots were then arranged, according to treatment group, randomly in a glasshouse and were repositioned on the first and second assessment days (7 and 14 days after treatment). The plant pots were watered from beneath with a controlled and regularly replenished water supply. Temperature and relative humidity were monitored continuously, and light conditions were automatically regulated.

### *Assessments*

The number of emerged seeds was assessed daily, until no more seeds emerged; after 50% of the seedlings in the control group emerged, the assessment of survival/mortality, phytotoxicity, and BBCH growth stage began and lasted 21 days. Survival/mortality, phytotoxicity, and BBCH growth stage were assessed 7, 14, and 21 days after treatment application. Phytotoxicity was assessed visually, by observing morphology, specifically chlorosis, necrosis, and other characteristics. Shoot height was measured on day 21, by measuring the length of the above-ground vegetation of each surviving plant, from the soil surface to the apical tip (oilseed rape, carrot, sugar beet, maize, ryegrass, oat, and onion) or highest aerial part (cucumber, tomato, and soybean). Shoot dry weight was recorded on day 21, by clipping each plant at its soil surface, drying it at 60°C, and weighing the dried plant.

Analytical verification of the stock product spray solution was conducted during the analytical phase of the study. Two samples (each 10 mL), one for the analytical rate verification (A) and one retain sample (R) were taken from the stock solution. Specimens were put in flasks, sealed tightly, labelled correspondingly, and placed into dry ice. A fortified solution at the same rate as the stock solution was prepared on the first application day by weighing 1.5425 g of CA3642 (azoxystrobin 150 g/L + prothioconazole 150 g/L SC) and adjusting the volume to 100 mL with water, two samples, one for the analytical verification (A) and one retain sample (R) (each 10 mL), were taken. Samples were put in flasks, sealed tightly, labelled and stored in dry ice.

### *Data analysis*

Data were statistically evaluated with the software ToxRat Solutions (ToxRat® Professional Version 3.3.0.). Data for mortality, shoot height, and shoot dry weight were statistically evaluated, and significant differences, relative to the control, were determined using  $\alpha = 0.05$  for all tests. Phytotoxicity and the BBCH growth stages were qualitatively tabulated for each species and were not analysed statistically.

Quantal data were analysed with a Fisher's exact binomial test, with Bonferroni corrections, while metric data was assessed for normality and homoscedasticity using Shapiro-Wilk's tests and Levene's test, respectively, before performing the appropriate statistical test. Metric data were analysed with a William's test ( $\alpha = 0.05$ ) or Dunnett's test ( $\alpha = 0.05$ ), when the data were normally distributed and had homogeneity of variance. A multiple sequentially rejective Welch t-test, with Bonferroni-Holm corrections ( $\alpha=0.05$ ), was used when data were normally distributed, but did not have homogeneity of variance. Multiple sequentially rejective U-tests, with Bonferroni-Holm corrections, or multiple sequentially rejective median (2x2 table) tests, with Bonferroni-Holm corrections, was performed, when data were not normally distributed.  $ER_x$  and  $LR_x$  values were estimated, rather than calculated, due to a lack of a rate-response curve.

## Results

### Analytical results

The HPLC -MS/MS method for the determination of prothioconazole and azoxystrobin in spray solution (tap water) was validated with regards to specificity, linearity, accuracy and precision in accordance with guideline SANTE/2020/12830 rev.1 24/02/2021.

Specificity in tap water was demonstrated by the absence of significant interference above 30% of the LOQ in control and blank samples. The linearity of the method was demonstrated using solvent calibration standards. The analytical method was shown to be linear ( $r > 0.99$ ) over the range from 10 to 100 ng/mL for both prothioconazole and azoxystrobin. Accuracy was confirmed with recovery of spiked samples at relevant concentrations of test item in tap water (1560 (LOQ) and 20350 mg test item/L). Mean recoveries were 89 and 103% for Azoxystrobin at 1560 and 20350 mg test item/L respectively (i.e., within 70-110%). Mean recoveries were 99% and 101% for prothioconazole at 1560 and 23500 mg test item/L respectively (i.e., within 70-110%). Precision was confirmed. For Azoxystrobin, the relative standard deviation ( $n = 5$  for each fortification level) was 17% and 1% at 1560 and 23500 mg test item/L respectively (i.e., within the guideline limit of  $\leq 20\%$ ). For Prothioconazole, the relative standard deviation ( $n = 5$  for each fortification level) was 4% and 2% at 1560 and 23500 mg test item/L respectively (i.e., within the guideline limit of  $\leq 20\%$ ).

