

REGISTRATION REPORT
Part B
Section 9
Ecotoxicology
Detailed summary of the risk assessment

Product code: CA3301
Product name(s): **JOUST 250 EC**
Chemical active substance:
Prothioconazole, 250 g/L

Central Zone
Zonal Rapporteur Member State: Poland

CORE ASSESSMENT
New Authorisation (Art.33)

Applicant: NUFARM Polska Sp. z o. o.
Submission date: 23/12/2021
MS Finalisation date: 08/2022

Version history

When	What
December 2021	First submission
August 2022	zRMS finalized dRR evaluation

Table of Contents

9	Ecotoxicology (KCP 10).....	6
9.1	Critical GAP and overall conclusions.....	8
9.1.1	Overall conclusions.....	14
9.1.1.1	Effects on birds (KCP 10.1.1), Effects on terrestrial vertebrates other than birds (KCP 10.1.2), and Effects on other terrestrial vertebrate wildlife (reptiles and amphibians) (KCP 10.1.3)	14
9.1.1.2	Effects on aquatic organisms (KCP 10.2).....	14
9.1.1.3	Effects on bees (KCP 10.3.1).....	15
9.1.1.4	Effects on arthropods other than bees (KCP 10.3.2)	15
9.1.1.5	Effects on non-target soil meso- and macrofauna (KCP 10.4), Effects on soil microbial activity (KCP 10.5)	16
9.1.1.6	Effects on non-target terrestrial plants (KCP 10.6)	16
9.1.1.7	Effects on other terrestrial organisms (flora and fauna) (KCP 10.7)	16
9.1.2	Grouping of intended uses for risk assessment.....	16
9.1.3	Consideration of metabolites	17
9.2	Effects on birds (KCP 10.1.1).....	18
9.2.1	Toxicity data	18
9.2.1.1	Justification for new endpoints	19
9.2.2	Risk assessment for spray applications.....	19
9.2.2.1	First-tier assessment (screening/generic focal species)	19
9.2.2.2	Higher-tier risk assessment.....	23
9.2.2.3	Drinking water exposure.....	23
9.2.2.4	Effects of secondary poisoning.....	24
9.2.2.5	Biomagnification in terrestrial food chains.....	27
9.2.3	Risk assessment for baits, pellets, granules, prills, or treated seed.....	27
9.2.4	Overall conclusions.....	27
9.3	Effects on terrestrial vertebrates other than birds (KCP 10.1.2).....	28
9.3.1	Toxicity data	28
9.3.1.1	Justification for new endpoints	29
9.3.2	Risk assessment for spray applications.....	29
9.3.2.1	First-tier assessment (screening/generic focal species)	29
9.3.2.2	Higher-tier risk assessment.....	31
9.3.2.3	Drinking water exposure.....	33
9.3.2.4	Effects of secondary poisoning.....	33
9.3.2.5	Biomagnification in terrestrial food chains.....	36
9.3.3	Risk assessment for baits, pellets, granules, prills or treated seed.....	36
9.3.4	Overall conclusions.....	36
9.4	Effects on other terrestrial vertebrate wildlife (reptiles and amphibians) (KCP 10.1.3)	37
9.5	Effects on aquatic organisms (KCP 10.2).....	37
9.5.1	Toxicity data	37
9.5.1.1	Justification for new endpoints	39
9.5.2	Risk assessment	40
9.5.3	Overall conclusions.....	58
9.6	Effects on bees (KCP 10.3.1).....	61
9.6.1	Toxicity data	61
9.6.1.1	Justification for new endpoints	62

9.6.2	Risk assessment	63
9.6.2.1	Hazard quotients and risk assessments for bees	63
9.6.2.2	Higher-tier risk assessment for bees (tunnel test, field studies).....	66
9.6.3	Effects on bumble bees	66
9.6.4	Effects on solitary bees	66
9.6.5	Overall conclusions.....	67
9.7	Effects on arthropods other than bees (KCP 10.3.2)	67
9.7.1	Toxicity data	67
9.7.1.1	Justification for new endpoints	69
9.7.2	Risk assessment	70
9.7.2.1	Risk assessment for in-field exposure.....	70
9.7.2.2	Risk assessment for off-field exposure	70
9.7.2.3	Additional higher-tier risk assessment.....	71
9.7.2.4	Risk-mitigation measures.....	72
9.7.3	Overall conclusions.....	72
9.8	Effects on non-target soil meso- and macrofauna (KCP 10.4)	72
9.8.1	Toxicity data	72
9.8.1.1	Justification for new endpoints	75
9.8.2	Risk assessment	75
9.8.2.1	First-tier risk assessment.....	75
9.8.2.2	Higher-tier risk assessment	76
9.8.3	Overall conclusions.....	76
9.9	Effects on soil microbial activity (KCP 10.5).....	76
9.9.1	Toxicity data	76
9.9.1.1	Justification for new endpoints	77
9.9.2	Risk assessment	77
9.9.3	Overall conclusions.....	78
9.10	Effects on non-target terrestrial plants (KCP 10.6)	78
9.10.1	Toxicity data	78
9.10.1.1	Justification for new endpoints	79
9.10.2	Risk assessment	79
9.10.2.1	Tier-1 risk assessment (based screening data)	79
9.10.2.2	Tier-2 risk assessment (based on dose-response data).....	80
9.10.2.3	Higher-tier risk assessment.....	81
9.10.2.4	Risk-mitigation measures.....	81
9.10.3	Overall conclusions.....	81
9.11	Effects on other terrestrial organisms (flora and fauna) (KCP 10.7)	81
9.12	Monitoring data (KCP 10.8)	81
9.13	Classification and Labelling	81
Appendix 1	Lists of data considered in support of the evaluation.....	84
Appendix 2	Detailed evaluation of the new studies	90
A 2.1	KCP 10.1 Effects on birds and other terrestrial vertebrates.....	90
A 2.1.1	KCP 10.1.1 Effects on birds	90
A 2.1.2	KCP 10.1.2 Effects on terrestrial vertebrates other than birds	90
A 2.1.3	KCP 10.1.3 Effects on other terrestrial vertebrate wildlife (reptiles and amphibians).....	90
A 2.2	KCP 10.2 Effects on aquatic organisms	90

A 2.2.1	KCP 10.2.1 Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes	90
A 2.2.2	KCP 10.2.2 Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms.....	113
A 2.2.3	KCP 10.2.3 Further testing on aquatic organisms	113
A 2.3	KCP 10.3 Effects on arthropods	113
A 2.3.1	KCP 10.3.1 Effects on bees	113
A 2.4	KCP 10.4 Effects on non-target soil meso- and macrofauna.....	219
A 2.4.1	KCP 10.4.1 Earthworms	219
A 2.4.2	KCP 10.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)	230
A 2.5	KCP 10.5 Effects on soil nitrogen transformation	243
A 2.6	KCP 10.6 Effects on terrestrial non-target higher plants	243
A 2.6.1	KCP 10.6.1 Summary of screening data	243
A 2.6.2	KCP 10.6.2 Testing on non-target plants	243
A 2.6.3	KCP 10.6.3 Extended laboratory studies on non-target plants	264
A 2.7	KCP 10.7 Effects on other terrestrial organisms (flora and fauna).....	264
A 2.8	KCP 10.8 Monitoring data	264

9 Ecotoxicology (KCP 10)

This application is in support of the registration of the new product CA3301, which is an EC formulation containing the active substance prothioconazole (250 g a.s./L), in the Central Zone (Article 33 application). This document reviews the ecotoxicological studies for the product CA3301. Prothioconazole was included to Annex I of Directive 91/414/EEC on 1 August 2008. Prothioconazole is currently undergoing renewal at EU level.

Prothioconazole is a fungicide, which is used to treat winter and spring varieties of cereals and oilseed rape. Prothioconazole is applied once or twice to cereals, as a foliar spray, at up to 200 g a.s./ha. Applications to oilseed rape may occur in autumn and/or spring, at rates of up to 175 g a.s./ha. The proposed GAP for this new product CA3301 includes the same crops (cereals and oilseed rape) and maximum application rates considered at the EU level for the active substance (a.s.) prothioconazole. In addition, two applications to flax and to mustard, cameline, and other seed-producing Brassicaceae, at rates up to 175 g a.s./ha, are included in the Central Zone GAP.

Where appropriate this document refers to the conclusions of the EU review of prothioconazole. 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:
 - *The protection of aquatic organisms. Risk-mitigation measures such, as buffer zones, shall be applied, where appropriate.*
 - *The protection of birds and small mammals. Risk-mitigation measures shall be applied where appropriate.*

These concerns have been addressed within the current submission.

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. 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 substance; existing EU-agreed endpoints, therefore, apply, unless further justification has been provided.

For the implementation of the uniform principles of Annex VI, the conclusions of the review report on prothioconazole (SANCO/3923/07 - final), the Conclusion on the peer review of the pesticide risk assessment of the active substance prothioconazole (EFSA Scientific Report (2007) 106, 1-98) shall be considered.

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 CA3301 can be found in the confidential dossier of this submission (Registration Report - Part C).

Review Comments:

This document describes the acceptable use conditions required for registration of Joust (CA3301), an emulsifiable concentration (EC) formulation containing prothioconazole 250 g/L, for use as a fungicide for controls a number of foliar and ear diseases in cereals and oilseed rape.

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

Since this document is based on the information provided by the applicant, all review comments, additions and corrections have been made using commenting boxes or highlighted in grey. Any incorrect data or text not evaluated by the zRMS has been crossed out.

9.1 Critical GAP and overall conclusions**Table 9.1-1: Table of critical GAPs**

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g., g saf- ener/ syner- gist 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	Soil organisms	Non-target plants
1	CEU SEU NEU	Barley winter	F	Leaf spot of Barley <i>Ramularia collo-cygni</i> (RAMUCC) Eyespot <i>Oculimacula acuformis</i> (PSDCHA) Brown Rust <i>Puccinia hordei</i> (PUCCHD) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Leaf Blotch <i>Rhynchosporium secalis</i> (RHYNSE) Fusarium ear blight <i>Fusarium spp.</i> (FUSASP) Net Blotch <i>Pyrenophora teres</i> (PYRNTE)	Foliar spray	BBCH 30 – 61 (Spring)	a) 1-2 b) 1-2	14 - 21	a) 0.6 b) 1.2	a) 150 b) 300	100-400	35								
2	CEU SEU NEU	Barley spring	F	Leaf spot of Barley <i>Ramularia collo-cygni</i> (RAMUCC) Eyespot <i>Oculimacula acuformis</i> (PSDCHA) 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	a) 1-2 b) 1-2	14 - 21	a) 0.6 b) 1.2	a) 150 b) 300	100-400	35								

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g., g saf- ener/ syner- gist 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	Soil organisms	Non-target plants
3	CEU SEU NEU	Oat (winter & spring)	F	Crown Rust <i>Puccinia coronata</i> (PUCCO) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Eyespot <i>Oculimacula acufomis</i> (PSDCHA)	Foliar spray	BBCH 30 – 61 (Spring)	a) 1-2 b) 1-2	14 - 21	a) 0.6 b) 1.2	a) 150 b) 300	100-400	35								
4	CEU SEU NEU	Wheat (winter & spring) Spelt Einkorn wheat Emmer Wheat Tritordeu m	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 tritici</i> (PUCCRT) Yellow Rust <i>Puccinia striiformis</i> (PUCST) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Eyespot <i>Oculimacula acufomis</i> (PSDCHA) Tan Spot <i>Pyrenophora tritici-repentis</i> (PYRNTR) Fusarium ear blight <i>Fusarium spp.</i> (FUSASP)	Foliar spray	BBCH 30 – 69 (Spring)	a) 1-2 b) 1-2	14 - 21	a) 0.6-0.8 b) 1.2-1.6	a) 150-200 b) 300-400	100-400	35	Risk envelope for uses 1-7							
5	CEU SEU NEU	Durum Wheat	F	Septoria leaf spot <i>Zymoseptoria tritici</i> <i>Mycosphaerella graminicola</i> (SEPTTR) Brown Rust <i>Puccinia recondita</i> <i>Puccinia tritici</i> (PUCCRT)	Foliar spray	BBCH 30 – 69 (Spring)	a) 1-2 b) 1-2	14 - 21	a) 0.6-0.8 b) 1.2-1.6	a) 150-200 b) 300-400	100-400	35								

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g., g saf- ener/ syner- gist 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	Soil organisms	Non-target plants
				Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Fusarium ear blight <i>Fusarium spp.</i> (FUSASP)																
6	CEU SEU NEU	Triticale (winter & spring)	F	Septoria leaf spot <i>Zymoseptoria tritici</i> <i>Mycosphaerella graminicola</i> (SEPTTR) Brown Rust <i>Puccinia recondite</i> <i>Puccinia tritici</i> (PUCCRT) Leaf blotch <i>Rhynchosporium secalis</i> (RHYNSE) Yellow Rust <i>Puccinia striiformis</i> (PUCCST) Glume blotch <i>Stagonospora nodorum</i> (LEPTNO) Powdery mildew <i>Blumeria graminis</i> (ERYSGR) Fusarium ear blight <i>Fusarium spp.</i> (FUSASP)	Foliar spray	BBCH 30 – 69 (Spring)	a) 1-2 b) 1-2	14 - 21	a) 0.6-0.8 b) 1.2-1.6	a) 150-200 b) 300-400	100-400	35								
7	CEU SEU NEU	Rye (winter & spring)	F	Septoria leaf spot <i>Zymoseptoria tritici</i> <i>Mycosphaerella graminicola</i> (SEPTTR) Leaf blotch <i>Rhynchosporium secalis</i> (RHYNSE) Crown Rust <i>Puccinia coronata</i> (PUCCCO) Eyespot <i>Oculimacula acuformis</i> (PSDCHA) Powdery mildew	Foliar spray	BBCH 30 – 69 (Spring)	a) 1-2 b) 1-2	14 - 21	a) 0.6-0.8 b) 1.2-1.6	a) 150-200 b) 300-400	100-400	35								

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g., g saf- ener/ syner- gist 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	Soil organisms	Non-target plants
				<i>Blumeria graminis</i> (ERYSGR)																
8	CEU SEU NEU	Oilseed Rape (winter)	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) and/or BBCH 20 – 69 (Spring)	a) 1-2 b) 1-2	90	a) 0.6-0.7 b) 1.2-1.4	a) 150-175 b) 300-350	100-400	56	Risk envelope for uses 8 and 11							
9	CEU SEU NEU	Oilseed Rape (winter)	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 20 – 69 (Spring)	a) 1-2 b) 1-2	14 - 28	a) 0.6-0.7 b) 1.2-1.4	a) 150-175 b) 300-350	100-400	56	Risk envelope for uses 9, 10 and 12							
10	CEU SEU NEU	Oilseed Rape (spring)	F	Phoma leaf spot/stem canker <i>Leptosphaeria maculans</i> (LEPTMA) Sclerotinia stem rot <i>Sclerotinia sclerotiorum</i> (SCLESC)	Foliar spray	BBCH 20 – 69	a) 1 b) 1	n/a	a) 0.6-0.7 b) 0.6-0.7	a) 150-175 b) 150-175	100-400	56								

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g., g saf- ener/ syner- gist 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	Soil organisms	Non-target plants
				Powdery mildew <i>Erysiphe cruciferarum</i> (ERYSCR) Alternaria leaf spot <i>Alternaria brassicae</i> (ALTEBA) Light leaf spot <i>Pyrenopeziza brassicae</i> (PYRPBR)																
None																				
11	CEU SEU NEU	Mustard, Cameline and other seed- producing Brassicaceae	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) BBCH 20 – 69 (Spring)	a) 1-2 b) 1-2	14 – 28 90	a) 0.6-0.7 b) 1.2-1.4	a) 150-175 b) 300-350	100-400	56								
12	FR, BE	Flax (for fiber production only)	F	Powdery mildew flax <i>Erysiphe spp</i> (ERYSPP)	Foliar spray	BBCH 33- 51	a) 1-2 b) 1-2	14 - 28	a) 0.6-0.7 b) 1.2-1.4	a) 150-175 b) 300-350	100-400	NA								
None																				

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

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

Explanation for column 15 – 21 “Conclusion”

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

Remarks table:	<p>(1) Numeration necessary to allow references</p> <p>(2) Use official codes/nomenclatures of EU</p> <p>(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)</p> <p>(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</p> <p>(5) Scientific names <u>and</u> 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</p> <p>(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</p>	<p>(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</p> <p>(8) The maximum number of application possible under practical conditions of use must be provided</p> <p>(9) Minimum interval (in days) between applications of the same product.</p> <p>(10) For specific uses other specifications might be possible, <i>e.g.</i>: g/m³ in case of fumigation of empty rooms. See also EPPO-Guideline PP 1/239 Dose expression for plant protection products</p> <p>(11) The dimension (g, kg) must be clearly specified. (Maximum) dose of a.s. per treatment (usually g, kg or L product / ha).</p> <p>(12) If water volume range depends on application equipments (<i>e.g.</i>, ULVA or LVA) it should be mentioned under “application: method/kind”.</p> <p>(13) PHI - minimum pre-harvest interval</p> <p>(14) Remarks may include: Extent of use/economic importance/restrictions</p>
-----------------------	---	---

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), and 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 (the active substance in the formulated product CA3301), and maximum residues occurring on food items, following applications according to the use pattern.

The screening acute TER (TER_a) values for birds and mammals are greater than the Commission Regulation (EU) No. 546/2011 trigger of 10, indicating that there are acceptable acute risks to birds and mammals from prothioconazole and its metabolite, prothioconazole-desthio, for the proposed use pattern of the product CA3301 in cereals and oilseed rape.

For birds, long-term/reproductive TER (TER_{lt}) values for prothioconazole and its metabolite prothioconazole-desthio are greater than the Commission Regulation (EU) No. 546/2011 trigger of 5 during the Screening step/Tier 1 risk assessment, indicating that the long-term risk to birds is acceptable according to the proposed use pattern of CA3301 in cereals and oilseed rape.

For mammals, the screening TER_{lt} value for prothioconazole is greater than the Commission Regulation (EU) No. 546/2011 trigger of 5, indicating that the long-term risk to mammals is acceptable, according to the proposed use pattern of CA3301 in cereals and oilseed rape. However, the screening TER_{lt} values for the metabolite prothioconazole-desthio are lower than the trigger of 5. Most long-term first-tier risk assessments for prothioconazole-desthio revealed TER_{lt} values greater than the trigger of 5, indicating that the long-term risk to mammals is acceptable according to the proposed use pattern. Only for use in cereals and oilseed rape at BBCH \geq 40 the TER_{lt} value is below the trigger of 5 for the generic focal species small herbivorous mammal “vole”. A higher-tier refinement is carried out, based on standard worst-case assumptions of a 100% grass diet, a FIR/bw value of 1.33, a RUD_m value of 54.2, and a MAF_m x TWA value of 1.4 x 0.53, as well as refined deposition factors of 0.1 (cereals) and 0.2 (oilseed rape), which result in acceptable TER_{lt} values for prothioconazole-desthio of 9.35 and 5.35, for use in cereals and oilseed rape, respectively.