The limit of quantification (LOQ) for tap water was 1560 mg test item/L (corresponding to 219 mg azoxystrobin/L and 216 mg prothioconazole/L). The limit of detection (LOD) was defined at 40 mg/L for both analytes.

**Table CP 10.6.2/02-01: Summary of the analytical results in the spray solutions.**

Substance analysed	Concentration (mg/L)		Recovery (% nominal)
	Nominal	Analysed	
Azoxystrobin	2168	2196	101
Prothioconazole	2132	2131	100

The analytical recoveries were all within  $\pm 20\%$  of nominal concentrations, therefore, results and endpoints are based on nominal concentrations or nominal rates.

### Biological results

#### *Seedling emergence*

The effects of CA3642 on seedling emergence, after 21 days of exposure, are presented in the table below.

**Table CP 10.6.2/02-02: The effects of CA3642 on the seedling emergence of 10 plant species, after 21 days of exposure.**

Treatment		<i>C. sativus</i>			<i>G. max</i>		
ID	L product/ha	No.	Mean $\pm$ SD (%)	CV (%)	No.	Mean $\pm$ SD (%)	CV (%)
C	0.00	17	85.00 $\pm$ 33.75	39.70	19	95.00 $\pm$ 15.81	16.64
T1	0.046	20	100.00 $\pm$ 0.00	0.00	19	95.00 $\pm$ 15.81	16.64
T2	0.128	20	100.00 $\pm$ 0.00	0.00	20	100.00 $\pm$ 0.00	0.00
T3	0.357	20	100.00 $\pm$ 0.00	0.00	20	100.00 $\pm$ 0.00	0.00
T4	1.00	19	95.00 $\pm$ 15.81	16.64	20	100.00 $\pm$ 0.00	0.00
T5	2.80	19	95.00 $\pm$ 15.81	16.64	18	90.00 $\pm$ 21.08	23.42
Treatment		<i>B. napus</i>			<i>Z. mays</i>		
ID	L product/ha	No.	Mean $\pm$ SD (%)	CV (%)	No.	Mean $\pm$ SD (%)	CV (%)
C	0.00	19	95.00 $\pm$ 15.81	16.64	20	100.00 $\pm$ 0.00	0.00
T1	0.046	20	100.00 $\pm$ 0.00	0.00	20	100.00 $\pm$ 0.00	0.00
T2	0.128	18	90.00 $\pm$ 21.08	23.42	20	100.00 $\pm$ 0.00	0.00