Risk assessments for exposure of birds and mammals, via drinking water, showed acceptable acute and long-term risks to prothioconazole and two of its metabolites – prothioconazole-desthio and prothioconazole-S-methyl. Risk assessments for exposure of birds and mammals, via secondary poisoning (fish- and earthworm-eating mammals), showed acceptable long-term risks, following exposure to prothioconazole, prothioconazole-desthio, and prothioconazole-S-methyl from the proposed use of CA3301.

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).

An acceptable risk is concluded for all aquatic organism groups using FOCUS Step-1 or -2 PEC_{sw} values, for the intended uses of CA3301 in cereals and oilseed rape, for the active substance prothioconazole and its metabolites, prothioconazole-S-methyl and 1,2,4-triazole, as well as the product CA3301.

However, for the metabolite prothioconazole-desthio, an acceptable aquatic risk can be demonstrated based

on FOCUS Step-4 PEC_{sw} values, for all relevant scenarios, considering the following mitigation measures:

- 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 2 applications on flax, cameline, mustard, and other seed-producing Brassicaceae; 2 applications to winter oilseed rape (autumn and spring application); and 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, and 1 and 2 applications to winter oilseed rape (spring application): 10-m no-spray-buffer zone and 10-m vegetative-filter strip.

9.1.1.3 Effects on bees (KCP 10.3.1)

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 ≤ 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, for all scenarios and intended BBCH stages. In oilseed rape, acceptable chronic oral risk assessment was demonstrated for all intended BBCH stages in weeds, field margin, adjacent crop, and succeeding crop scenarios. However, the ETR_{oral} values of the first-tier chronic oral risk assessment for adult honeybees are above the trigger values, for the proposed uses in oilseed rape, for the scenario treated crop (all intended BBCH stages). Nevertheless, the available semi-field tunnel data show no significant effects by prothioconazole on adult, pupal, and larval honey bees. Therefore, the higher tier data demonstrates an acceptable acute and chronic risk to bees from the proposed use of CA3301.

The risk assessment based on the EFSA Guidance (2013) is not yet approved and certain parts are currently under revision. As there is not harmonized approach for the chronic risk assessment for bees, therefore, Concerned Member States must decide on the acceptability of EFSA Guidance (2013) on national level.

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 of CA3301 to non-target arthropods was assessed in first-tier assessments, from hazard quotients, between toxicity endpoints that were estimated from laboratory studies with CA3301 and crop-specific use patterns. The assessment was conducted for the worst-case application patterns of 2 x 200 g a.s./ha (14-d interval), covering the risk for non-target arthropods from all other intended uses.

Acceptable in-field risks were not demonstrated for *T. pyri* and *A. rhopalosiphi* at the first tier, and a refined risk assessment, using extended laboratory studies, was conducted. However, risk assessments considering the above-mentioned refinement still showed potential risk to *T. pyri*, although not to *A. rhopalosiphi*, *C. carnea*, and *A. bilineata*. Consequently, extended-laboratory (aged-residue) studies with *T. pyri* and *C. carnea* were conducted, which demonstrated that there is an acceptable potential for recovery within an ecologically relevant period as mortality and reproductive effects at 0 DAT and 7 DAT were minimal at 9.2% and -1.1% corrected mortality (*T. pyri*), respectively, and no adverse effects on mortality in either bioassay on *C. carnea* (i.e., significantly less than 50%).

Acceptable off-field risks were demonstrated for *T. pyri* and *A. rhopalosiphi* at the first tier, therefore, the off-field risk to non-target arthropods was shown to be acceptable.

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 prothioconazole (the active substance in CA3301) and its metabolites – prothioconazole-desthio and prothioconazole-S-methyl was assessed and demonstrated to be acceptable, when the maximum predicted concentration in soil was used. Worst-case soil exposure following autumn application to winter oilseed rape was used in a risk envelope approach. All TER_{it} values were above the trigger of 5.

No significant effects (<25%) on soil microorganisms were shown for the proposed uses of CA3301 at concentrations greater than the predicted maximum soil concentrations. Therefore, the risk to soil microorganisms was considered acceptable.

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 prothioconazole, using data from new vegetative-vigour and seedling-emergence studies. No adverse effects are expected from the worst-case GAP (200 g a.s./ha x 2), since worst-case TER values for vegetative vigour and seedling emergence were >50.3 and >45.0, respectively (i.e., greater than the trigger value of 5).

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 CA3301 grouped according to crop type

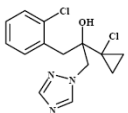
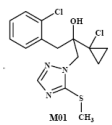
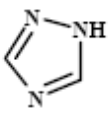
Group	Intended uses	Maximum rate per application	Maximum number of applications	Minimum interval between applications	Maximum total rate per season
Cereals	BBCH 30-69	200 g a.s./ha	2	14 d	400 g a.s./ha
Oilseed rape	BBCH 14-18 (autumn use, first application) BBCH 20-69 (spring use)	175 g a.s./ha	2	90 d (autumn and spring applications) 14 d (given two spring applications)	350 g a.s./ha
Flax (linseed)*	BBCH 33-51	175 g a.s./ha	2	14 d	350 g a.s./ha
Mustard, cameline, and other seed-producing Brassicaceae*	BBCH 14-18 (autumn use) BBCH 20-69 (spring use)	175 g a.s./ha	2	14 d	350 g a.s./ha

*The intended use in flax and in mustard, cameline, and other seed-producing Brassicaceae is covered by the risk assessments for the intended uses in oilseed rape.

9.1.3 Consideration of metabolites

A list of metabolites found in environmental compartments is provided below. The need for conducting a metabolite-specific risk assessment in the context of the evaluation of CA3301 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. 26.9% at 14d) Water/sediment system (54.6% at 7d)	Yes (soil and aquatic)
Prothioconazole-S-methyl (via soil)		358.3	Soil (max. 14.6% at 7d)	Yes (soil and aquatic)
1,2,4-triazole (via water/sediment)		69.1	Water (max. 37.2% at 121d) Sediment (max. 6.1% at 121d) Water/sediment system (max. 41.8% at 121d)	Yes (aquatic)

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

Avian toxicity studies have been carried out with prothioconazole and its relevant metabolites. Full details of these studies are provided in the respective EU DAR for prothioconazole (2005) and related documents.

Effects on birds of CA3301 were not evaluated as part of the EU assessment of prothioconazole. No new data are submitted with this application.

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

Species	Substance	Exposure System	Results	Reference
Active substance				
Northern bobwhite (<i>Colinus virginianus</i>)	Prothioconazole	Acute	LD₅₀ > 2000 mg/kg bw (worst case)	EFSA Sci. Report. 2007; 106, 1-98
Bobwhite quail (<i>Colinus virginianus</i>)		5 d dietary	LC50 > 5000 mg a.s./kg diet calc. LD50 > 1413 mg a.s./kg 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	LD₅₀ > 2000 mg/kg bw	EFSA Sci. Report. 2007; 106, 1-98
		Short-term, dietary	LD₅₀ > 297 mg /kg bw/d	
		Reproductive, dietary	NOEL = 14.8 mg/kg bw/d	

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 of two applications of 200 g a.s./ha (minimum of a 14-d interval, at BBCH 30-69) for cereals; two applications of 175 g a.s./h to winter oilseed rape (minimum of 90-day interval), once in autumn (at BBCH 14-18) and once in spring (at BBCH 20-69); and two spring applications of 175 g a.s./h to winter and spring oilseed rape (minimum of 14-day interval, at BBCH 20-69). For the risk assessment, the risk-envelope approach has been used for flax (linseed; with two applications of 175 g a.s./h, with a minimum 14-d interval, at BBCH 33-51) and for mustard, cameline, and other seed-producing Brassicaceae (with two applications of 175 g a.s./h, application timings and crop stage as described for oilseed rape). The application rates are the same for the intended uses in oilseed rape, flax (linseed), and seed-producing Brassicaceae; therefore, the calculations with oilseed rape also cover the application in flax and in seed-producing Brassicaceae.

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.

Table 9.2-2: Screening assessment of the acute and long-term/reproductive risk for birds due to the use of CA3301 in cereals and oilseed rape - prothioconazole.

Intended use	Cereals and oilseed rape				
Active substance	Prothioconazole				
Application rate (g/ha)	2 x 200 g a.s./ha (14-d interval); BBCH 14-69				
Acute toxicity (mg/kg bw)	>2000 (worst-case) / 1413*				
TER criterion	10				
Crop scenario Growth stage	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a
Screening- cereals and oilseed rape	Small omnivorous bird	158.8	1.2	38.1	>52.5 / 37.1
Reprod. toxicity (mg/kg bw/d)	78				
TER criterion	5				
Crop scenario Growth stage	Indicator/generic focal species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{lt}
Screening- cereals and oilseed rape	Small omnivorous bird	64.8	1.4 x 0.53	9.62	8.1

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.

*lowest available endpoint (dietary toxicity)

Acceptable acute and long-term risks to birds are concluded for the active substance, prothioconazole, at the screening step, for the intended uses of CA3301 in cereals and oilseed rape. No further avian risk assessment or data are 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 assumption.

Table 9.2-3: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of CA3301 in cereals - prothioconazole-desthio.

Intended use	Cereals				
Metabolite	Prothioconazole-desthio				
Application rate (g/ha)	2 x 200 g a.s./ha* (14-d interval); BBCH 30-69				
Acute toxicity (mg/kg bw)	>297(worst-case, based on short-term dietary data)				
TER criterion	10				
Crop scenario Growth stage	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a
Screening- cereals	Small omnivorous bird	158.8	1.2	38.1	>7.8
Cereals BBCH 30-39	Small omnivorous bird "lark"	12.0	1.2	2.88	>103.1
Cereals BBCH ≥40	Small omnivorous bird "lark"	7.2	1.2	1.73	>171.9
Reprod. toxicity (mg/kg bw/d)	14.8				
TER criterion	5				
Crop scenario Growth stage	Indicator/generic focal species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{lt}
Screening- cereals	Small omnivorous bird	64.8	1.4 x 0.53	9.62	1.5
Cereals BBCH 30-39	Small omnivorous bird "lark"	5.4		0.801	18.5
Cereals BBCH ≥40	Small omnivorous bird "lark"	3.3		0.490	30.2

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.

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 CA3301 in cereals. No further avian risk assessment or data are necessary.

Table 9.2-4: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of CA3301 in oilseed rape - prothioconazole-desthio.

the use of Prothioconazole in oilseed rape - prothioconazole-desthio.						
Intended use	Oilseed rape					
Metabolite	Prothioconazole-desthio					
Application rate (g/ha)	2 x 175 g a.s./ha* (14-d interval); BBCH 14-69					
Acute toxicity (mg/kg bw)	>297 (worst-case based on short-term dietary data)					
TER criterion	10					
Crop scenario	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀	TER _a	
Growth stage				(mg/kg bw/d)		
Screening - oilseed rape	Small omnivorous bird	158.8	1.2	33.3	8.9	
Oilseed rape BBCH 30-99	Small insectivorous bird "dunnock"	7.4		1.55	191.1	
Oilseed rape BBCH 10-29 19	Large herbivorous bird "goose"	39.0		8.19	36.3	
Oilseed rape BBCH 10-29	Small omnivorous bird "lark"	24.0		5.04	58.9	
Oilseed rape BBCH 30-39	Small omnivorous bird "lark"	7.2		1.51	196.4	
Oilseed rape BBCH ≥40	Small omnivorous bird "lark"	6.0		1.26	235.7	
Oilseed rape BBCH 10-19	medium herbivorous/granivorous bird "pigeon"	55.6		11.68	25.4	
Oilseed rape BBCH 20-29	medium herbivorous/granivorous bird "pigeon"	4.0		0.84	353.6	
Oilseed rape BBCH 30-39	medium herbivorous/granivorous bird "pigeon"	2.4		0.50	589.3	
Oilseed rape BBCH ≥40	medium herbivorous/granivorous bird "pigeon"	2.0		0.42	707.1	
Oilseed rape BBCH 10-19	Small insectivorous bird "wagtail"	10.9		2.29	129.8	
Oilseed rape BBCH 20-29	Small insectivorous bird "wagtail"	7.7		1.62	183.7	
Reprod. toxicity (mg/kg bw/d)	14.8					
TER criterion	5					
Crop scenario	Indicator/generic focal species	SV _m	MAF _m × TWA	DDD _m	TER _{lt}	
Growth stage				(mg/kg bw/d)		
Screening- oilseed rape	Small omnivorous bird	64.8	1.4 x 0.53	8.41	1.8	
Oilseed rape BBCH 30-99	Small insectivorous bird "dunnock"	2.7		0.351	42.2	
Oilseed rape BBCH 10-29 19	Large herbivorous bird "goose"	15.9		2.07	7.2	
Oilseed rape BBCH 10-29	Small omnivorous bird "lark"	10.9		1.42	10.0	
Oilseed rape BBCH 30-39	Small omnivorous bird "lark"	3.3		0.429	10.5	
Oilseed rape BBCH ≥40	Small omnivorous bird "lark"	2.7		0.351	34.5	
Oilseed rape BBCH 10-19	medium herbivorous/granivorous bird "pigeon"	22.7		2.95	42.2	
Oilseed rape BBCH 20-29	medium herbivorous/granivorous bird "pigeon"	3.5		0.454	5.0	
Oilseed rape BBCH 30-39	medium herbivorous/granivorous bird "pigeon"	1.1		0.143	32.6	

Oilseed rape BBCH ≥ 40	medium herbivorous/granivorous bird "pigeon"	0.9		0.117	103.6
Oilseed rape BBCH 10-19	Small insectivorous bird "wagtail"	5.9		0.766	126.6
Oilseed rape BBCH 20-29	Small insectivorous bird "wagtail"	2.8		0.364	19.3

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.

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 CA3301 in oilseed rape. No further avian risk assessment or data are necessary.

The intended uses of CA3301 in flax (linseed) and in mustard, cameline, and other seed-producing Brassicaceae are considered covered by the above risk assessments for the intended uses in oilseed rape [linseed, mustard, cameline, and other seed-producing Brassicaceae are listed within the oilseed rape crop group in the EFSA Guidance Document on Risk Assessment for Birds and Mammals (EFSA Journal 2009; 7(12): 1438)].

9.2.2.2 Higher-tier risk assessment

The risk assessments presented above concluded acceptable risks to birds at the screening step, for the active substance prothioconazole, for use in both cereals and oilseed rape. Acceptable risk from the metabolite prothioconazole-desthio was also concluded using first-tier risk assessments, for the proposed uses in cereals and oilseed rape. The risk for the proposed use in flax (linseed) and in mustard, cameline, and other seed-producing Brassicaceae are considered covered by the risk assessment for the proposed use in oilseed rape. Therefore, no further studies or assessments are considered necessary.

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.* Section 5.5 and Appendix K of EFSA/2009/1438).

Leaf scenario

Since CA3301 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 $K_{(f)oc}$ of 1765 L/kg (EFSA Sci. Report. 2007; 106, 1-98), prothioconazole belongs to the group of more sorptive substances.

Effective application rate (g/ha)	400*	Quotient
Acute toxicity (mg/kg bw)	>2000 / 1413	<0.200 / 0.283
Reprod. toxicity (mg/kg bw/d)	78	5.13

*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 $K_{(f)oc}$ of 523-625 L/kg (EFSA Sci. Report. 2007; 106, 1-98), prothioconazole-desthio belongs to the group of more sorptive substances.

Effective application rate (g/ha)	400* [#]	Quotient
Acute toxicity (mg/kg bw)	>297	<1.35
Reprod. toxicity (mg/kg bw/d)	14.8	27.0

*Total maximum seasonal application rate of the a.s. used here as a worst-case. [#] 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.

9.2.2.4 Effects of secondary poisoning

Bioconcentration assessments are triggered for prothioconazole, prothioconazole-desthio, and prothioconazole-S-methyl, as these substances have log K_{OW} values >3.0. The log P_{OW} values for prothioconazole, prothioconazole-desthio, and prothioconazole-S-methyl are 4.16, 3.04, and 4.19, respectively. The log P_{OW} value for 1,2,5-triazole is <3 and, therefore, bioconcentration is not expected for this metabolite.

Risk assessment for earthworm-eating birds, via secondary poisoning

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

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 for birds from all other intended uses in groups cereals, oilseed rape, flax (linseed), mustard, cameline, and other seed-producing Brassicaceae.

Table 9.2-5: Assessment of the risk for earthworm-eating birds due to exposure to prothioconazole, via bioaccumulation in earthworms (secondary poisoning), for the intended use in cereals and oilseed rape.

Parameter	Prothioconazole	Comments
PEC _{soil} (mg/kg soil)	0.1400	Worst-case initial PEC _{soil} for winter oilseed rape (see dRR B8, Environmental Fate, section 8.7.2)
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 _{OC}	0.02	Default
BCF _{worm}	4.94	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + 0.012P_{OW}) / f_{OC} \times K_{OC}$
PEC _{worm}	0.691	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.726	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	78.0	EFSA Sci. Report. 2007; 106, 1-98
TER _{It}	107.5	Acceptable risk demonstrated

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

Table 9.2-6: Assessment of the risk for earthworm-eating birds due to exposure to prothioconazole-desthio, via bioaccumulation in earthworms (secondary poisoning), for the intended use in cereals and oilseed rape.

Parameter	Prothioconazole-desthio	Comments
PEC _{soil} (mg/kg soil)	0.0725	Worst-case initial PEC _{soil} for winter oilseed rape (see dRR B8, Environmental Fate, section 8.7.2)
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}	523-625	EFSA Sci. Report. 2007; 106, 1-98
F _{OC}	0.02	Default
BCF _{worm}	1.12-1.34	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + 0.012P_{OW}) / f_{OC} \times K_{OC}$
PEC _{worm}	0.081-0.097	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.086-0.102	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	14.8	EFSA Sci. Report. 2007; 106, 1-98
TER _{It}	173.1-144.8	Acceptable risk demonstrated

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

Table 9.2-7: 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 in cereals and oilseed rape.

Parameter	Prothioconazole-S-methyl	Comments
PEC _{soil} (mg/kg soil)	0.0213	Worst-case initial PEC _{soil} for winter oilseed rape (see dRR B8, Environmental Fate, section 8.7.2)
log P _{OW} / P _{OW}	4.19 / 15500	EFSA Sci. Report. 2007; 106, 1-98
K _{OC}	1974-2995	EFSA Sci. Report. 2007; 106, 1-98
F _{OC}	0.02	Default
BCF _{worm}	3.12-4.73	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + 0.012P_{OW}) / f_{OC} \times K_{OC}$
PEC _{worm}	0.066-0.101	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.070-0.106	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	7.8	Conservative estimate of parent NOEL/10
TER _{It}	73.7-111.8	Acceptable risk demonstrated

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

Even using worst-case, conservative initial PEC_{soil} values (as opposed to 21-d TWA values), acceptable risks are concluded for earthworm-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).