T3	0.357	18	90.00 ± 31.62	35.14	20	100.00 ± 0.00	0.00
T4	1.00	17	85.00 ± 24.15	28.41	19	95.00 ± 15.81	16.64
T5	2.80	19	95.00 ± 15.81	16.64	19	95.00 ± 15.81	16.64
<b>Treatment</b>		<b><i>D. carota</i></b>			<b><i>L. perenne</i></b>		
<b>ID</b>	<b>L product/ha</b>	<b>No.</b>	<b>Mean ± SD (%)</b>	<b>CV (%)</b>	<b>No.</b>	<b>Mean ± SD (%)</b>	<b>CV (%)</b>
C	0.00	17	85.00 ± 24.15	28.41	18	90.00 ± 13.69	15.21
T1	0.046	16	80.00 ± 25.82	32.27	19	90.00 ± 22.36	24.85
T2	0.128	17	85.00 ± 24.15	28.41	18	90.00 ± 22.36	24.85
T3	0.357	18	90.00 ± 21.08	23.42	16	80.00 ± 20.92	26.15
T4	1.00	17	85.00 ± 24.15	28.41	18	90.00 ± 22.36	24.85
T5	2.80	17	85.00 ± 24.15	28.41	19	95.00 ± 11.18	11.77
<b>Treatment</b>		<b><i>L. esculentum</i></b>			<b><i>A. sativa</i></b>		
<b>ID</b>	<b>L product/ha</b>	<b>No.</b>	<b>Mean ± SD (%)</b>	<b>CV (%)</b>	<b>No.</b>	<b>Mean ± SD (%)</b>	<b>CV (%)</b>
C	0.00	20	100.00 ± 0.00	0.00	18	90.00 ± 13.69	15.21
T1	0.046	20	100.00 ± 0.00	0.00	20	100.00 ± 0.00	0.00
T2	0.128	20	100.00 ± 0.00	0.00	19	95.00 ± 11.18	11.77
T3	0.357	20	100.00 ± 0.00	0.00	20	100.00 ± 0.00	0.00
T4	1.00	20	100.00 ± 0.00	0.00	18	90.00 ± 13.69	15.21
T5	2.80	20	100.00 ± 0.00	0.00	19	95.00 ± 11.18	11.77
<b>Treatment</b>		<b><i>B. vulgaris</i></b>			<b><i>A. cepa</i></b>		
<b>ID</b>	<b>L product/ha</b>	<b>No.</b>	<b>Mean ± SD (%)</b>	<b>CV (%)</b>	<b>No.</b>	<b>Mean ± SD (%)</b>	<b>CV (%)</b>
C	0.00	18	90.00 ± 21.08	23.42	15	75.00 ± 17.68	23.57
T1	0.046	19	95.00 ± 15.81	16.64	15	75.00 ± 17.68	23.57
T2	0.128	20	100.00 ± 0.00	0.00	16	80.00 ± 20.92	26.15
T3	0.357	20	100.00 ± 0.00	0.00	15	75.00 ± 17.68	23.57
T4	1.00	20	100.00 ± 0.00	0.00	11	55.00 ± 20.92	38.03
T5	2.80	20	100.00 ± 0.00	0.00	14	70.00 ± 11.18	15.97

No.: number; SD: standard deviation; CV: coefficient of variation.

No statistically significant differences in seedling emergence, relative to the control, were observed for all tested plant species. Therefore, the 21-day ER<sub>50</sub> value, for seedling emergence, was estimated to be >2.80 L product/ha (equivalent to >426.24 g prothioconazole/ha and >433.52 g azoxystrobin/ha, respectively). The corresponding 21-day NOER (seedling emergence) value was estimated to be ≥2.80 L product/ha (equivalent to ≥426.24 g prothioconazole/ha and ≥433.52 g azoxystrobin/ha, respectively).

### Survival and mortality

The effects of CA3642 on survival and mortality, after 21 days of exposure, is presented in the table below.

**Table CP 10.6.2/02-03: The effects of CA3642 on the mortality of 10 plant species, after 21 days of exposure.**

<b>Treatment</b>		<b><i>C. sativus</i></b>			<b><i>G. max</i></b>		
<b>ID</b>	<b>L product/ha</b>	<b>No.</b>	<b>Mean ± SD (%)</b>	<b>CV (%)</b>	<b>No.</b>	<b>Mean ± SD (%)</b>	<b>CV (%)</b>
C	0.00	0	0.00 ± 0.00	-	0	0.00 ± 0.00	-
T1	0.046	0	0.00 ± 0.00	-	1	5.00 ± 15.81	316.23
T2	0.128	0	0.00 ± 0.00	-	1	5.00 ± 15.81	316.23
T3	0.357	0	0.00 ± 0.00	-	0	0.00 ± 0.00	-
T4	1.00	0	0.00 ± 0.00	-	0	0.00 ± 0.00	-
T5	2.80	0	0.00 ± 0.00	-	1	5.00 ± 15.81	316.23
<b>Treatment</b>		<b><i>B. napus</i></b>			<b><i>Z. mays</i></b>		
<b>ID</b>	<b>L product/ha</b>	<b>No.</b>	<b>Mean ± SD (%)</b>	<b>CV (%)</b>	<b>No.</b>	<b>Mean ± SD (%)</b>	<b>CV (%)</b>
C	0.00	0	0.00 ± 0.00	-	0	0.00 ± 0.00	-
T1	0.046	0	0.00 ± 0.00	-	0	0.00 ± 0.00	-
T2	0.128	0	0.00 ± 0.00	-	0	0.00 ± 0.00	-
T3	0.357	0	0.00 ± 0.00	-	0	0.00 ± 0.00	-



T4	1.00	0	0.00 ± 0.00	-	0	0.00 ± 0.00	-
T5	2.80	0	0.00 ± 0.00	-	0	0.00 ± 0.00	-
<b>Treatment</b>		<b><i>D. carota</i></b>			<b><i>L. perenne</i></b>		
<b>ID</b>	<b>L product/ha</b>	<b>No.</b>	<b>Mean ± SD (%)</b>	<b>CV (%)</b>	<b>No.</b>	<b>Mean ± SD (%)</b>	<b>CV (%)</b>
C	0.00	0	0.00 ± 0.00	-	1	6.67 ± 14.91	223.61
T1	0.046	0	0.00 ± 0.00	-	0	0.00 ± 0.00	-
T2	0.128	0	0.00 ± 0.00	-	0	0.00 ± 0.00	-
T3	0.357	0	0.00 ± 0.00	-	0	0.00 ± 0.00	-
T4	1.00	0	0.00 ± 0.00	-	0	0.00 ± 0.00	-
T5	2.80	0	0.00 ± 0.00	-	0	0.00 ± 0.00	-
<b>Treatment</b>		<b><i>L. esculentum</i></b>			<b><i>A. sativa</i></b>		
<b>ID</b>	<b>L product/ha</b>	<b>No.</b>	<b>Mean ± SD (%)</b>	<b>CV (%)</b>	<b>No.</b>	<b>Mean ± SD (%)</b>	<b>CV (%)</b>
C	0.00	1	5.00 ± 15.81	316.23	0	0.00 ± 0.00	-
T1	0.046	0	0.00 ± 0.00	-	0	0.00 ± 0.00	-
T2	0.128	1	5.00 ± 15.81	316.23	0	0.00 ± 0.00	-
T3	0.357	0	0.00 ± 0.00	-	1	5.00 ± 11.18	223.61
T4	1.00	0	0.00 ± 0.00	-	0	0.00 ± 0.00	-
T5	2.80	0	0.00 ± 0.00	-	0	0.00 ± 0.00	-
<b>Treatment</b>		<b><i>B. vulgaris</i></b>			<b><i>A. cepa</i></b>		
<b>ID</b>	<b>L product/ha</b>	<b>No.</b>	<b>Mean ± SD (%)</b>	<b>CV (%)</b>	<b>No.</b>	<b>Mean ± SD (%)</b>	<b>CV (%)</b>
C	0.00	0	0.00 ± 0.00	-	0	0.00 ± 0.00	-
T1	0.046	0	0.00 ± 0.00	-	0	0.00 ± 0.00	-
T2	0.128	0	0.00 ± 0.00	-	0	0.00 ± 0.00	-
T3	0.357	0	0.00 ± 0.00	-	1	6.67 ± 14.91	223.61
T4	1.00	0	0.00 ± 0.00	-	0	0.00 ± 0.00	-
T5	2.80	0	0.00 ± 0.00	-	0	0.00 ± 0.00	-

No.: number; SD: standard deviation; CV: coefficient of variation.

No statistically significant changes to mortality, relative to the control, were observed, for all tested plant species. Therefore, the 21-day LR<sub>50</sub> (mortality) value was estimated to be >2.80 L product/ha (equivalent to >426.24 g prothioconazole/ha and >433.52 g azoxystrobin/ha, respectively). The corresponding 21-day NOER (mortality) value was estimated to be ≥2.80 L product/ha (equivalent to ≥426.24 g prothioconazole/ha and ≥433.52 g azoxystrobin/ha, respectively).

#### Shoot height and shoot dry weight

A summary of the effects of CA3642 on shoot height and dry shoot weight, after 21 days of exposure, is presented in tables below.