Risk assessment for fish-eating birds, via secondary poisoning

According to EFSA/2009/1438, the risk for piscivorous birds is assessed for a bird of 1000 g body weight, with a daily food consumption of 159 g. Bioaccumulation in fish is estimated based on predicted concentrations in surface water for aquatic organisms and BCF values for prothioconazole, prothioconazole-desthio, and prothioconazole-S-methyl.

Table 9.2-8: Assessment of the risk for fish-eating birds due to exposure to prothioconazole, via bioaccumulation in fish (secondary poisoning), for the intended use in cereals and oilseed rape.

Parameter	Prothioconazole	Comments
PEC _{sw} (mg/L)	0.00184	Worst-case FOCUS Step-2 PEC _{sw} for single applications to spring or winter cereals (see dRR B8, Environmental Fate, section 8.9)
BCF _{fish}	19.7	EFSA Sci. Report. 2007; 106, 1-98
BMF	Not applicable	biomagnification factor (relevant for BCF ≥ 2000)
PEC _{fish}	0.036	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.006	$DDD = PEC_{fish} \times 0.159$
NOEL (mg/kg bw/d)	78.0	EFSA Sci. Report. 2007; 106, 1-98
TER _{It}	13534	Acceptable risk demonstrated

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

Table 9.2-9: Assessment of the risk for fish-eating birds due to exposure to prothioconazole-desthio, via bioaccumulation in fish (secondary poisoning), for the intended use in cereals and oilseed rape.

Parameter	Prothioconazole-desthio	Comments
PEC _{sw} (mg/L)	0.00623	Worst-case FOCUS Step-2 PEC _{sw} for autumn and spring applications to oilseed rape (see dRR B8, Environmental Fate, section 8.9)
BCF _{fish}	65	EFSA Sci. Report. 2007; 106, 1-98
BMF	Not applicable	biomagnification factor (relevant for BCF ≥ 2000)
PEC _{fish}	0.405	PEC _{fish} = PEC _{water} × BCF _{fish}
Daily dietary dose (mg/kg bw/d)	0.064	DDD = PEC _{fish} × 0.159
NOEL (mg/kg bw/d)	14.8	EFSA Sci. Report. 2007; 106, 1-98
TER _{lt}	229.86	Acceptable risk demonstrated

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

Table 9.2-10: 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 in cereals and oilseed rape.

Parameter	Prothioconazole-S-methyl	Comments
PEC _{sw} (mg/L)	0.00052	Worst-case FOCUS Step-2 PEC _{sw} for autumn and spring applications to oilseed rape (see dRR B8, Environmental Fate, section 8.9)
BCF _{fish}	5.08	Estimated with EPI Suite*
BMF	Not applicable	biomagnification factor (relevant for BCF ≥ 2000)
PEC _{fish}	0.00264	PEC _{fish} = PEC _{water} × BCF _{fish}
Daily dietary dose (mg/kg bw/d)	0.000420	DDD = PEC _{fish} × 0.159
NOEL (mg/kg bw/d)	7.8	Conservative estimate of parent NOEL/10
TER _{lt}	18571	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.

Even using worst-case, conservative inputs and FOCUS Step-2 initial PEC_{sw} 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). Therefore, no further avian risk assessments or mitigation is required.

9.2.2.5 Biomagnification in terrestrial food chains

Not relevant, as the mammalian toxicity and goat metabolism data did not show the potential for accumulation (EFSA Conclusion, 2007).

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

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

9.2.4 Overall conclusions

Acceptable risks to birds can be concluded for all intended uses of CA3301 at the first tier, with the available data. Therefore, no further avian risk assessments are required. The additional intended minor uses of

CA3301 in flax and in mustard, cameline, and other seed-producing Brassicaceae are considered to be covered by the risk assessments for use on oilseed rape.

Review Comments:

The acute and chronic risks of CA3301 to birds were assessed from toxicity exposure ratios between toxicity endpoints, estimated from study with active ingredient, its metabolites, and maximum residues occurring on food items. No acute toxicity test with the formulation was required.

All TER values exceed the relevant triggers indicating that CA3301 does not pose an unacceptable risk to birds following applications according to recommended use pattern.

Evaluation of exposing to birds through the drinking water demonstrated the acceptable risk. The risk to earthworm- and fish-eating animals from secondary poisoning is low.

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

9.3.1 Toxicity data

Mammalian toxicity studies have been carried out with prothioconazole and its 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 of CA3301 were not evaluated as part of the EU assessment of prothioconazole. No new data are submitted with this application.

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

Species	Substance	Exposure System	Results	Reference
Active substance				
Rat	Prothioconazole	Oral, acute	LD₅₀ > 6200 mg/kg bw	EFSA Sci. Report. 2007; 106, 1-98
		Dietary, reproductive toxicity, multiple-generation study	NOAEL = 95.6 mg/kg bw/d (reduced pup weight gain, reduced litter size)	
Prothioconazole-desthio				
Mouse	Prothioconazole-desthio	Oral, acute	LD₅₀ = 2235 mg/kg bw (males)	EFSA Sci. Report. 2007; 106, 1-98
Rat		Dietary, reproductive toxicity, multiple-generation study	NOAEL = 10 mg/kg bw/d (reproductive effects)	

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 of two applications of 200 g a.s./ha (minimum of a 14-d interval, at BBCH 30-69) for cereals and two applications of 175 g a.s./ha (minimum of 14-d interval, at BBCH 14-18, for autumn use, and at BBCH 20-69, for spring use) for oilseed rape. For the risk assessment, the risk-envelope approach has been used for flax (linseed; with two applications of 175 g a.s./h, with a minimum 14-d interval, at BBCH 33-51) and for mustard, cameline, and other seed-producing Brassicaceae (with two applications of 175 g a.s./h, with a minimum 14-d interval, at BBCH 14-18 and BBCH 20-69). The application rates are the same for the intended uses in oilseed rape, flax (linseed), and seed-producing Brassicaceae; therefore, the calculations with oilseed rape also cover the application in flax and in seed-producing Brassicaceae.

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

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

Table 9.3-2: Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of CA3301 in cereals and oilseed rape- prothioconazole.

Intended use	Cereals and oilseed rape				
Active substance	Prothioconazole				
Application rate (g/ha)	2 x 200 g a.s./ha (14-d interval); BBCH 14-69				
Acute toxicity (mg/kg bw)	>6200				
TER criterion	10				
Crop scenario Growth stage	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a
Screening- cereals and oilseed rape	Small herbivorous mammal	118.4	1.2	28.4	>218
Reprod. toxicity (mg/kg bw/d)	95.6				
TER criterion	5				
Crop scenario Growth stage	Indicator/generic focal species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{lt}
Screening- cereals and oilseed rape	Small herbivorous mammal	48.3	1.4 x 0.53	7.17	13.3

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.

Acceptable acute and long-term risks to mammals are concluded for the active substance, prothioconazole, at the screening step, for intended uses of CA3301 in cereals and oilseed rape. 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 CA3301 in cereals- prothioconazole-desthio.

Intended use	Cereals				
Metabolite	Prothioconazole-desthio				
Application rate (g/ha)	2 x 200 g a.s./ha* (14-d interval); BBCH 30-69				
Acute toxicity (mg/kg bw)	2235				
TER criterion	10				
Crop scenario Growth stage	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a
Screening- cereals and oilseed rape	Small herbivorous mammal	118.4	1.2	28.4	78.7
Reprod. toxicity (mg/kg bw/d)	10				
TER criterion	5				
Crop scenario Growth stage	Indicator/generic focal species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{lt}
Screening- cereals	Small herbivorous mammal	48.3	1.4 x 0.53	7.17	1.4
Cereals BBCH ≥20	Small insectivorous mammal “shrew”	1.9		0.282	35.5
Cereals BBCH ≥40	Small herbivorous mammal “vole”	21.7		3.22	3.1
Cereals BBCH 30-39	Small omnivorous mammal “mouse”	3.9		0.579	17.3
Cereals BBCH ≥40	Small omnivorous mammal “mouse”	2.3		0.341	29.3

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 > trigger 10) for the proposed use on cereals.

The reproductive risk assessment for the majority of generic focal species at the first tier is acceptable (TER values > trigger of 5). A higher-tier risk assessment is required to address the long-term reproductive risk to mammals from the metabolite prothioconazole-desthio, for the proposed use of CA3301 in cereals at BBCH ≥ 40 (scenario: small herbivorous mammal “vole”). See further discussion 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 CA3301 in oilseed rape - prothioconazole-desthio.

Intended use	Oilseed rape				
Metabolite	Prothioconazole-desthio				
Application rate (g/ha)	2 x 175 g a.s./ha* (14-d interval); BBCH 14-69				
Acute toxicity (mg/kg bw)	2235				
TER criterion	10				
Crop scenario Growth stage	Indicator/generic focal species	SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a
Screening- cereals and oilseed rape	Small herbivorous mammal	118.4	1.2	24.9	89.8
Reprod. toxicity (mg/kg bw/d)	10				
TER criterion	5				
Crop scenario Growth stage	Indicator/generic focal species	SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{lt}
Screening- oilseed rape	Small herbivorous mammal	48.3	1.4 x 0.53	6.27	1.6
Oilseed rape BBCH 10-19	Small insectivorous mammal “shrew”	4.2		0.545	18.3
Oilseed rape BBCH ≥20	Small insectivorous mammal “shrew”	1.9		0.247	40.5
Oilseed rape BBCH ≥40	Small herbivorous mammal “vole”	18.1		2.35	4.3
Oilseed rape All season	Large herbivorous mammal “lagomorph”	14.3		1.86	5.4
Oilseed rape BBCH 10-29	Small omnivorous mammal “mouse”	7.8		1.01	9.9
Oilseed rape BBCH 30-39	Small omnivorous mammal “mouse”	2.3		0.299	33.5
Oilseed rape BBCH ≥40	Small omnivorous mammal “mouse”	1.9		0.247	40.5

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 > trigger 10) for the proposed use on oilseed rape.

The reproductive risk assessment for the majority of generic focal species at the first tier is acceptable (TER values > trigger of 5). A higher-tier risk assessment is required to address the long-term reproductive risk to mammals from the metabolite prothioconazole-desthio, for the proposed use of CA3301 on oilseed rape at BBCH ≥ 40 (scenario: small herbivorous mammal “vole”). See further discussion below.

9.3.2.2 Higher-tier risk assessment

The first-tier risk assessments use worst-case default deposition factors (DF) of 0.3 (cereals, at BBCH ≥40) and 0.25 (oilseed rape, at BBCH ≥40), from Appendix A of EFSA/2009/1438, for the vole food item of 100% grass. These default DF values are based on the interception values for cereals and oilseed rape, taken from the FOCUS groundwater guidance (2001). However, refined DF values of 0.1 (cereals BBCH ≥40) and 0.2 (oilseed rape BBCH ≥40), for grass under the 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 these refined DF values.

Review Comments:

The Applicant's proposal to refine a deposition factor following the guidance for FOCUS ground water assessments (EFSA, 2014) was accepted.

Table 9.3-5: Higher-tier assessment of the long-term/reproductive risk for mammals due to the use of CA3301 in cereals – prothioconazole-desthio – refined deposition factor (DF)*.

Intended use		Cereals						
Metabolite		Prothioconazole-desthio						
Application rate (g/ha)		2 x 200 g a.s./ha [#] (14-d interval); BBCH 40-69						
Reprod. toxicity (mg/kg bw/day)		10						
TER criterion		5						
Generic focal species	Food category, % in diet	FIR/bw	RUD _m	DF	MAF _m × TWA	PT	DDD _m (mg/kg bw/d)	TER _{lt}
Small herbivorous mammal "vole"	Grass, 100%	1.33	54.2	0.1*	1.4 x 0.53	1	1.07	9.35

TER values shown in **bold** fall below the relevant trigger. [#]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_{lt} 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, following the intended use of CA3301 in cereals, at BBCH 40-69. No further refinements or data are considered necessary.

Table 9.3-6: Higher-tier assessment of the long-term/reproductive risk for mammals due to the use of CA3301 in oilseed rape – prothioconazole-desthio – refined deposition factor (DF)*.

Intended use		Oilseed rape						
Metabolite		Prothioconazole-desthio						
Application rate (g/ha)		2 x 175 g a.s./ha [#] (14-d interval); BBCH 40-69						
Reprod. toxicity (mg/kg bw/day)		10						
TER criterion		5						
Generic focal species	Food category, % in diet	FIR/bw	RUD _m	DF	MAF _m × TWA	PT	DDD _m (mg/kg bw/d)	TER _{lt}
Small herbivorous mammal "vole"	Grass, 100%	1.33	54.2	0.2*	1.4 x 0.53	1	1.87	5.35

TER values shown in **bold** fall below the relevant trigger. [#]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 0.2, the TER_{ik} 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, following the intended use of CA3301 in oilseed rape, at BBCH 40-69. No further refinements or data are considered necessary.

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. Section 5.5 and Appendix K of EFSA/2009/1438).

Puddle scenario

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

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

Effective application rate (g/ha)	400*	Quotient
Acute toxicity (mg/kg bw)	>6200	<0.065
Reprod. toxicity (mg/kg bw/d)	95.6	4.18

*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 $K_{(f)oc}$ of 523-625 L/kg (EFSA Sci. Report. 2007; 106, 1-98), the metabolite prothioconazole-desthio belongs to the group of more sorptive substances.

Effective application rate (g/ha)	400* [#]	Quotient
Acute toxicity (mg/kg bw)	2235	0.179
Reprod. toxicity (mg/kg bw/d)	10	40.0

*Total maximum seasonal application rate used here as a worst-case. [#]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.

9.3.2.4 Effects of secondary poisoning

Bioconcentration assessments are triggered for prothioconazole, prothioconazole-desthio, and prothioconazole-S-methyl, as these substances have log K_{ow} values >3.0. The log P_{ow} values for prothioconazole, prothioconazole-desthio, and prothioconazole-S-methyl are 4.16, 3.04, and 4.19, respectively. The log P_{ow} value for 1,2,5-triazole is <3 and, therefore, bioconcentration is not expected for this metabolite.

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 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 for mammals from all other intended uses.

Table 9.3-7: Assessment of the risk for earthworm-eating mammals due to exposure to prothioconazole via bioaccumulation in earthworms (secondary poisoning) for the intended use in cereals and oilseed rape.

Parameter	Prothioconazole	Comments
PEC _{soil} (mg/kg soil)	0.1400	Worst-case initial PEC _{soil} for winter oilseed rape (see dRR B8, Environmental Fate, section 8.7.2, Table 8.7-4)
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 _{OC}	0.02	Default
BCF _{worm}	4.94	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + 0.012P_{ow}) / f_{OC} \times K_{OC}$
PEC _{worm}	0.691	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.885	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	95.6	EFSA Sci. Report. 2007; 106, 1-98
TER _{It}	108.1	Acceptable risk demonstrated

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

Table 9.3-8: Assessment of the risk for earthworm-eating mammals due to exposure to prothioconazole-desthio via bioaccumulation in earthworms (secondary poisoning) for the intended use in cereals and oilseed rape.

Parameter	Prothioconazole-desthio	Comments
PEC _{soil} (mg/kg soil)	0.0725	Worst-case initial PEC _{soil} for winter oilseed rape (see dRR B8, Environmental Fate, section 8.7.2)
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}	523-625	EFSA Sci. Report. 2007; 106, 1-98
f _{OC}	0.02	Default
BCF _{worm}	1.12-1.34	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + 0.012P_{ow}) / f_{OC} \times K_{OC}$
PEC _{worm}	0.081-0.097	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.104-0.125	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	10	EFSA Sci. Report. 2007; 106, 1-98
TER _{It}	80.3-95.9	Acceptable risk demonstrated

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

Table 9.3-9: Assessment of the risk for earthworm-eating mammals due to exposure to prothioconazole-S-methyl via bioaccumulation in earthworms (secondary poisoning) for the intended use in cereals and oilseed rape.

Parameter	Prothioconazole-S-methyl	Comments
PEC _{soil} (mg/kg soil)	0.0213	Worst-case initial PEC _{soil} for winter oilseed rape (see dRR B8, Environmental Fate, section 8.7.2)
log P _{ow} / P _{ow}	4.19 / 15500	EFSA Sci. Report. 2007; 106, 1-98
K _{oc}	1974-2995	EFSA Sci. Report. 2007; 106, 1-98
f _{oc}	0.02	Default
BCF _{worm}	3.12-4.73	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) = (0.84 + 0.01P_{ow}) / f_{oc} \times K_{oc}$
PEC _{worm}	0.066-0.101	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.085-0.129	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	9.56	Conservative estimate of parent NOEL/10
TER _{It}	74.1-112.4	Acceptable risk demonstrated

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

Even using worst-case, conservative initial PEC_{soil} 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 (TER values well above the trigger of 5).

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 9.3-10: Assessment of the risk for fish-eating mammals due to exposure to prothioconazole via bioaccumulation in fish (secondary poisoning) for the intended use in cereals and oilseed rape.

Parameter	Prothioconazole	Comments
PEC _{sw} (mg/L)	0.00184	Worst-case FOCUS Step-2 PEC _{sw} for autumn and spring applications to oilseed rape (see dRR B8, Environmental Fate, section 8.9)
BCF _{fish}	19.7	EFSA Sci. Report. 2007; 106, 1-98
BMF	Not applicable	biomagnification factor (relevant for BCF ≥ 2000)
PEC _{fish}	0.036	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.005	$DDD = PEC_{fish} \times 0.142$
NOEL (mg/kg bw/d)	95.6	EFSA Sci. Report. 2007; 106, 1-98
TER _{It}	18573	Acceptable risk demonstrated

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

Table 9.3-11: Assessment of the risk for fish-eating mammals due to exposure to prothioconazole-desthio via bioaccumulation in fish (secondary poisoning) for the intended use in cereals and oilseed rape.

Parameter	Prothioconazole-desthio	Comments
PEC _{sw} (mg/L)	0.00623	Worst-case FOCUS Step-2 PEC _{sw} for autumn and spring applications to oilseed rape (see dRR B8, Environmental Fate, section 8.9)
BCF _{fish}	65	EFSA Sci. Report. 2007; 106, 1-98
BMF	Not applicable	biomagnification factor (relevant for BCF ≥ 2000)
PEC _{fish}	0.405	PEC _{fish} = PEC _{water} × BCF _{fish}
Daily dietary dose (mg/kg bw/d)	0.058	DDD = PEC _{fish} × 0.142
NOEL (mg/kg bw/d)	10.0	EFSA Sci. Report. 2007; 106, 1-98
TER _{It}	173.9	Acceptable risk demonstrated

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

Table 9.3-12: Assessment of the risk for fish-eating mammals due to exposure to prothioconazole-S-methyl via bioaccumulation in fish (secondary poisoning) for the intended use in cereals and oilseed rape.

Parameter	Prothioconazole-S-methyl	Comments
PEC _{sw} (mg/L)	0.00052	Worst-case FOCUS Step-2 PEC _{sw} for multiple applications in cereals (see dRR B8, section 8.9)
BCF _{fish}	5.08	Estimated using EPI Suite ¹
BMF	Not applicable	biomagnification factor (relevant for BCF ≥ 2000)
PEC _{fish}	0.00264	PEC _{fish} = PEC _{water} × BCF _{fish}
Daily dietary dose (mg/kg bw/d)	0.000375	DDD = PEC _{fish} × 0.142
NOEL (mg/kg bw/d)	9.6	Conservative estimate of parent NOEL/10
TER _{It}	25486	Acceptable risk demonstrated

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

Even using worst-case, conservative inputs and FOCUS Step-2 actual PEC_{sw} 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 (TER values well above the trigger of 5). Therefore, no further mammalian risk assessments or mitigation are required.

9.3.2.5 Biomagnification in terrestrial food chains

Not relevant, as the mammalian toxicity and goat metabolism data did not show the potential for accumulation (EFSA Conclusion, 2007).

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

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

9.3.4 Overall conclusions

Acceptable risks to mammals can be concluded for all intended uses of CA3301 at the screening and first

¹US EPA. 2021. Estimation Programs Interface Suite™ for Microsoft® Windows, v 4.11. United States Environmental Protection Agency, Washington, DC, USA.

tier with the available data, apart from the long-term risk from prothioconazole-desthio at BBCH ≥ 40 for the scenario small herbivorous mammal “vole.” 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. Therefore, no further mammalian risk assessments or data are considered necessary.

Review Comments:

The acute and chronic risks of CA3301 to mammals were assessed from toxicity exposure ratios between toxicity endpoints, estimated from study with active ingredient, its metabolites, and maximum residues occurring on food items. No acute toxicity test with the formulation was required.

All TER values exceed the relevant triggers indicating that CA3301 does not pose an unacceptable risk to mammals following applications according to recommended use pattern.

Evaluation of exposing to mammals through the drinking water demonstrated the acceptable risk. The risk to earthworm- and fish-eating animals from secondary poisoning is low.

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 and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents, as well as in Appendix 2 of this document (new studies).

Effects on aquatic organisms of CA3301 were not evaluated as part of the EU assessment of prothioconazole. New data for this product are submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

Species	Substance	Exposure System	Results	RAC (µg/L)	Reference
Active substance					
<i>Oncorhynchus mykiss</i>	Prothioconazole	96-h, s	LC ₅₀ = 1.83 mg a.s./L _{mm}	18.3	EFSA Sci. Report. 2007; 106, 1-98
		97-d ELS	NOEC = 0.308 mg a.s./L _{mm}	30.8	
<i>Daphnia magna</i>		48-h, s	EC ₅₀ = 1.3 mg a.s./L _{mm}	13	
		21-d, ss	NOEC = 0.56 mg a.s./L _{nom}	56	
<i>Pseudokirchneriella subcapitata</i>		96-h, s	72 h E _r C ₅₀ = 2.18 mg a.s./L _{im}	218	
Prothioconazole-desthio					
<i>Oncorhynchus mykiss</i>	Prothioconazole-desthio	96-h, s	LC ₅₀ = 6.63 mg/L _{nom}	66.3	EFSA Sci. Report. 2007; 106, 1-98
		96-d ELS	NOEC = 0.00334 mg/L _{mm}	0.334	
<i>Daphnia magna</i>		48-h, s	EC ₅₀ > 10 mg/L _{mm}	>100	
		21-d, ss	NOEC = 0.10 mg/L _{nom}	10	
<i>Chironomus riparius</i>		28-d, s, spiked water	NOEC = 2.0 mg/L _{nom}	200	
<i>Scenedesmus subspicatus</i>		96-h, s	E _r C ₅₀ = 0.55 mg/L _{nom}	55	
<i>Desmodesmus subspicatus</i>		96-h, s	E _r C ₅₀ = 0.955 mg/L E ₅₀ = 0.0811 mg/L NOEC < 0.00596 mg/L	95.5	
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 ₅₀ = 1.8 mg/L _{mm}	18.0	EFSA Sci. Report. 2007; 106, 1-98
<i>Daphnia magna</i>		48-h, s	EC ₅₀ = 2.8 mg/L _{nom}	28	
<i>Pseudokirchneriella subcapitata</i>		72 h, s	E _r C ₅₀ = 47.4 mg/L _{im}	4740	
1,2,4-triazole					
<i>Oncorhynchus</i>	1,2,4-triazole	96-h, s	LC ₅₀ = 498 mg/L	4980	EFSA Sci.

<i>mykiss</i>		97-d ELS	NOEC = 3.2 mg/L	320	Report. 2007; 106, 1-98
<i>Daphnia magna</i>		48-h, s	EC ₅₀ = 900 mg/L	9000	
		48-h, s	EC ₅₀ >100 mg/L	>1000	
<i>Pseudokirchneriella subcapitata</i>		72-h, s	E _r C ₅₀ = 22.5 mg/L	2250	
EC 250 formulation					
<i>Oncorhynchus mykiss</i>	EC 250 formulation	96-h, s	LC ₅₀ = 1.00 mg a.s./L	10.0	EFSA Sci. Report. 2007; 106, 1-98
<i>Daphnia magna</i>		48-h, s	EC ₅₀ = 0.71 mg a.s./L	7.1	
<i>Pseudokirchneriella subcapitata</i>		72-h, s	E _r C ₅₀ = 1.11 mg a.s./L	111	
New Product – CA3301					
<i>Daphnia magna</i>	CA3301	48-h, ss	EC ₅₀ = 0.28 mg a.s./L (1.1 mg product/L) _{mm}	2.8	KCP 10.2.1/01
<i>Lemna gibba</i>		7-d, ss	E_rC₅₀ (frond growth rate) = 0.288 mg a.s./L (1.1 mg product/L) _{mm} E _r C ₅₀ (frond growth rate) > 0.444 mg a.s./L (>1.7 mg product/L) _{mm} E _r C ₅₀ (dry weight) > 0.444 mg a.s./L (>1.74 mg product/L) _{mm} E _y C ₅₀ (frond number; yield) = 0.342 mg a.s./L (1.3 mg product/L) _{mm} E _y C ₅₀ (dry weight) > 0.444 mg a.s./L (>1.7 mg product/L) _{mm}	28.8 >44.4	KCP 10.2.1/02
<i>Skeletonema</i> sp., Strain No. CCAP 1077/1C; formerly <i>Skeletonema costatum</i>		72-h, ss	E _r C ₅₀ = 0.084 mg a.s./L (0.328 mg product/L) _{mm} E _y C ₅₀ = 0.020 mg a.s./L (0.078 mg product/L) _{mm}	8.4	KCP 10.2.1/03

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

9.5.1.1 Justification for new endpoints

To comply with data requirements of Regulation (EU) 284/2013, three new studies with algae, invertebrates, and macrophytes were conducted with the new formulation CA3301. The choice of testing was determined based on the draft renewal information for prothioconazole, which indicated that the worst-case RAC value for prothioconazole would result from the algal study with the species *Skeletonema costatum* (E_rC₅₀ of 0.033 mg a.s./L mm; this endpoint is yet to be agreed at the EU level). This E_rC₅₀ value is lower than the currently agreed E_rC₅₀ value of 1.11 mg a.s./L for *P. subcapitata* and the lowest RAC value for the EU representative EC formulation from the acute *Daphnia magna* study. To reduce vertebrate

testing, no new studies with fish were performed. In comparison of the active substance, the EU representative 250 EC formulation and the proposed 250 EC formulation CA3301 show comparable toxicity. Differences in endpoints for acute toxicity to *D. magna* and *S. costatum* are between a factor of 2.5 and 4.6 (*D. magna*: EC₅₀ = 1.3 mg a.s./L for the active substance, 0.71 mg a.s./L for the EU representative formulation, and 0.28 mg a.s./L for CA3301; for *S. costatum*: E_rC₅₀ = 0.033 mg a.s./L for the active substance and 0.084 mg a.s./L for CA3301), which are well within the factor of 5 that accounts for biological background variability, which accounts for intra-species and inter-laboratory variability. Based on the available data, it is considered that the new data for CA3301 do not show any significant increase in aquatic toxicity compared to the active substance and the representative EU formulation, but clearly indicate that primary producers are the most sensitive aquatic organism species. Therefore, it is considered that the toxicity to fish will be adequately covered by the risk assessment using the available aquatic ecotoxicity data. As a worst case, the EU 250 EC endpoint for fish will be used in the risk assessment.

Table 9.5-2: Comparison of critical acute aquatic toxicity data for the active substance and formulation (endpoints in mg a.s./L)

Endpoint	EU-agreed a.s value	EU-agreed value for the 250 EC formulation	New product data for CA3301
Fish acute LC ₅₀	1.83 (mm)	1.00	-
<i>Daphnia</i> acute EC ₅₀	1.3 (mm)	0.71	0.28 (mm)
<i>S. costatum</i> E _r C ₅₀	0.033 (mm)*	-	0.084 (mm)

*Endpoint is yet to be agreed at the EU level. mm, mean measured.

Review Comments:

The applicant's justification was accepted. An acute toxicity study for formulation is not required.

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 (i.e., lowest of a.s. or relevant product data are used for the derivation of RAC values to use in the risk assessment). The relevant global maximum FOCUS Step-1, -2, and -3 PEC_{sw} values for risk assessments covering the proposed use pattern (see Part B8 for full details of the PEC_{sw} value) and the resulting PEC/RAC ratios are presented in the tables below.

Intended use of CA3301 in cereals (2 x 200 g a.s./ha, minimum 14-d interval; BBCH 30-69)

The risk assessments presented below are based on PEC_{sw} values for the intended use of CA3301 in spring and winter cereals (see dRR B8, section 8.9), which are considered worst-case. The toxicity endpoints are worst case, coming either from active-substance studies or derived from the new product (CA3301) studies. The ratios between predicted environmental concentrations in surface water bodies (PEC_{sw}) and regulatory acceptable concentrations (RAC) for aquatic organisms are given per intended use for each FOCUS scenario and each organism group.

Table 9.5-3: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prothioconazole for each organism group based on FOCUS Steps-1 and -2 calculations for the use of CA3301 in spring and winter cereals (worst-case scenario, for single applications of 200 g a.s./ha)

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Macrophyte	Algae	Algae
Test species		<i>Oncorhynchus mykiss</i>	<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Daphnia magna</i>	<i>Lemna gibba</i>	<i>P. subcapitata</i>	<i>S. costatum</i>
Endpoint (µg a.s./L)		LC ₅₀ *	NOEC*	EC ₅₀ [#]	NOEC*	E _r C ₅₀ [#]	E _r C ₅₀ *	E _r C ₅₀ [#]
		1000	308	280	560	288 >444	2180	84
AF		100	10	100	10	10	10	10
RAC (µg a.s./L)		10	30.8	2.8	56	28.8 >44.4	218	8.4
FOCUS Scenario	PEC _{gl-max} (µg a.s./L)	PEC/RAC						
Step 1								
Worst case	21.72	2.17	0.71	7.76	0.39	0.75 0.49	0.10	2.59
Step 2 – worst case								
N-Europe	1.84	0.18	-	0.66	-	-	-	0.22
S-Europe	1.84	0.18	-	0.66	-	-	-	0.22

PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**. *EFSA-agreed endpoints used (EFSA Sci. Report. 2007; 106, 1-98), when the a.s. value is lower than the a.s. value derived from the product study. [#]Endpoints derived from new studies with the proposed formulation CA3301 used, when a.s. value is lower than the EFSA-agreed endpoint. AF, assessment factor; PEC, predicted environmental concentration; RAC, regulatory acceptable concentration.

For the intended uses of CA3301, calculated PEC/RAC ratios for the active substance prothioconazole indicate an acceptable risk for the most sensitive group of aquatic organisms (fish, daphnids, and algae) using worst-case PEC_{sw} values at FOCUS Step 2. Therefore, no further assessment or mitigation is necessary.

Table 9.5-4: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the metabolite prothioconazole-desthio for each organism group based on FOCUS Steps-1, -2, and -3 PEC_{sw} values for the use of CA3301 in winter cereals (worst-case scenario, for multiple and single applications of 200 g a.s./ha).

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Sed.-dwell. chronic	Algae
Test species		<i>Oncorhynchus mykiss</i>	<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Daphnia magna</i>	<i>Chironomus riparius</i>	<i>Scenedesmus subspicatus</i>
Endpoint* (µg metabolite/L)		LC ₅₀ 6630	NOEC 3.34	EC ₅₀ >10000	NOEC 100	NOEC 2000	ErC ₅₀ 550
AF		100	10	100	10	10	10
RAC (µg metabolite/L)		66.3	0.334	>100	10	200	55
FOCUS Scenario		PEC _{gl-max} (µg/L)					
Step 1		PEC/RAC					
Worst case	78.36	1.18	235	<0.78	7.84	0.39	1.42
Step 2 – worst case							
N-Europe	5.93	0.09	17.75	-	0.59	-	0.11
S-Europe	10.81	0.16	32.37	-	1.08	-	0.20
Step 3 (Multiple applications)							
D3/ditch	0.1055	-	0.32	-	0.01	-	-
D4/pond	0.03537	-	0.11	-	<0.01	-	-
D4/stream	0.06452	-	0.19	-	0.01	-	-
D5/pond	0.04375	-	0.13	-	<0.01	-	-
D5/stream	0.1072	-	0.32	-	0.01	-	-
R1/pond	0.1397	-	0.42	-	0.01	-	-
R1/stream	1.058	-	3.17	-	0.11	-	-
R3/stream	1.136	-	3.40	-	0.11	-	-

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Sed.-dwell. chronic	Algae
Test species		<i>Oncorhynchus mykiss</i>	<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Daphnia magna</i>	<i>Chironomus riparius</i>	<i>Scenedesmus subspicatus</i>
R4/stream	0.7137	-	2.14	-	0.07	-	-
Step 3 (Single application)							
D3/ditch	0.05982	-	0.18	-	0.01	-	-
D4/pond	0.02054	-	0.06	-	<0.01	-	-
D4/stream	0.0721	-	0.22	-	0.01	-	-
D5/pond	0.02574	-	0.08	-	<0.01	-	-
D5/stream	0.1110	-	0.33	-	0.01	-	-
R1/pond	0.05443	-	0.16	-	0.01	-	-
R1/stream	0.3520	-	1.05	-	0.04	-	-
R3/stream	0.4567	-	1.37	-	0.05	-	-
R4/stream	0.2455	-	0.74	-	0.02	-	-

PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**. *All values are EFSA-agreed endpoints (EFSA Sci. Report. 2007; 106, 1-98). AF, assessment factor; PEC, predicted environmental concentration; RAC, regulatory acceptable concentration.

An acceptable risk is not concluded for prothioconazole-desthio for all FOCUS Step-3 scenarios, for the intended uses of CA3301 in winter cereals. A further assessment is presented below, with consideration of risk-mitigation measures for the relevant scenarios that did not pass at FOCUS Step 3.

Table 9.5-5: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prothioconazole-desthio for the critical organism group based on FOCUS Step-4 calculations for the use of CA3301 in winter cereals.

Substance Critical aquatic RAC	Prothioconazole-desthio 0.334 (chronic fish)					
FOCUS Step-4 scenario	Winter cereals (two applications)				Winter cereals (single application)	
	10-m NSBZ + 10-m VFS		20-m NSBZ + 20-m VFS		10-m NSBZ + 10-m VFS	
	PEC _{sw} (µg/L)	PEC/RAC	PEC _{sw} (µg/L)	PEC/RAC	PEC _{sw} (µg/L)	PEC/RAC
R1/stream	0.4807	1.44	0.2517	0.75	0.1599	0.48
R3/stream	0.5185	1.55	0.2720	0.81	0.2084	0.62
R4/stream	0.3220	0.96	-	-	-	-

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 runoff); DRT, drift-reduction technology (to mitigate drift); PEC, predicted environmental concentration; RAC, regulatory acceptable concentration.

For the intended use of CA3301 in winter cereals, acceptable risks to the metabolite prothioconazole-desthio can be demonstrated with the inclusion of the following mitigation for each FOCUS scenario:

FOCUS Scenario	Mitigation required to conclude an acceptable aquatic risk to prothioconazole-desthio for the intended uses of CA3301 in winter cereals
D3/ditch	-
D4/pond	-
D4/stream	-
D5/pond	-
D5/stream	-
R1/pond	-
R1/stream	Acceptable risk for two applications, with 20-m NSBZ + 20-m VFS, and for a single application, with 10-m NSBZ + 10-m VFS
R3/stream	Acceptable risk for two applications, with 20-m NSBZ + 20-m VFS, and for a single application, with 10-m NSBZ + 10-m VFS
R4/stream	Acceptable risk for two applications, with 10-m NSBZ + 10-m VFS

Dashes (-) indicate no required mitigation (acceptable risk at FOCUS Step 3). NSBZ, no-spray-buffer zone (to mitigate drift); VFS, vegetated filter strip (to mitigate runoff).

Table 9.5-6: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the metabolite prothioconazole-desthio for each organism group based on FOCUS Steps-1, -2, and -3 calculations for the use of CA3301 in spring cereals (worst-case scenario, for multiple and single applications of 200 g a.s./ha).

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Sed.-dwell. chronic	Algae
Test species		<i>Oncorhynchus mykiss</i>	<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Daphnia magna</i>	<i>Chironomus riparius</i>	<i>Scenedesmus subspicatus</i>
Endpoint* (µg metabolite/L)		LC ₅₀ 6630	NOEC 3.34	EC ₅₀ >10000	NOEC 100	NOEC 2000	E _r C ₅₀ 550
AF		100	10	100	10	10	10
RAC (µg metabolite/L)		66.3	0.334	>100	10	200	55
FOCUS Scenario	PEC _{gl-max} (µg/L)	PEC/RAC					
Step 1							
Worst case	78.36	1.18	235	<0.78	7.84	0.39	1.42
Step 2 – worst case							
N-Europe	5.93	0.09	17.75	-	0.59	-	0.11
S-Europe	10.81	0.16	32.37	-	1.08	-	0.20
Step 3 (Multiple applications)							
D3/ditch	0.1103	-	0.33	-	0.01	-	-
D4/pond	0.04453	-	0.13	-	<0.01	-	-
D4/stream	0.07733	-	0.23	-	0.01	-	-
D5/pond	0.04304	-	0.13	-	<0.01	-	-
D5/stream	0.1054	-	0.32	-	0.01	-	-
R4/stream	1.315	-	3.94	-	0.13	-	-
Step 3 (Single application)							
D3/ditch	0.1218	-	0.36	-	0.01	-	-
D4/pond	0.02803	-	0.08	-	<0.01	-	-

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Sed.-dwell. chronic	Algae
Test species		<i>Oncorhynchus mykiss</i>	<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Daphnia magna</i>	<i>Chironomus riparius</i>	<i>Scenedesmus subspicatus</i>
D4/stream	0.08232	-	0.25	-	0.01	-	-
D5/pond	0.02639	-	0.08	-	<0.01	-	-
D5/stream	0.1169	-	0.35	-	0.01	-	-
R4/stream	0.6792	-	2.03	-	0.07	-	-

PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**. *All values are EFSA-agreed endpoints (EFSA Sci. Report. 2007; 106, 1-98). AF, assessment factor; PEC, predicted environmental concentration; RAC, regulatory acceptable concentration.