**Table CP 10.6.2/02-04: The effects of CA3642 on shoot height of 10 plant species, after 21 days of exposure.**

<b>Treatment</b>		<b><i>C. sativus</i></b>			<b><i>G. max</i></b>		
<b>ID</b>	<b>L product/ha</b>	<b>Mean ± SD (cm)</b>	<b>CV (%)</b>	<b>Inhibition (% control)</b>	<b>Mean ± SD (cm)</b>	<b>CV (%)</b>	<b>Inhibition (% control)</b>
C	0.00	92.06 ± 8.27	8.98	0.00	51.55 ± 4.56	8.85	0.00
T1	0.046	86.15 ± 12.12	14.06	6.42	55.75 ± 6.19	11.11	-8.15
T2	0.128	82.4 ± 13.94	16.92	10.49	53.5 ± 3.25	6.07	-3.78
T3	0.357	<b>74.6 ± 14.96*</b>	20.05	18.97	49.18 ± 3.08	6.27	4.60
T4	1.00	<b>71.2 ± 10.27*</b>	14.42	22.66	53.2 ± 4.7	8.84	-3.20
T5	2.80	83.6 ± 6.19	7.40	9.19	52.83 ± 4.17	7.89	-2.48
<b>Treatment</b>		<b><i>B. napus</i></b>			<b><i>Z. mays</i></b>		
<b>ID</b>	<b>L product/ha</b>	<b>Mean ± SD (cm)</b>	<b>CV (%)</b>	<b>Inhibition (% control)</b>	<b>Mean ± SD (cm)</b>	<b>CV (%)</b>	<b>Inhibition (% control)</b>
C	0.00	35.18 ± 3.84	10.92	0.00	127.45 ± 5.29	4.15	0.00
T1	0.046	35.75 ± 2.95	8.24	-1.62	126.1 ± 6.85	5.43	1.06
T2	0.128	34.65 ± 2.4	6.94	1.51	<b>119.4 ± 9.68*</b>	8.11	6.32
T3	0.357	34.67 ± 3.12	9.01	1.45	121.8 ± 4.37	3.59	4.43
T4	1.00	38.18 ± 2.32	6.08	-8.53	123 ± 6.21	5.05	3.49

T5	2.80	35.88 ± 2.77	7.73	-1.99	122.5 ± 5.56	4.54	3.88
<b>Treatment</b>		<b><i>D. carota</i></b>			<b><i>L. perenne</i></b>		
<b>ID</b>	<b>L product/ha</b>	<b>Mean ± SD (cm)</b>	<b>CV (%)</b>	<b>Inhibition (%) control)</b>	<b>Mean ± SD (cm)</b>	<b>CV (%)</b>	<b>Inhibition (%) control)</b>
C	0.00	16.8 ± 5.54	33.00	0.00	17.95 ± 2.03	11.33	0.00
T1	0.046	15.2 ± 2.85	18.75	9.52	20.33 ± 1.97	9.71	-13.26
T2	0.128	16.8 ± 4.07	24.23	0.00	16.1 ± 3.78	23.46	10.31
T3	0.357	16.65 ± 1.38	8.26	0.89	19.27 ± 2.65	13.74	-7.35
T4	1.00	15.6 ± 2.46	15.76	7.14	19.2 ± 2.14	11.14	-6.96
T5	2.80	17.35 ± 2.59	14.95	-3.27	18.77 ± 2.28	12.15	-4.57
<b>Treatment</b>		<b><i>L. esculentum</i></b>			<b><i>A. sativa</i></b>		
<b>ID</b>	<b>L product/ha</b>	<b>Mean ± SD (cm)</b>	<b>CV (%)</b>	<b>Inhibition (%) control)</b>	<b>Mean ± SD (cm)</b>	<b>CV (%)</b>	<b>Inhibition (%) control)</b>
C	0.00	28.6 ± 2.34	8.19	0.00	33.78 ± 2.51	7.42	0.00
T1	0.046	30.6 ± 2.09	6.84	-6.99	34.1 ± 3.42	10.02	-0.95
T2	0.128	31.7 ± 2.75	8.68	-10.84	<b>28.02 ± 2.63*</b>	9.38	17.05
T3	0.357	28.05 ± 2.45	8.75	1.92	30.17 ± 1.99	6.60	10.69
T4	1.00	31.65 ± 2.4	7.60	-10.66	31.35 ± 3.13	9.99	7.19
T5	2.80	29.55 ± 2.67	9.04	-3.32	30.68 ± 3.57	11.63	9.18
<b>Treatment</b>		<b><i>B. vulgaris</i></b>			<b><i>A. cepa</i></b>		
<b>ID</b>	<b>L product/ha</b>	<b>Mean ± SD (cm)</b>	<b>CV (%)</b>	<b>Inhibition (%) control)</b>	<b>Mean ± SD (cm)</b>	<b>CV (%)</b>	<b>Inhibition (%) control)</b>
C	0.00	26.23 ± 1.91	7.28	0.00	21.18 ± 2.17	10.24	0.00
T1	0.046	24.98 ± 2.06	8.23	4.77	20.88 ± 2.14	10.24	1.42
T2	0.128	25.2 ± 1.49	5.93	3.93	22.7 ± 1.33	5.85	-7.18
T3	0.357	<b>24.08 ± 1.92*</b>	7.98	8.20	21.55 ± 1.91	8.84	-1.75
T4	1.00	25.8 ± 1.65	6.41	1.64	23.6 ± 2.17	9.22	-11.43
T5	2.80	24.55 ± 2.05	8.34	6.40	23.03 ± 1.69	7.35	-8.73