An acceptable risk is not concluded for prothioconazole-desthio for all FOCUS Step-3 scenarios, for the intended uses of CA3301 in spring cereals. A further assessment is presented below, with consideration of risk-mitigation measures for the scenario that did not pass at FOCUS Step 3 (R4/stream).

Table 9.5-7: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prothioconazole-desthio for the critical organism group based on FOCUS Step-4 calculations for the use of CA3301 in spring cereals.

Substance Critical aquatic RAC	Prothioconazole-desthio 0.334 (chronic fish)					
FOCUS Step-4 scenario	Spring cereals (two applications)				Spring cereals (single application)	
	10-m NSBZ + 10-m VFS		20-m NSBZ + 20-m VFS		10-m NSBZ + 10-m VFS	
	PEC _{Sw} (µg/L)	PEC/RAC	PEC _{Sw} (µg/L)	PEC/RAC	PEC _{Sw} (µg/L)	PEC/RAC
R4/stream	0.5917	1.77	0.3087	0.92	0.3089	0.92

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 runoff); DRT, drift-reduction technology (to mitigate drift); PEC, predicted environmental concentration; RAC, regulatory acceptable concentration.

For the intended use of CA3301 in spring cereals, acceptable risks to the metabolite prothioconazole-desthio can be demonstrated with the inclusion of the following mitigation for each FOCUS scenario:

FOCUS Scenario	Mitigation required to conclude an acceptable aquatic risk to prothioconazole-desthio for the intended uses of CA3301 in spring cereals
D3/ditch	-
D4/pond	-
D4/stream	-
D5/pond	-
D5/stream	-
R1/pond	-
R1/stream	-
R3/stream	-
R4/stream	Acceptable risk for two applications, with 20-m NSBZ + 20-m VFS, and for a single application, with 10-m NSBZ + 10-m VFS

Dashes (-) indicate no required mitigation (acceptable risk at FOCUS Step 3). NSBZ, no-spray-buffer zone (to mitigate drift); VFS, vegetated filter strip (to mitigate runoff).

For the intended use of CA3301 in winter and spring cereals, acceptable risks to the metabolite prothioconazole-desthio can be demonstrated with the inclusion of the following mitigation for each FOCUS scenario:

FOCUS Scenario	Mitigation required to conclude an acceptable aquatic risk to prothioconazole-desthio for the intended uses	
	Winter cereals	Spring cereals
D3/ditch	-	-
D4/pond	-	-
D4/stream	-	-
D5/pond	-	-
D5/stream	-	-
R1/pond	-	-
R1/stream	Acceptable risk for two applications, with 20-m NSBZ + 20-m VFS, and for a single application, with 10-m NSBZ + 10-m VFS	-
R3/stream	Acceptable risk for two applications, with 20-m NSBZ + 20-m VFS, and for a single application, with 10-m NSBZ + 10-m VFS	-
R4/stream	Acceptable risk for two applications, with 10-m NSBZ + 10-m VFS	Acceptable risk for two applications, with 20-m NSBZ + 20-m VFS, and for a single application, with 10-m NSBZ + 10-m VFS

Dashes (-) indicate no required mitigation (acceptable risk at FOCUS Step 3). NSBZ, no-spray-buffer zone (to mitigate drift); VFS, vegetated filter strip (to mitigate runoff).

Table 9.5-8: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the metabolite prothioconazole-S-methyl for each organism group, based on FOCUS Step-1 calculations for the use of CA3301 in spring and winter cereals (worst-case, considering two applications of 200 g a.s./ha).

Group		Fish acute	Inverteb. acute	Algae
Test species		<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>P. subcapitata</i>
Endpoint* (µg metabolite/L)		LC ₅₀ 1790	EC ₅₀ 2800	ErC ₅₀ 47400
AF		100	100	10
RAC (µg metabolite/L)		17.9	28	4740
FOCUS Step-1 Scenario	PEC _{gl-max} (µg/L)	PEC/RAC		
Worst case	4.64	0.26	0.17	<0.01

PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**. *All values are EFSA-agreed endpoints (EFSA Sci. Report. 2007; 106, 1-98). #Surrogate toxicity endpoint derived by assuming that worst-case toxicity of prothioconazole-S-methyl is 10x greater than the parent. AF, assessment factor; PEC, predicted environmental concentration; RAC, regulatory acceptable concentration.

Table 9.5-9: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for 1,2,4-triazole for each organism group, based on FOCUS Step-1 calculations for the use of CA3301 in spring and winter cereals (worst-case, considering two applications of 200 g a.s./ha).

For the use of CRISPR in spring and winter cereals (worst case, considering two applications of 200 g a.i.s./ha).						
Group		Fish acute	Fish chronic	Inverteb. acute	Algae	
Test species		<i>Oncorhynchus mykiss</i>	<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>P. subcapitata</i>	
Endpoint* (µg metabolite/L)		LC ₅₀	EC ₅₀	EC ₅₀	E _r C ₅₀	
		498000	3200	900000	22500	
AF		100	10	100	10	
RAC (µg/L)		4980	320	9000	2250	
FOCUS Step-1 Scenario		PEC _{gl-max} (µg/L)	PEC/RAC			
Worst case		6.65	<0.01	0.02	<0.01	<0.01

PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**. *All values are EFSA-agreed endpoints (EFSA Sci. Report. 2007; 106, 1-98). AF, assessment factor; PEC, predicted environmental concentration; RAC, regulatory acceptable concentration.

For the intended uses of CA3301, calculated PEC/RAC ratios indicate an acceptable risk for aquatic organisms to the metabolites prothioconazole-S-methyl and 1,2,4-triazole using FOCUS Step 1 PEC_{sw} values. Therefore, no further assessments or mitigation are necessary for these metabolites.

Intended use of CA3301 in spring and winter oilseed rape (2 x 175 g a.s./ha, minimum 14-d interval; BBCH 14-69).

Worst-case PEC_{sw} values from the environmental fate section (see dRR B8, section 8.9) are used in the risk assessment shown below. The toxicity endpoints are worst case, coming either from active-substance studies or derived from the new product (CA3301) studies. In the tables below, the ratios between predicted environmental concentrations in surface water bodies (PEC_{sw}) and regulatory acceptable concentrations (RAC) for aquatic organisms are given per intended use for each relevant FOCUS scenario and each organism group.

Table 9.5-10: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prothioconazole for each organism group based on FOCUS Steps-1 and -2 calculations for the use of CA3301 in spring and winter oilseed rape.

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Macrophyte	Algae	Algae
Test species		<i>Oncorhynchus mykiss</i>	<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Daphnia magna</i>	<i>Lemna gibba</i>	<i>P. subcapitata</i>	<i>S. costatum</i>
Endpoint (µg/L)		LC ₅₀ *	NOEC*	EC ₅₀ [#]	NOEC*	E _r C ₅₀ [#]	E _r C ₅₀ *	E _r C ₅₀ [#]
		1000	308	280	560	288 >444	2180	84
AF		100	10	100	10	10	10	10
RAC (µg/L)		10	30.8	2.8	56	28.8 >44.4	218	8.4
FOCUS Scenario	PEC _{gl-max} (µg/L)	PEC/RAC						
Step 1								
Worst case	19.01	1.90	0.62	6.79	0.34	0.66 0.43	0.09	2.26
Step 2 – worst case								
N-Europe	1.61	0.16	-	0.58	-	-	-	0.19
S-Europe	1.61	0.16	-	0.58	-	-	-	0.19

PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**. *EFSA-agreed endpoints used (EFSA Sci. Report. 2007; 106, 1-98), when the a.s. value is lower than the a.s. value derived from the product study. [#]Endpoints derived from new studies with the proposed formulation CA3301 used, when a.s. value is lower than the EFSA-agreed endpoint. AF, assessment factor; PEC, predicted environmental concentration; RAC, regulatory acceptable concentration.

For the intended uses of CA3301, calculated PEC/RAC ratios for the active substance prothioconazole indicate an acceptable risk for all aquatic organism groups, using FOCUS Step-1 or -2 PEC_{sw} values. Therefore, no further assessment and no aquatic mitigation measures are necessary.

Table 9.5-11: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the metabolite prothioconazole-desthio for each organism group based on FOCUS Steps-1, -2, and -3 calculations for the use of CA3301 in winter oilseed rape.

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Sed.-dwell. chronic	Algae
Test species		<i>Oncorhynchus mykiss</i>	<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Daphnia magna</i>	<i>Chironomus riparius</i>	<i>P. subcapitata</i>
Endpoint* (µg metabolite/L)		LC ₅₀ 6630	NOEC 3.34	EC ₅₀ >10000	NOEC 100	NOEC 2000	E _r C ₅₀ 550
AF		100	10	100	10	10	10
RAC (µg metabolite/L)		66.3	0.334	>100	10	200	55
FOCUS Scenario	PEC _{gl-max} (µg/L)	PEC/RAC					
Step 1							
Worst case	68.56	1.03	205.27	<0.69	6.86	0.34	1.25
Step 2 – worst case							
N-Europe	6.23	0.09	18.65	-	0.62	-	0.11
S-Europe	5.16	0.08	15.45	-	0.52	-	0.09
Step 3 (autumn and spring applications)							
D3/ditch	0.1337	-	0.40	-	-	-	-
D4/pond	0.0229	-	0.07	-	-	-	-
D4/stream	0.07624	-	0.23	-	-	-	-
D5/pond	0.02225	-	0.07	-	-	-	-
D5/stream	0.1145	-	0.34	-	-	-	-
R1/pond	0.06451	-	0.19	-	-	-	-
R1/stream	0.4671	-	1.40	-	-	-	-
R3/stream	0.7388	-	2.21	-	-	-	-
Step 3 (single autumn application)							

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Sed.-dwell. chronic	Algae
Test species		<i>Oncorhynchus mykiss</i>	<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Daphnia magna</i>	<i>Chironomus riparius</i>	<i>P. subcapitata</i>
D3/ditch	0.1531	-	0.46	-	-	-	-
D4/pond	0.02426	-	0.07	-	-	-	-
D4/stream	0.08817	-	0.26	-	-	-	-
D5/pond	0.02535	-	0.08	-	-	-	-
D5/stream	0.1411	-	0.42	-	-	-	-
R1/pond	0.03322	-	0.10	-	-	-	-
R1/stream	0.2701	-	0.81	-	-	-	-
R3/stream	0.7388	-	2.21	-	-	-	-
Step 3 (two spring applications)							
D3/ditch	0.03673	-	0.11	-	-	-	-
D4/pond	0.02883	-	0.09	-	-	-	-
D4/stream	0.05739	-	0.17	-	-	-	-
D5/pond	0.03415	-	0.10	-	-	-	-
D5/stream	0.08374	-	0.25	-	-	-	-
R1/pond	0.08332	-	0.25	-	-	-	-
R1/stream	0.5814	-	1.74	-	-	-	-
R3/stream	0.4811	-	1.44	-	-	-	-
Step 3 (single spring application only)							
D3/ditch	0.03272	-	0.10	-	-	-	-
D4/pond	0.0186	-	0.06	-	-	-	-
D4/stream	0.06637	-	0.20	-	-	-	-
D5/pond	0.0204	-	0.06	-	-	-	-

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Sed.-dwell. chronic	Algae
Test species		<i>Oncorhynchus mykiss</i>	<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Daphnia magna</i>	<i>Chironomus riparius</i>	<i>P. subcapitata</i>
D5/stream	0.07887	-	0.24	-	-	-	-
R1/pond	0.04588	-	0.14	-	-	-	-
R1/stream	0.2787	-	0.83	-	-	-	-
R3/stream	0.4811	-	1.44	-	-	-	-

PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**. *All values are EFSA-agreed endpoints (EFSA Sci. Report. 2007; 106, 1-98). AF, assessment factor; PEC, predicted environmental concentration; RAC, regulatory acceptable concentration; NSBZ, no-spray-buffer zone (to mitigate drift); VFS, vegetated filter strip (to mitigate runoff); DRT, drift-reduction technology (to mitigate drift).

An acceptable risk is not concluded for prothioconazole-desthio for all FOCUS Step-3 scenarios, for the intended uses of CA3301 in winter oilseed rape. Further assessments, with risk-mitigation measures for the scenarios that did not pass at FOCUS Step 3 are shown below.

Table 9.5-12: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prothioconazole-desthio for the critical organism group based on FOCUS Step-4 calculations for the use of CA3301 in winter oilseed rape.

Substance	Prothioconazole-desthio							
Critical aquatic RAC	0.334 µg metabolite/L (chronic fish)							
FOCUS Step-4 scenario	Autumn and spring applications				Single autumn application			
	10-m NSBZ + 10-m VFS		20-m NSBZ + 20-m VFS		10-m NSBZ + 10-m VFS		20-m NSBZ + 20-m VFS	
	PEC _{sw} (µg/L)	PEC/RAC	PEC _{sw} (µg/L)	PEC/RAC	PEC _{sw} (µg/L)	PEC/RAC	PEC _{sw} (µg/L)	PEC/RAC
R1/stream	0.2123	0.64	-	-	-	-	-	-
R3/stream	0.3362	1.01	0.1762	0.53	0.3362	1.01	0.1762	0.53
FOCUS Step-4 scenario	Two spring applications				Single spring application			
	10-m NSBZ + 10-m VFS		20-m NSBZ + 20-m VFS		10-m NSBZ + 10-m VFS		20-m NSBZ + 20-m VFS	
	PEC _{sw} (µg/L)	PEC/RAC	PEC _{sw} (µg/L)	PEC/RAC	PEC _{sw} (µg/L)	PEC/RAC	PEC _{sw} (µg/L)	PEC/RAC
R1/stream	0.2637	0.79	-	-	-	-	-	-
R3/stream	0.2126	0.64	-	-	0.2126	0.64	-	-

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 runoff); DRT, drift-reduction technology (to mitigate drift); PEC, predicted environmental concentration; RAC, regulatory acceptable concentration.

For the intended use of CA3301 in winter oilseed rape, acceptable risks to the metabolite prothioconazole-desthio can be demonstrated with the inclusion of the following mitigation for each FOCUS scenario:

FOCUS Scenario	Mitigation required to conclude an acceptable aquatic risk to prothioconazole-desthio for the intended uses of CA3301 in winter oilseed rape
D3/ditch	-
D4/pond	-
D4/stream	-
D5/pond	-
D5/stream	-
R1/pond	-
R1/stream	Acceptable risk for all multiple application scenarios applications, with 10-m NSBZ + 10-m VFS
R3/stream	Acceptable risk for autumn & spring application and single autumn application, with 20-m NSBZ + 20-m VFS, and for single spring and two spring applications, with 10-m NSBZ + 10-m VFS

Dashes (-) indicate no required mitigation (acceptable risk at FOCUS Step 3). NSBZ, no-spray-buffer zone (to mitigate drift); VFS, vegetated filter strip (to mitigate runoff).

Table 9.5-13: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prothioconazole-desthio for each organism group based on FOCUS Steps-1, -2, and -3 calculations for the use of CA3301 in spring oilseed rape.

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Sed.-dwell. chronic	Algae
Test species		<i>Oncorhynchus mykiss</i>	<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Daphnia magna</i>	<i>Chironomus riparius</i>	<i>P. subcapitata</i>
Endpoint* (µg metabolite/L)		LC ₅₀ 6630	NOEC 3.34	EC ₅₀ >10000	NOEC 100	NOEC 2000	E _r C ₅₀ 550
AF		100	10	100	10	10	10
RAC (µg metabolite/L)		66.3	0.334	>100	10	200	55
FOCUS Scenario	PEC _{gl-max} (µg/L)	PEC/RAC					
Step 1							
Worst case	68.56	1.03	205.27	<0.69	6.86	0.34	1.25
Step 2 – worst case							
N-Europe	2.53	0.04	7.57	-	0.25	-	0.05
S-Europe	4.12	0.06	12.34	-	0.41	-	0.07
Step 3 (two applications) [#]							
D3/ditch	0.1009	-	0.30	-	-	-	-
D4/pond	0.0389	-	0.12	-	-	-	-
D4/stream	0.06898	-	0.21	-	-	-	-
D5/pond	0.03767	-	0.11	-	-	-	-
D5/stream	0.09166	-	0.27	-	-	-	-
R1/pond	0.1229	-	0.37	-	-	-	-
R1/stream	1.073	-	3.21	-	-	-	-
Step 3 (single application)							
D3/ditch	0.1115	-	0.33	-	-	-	-
D4/pond	0.02449	-	0.07	-	-	-	-

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Sed.-dwell. chronic	Algae
Test species		<i>Oncorhynchus mykiss</i>	<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Daphnia magna</i>	<i>Chironomus riparius</i>	<i>P. subcapitata</i>
D4/stream	0.07228	-	0.22	-	-	-	-
D5/pond	0.02225	-	0.07	-	-	-	-
D5/stream	0.09664	-	0.29	-	-	-	-
R1/pond	0.05223	-	0.16	-	-	-	-
R1/stream	0.4081	-	1.22	-	-	-	-

PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**. *All values are EFSA-agreed endpoints (EFSA Sci. Report. 2007; 106, 1-98). #Given that the risk-envelope approach is applied, the PEC values can be used as surrogates for the intended uses of CA3301 in flax and in mustard, cameline, and other seed-producing Brassicaceae. AF, assessment factor; PEC, predicted environmental concentration; RAC, regulatory acceptable concentration; NSBZ, no-spray-buffer zone (to mitigate drift); VFS, vegetated filter strip (to mitigate runoff); DRT, drift-reduction technology (to mitigate drift).

An acceptable risk is not concluded for the metabolite prothioconazole-desthio for all FOCUS Step-3 scenarios, for the intended uses of CA3301 in spring oilseed rape. Further assessments, with risk-mitigation measures for the scenario that did not pass at FOCUS Step 3 (R1/stream), are shown below.

Table 9.5-14: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prothioconazole-desthio for the critical organism group based on FOCUS Step-4 calculations for the use of CA3301 in spring oilseed rape.

Substance	Prothioconazole-desthio							
Critical aquatic RAC	0.334 µg metabolite/L (chronic fish)							
FOCUS Step-4 scenario	Two applications [#]				Single application			
	10-m NSBZ + 10-m VFS		20-m NSBZ + 20-m VFS		10-m NSBZ + 10-m VFS		20-m NSBZ + 20-m VFS	
	PEC _{sw} (µg/L)	PEC/RAC	PEC _{sw} (µg/L)	PEC/RAC	PEC _{sw} (µg/L)	PEC/RAC	PEC _{sw} (µg/L)	PEC/RAC
R1/stream	0.4871	1.46	0.255	0.76	0.1852	0.55	-	-

PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**. #Given that the risk-envelope approach is applied, the PEC values can be used as surrogates for the intended uses of CA3301 in flax and in mustard, cameline, and other seed-producing Brassicaceae. NSBZ, no-spray-buffer zone (to mitigate drift); VFS, vegetated filter strip (to mitigate runoff); DRT, drift-reduction technology (to mitigate drift); PEC, predicted environmental concentration; RAC, regulatory acceptable concentration.

For the intended use of CA3301 in spring oilseed rape, acceptable risks to the metabolite prothioconazole-desthio can be demonstrated with the inclusion of the following mitigation for each FOCUS scenario:

FOCUS Scenario	Mitigation required to conclude an acceptable aquatic risk to prothioconazole-desthio for the intended uses of CA3301 in spring oilseed rape (1-2 spring application with 14 d interval)
D3/ditch	-
D4/pond	-
D4/stream	-
D5/pond	-
D5/stream	-
R1/pond	-
R1/stream	Acceptable risk for two applications*, with 20-m NSBZ + 20-m VFS, and for a single application, with 10-m NSBZ + 10-m VFS

Dashes (-) indicate no required mitigation (acceptable risk at FOCUS Step 3). *Given that the risk-envelope approach is applied, these mitigation measures can be used as surrogates for the intended uses of CA3301 in flax (Use no. 12) and in mustard, cameline, and other seed-producing Brassicaceae. NSBZ, no-spray-buffer zone (to mitigate drift); VFS, vegetated filter strip (to mitigate runoff).

Based on the currently available data, an acceptable aquatic risk to the metabolite prothioconazole-desthio can be demonstrated with appropriate mitigation for the R1/stream and R3/stream scenarios.

For the intended use of CA3301 in winter and spring oilseed rape, acceptable risks to the metabolite prothioconazole-desthio can be demonstrated with the inclusion of the following mitigation for each FOCUS scenario:

FOCUS Scenario	Mitigation required to conclude an acceptable aquatic risk to prothioconazole-desthio for the intended uses	
	Winter oilseed rape	Spring oilseed rape
D3/ditch	-	-
D4/pond	-	-
D4/stream	-	-
D5/pond	-	-
D5/stream	-	-
R1/pond	-	-
R1/stream	Acceptable risk for all multiple application scenarios applications, with 10-m NSBZ + 10-m VFS**	Acceptable risk for two applications*, with 20-m NSBZ + 20-m VFS, and for a single application, with 10-m NSBZ + 10-m VFS
R3/stream	Acceptable risk for autumn & spring application and single autumn application, with 20-m NSBZ + 20-m VFS, and for single spring and two spring applications, with 10-m NSBZ + 10-m VFS **	-

Dashes (-) indicate no required mitigation (acceptable risk at FOCUS Step 3). *Given that the risk-envelope approach is applied, these mitigation measures can be used as surrogates for the intended uses of CA3301 in flax (two spring applications) and **surrogates for the intended uses of CA3301 in mustard, cameline, and other seed-producing Brassicaceae (autumn & spring application). NSBZ, no-spray-buffer zone (to mitigate drift); VFS, vegetated filter strip (to mitigate runoff).

Table 9.5-15: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the metabolite prothioconazole-S-methyl for each organism group, based on FOCUS Step-1 calculations for the use of CA3301 in spring and winter oilseed rape.

Group		Fish acute	Inverteb. acute	Algae
Test species		<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Pseudokirchneriella subcapitata</i>
Endpoint* (µg metabolite/L)		LC ₅₀ 1790	EC ₅₀ 2800	E _r C ₅₀ 47400
AF		100	100	10
RAC (µg metabolite/L)		17.9	28	4740
FOCUS Step-1 Scenario	PEC _{gl-max} (µg/L)	PEC/RAC		
Worst case	4.06	0.23	0.15	<0.01

PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**. *All values are EFSA-agreed endpoints (EFSA Sci. Report. 2007; 106, 1-98). AF, assessment factor; PEC, predicted environmental concentration; RAC, regulatory acceptable concentration.

Table 9.5-16: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the metabolite 1,2,4-triazole for each organism group, based on FOCUS Step-1 calculations for the use of CA3301 in spring and winter oilseed rape.

Group			Fish acute	Fish chronic	Inverteb. acute	Algae
Test species			<i>Oncorhynchus mykiss</i>	<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>P. subcapitata</i>
Endpoint* (µg metabolite/L)			LC ₅₀ 498000	EC ₅₀ 3200	EC ₅₀ 900000	ErC ₅₀ 22500
AF			100	10	100	10
RAC (µg metabolite/L)			4980	320	9000	2250
FOCUS Step-1 Scenario	PEC _{gl-max} (µg/L)	PEC/RAC				
Worst case	5.82		<0.01	0.02	<0.01	<0.01

PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**. *All values are EFSA-agreed endpoints (EFSA Sci. Report. 2007; 106, 1-98). AF, assessment factor; PEC, predicted environmental concentration; RAC, regulatory acceptable concentration.

For the intended uses of CA3301 on spring and winter oilseed rape, the calculated PEC/RAC ratios indicate an acceptable risk for aquatic organisms exposed to the metabolites prothioconazole-S-methyl and 1,2,4-triazole in FOCUS Step 1. Therefore, no further assessments or mitigation measures are necessary.

9.5.3 Overall conclusions

An acceptable risk is concluded for all aquatic organism groups using FOCUS Step-1 or -2 PEC_{sw} values, for the intended uses of CA3301 in cereals and oilseed rape, for the active substance prothioconazole and the metabolites prothioconazole-S-methyl and 1,2,4-triazole.

The critical area of the aquatic risk assessment is for the metabolite prothioconazole-desthio, with the risk driven by the critical chronic fish RAC_{sw} value of 0.334 µg/L. FOCUS Step-4 PEC_{sw} modelling is required for this metabolite, with consideration of risk-mitigation measures.

Step-4 mitigation required for use on cereals and oilseed rape

In conclusion, based on the currently available data, an acceptable aquatic risk to the metabolite prothioconazole-desthio can be demonstrated for all relevant FOCUS scenarios, following the intended use of CA3301 in cereals and oilseed rape considering the following mitigation measures:

Mitigation required to conclude an acceptable aquatic risk to prothioconazole-desthio for the intended uses in:		
FOCUS Scenario	Cereals	
	Winter	Spring
D3/ditch	-	-
D4/pond	-	-
D4/stream	-	-
D5/pond	-	-
D5/stream	-	-
D6/stream	-	-
R1/pond	-	-
R1/stream	Acceptable risk for two applications, with 20-m NSBZ + 20-m VFS, and for a single application, with 10-m NSBZ + 10-m VFS	-
R3/stream	Acceptable risk for two applications, with 20-m NSBZ + 20-m VFS, and for a single application, with 10-m NSBZ + 10-m VFS	-
R4/stream	Acceptable risk for two applications, with 10-m NSBZ + 10-m VFS	Acceptable risk for two applications, with 20-m NSBZ + 20-m VFS, and for a single application, with 10-m NSBZ + 10-m VFS
FOCUS Scenario	Oilseed rape	
	Winter	Spring
D3/ditch	-	-
D4/pond	-	-
D4/stream	-	-
D5/pond	-	-
D5/stream	-	-
R1/pond	-	-
R1/stream	Acceptable risk for all multiple application scenarios applications, with 10-m NSBZ + 10-m VFS	Acceptable risk for two applications*, with 20-m NSBZ + 20-m VFS, and for a single application, with 10-m NSBZ + 10-m VFS
R3/stream	Acceptable risk for autumn & spring application and single autumn application, with 20-m NSBZ + 20-m VFS, and for single spring and two spring applications, with 10-m NSBZ + 10-m VFS	-

Dashes (-) indicate no required mitigation (acceptable risk at FOCUS Step 3). *Given that the risk-envelope approach is applied, these mitigation measures can be used as surrogates for the intended uses of CA3301 in flax and in mustard, cameline, and other seed-producing Brassicaceae. NSBZ, no-spray-buffer zone (to mitigate drift); VFS, vegetated filter strip (to mitigate runoff).

Review Comments:

The submitted risk assessment has been accepted.

The relevant predicted environmental concentrations in water (PEC_{sw}) for risk assessments covering the proposed use pattern are taken from Part B Section 8 (Environmental Fate). The initial risk assessment was based on the worst case PEC_{sw} values and the results of laboratory toxicity testing. The PEC_{sw} Step 1-2 (for prothioconazole, the metabolites prothioconazole-S-methyl, 1,2,4-triazole and for formulation) and Step 3 and 4 (for the metabolite prothioconazole-desthio) were used.

CA3301 (JOUST) applications close to surface water pose acceptable risk to aquatic organisms with appropriate mitigation measures (no-spray buffer zones and vegetative buffer strips).

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.

9.6.1 Toxicity data

Studies on the toxicity to bees have been carried out with prothioconazole. Full details of these studies are provided in the respective EU DAR and related documents.

The effects on bees of CA3301 were not evaluated as part of the EU assessment of prothioconazole. 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
Active substance				
<i>Apis mellifera</i> (honey bee)	Prothioconazole	Acute oral, 48 h	LD ₅₀ > 71 µg a.s./bee	EFSA Sci Report. 2007 106, 1-98
		Acute contact, 48 h	LD ₅₀ > 200 µg a.s./bee	
Product – CA3301				
<i>Apis mellifera</i> (honey bee)	CA3301	Acute oral, 48 h	LD₅₀ = 109.11 µg a.s./bee , equivalent to 427.7 µg product/bee	KCP 10.3.1.1/01
		Acute contact, 48 h	LD₅₀ = 20.43 µg a.s./bee , equivalent to 80.1 µg product/bee	
<i>Bombus terrestris</i> (bumble bee)		Acute oral, 48 h	LD₅₀ > 200 µg a.s./bee , equivalent to >782.17 µg product/bee	KCP 10.3.1.1/02
		Acute contact, 48 h	LD₅₀ > 100 µg a.s./bee , equivalent to >391.08 µg product/bee	
<i>Osmia bicornis</i> (solitary bee)		Acute contact, 96 h	LD ₅₀ = 11.63 µg a.s./bee, equivalent to 45.21 µg product/bee	KCP 10.3.1.1/03
<i>Apis mellifera</i> (honey bee)		Chronic oral, 10 d	LDD₅₀ = 13.82 µg a.s./bee/day , equivalent to 54.05 µg product/bee/day	KCP 10.3.1.2/01
		Larval, 22 d	NOED = 10.0 µg a.s./larva/dev. per. , equivalent to 39.11 µg product/larva/dev. per. ED ₁₀ > 10.0 µg a.s./larva/dev. per., equivalent to >39.11 µg product/larva/dev. per.	KCP 10.3.1.3/01
		Semi-field tunnel, <i>Phacelia</i>	Two applications of 200 g a.s./ha:	KCP 10.3.1.5/02

Species	Substance	Exposure System	Results	Reference
		<i>tanacetifolia</i> , 14-day interval between applications	- No effect on adult mortality, foraging activity, and behaviour.	Note to RMS: these are interim results, which will be updated when possible. The study was stopped, due to poor weather conditions and will recommence in 2022.
EC 250 formulation				
<i>Apis mellifera</i> (honey bee)	EC 250 formulation	Acute oral, 48 h	LD ₅₀ > 48.7 µg a.s./bee	EFSA Sci. Report. 2007; 106, 1-98
		Acute contact, 48 h	LD ₅₀ > 200 µg a.s./bee	
	Prothioconazole EC 250 G	Semi-field tunnel; <i>Phacelia tanacetifolia</i> ; Assessment of effects for 26 d post-treatment	Target application of 187.5 g a.s./ha; (measured application 199.2 g a.s./ha) —No effect on adult mortality, flight intensity, and behaviour. —No effect on pupal mortality. —No effect on amount of brood and brood development. —No adverse effects on colony strength (mean number of bees per colony). —Overall conclusion from the RMS Poland: No treatment related effects were shown for any measured parameter in this study at the measured concentration 199.2 g prothioconazole/ha.	2018 RAR, prothioconazole, (KCA 8.3.1.3 /02; Hein, R., 2015) KCP 10.3.1.5/01 Note to RMS: this study is currently under review in the EU approval renewal process for prothioconazole.

Values in **bold** are used in the risk assessments.

9.6.1.1 Justification for new endpoints

New studies have been provided with CA3301 – three acute oral and contact studies on honey bee, bumblebee, and solitary bee (KCP 10.3.1.1/01, KCP 10.3.1.1/02, and KCP 10.3.1.1/03) and three chronic studies – one chronic oral test with adults, one chronic test with larval honey bees, and one higher-tier, semi-field tunnel test (KCP 10.3.1.2/01, KCP 10.3.1.3/01, and the KCP 10.3.1.5/02, respectively). The studies are submitted to satisfy new data requirements under Regulation (EU) No 284/2013. In addition, a further study (KCP 10.3.1.5/01) is a protected study to which the Sponsor, Nufarm, has been given access, and is submitted as a higher-tier study (semi-field tunnel test). It is noted that the Bayer study is currently being considered as part of the EU approval renewal process for prothioconazole. A full summary of these new studies is provided in Appendix 2, points A 2.3.1.1.1, A 2.3.1.1.2, A 2.3.1.2, A 2.3.1.3, and A 2.3.1.5.

9.6.2 Risk assessment

In the first instance, hazard quotients are calculated for the acute risks to honey and bumble bees, in accordance with SANCO/10329/2002. For completeness, the evaluation of the risk for bees was performed in accordance with the recommendations of the un-noted EFSA bee guidance (EFSA Journal 2013:11(7):3295; hereafter referred to as EFSA/2013/3295).

As test methods for acute solitary bee testing and for chronic oral and larval testing of bumble and solitary bees are not yet developed and regarded as long-term research projects (EC, 2014), the current 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)

Areas where much uncertainty in the approach still exist (e.g., HPG assessment) have not been addressed. Solitary bee data are available, but, as no adopted OECD guidelines for solitary bee testing exist, data need to be taken as provisionally and are associated with some uncertainties regarding the methodology and are presented as additional information only.

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 200 g a.s./ha) also covers the risk for bees from all other intended uses in the oilseed rape crop group.

9.6.2.1 Hazard quotients and risk assessments for bees

Table 9.6-2: First-tier assessment of the acute contact risk to adult honey bees and bumble bees, due to the use of CA3301 at 200 g a.s./ha (in accordance with SANCO/10329/2002) – prothioconazole.

Intended use		Cereals and oilseed rape			
Active substance		Prothioconazole			
Application rate (g a.s. /ha)		2 x 200 g a.s./ha (14-d interval); BBCH 14-69			
Species	Type of assessment	LD ₅₀ (µg/bee)	Single application rate (g/ha)	HQ*	Acceptable if HQ is:
<i>Apis mellifera</i>	Acute contact exposure	20.43	200	9.8	≤50
<i>Bombus terrestris</i>		>100		<2.0	
<i>Apis mellifera</i>	Acute oral exposure	109.11		1.83	
<i>Bombus terrestris</i>		>200		<1	

*HQ values calculated in accordance with SANCO/10329/2002.

The hazard quotients (HQ) values are well below the trigger of 50, indicating an acceptable acute oral and contact risk to honey and bumble bees, following the proposed use of CA3301 in cereals and oilseed rape.

Table 9.6-3: Screening assessment of the acute contact risk to adult bees, due to the use of CA3301 at 200 g a.s./ha (in accordance with EFSA/2013/3295) – prothioconazole.

Intended use		Cereals and oilseed rape			
Active substance		Prothioconazole			
Application rate (g a.s. /ha)		2 x 200 g a.s./ha (14-d interval); BBCH 14-69			
Species	Type of assessment	LD ₅₀ (µg/bee)	Single application rate (g/ha)	HQ	Trigger value for downward spray. Acceptable if HQ is:
<i>Apis mellifera</i>	Acute contact exposure	20.43	200	9.8	≤42
<i>Bombus terrestris</i>		>100		<2.0	≤7

An acceptable acute contact risk to honey bees and bumblebees for prothioconazole is concluded at the screening step, with a large margin of safety, for the intended uses of CA3301, at a maximum, single application rate of 200 g a.s./ha in cereals and oilseed rape.

Table 9.6-5: Screening assessment of the oral risk to bees, due to the use of CA3301 maximum application rate (in accordance with EFSA/2013/3295) – prothioconazole.

Intended use		Cereals and oilseed rape				
Active substance		Prothioconazole				
Application rate (g a.s./ha)		2 x 200 g a.s./ha (14-d interval); BBCH 14-69				
Species	Type of assessment	LD ₅₀ (µg/bee)	Single application rate (g/ha)	EF x SV	ETR	Trigger value for downward spray. Acceptable if HQ is:
<i>Apis mellifera</i>	Acute oral	109.11	200	7.6	0.01	0.2
<i>Bombus terrestris</i>		>200		11.2	<0.01	0.036
<i>Apis mellifera</i>	Chronic adult	13.82		7.6	0.11	0.03
	Chronic larval	10.0		4.4	0.09	0.2

ETR values in **bold** are above the trigger.

Acceptable acute oral and larval risks to honey bees and acute oral risk to bumblebees, for prothioconazole, are concluded at the screening step, with large margins of safety, for the intended uses of CA3301, at a maximum application rate of 200 g a.s./ha. However, in the case of the chronic oral exposure to adult honey bees, the risk is not acceptable at the screening step and a first-tier assessment is presented in Tables 9.6-6 and 9.6-7 below.

Table 9.6-6: First-tier assessment of the chronic oral risks to adult honey bees, due to the use of CA3301 in cereals (in accordance with EFSA/2013/3295) – prothioconazole.

Intended use		Cereals						
Active substance		Prothioconazole						
Application rate (g/ha)		2 x 200 g a.s./ha (14-d interval); BBCH 30-69						
Scenario	BBCH	LDD ₅₀ (µg a.s./bee)	EF	SV (downward spray)	TWA	ETR _{oral}	Trigger	Acceptable risk?
Treated crop	30 - 39	13.82	1	0.92	0.72	0.010	0.03	Yes
	40 - 69		1	0.92	0.72	0.010	0.03	Yes
Weeds	30 - 39	13.82	0.5	2.9	0.72	0.015	0.03	Yes
	40 - 69		0.3	2.9	0.72	0.009	0.03	Yes
Field margin	30 - 39	13.82	0.0092	2.9	0.72	0.0003	0.03	Yes
	40 - 69		0.0092	2.9	0.72	0.0003	0.03	Yes
Adjacent crop	30 - 39	13.82	0.0033	5.8	0.72	0.0002	0.03	Yes
	40 - 69		0.0033	5.8	0.72	0.0002	0.03	Yes
Next crop	30 - 39	13.82	1	0.54	0.72	0.006	0.03	Yes
	40 - 69		1	0.54	0.72	0.006	0.03	Yes

Table 9.6-7: First-tier assessment of the chronic oral risks to adult honey bees, due to the use of CA3301 in oilseed rape (in accordance with EFSA/2013/3295) – prothioconazole.