\*: Significant differences in shoot height, relative to the control (in **bold**).

Positive and negative inhibition values indicate a decrease and increase in shoot height, respectively, relative to the control. No.: number; SD: standard deviation; CV: coefficient of variation.

Statistically significant differences in mean shoot height, relative to the control, were observed 21 days after treatment with CA3642, in *Cucumis sativus* (treatments T3 and T4), *Beta vulgaris* (treatment T3), and *Zea mays* (treatment T2). The lack of a dose-related response in these species is considered likely attributed to natural biological variability, as no statistically significant reductions were observed with the highest product treatment group. Consequently, the NOER (mean shoot height) value was estimated to be ≥2.80 L product/ha, equivalent to ≥426.24 g prothioconazole/ha and ≥433.52 g azoxystrobin/ha, respectively. The ER<sub>10</sub>, ER<sub>20</sub>, ER<sub>25</sub>, and ER<sub>50</sub> values for mean shoot height were estimated to be >2.80 L test item/ha, equivalent to >426.24 g prothioconazole/ha and >433.52 g azoxystrobin/ha, respectively, for all the test species.

**Table CP 10.6.2/02-05: The effects of CA3642 on shoot dry weight of 10 plant species, after 21 days of exposure.**

<b>Treatment</b>		<b><i>C. sativus</i></b>			<b><i>G. max</i></b>		
<b>ID</b>	<b>L product/ha</b>	<b>Mean ± SD (g)</b>	<b>CV (%)</b>	<b>Inhibition (%) control)</b>	<b>Mean ± SD (g)</b>	<b>CV (%)</b>	<b>Inhibition (%) control)</b>
C	0.00	9.26 ± 2.54	2.54	0.00	8.45 ± 0.93	0.93	0.00
T1	0.046	9.39 ± 0.89	0.89	-1.40	7.88 ± 1.37	1.37	6.75
T2	0.128	7.98 ± 1.45	1.45	13.82	7.48 ± 1.08	1.08	11.48
T3	0.357	8.64 ± 1.87	1.87	6.70	7.8 ± 0.71	0.71	7.69
T4	1.00	8.55 ± 2.55	2.55	7.67	<b>6.9 ± 1.01*</b>	1.01	18.34
T5	2.80	8.35 ± 1.51	1.51	9.83	7.73 ± 1.15	1.15	8.52
<b>Treatment</b>		<b><i>B. napus</i></b>			<b><i>Z. mays</i></b>		
<b>ID</b>	<b>L product/ha</b>	<b>Mean ± SD (g)</b>	<b>CV (%)</b>	<b>Inhibition (%) control)</b>	<b>Mean ± SD (g)</b>	<b>CV (%)</b>	<b>Inhibition (%) control)</b>
C	0.00	5.53 ± 1.6	1.60	0.00	10.33 ± 0.69	0.69	0.00
T1	0.046	5.7 ± 0.74	0.74	-3.07	10.91 ± 1.27	1.27	-5.61
T2	0.128	6.61 ± 2.89	2.89	-19.53	9.88 ± 2.9	2.90	4.36
T3	0.357	4.66 ± 0.73	0.73	15.73	9.7 ± 1.07	1.07	6.10
T4	1.00	5.02 ± 0.79	0.79	9.22	<b>8.59 ± 1.18*</b>	1.18	16.84

\*: Significant differences in shoot height, relative to the control (in **bold**). Positive and negative inhibition values indicate a decrease and increase in shoot height, respectively, relative to the control. No.: number; SD.: standard deviation; CV: coefficient of variation.