Intended use		Oilseed rape						
Active substance		Prothioconazole						
Application rate (g/ha)		2 x 175 g a.s./ha (14-d interval); BBCH 14-69						
Scenario	BBCH	LDD ₅₀ (µg a.s./bee)	EF	SV (downward spray)	TWA	ETR _{oral}	Trigger	Acceptable risk?
Treated crop	10 - 29	13.82	1	5.8	0.72	0.053	0.03	No
	30 - 39		1	5.8	0.72	0.053	0.03	No
	40 - 69		1	5.8	0.72	0.053	0.03	No
Weeds	10 - 29	13.82	1	5.8	0.72	0.026	0.03	Yes
	30 - 39		0.3	5.8	0.72	0.008	0.03	Yes
	40 - 69		0.25	5.8	0.72	0.007	0.03	Yes
Field margin	10 - 29	13.82	0.0092	5.8	0.72	<0.001	0.03	Yes
	30 - 39		0.0092	5.8	0.72	<0.001	0.03	Yes
	40 - 69		0.0092	5.8	0.72	<0.001	0.03	Yes
Adjacent crop	10 - 29	13.82	0.0033	5.8	0.72	<0.001	0.03	Yes
	30 - 39		0.0033	5.8	0.72	<0.001	0.03	Yes
	40 - 69		0.0033	5.8	0.72	<0.001	0.03	Yes
Next crop	10 - 29	13.82	1	5.8	0.72	0.005	0.03	Yes
	30 - 39		1	5.8	0.72	0.005	0.03	Yes
	40 - 69		1	5.8	0.72	0.005	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 acceptable risk was shown for all relevant scenarios and BBCH growth stages, except for the “treated crop” scenario. Therefore, further refinement is required. Oilseed rape is likely to be attractive to bees when flowering and requires pollination to propagate¹. However, since oilseed rape only flowers at growth stages of BBCH 60-69², it is unlikely

¹Attractiveness of Agricultural Crops to Pollinating Bees for the Collection of Nectar and/or Pollen. 2017. USDA

²Growth stages of mono- and dicotyledonous plants. BBCH Monograph, 2001. 2nd Ed, Uwe Meier, Federal Biological Research Centre for Agriculture and Forestry

that bees will be at significant risk in the “treated crop” scenarios for BBCH <30 and 30-39. In these cases, ETR values will be much lower, as likely exposure will be minimal when oilseed rape is not flowering. Consequently, a significant unacceptable chronic risk to bees remains only in the “treated crop” scenario for BBCH ≥ 40 , and further risk assessment is required (see section 9.6.2.2).

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 semi-field tunnel study is available for the representative EU EC formulation (Prothioconazole EC 250 G), which applied prothioconazole at a rate of 199.2 g a.s./ha, on full-flowering *Phacelia tanacetifolia*, during honey-bee foraging activity. This study was conducted to demonstrate the safe use of prothioconazole in an ecologically relevant setting and period of time (26 days postexposure).

Full details of this study are provided in Appendix 2 (point A 2.3.1.5, study 1, KCP 10.3.1.5).

The effects on honey bee colonies, under confined conditions, considering mortality, flight intensity, behaviour, colony strength, amount of brood, and brood cell development, were evaluated. Prothioconazole had no adverse effects on adult mortality, flight intensity, and behaviour, as well as no biologically relevant effect on pupal mortality. The quantitative assessments of brood development in individually marked cells containing eggs did not result in statistically significant differences on honey bee brood development. No product-related adverse effects on colony strength (mean number of bees per colony), amount of brood (mean number of cells covered with the different types of brood), or on the development of the food storage area were observed.

An additional study with CA3301 – the relevant formulation of this submission – was initiated (applied at 2 x 200 g a.s./ha, 12-d interval, to flowering *Phacelia tanacetifolia*). The interim results for this semi-field tunnel study with CA3301 (KCP 10.3.1.5 Cage and tunnel tests, Study 2) show that there were no significant differences in mortality between the control and product treatment groups, after the second application of treatment. Mean adult honey bee mortality during the exposure phase, from 0DAA to 7DAA, was 110.0 ± 59.4 and 118.9 ± 49.0 dead bees/colony/day, in the control and product treatment group, respectively. During the monitoring phase, from 8DAA to 15DAA, honey bee mortality was 14.5 ± 7.6 and 17.5 ± 7.9 dead bees/colony/day, in the control and product treatment group, respectively. Additionally, there were no product-related treatment effects on honey bee foraging activity and behaviour.

The study performed with the representative EU formulation (Prothioconazole EC 250 G) that demonstrated no significant effects, following application of 199.2 g a.s./ha, can be extrapolated to support the proposed use of CA3301. The interim results for new the semi-field study with CA3301, which had to be terminated during 2021, due to poor weather conditions, clearly demonstrated no significant effects on honey bee mortality, behaviour, and flight activity, following two applications (200 g a.s. ha, with a 14-day interval between applications) despite unfavorable weather conditions. Together these data confirm that no significant effects on honey bees or other bees are anticipated from the proposed use of CA3301. Therefore, no further assessment is required.

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 CA3301. As adopted OECD guidelines for bumble bees exist, the risk assessment was conducted above, in Tables 9.6-2 to 9.6-7. For full details of the acute toxicity study results, refer to Appendix 2, section A 2.3.1.1.1, KCP 10.3.1.1, Study 2 (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. Nonetheless, acute contact toxicity to the mason bee species *Osmia bicornis* has been tested for CA3301. As no adopted OECD test guidelines for solitary bees exist so far, data need to be taken as provisionally and are associated with some uncertainties regarding the methodology and are therefore provided as additional information only. For full details of the acute toxicity study results, refer to Appendix 2, section A 2.3.1.1.1, KCP 10.3.1.1, Study 3 (KCP 10.3.1.1/03).

9.6.5 Overall conclusions

The first-tier risk assessment, conducted in accordance with SANCO/10329/2002, indicated acceptable acute contact and oral risks to honey and bumble bees. The screening risk assessment, conducted according to EFSA/2013/3295, indicated acceptable acute oral and contact risks to honey bees and bumble bees and acceptable chronic oral risks to honey bee larvae, but not to adult honey bees. 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, an acceptable chronic oral risk was demonstrated for all intended BBCH stages in weeds, field margin, adjacent crop, and succeeding crop scenarios. However, the ETR_{oral} values are above the trigger values for the “treated crop” scenario (all intended BBCH stages). However, the available semi-field tunnel data show no significant effects of prothioconazole on adult, pupal, and larval honey bees. Therefore, the higher-tier data demonstrates an acceptable risk to bees from the proposed use of CA3301.

Review Comments:

The evaluation of the acute risk for bees was performed in accordance with the recommendations of the “Guidance Document on Terrestrial Ecotoxicology”, as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002). The submitted risk assessment, based on laboratory studies, has been accepted. It can therefore be concluded that there will be negligible acute risk associated with the exposure of *Apis mellifera* and *Bombus terrestris* to CA3301.

The data requirements in accordance with Commission Regulation (EU) No 284/2013 for the chronic toxicity to adult honeybees and honeybee larvae are fulfilled.

The risk assessment based on the EFSA Guidance (2013) is not yet approved and certain parts are currently under revision. As there is not harmonized approach for the chronic risk assessment for bees, therefore, Concerned Member States must decide on the acceptability of EFSA Guidance (2013) on national level.

9.7 Effects on arthropods other than bees (KCP 10.3.2)

9.7.1 Toxicity data

Studies on the toxicity to non-target arthropods have been carried out with prothioconazole. Full details of these studies are provided in the respective EU DAR and related documents, as well as in Appendix 2 of this document (new studies).

Effects on non-target arthropods of CA3301 were not evaluated as part of the EU assessment of prothioconazole. 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
Standard laboratory tests				
<i>Typhlodromus pyri</i>	CA3301	Laboratory test, glass plates (2D)	LR₅₀ = 52.3 g a.s./ha ER₅₀ >37.5 g a.s./ha	KCP 10.3.2/01
<i>Aphidius rhopalosiphi</i>		Laboratory test, glass plates (2D)	LR₅₀ = 14.2 g a.s./ha ER₅₀ 10.8 g a.s./ha	KCP 10.3.2/02
Extended laboratory tests				
<i>Typhlodromus pyri</i>	CA3301	Extended laboratory test, pepper-plant leaves (2D)	LR₅₀ = 219.14 g a.s./ha Corrected mortality: 20% at 133 g a.s./ha 49.1% at 216 g a.s./ha 80.6% at 346 g a.s./ha 95.4% at 555 g a.s./ha 81.5% at 885 g a.s./ha ER₅₀ > 216.2 g a.s./ha Red. of reproduction: 20% at 133 g a.s./ha -39.5% at 216 g a.s./ha	KCP 10.3.2/03
<i>Aphidius rhopalosiphi</i>		Extended laboratory test, barley seedlings (3D)	LR₅₀ > 864.9 g a.s./ha Corrected mortality: 4.8% at 23 g a.s./ha -3.44% at 56 g a.s./ha 20.7% at 140 g a.s./ha 0.7% at 346 g a.s./ha 24.1% at 865 g a.s./ha ER₅₀ > 864.9 g a.s./ha Red. of reproduction: 8.3% at 23 g a.s./ha 4.2% at 56 g a.s./ha 12.2% at 140 g a.s./ha 4.0% at 346 g a.s./ha 5.6% at 865 g a.s./ha	KCP 10.3.2/04
<i>Chrysoperla carnea</i>		Extended laboratory test, pepper-plant leaves (2D)	LR₅₀ = 406.98 g a.s./ha Corrected mortality: -6.3% at 56 g a.s./ha -11% at 140 g a.s./ha 79.4% at 346 g a.s./ha 48.5% at 865 g a.s./ha 91.2% at 2162 g a.s./ha ER₅₀ > 2162 g a.s./ha Red. of reproduction: 1.2% at 56 g a.s./ha -34.6% at 140 g a.s./ha -3.8% at 346 g a.s./ha -17.1% at 865 g a.s./ha 47.9% at 2162 g a.s./ha	KCP 10.3.2/05

Species	Substance	Exposure System	Results	Reference
<i>Aleochara bilineata</i>		Extended laboratory test, exposure of soil in each test unit	<p>LR₅₀ > 864.9 g a.s./ha Corrected mortality: 6.7% at 23 g a.s./ha -6.7% at 56 g a.s./ha 13.3% at 140 g a.s./ha 6.7% at 346 g a.s./ha 5.3% at 865 g a.s./ha</p> <p>ER₅₀ > 864.9 g a.s./ha Red. of reproduction: -3.3% at 23 g a.s./ha -3.9% at 56 g a.s./ha -3.6% at 140 g a.s./ha 5.5% at 346 g a.s./ha -3.1% at 865 g a.s./ha</p>	KCP 10.3.2/06
Aged-residue tests				
<i>Typhlodromus pyri</i>	CA3301	<p>Aged-residue study with French bean leaves sprayed (3D) and then exposure to treated leaves</p> <p>7 days' aging following two applications of product at nominally 200.4 g a.s./ha (14-d interval)</p>	<p>7-d corrected mortality at a target application of 200.4 g a.s./ha (measured: 186.4 g a.s./ha): 9.2% at 0 DAT -1.1% at 7 DAT</p> <p>Reduction in reproduction at a target application of 200.4 g a.s./ha (measured: 186.4 g a.s./ha): 11.9% at 0 DAT 7.5% at 7 DAT</p> <p>Note to RMS: It is noted that the application rate was slightly lower than planned due to a calculation error. The actual rate applied was 93% of the proposed application rate (200 g a.s./ha). However, this small reduction in application rate is not expected to significantly alter the results especially as such low level of effects were seen even at 0DAT.</p>	KCP 10.3.2/07
<i>Chrysoperla carnea</i>		Aged-residue study with French bean leaves sprayed (3D) and then exposure to treated leaves	<p>7-d corrected mortality at a target application of 200.4 g a.s./ha: No adverse effects at 0 DAT and 7 DAT</p> <p>Reduction in reproduction at a target application of 200.4 g a.s./ha: No adverse effects at 0 DAT and 7 DAT</p>	KCP 10.3.2/08

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. Therefore, it is not appropriate to rely on the data from the solo formulation, submitted as representative formulation for the EU review, for the risk assessment for non-target arthropods. New studies are available for the Joust (CA3301, an EC 250 formulation).

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.

Table 9.7-2: First- and second-tier assessment of the in-field risk for non-target arthropods due to the use of CA3301 in cereals and oilseed rape.

Intended use	Cereals and oilseed rape		
Active substance	Prothioconazole		
Application rate (g/ha)	2 x 200 g a.s./ha (14-d interval); BBCH 14-69		
MAF	1.7 (Appendix V of ESCORT 2) – foliar NTA MAF, as use is not pre-emergence		
Test species First tier	LR ₅₀ (lab.) (g a.s./ha)	PER _{in-field} (g a.s./ha)	HQ _{in-field} criterion: HQ ≤ 2
<i>T. pyri</i>	52.3	340	6.5
<i>A. rhopalosiphi</i>	14.2		23.9
Test species Higher tier (extended lab.)	Rate with ≤ 50% effect (g a.s./ha)*	PER _{in-field} (g a.s./ha)	PER _{in-field} below rate with ≤ 50% effect?
<i>T. pyri</i>	219.14	340	No
<i>A. rhopalosiphi</i>	>864.9		Yes
<i>C. carnea</i>	406.98		Yes
<i>A. bilineata</i>	>864.9		Yes

Values in **bold** are above either the trigger value or the PER_{in-field} value. *If an LR₅₀ or ER₅₀ from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50% effect

An acceptable in-field risk is not concluded at the first-tier assessment, based on the glass-plate laboratory data, for *T. pyri* and *A. rhopalosiphi*, when considering the two applications of 200 g a.s./ha, with a MAF of 1.7. Therefore, extended laboratory data are included and demonstrate an acceptable in-field risk for *A. rhopalosiphi*, *C. carnea*, and *A. bilineata*. The in-field PER values in these cases are below the application rates with ≥ 50% effect on mortality. However, an acceptable in-field risk is still not concluded for *T. pyri*, as the PER_{in-field} is greater than the rate with 50% effects. Overall, an acceptable in-field risk to non-target arthropods is not concluded and requires further refinement with a higher-tier, aged-residue study, which is detailed in section 9.7.2.3 below.

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 groups oilseed rape.

Table 9.7-3: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of CA3301 in cereals and oilseed rape.

Intended use	Cereals and oilseed rape				
Active substance	Prothioconazole				
Application rate (g/ha)	2 x 200 g a.s./ha (14-d interval); BBCH 14-69				
MAF	1.7 (foliar); 1.9 (soil) (Appendix V of ESCORT 2)				
VDF	10 for first-tier studies, 5 for second- and higher-tier studies (EFSA supporting publication 2019: EN-1673)				
Test species	LR ₅₀ (lab.)	DF*	CF	PER _{off-field} (corr.)	HQ _{in-field}
First tier	(g a.s./ha)			(g a.s./ha)	criterion: HQ ≤ 2
Foliar					
T. pyri	52.3	2.38%	10	8.09	0.15
A. rhopalosiphi	14.2				0.57
Soil					
T. pyri	52.3	2.38%	10	8.09 9.04	0.17
A. rhopalosiphi	14.2				0.64

HQ_{off-field} values in **bold** are above the trigger. *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 (corr.), corrected predicted environmental rate; CF, correction factor; HQ, hazard quotient.

Based on the risk assessment presented above, an acceptable off-field risk can be concluded for the two standard indicator species, *T. pyri* and *A. rhopalosiphi*.

9.7.2.3 Additional higher-tier risk assessment

The in-field risk assessment, using standard glass plate and extended laboratory non-target arthropod studies, indicated a potential risk to *T. pyri*. Additional higher-tier (aged-residue) studies with *T. pyri* and *C. carnea* were conducted to demonstrate that there is an acceptable potential for recovery within an ecologically relevant period, as effects on non-target arthropods rapidly decrease over time.

Full details of these studies are provided in Appendix 2.

The aged residue study on *T. pyri* included two applications of the product at nominally 800 mL/ha, equivalent to 200.4 g a.s./ha (actual value: 186.4 g a.s./L), which were made 14 days apart (as per the maximum application listed in the GAP). It determined that both the 0- and 7-DAT bioassays exhibited <50% effects on mortality, demonstrating strong evidence that recolonization of non-target arthropods can occur within 1 year. Indeed, treatment with CA3301 resulted in 9.2% and -1.1% corrected mortality in the 0-DAT and 7-DAT bioassays, respectively.

Table 9.7-4: Higher-tier assessment of the in-field risk for non-target arthropods due to the use of CA3301 in cereals and oilseed rape.

Intended use	Cereals and oilseed rape		
Active substance	Prothioconazole		
Application rate (g/ha)	2 x 200 g a.s./ha (14-d interval); BBCH 14-69		
Test species Higher tier (aged residue)	Effects following exposure to leaves from plants treated with 2 x 186.4 g a.s./ha	Worst-case GAP:	≤ 50% effect?
<i>T. pyri</i>	Mortality 9.2% at 0 DAT -1.1% at 7 DAT Reduction in reproduction: 11.9% at 0 DAT 7.5% at 7 DAT	200 g a.s/ha (14-day interval)	Yes

Based on the risk assessment presented above, an acceptable in-field risk for non-target arthropods can be concluded.

9.7.2.4 Risk-mitigation measures

No risk mitigation for non-target arthropods is needed for the proposed use of CA3301.

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.

Acceptable in-field risks were demonstrated for *A. rhopalosiphi*, *C. carnea*, and *A. bilineata* at the first tier based on glass plate and extended laboratory tests. However, Tier 1 risk assessments showed a potential risk to *T. pyri*. Consequently, aged-residue studies with *T. pyri* and *C. carnea* were conducted, which demonstrated that there is an acceptable potential for recovery within an ecologically relevant period, as mortality and reproductive effects at 0 DAT and 7 DAT were minimal and thus significantly below the trigger value of 50% effect.

For the off-field risk of CA3301 acceptable risk was demonstrated for *T. pyri* and *A. rhopalosiphi* at the first tier, therefore, the off-field risk to non-target arthropods was shown to be acceptable.

Review Comments:

The submitted risk assessment has been accepted.

Based on the results of the conducted first and higher-tier risk assessment it can be concluded that no in-field and off-field risk for non-target arthropods is expected from use of CA3301. No mitigation measures are required.

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 and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents, as well as in Appendix 2 of this document (new studies).

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of CA3301 were not evaluated as part of the EU assessment of prothioconazole. 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).