### Phytotoxicity

A summary of the phytotoxic effects of CA3642 after 21 days of exposure, is presented in table below.

Treatment		Stunted	Chlorosis	Necrosis	Leaf/stem deformities	Wilting	Rolled leaves	Dead	Stunted	Chlorosis	Necrosis	Leaf/stem deformities	Wilting	Rolled leaves	Dead
ID	L product/ha	<i>Cucumis sativus</i>							<i>Glycine max</i>						
C	0.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T1	0.046	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T2	0.128	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T3	0.357	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T4	1.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T5	2.8	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Treatment		<i>Brassica napus</i>							<i>Zea mays</i>						
C	0.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

T1	0.046	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T2	0.128	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T3	0.357	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T4	1.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T5	2.8	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<b>Treatment</b>		<b><i>Daucus carota (carrot)</i></b>							<b><i>Lolium perenne</i></b>						
C	0.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T1	0.046	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T2	0.128	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T3	0.357	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T4	1.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T5	2.8	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<b>Treatment</b>		<b><i>Lycopersicon esculentum</i></b>							<b><i>Avena sativa</i></b>						
C	0.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T1	0.046	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T2	0.128	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T3	0.357	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T4	1.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T5	2.8	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<b>Treatment</b>		<b><i>Beta vulgaris</i></b>							<b><i>Allium cepa</i></b>						
C	0.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T1	0.046	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T2	0.128	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T3	0.357	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T4	1.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T5	2.8	0	0	0	0	0	0	0	0	0	0	0	0	0	0

\*: Statistically significant differences, relative to the control group, ( $P < 0.05$ ).

C: control; Tn: test-item treatment code.

No statistically significant phytotoxic effects, relative to the control, were observed for all tested plant species. Consequently, the NOER value was estimated to be  $\geq 2.80$  L CA3642/ha, equivalent to  $\geq 426.24$  g prothioconazole/ha and  $\geq 433.52$  g azoxystrobin/ha, respectively. The ER<sub>10</sub>, ER<sub>20</sub>, ER<sub>25</sub>, and ER<sub>50</sub> values for mean shoot dry weight were estimated to be  $> 2.80$  L product/ha, equivalent to  $> 426.24$  g prothioconazole/ha and  $> 433.52$  g azoxystrobin/ha, respectively, for all the species tested.

## Validity

All validity criteria were met in accordance with the OECD 208 (2006) test guideline:

- **Seedling emergence:** The control seedling emergence was  $\geq 70.0\%$  (actual values: between 75.0% and 100.0%).
- **Phytotoxicity:** The control seedlings of each species did not exhibit visible phytotoxic effects and control plants exhibited only normal variation in growth and morphology for that particular species.
- **Mean Survival:** The mean survival of emerged control seedlings was  $\geq 90.0\%$  (actual values: between 93.33% and 100.0%).
- **Cultivation Conditions:** The environmental conditions for each particular species were identical and growing media contained the same amount of soil matrix, support media, or substrate from the same source.

## Conclusion

A 21-day laboratory rate-response test was conducted to assess the effects of CA3642 on seedling emergence of ten non-target terrestrial plant species, according to OECD test guidelines 208 (2006).

The 21-day ER<sub>50</sub> (seedling emergence, mean shoot height, and mean shoot dry weight) value was:  $> 2.80$  L product/ha, equivalent to  $> 426.24$  g prothioconazole/ha and  $> 433.52$  g azoxystrobin/ha, respectively, based on nominal concentrations. for all the species tested

This study is considered acceptable and valid.

## A 2.8 KCP 10.8 Monitoring data