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 _{corr} ≥ 32 mg a.s./kg dw *	EFSA Sci. Report. 2007; 106, 1-98
<i>Hypoaspis aculeifer</i>		Mixed into substrate, 34 d, chronic, LUFA 2.1 soil	NOEC ≥ 100 mg a.s./kg dw NOEC _{corr} ≥ 50 mg a.s./kg dw *	
Product				
<i>Eisenia fetida</i>	CA3301	Mixed into substrate, 56 d, chronic.	NOEC = 139 143 mg a.s./kg dw NOEC _{corr} = 69.5 71.5 mg a.s./kg dw* EC ₁₀ = 23.7 24.3 mg a.s./kg dw EC _{10,corr} = 11.85 12.16 mg a.s./kg dw*	KCP 10.4.1.1/01
<i>Folsomia candida</i>		Mixed into substrate, 28 d, chronic 5% peat content	NOEC = 15.44 mg a.s./kg dw NOEC _{corr} = 7.72 mg a.s./kg dw* EC ₁₀ = 22.29 mg a.s./kg dw	KCP 10.4.2.1/01
<i>Hypoaspis aculeifer</i>		Mixed into substrate, 14 d, chronic 5% peat content	NOEC = 15.44 mg a.s./kg dw NOEC _{corr} = 7.72 mg a.s./kg dw* EC ₁₀ = 23.03 mg a.s./kg dw EC _{10,corr} = 11.52 mg a.s./kg dw*	KCP 10.4.2.1/02
Prothioconazole-desthio				
<i>Eisenia fetida</i>	Prothioconazole-desthio	Mixed into substrate, 56 d, chronic, 10% peat content	NOEC = 1 mg/kg dw NOEC _{corr} = 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 _{corr} = 31.25 mg/kg dw*	
Prothioconazole-S-methyl				
<i>Eisenia fetida</i>	Prothioconazole-S-methyl	Mixed into substrate, 56 d, chronic, 10% peat content	NOEC = 100 mg/kg dw NOEC _{corr} = 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 _{corr} ≥ 15.8 mg/kg dw*	

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.

9.8.1.1 Justification for new endpoints

New studies are available for CA3301, 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 1 and detailed study summaries are presented 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).

9.8.2.1 First-tier risk assessment

The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2, Table 8.7-3. According to the assessment of environmental-fate data, multi-annual accumulation in soil does not need to be considered for prothioconazole, given that it has a $DT_{90} < 1$ year and, therefore, does not accumulate (see dRR, Part B8).

To achieve a concise risk assessment, PEC_{soil} values have been calculated for three uses (cereals, spring oilseed rape, and winter oilseed rape), providing a risk envelope that covers the risk for earthworms and other non-target soil organisms (meso- and macrofauna) from all intended uses in the GAP. The worst-case use is autumn application to winter oilseed rape, due to the earlier crop growth stage and lower crop interception.

Table 9.8-2: First-tier assessment of the acute and chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of CA3301 in winter oilseed rape - prothioconazole and relevant soil metabolites.

Oilseed rape – prothioconazole and relevant soil metabolites			
Intended use	Winter oilseed rape (worst-case use, autumn application, given earlier crop growth stage and lower crop interception)		
Product/active substance	CA3301/prothioconazole and its metabolites		
Application rate (g a.s./ha)	2 x 175 g a.s./ha		
Species	NOEC _{corr} or EC ₁₀ corr (mg a.s. or metabolite/kg dw)	PEC _{soil} (mg/kg dw)*	TER _{lt} (criterion TER ≥ 5)
CA3301			
<i>Eisenia fetida</i>	11.85 12.16	0.1400	84.6 86.9
<i>Folsomia candida</i>	7.72		55.1
<i>Hypoaspis aculeifer</i>	7.72		55.1
Prothioconazole-desthio			
<i>Eisenia fetida</i>	0.5	0.0725	6.9
<i>Folsomia candida</i>	31.25		431.0
Prothioconazole-S-methyl			
<i>Eisenia fetida</i>	50	0.0213	2347.4
<i>Folsomia candida</i>	≥15.8		741.8

* PEC_{soil} values are worst case for all intended uses of CA3301.

An acceptable risk is concluded at the first tier for earthworms and other soil meso/macro- fauna, for the intended uses of CA3301 in winter oilseed rape from autumn application, in consideration of the active

substance prothioconazole and its metabolites, prothioconazole-desthio and prothioconazole-S-methyl. As the PEC_{soil} values from autumn application on winter oilseed rape were worst case, it is concluded that there should be no unacceptable risk from any of the proposed uses of CA3301.

9.8.2.2 Higher-tier risk assessment

Based on the results of the first-tier risk assessment, no further studies are considered necessary.

9.8.3 Overall conclusions

An acceptable risk from CA3301 and from the active substance prothioconazole and its relevant soil metabolites is concluded at the first tier, for earthworms and other soil meso/macro- fauna, for all intended uses of CA3301, following worst-case soil exposure from autumn application to winter oilseed rape. The intended uses of are considered covered by the risk assessments for the intended use for autumn application on winter oilseed rape.

Review Comments:

The long-term risks of CA3301 to soil meso- and macro-organisms were assessed from toxicity exposure ratios between toxicity endpoints and maximum PEC_{soil} . The relevant predicted environmental concentrations in soil (PEC_{soil}) for risk assessments covering the proposed use pattern are taken from Part B Section 8 (Environmental Fate).

The submitted risk assessment, based on several laboratory studies, has been accepted. It can therefore be concluded that there will be negligible risk associated with the exposure of beneficial soil organisms to CA3301 following proposed use pattern.

9.9 Effects on soil microbial activity (KCP 10.5)

9.9.1 Toxicity data

Studies on effects of soil microorganisms have been carried out with prothioconazole and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents, as well as in Appendix 2 of this document (new studies).

Effects on soil microorganisms of CA3301 were not evaluated as part of the EU assessment of prothioconazole. No new data are submitted with this application as the risk from the active substance prothioconazole and its metabolites, prothioconazole-desthio and prothioconazole-S-methyl, can be assessed using the available active substance and metabolite data.

Table 9.9-1: Endpoints and effect values relevant for the risk assessment for soil microorganisms.

Endpoint	Substance	Exposure System	Results (mg a.s. or metabolite /kg soil dw)	Reference
N-mineralisation	Prothioconazole	28 d, 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	

*Endpoints in terms of mg/kg soil dw are derived from the applications rates listed in EFSA Conclusion 2007.

9.9.1.1 Justification for new endpoints

Not relevant. No new data submitted. Product studies are not required, in accordance with regulation (EU) No. 284/2013, as the toxicity of the plant protection product CA3301 can be predicted based on the available data for the active substance and its metabolites.

9.9.2 Risk assessment

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

The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2, Table 8.7-3 and were already used in the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) (see 9.8).

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the autumn use on winter oilseed rape also covers the risk for the soil microorganisms from all other intended uses.

Table 9.9-2: Assessment of the risk for effects on soil microorganisms due to the use of CA3301 worst case autumn use on winter oilseed rape.

Intended use	Cereals and oilseed rape		
N-mineralisation			
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 d)	0.1400 (Section B8, Table 8.7-4)	Yes
Prothioconazole-desthio	1.37 (at 28 d)	0.0725 (Section B8, Table 8.7-10)	Yes
Prothioconazole-S-methyl	2.69 (at 28 d)	0.0213 (Section B8, Table 8.7-7)	Yes

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 use of CA3301, assuming the worst-case PEC_{soil} value from autumn application to winter oilseed rape. The risk-envelope approach confirms that all intended uses will lead to PEC_{soil} values lower than the worst-case value assumed and, thus, no unacceptable risk to soil microorganisms from the proposed uses is expected. No further risk assessments or mitigation are considered necessary.

Review Comments:

Based on the results of the conducted first tier risk assessment it can be concluded that no risk for soil micro-organisms is expected from use of CA3301.

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

9.10.1 Toxicity data

Studies on the toxicity to non-target terrestrial plants have been carried out with prothioconazole. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on non-target terrestrial plants of CA3301 were not evaluated as part of the EU assessment of prothioconazole. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

Table 9.10-1: Endpoints and effect values relevant for the risk assessment for non-target terrestrial plants

Species			Substance	Exposure System	Results, ER ₅₀	Reference				
Active substance										
<i>Amaranthus retroflexus</i> (most sensitive)			Prothioconazole	21-d, Seedling emergence	>200 g/ha*	EFSA Sci. Report. 2007; 106, 1-98				
<i>Amaranthus retroflexus</i> , <i>Beta vulgaris</i> (most sensitive)				21-d, Vegetative vigour	>250 g/ha*					
Product										
Monocot	Ryegrass	<i>Lolium perenne</i> L.	CA3301	21-d, Vegetative vigour	ER ₅₀ > 406.99 g a.s./ha	KCP 10. 6.2/01				
	Sorghum	<i>Sorghum sudanense</i> L.								
	Onion	<i>Allium cepa</i> L.								
	Corn	<i>Zea mays</i> L.								
Dicot	Carrot	<i>Daucus carota</i> L.								
	Radish	<i>Rafanus sativus</i> L.								
	Soybean	<i>Glycine max</i> L.								
	Tomato	<i>Solanum lycopersicum</i> L.								
	Cucumber	<i>Cucumis sativus</i> L.								
	Lettuce	<i>Lactuca sativa</i> L.								
Monocot	Corn	<i>Zea mays</i> L.	CA3301	21-day, seedling emergence	ER ₅₀ > 406.99 g a.s./ha	KCP 10. 6.2/02				
	Ryegrass	<i>Lolium perenne</i> L.								
	Sorghum	<i>Sorghum sudanense</i> L.								
	Onion	<i>Allium cepa</i> L.								
Dicot	Carrot	<i>Daucus carota</i> L.								
	Cucumber	<i>Cucumis sativus</i> L.								
	Lettuce	<i>Lactuca sativa</i> L.								
	Radish	<i>Rafanus sativus</i> L.								
	Soybean	<i>Glycine max</i> L.								
	Tomato	<i>Solanum lycopersicum</i> L.								

*Application rate in the study.

9.10.1.1 Justification for new endpoints

In accordance with Regulation (EU) No. 284/2013, studies with non-target terrestrial plants have been conducted with a formulated product.

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)

The new studies on vegetative vigour and seedling emergence with CA3301 were conducted as limit tests, with an exposure rate of 406.99 g a.s./ha. The evaluation of the risk for 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, grouping was done according to crop groups covering relevant maximum application patterns and considering maximum annual application rates (see section 9.1.2).

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 CA3301 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).

For the exposure estimate (PER), multiple application factors for two applications were considered to take into account the potential build-up of applied substances between applications, according to ESCORT 2 (2000), Appendix V. For effects on vegetative vigour, default MAF values for foliar substrates were used, as tests were conducted by spraying emerged plants.

MAF = 1.7 for two applications (foliar default of DT₅₀: interval 2.3:1), MAF = 1.9 for two applications (soil-substrate default of DT₅₀: interval 6:1).

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 given for this fungicide and, 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 for the vegetative-vigour and seedling emergence studies.

The risk assessment is provided below.

Table 9.10-2: Assessment of the risk for non-target plants due to the use of CA3301 in cereals and oilseed rape – prothioconazole.

Intended use	Cereals and oilseed rape			
Active substance	Prothioconazole			
Application rate (g/ha)	2 x 200 g/ha (14-day interval)			
MAF	1.7 (foliar), 1.9 (soil); default values, ESCORT 2 **			
Study	ER ₅₀ (g a.s./ha)	Drift rate	PER _{off-field} (g/ha)	TER criterion: TER ≥ 5
Foliar				
Vegetative vigour	>406.99*	0.0238	8.09	>50.3
Soil				
Seedling emergence	>406.99*	0.0238	9.04	>45.0

TER values shown in **bold** fall below the relevant trigger. MAF, multiple application factor; PER, predicted environmental rate; TER, toxicity-to-exposure ratio. *Exposure rate of the limit test.

** accepted as represent worst-case exposure scenario; nevertheless, according SANCO/10329/2002 rev.2 final, 2002 the MAF value is not required for the risk assessment for NTTP

Based on the available non-target terrestrial plant toxicity data for CA3301, an acceptable off-field risk is

demonstrated for the proposed worst-case use (2 x 200 g a.s./ha) on cereals. The other intended uses of CA3301 in the oilseed rape crop grouping (2 x 175 g a.s./ha) will also have an acceptable risk assessment to non-target terrestrial plants, 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 studies are considered necessary.

9.10.2.4 Risk-mitigation measures

No risk mitigation for non-target terrestrial plants is needed.

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 the product CA3301, is considered acceptable without the need for any mitigation measures.

Review Comments:

Based on the risk assessment it can be concluded that the proposed use of CA3301 poses no unacceptable risk to non-target plants, if applied according to the recommended use pattern. Particular precautions to reduce the environmental concentrations resulting from CA3301 applications are not required for the protection of terrestrial non-target plants.

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 CA3301, 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 formulation CA3301 are summarized below.

Available formulation data for CA3301 for classification to the aquatic environment

96-hour LC ₅₀ , fish	Extrapolated LC ₅₀ = 15.6 mg product/L (4.0 mg a.s./L); see section 9.5 for details of extrapolation.
48-hour EC ₅₀ , crustacea	CA3301 EC ₅₀ = 1.1 mg product/L (0.28 mg a.s./L)
72-hour EC ₅₀ , algae <i>S. costatum</i>	CA3301 E _r C ₅₀ = 0.328 mg product/L (0.084 mg a.s./L) NOEC <0.00093 mg product/L E _r C ₁₀ 0.104 mg product/L (mm)
7-day EC ₅₀ <i>Lemna</i>	CA3301 E _r C ₅₀ = 1.1 mg product/L (0.288 mg a.s./L) >1.7 mg product/L (0.444 mg a.s./L) NOEC (growth rate) 0.0014 mg product/L E _r C ₁₀ 0.11 mg product/L (mm)

Available data for prothioconazole

97-day NOEC, fish CA3301 NOEC = 0.308 mg a.s./L
21-day NOEC, crustacea CA3301 NOEC = 0.56 mg a.s./L

CA3301 contains one active substance, 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'

Based on Regulation (EC) 1272/2008, the following classification for CA3301 is proposed, which considers the above aquatic endpoints for CA3301 and the harmonised classification for prothioconazole:

Conclusion: short-term (acute) aquatic hazard

The available acute toxicity of CA3301 as a formulation can be used for classification of the mixture. CA3301 is classified for short-term (acute) hazard as Acute Category 1, based on the lowest EC₅₀ value of 0.328 mg product/L for algae (i.e., ≤1 mg a.s./L)

Conclusion: long-term (chronic) aquatic hazard

Prothioconazole is not considered to be rapidly degradable due to formation of toxic relevant metabolites in the aquatic environment. Only long term (NOEC/EC₁₀) values for algae and *Lemna* are available for the formulation CA3301. As adequate chronic toxicity data for all aquatic organism groups are not available the product should be classified as Chronic category 1 as EC₅₀ values are not all ≥1 mg product/L. The proposed environmental classification and labelling of CA3301 according to the CLP Regulation (EC) No 1272/2008 is presented below.

Table 9.13-1: Proposed environmental classification and labelling of CA3301 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	P273, P391, & P501.

Review Comments:

The submitted CA3301 propose of classification has been accepted.

Classification and labelling of the product CA3301 (JOUST) has been carried out applying the criteria outlined in the Regulation No 1272/2008 of the European Parliament and of the council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006 for environmental hazards.

Taking into account all of the ecotoxicological data classification and labelling is:

Signal word: Warning



GHS pictogram 09:

H410: Very toxic to aquatic life with long lasting effects

EUH401: To avoid risks to human health and the environment, comply with the instructions for use.

P391: Collect spillage/liquid.

P501: Dispose of contents/container in accordance with national regulations.

SP1: Do not contaminate water with the product or its container (Do not clean application equipment near surface water/ Avoid contamination via drains from farmyards and roads)

To protect the aquatic organisms, the specific mitigation measures must be applied (label need to be adapted according to the scenarios relevant in each country where the product will be submitted).

CA3301 / JOUST	Page 84 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

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	Dupont, A.	2021	250 EC Prothioconazole (CA3301) – Acute Toxicity to <i>Daphnia magna</i> in a 48-Hour Immobilization Test Report no. 20190455 IES, Ltd., Witterswil, Switzerland GLP Unpublished	N	Nufarm
KCP 10.2.1/02	Dupont, A.	2021	250 EC Prothioconazole (CA3301) – Effect on the Aquatic Higher Plant <i>Lemna gibba</i> in a 7-Day Growth Inhibition Test Report no. 20190456 IES, Ltd., Witterswil, Switzerland GLP Unpublished	N	Nufarm
KCP 10.2.1/03	Dupont, A.	2021	250 EC Prothioconazole (CA3301) - Effect on <i>Skeletonema sp.</i> in a 72-Hour Algal Growth Inhibition Test Report no. 20190454 IES, Ltd., Witterswil, Switzerland GLP Unpublished	N	Nufarm
KCP 10.2.1/04	Kuhl, K.	2018	<i>Desmodesmus subspicatus</i> growth inhibition test with AE1194888 (BCS-AA53879) Report no. M 630874 01-1 Bayer AG, Crop Science Division, Monheim, Germany GLP Unpublished Note to RMS: This study is protected, but the Owner (Bayer) has provided the Sponsor (Nufarm) access. However, since Nufarm does not have access to a copy of the final study report, this study summary is prepared using only the summary available in the 2018 RAR for prothioconazole.	N	Bayer
KCP 10.3.1.1/01	Couture, E.	2021	CA3301 – Acute Oral and Contact Toxicity to the Honey Bee, <i>Apis mellifera</i> L. (Hymenoptera: Apidae) under Laboratory Conditions Report no. 029SRFR20C01 SynTech Research, Chapelle de Guinchay, France GLP Unpublished	N	Nufarm

CA3301 / JOUST	Page 85 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study (Y/N)	Owner
KCP 10.3.1.1/02	Couture, E.	2021	CA3301 – A laboratory study to determine the acute oral and contact toxicity on the adult <i>Bombus terrestris</i> L. (Hymenoptera: Apidae) Report no. 029SRFR20C02 SynTech Research, Chapelle de Guinchay, France GLP Unpublished	N	Nufarm
KCP 10.3.1.1/03	Couture, E.	2021	CA3301 – A laboratory study to determine the acute contact toxicity on the adult <i>Osmia bicornis</i> (Hymenoptera: Megachilidae) Report no. 029SRFR20C03 SynTech Research, Chapelle de Guinchay, France GLP Unpublished	N	Nufarm
KCP 10.3.1.2/01	Ansaloni, T.	2021	CA3301 (Prothioconazole 250 EC, NUL 3390): Honey Bee (<i>Apis mellifera</i> L.) Chronic Oral Toxicity Test (10-Day Feeding) under Laboratory Conditions Report no. S20-09402 Eurofins Trialcamp S.L.U., Valencia, Spain GLP Unpublished	N	Nufarm
KCP 10.3.1.3/01	Ansaloni, T.	2021	CA3301 (Prothioconazole 250 EC, NUL 3390): Honey Bee (<i>Apis mellifera</i> L.) Larval Toxicity Test following Repeated Exposure under laboratory conditions Report no. S20-09403 Eurofins Trialcamp S.L.U., Valencia, Spain GLP Unpublished	N	Nufarm
KCP 10.3.1.5/01	Hein, R.	2015	Assessment of side effects of prothioconazole EC 250 G on the honey bee (<i>Apis mellifera</i> L.) in the semi field after one application on <i>Phacelia tanacetifolia</i> in Germany 2015 Report no. S15-02997 Eurofins Agrosience Services EcoChem GmbH, Niefern-Oeschelbronn, Germany GLP Unpublished	N	Bayer
KCP 10.3.1.5/02	Kaiser, F.	2021	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 <i>Phacelia tanacetifolia</i> in Germany 2021 Report no. S21-00461 Eurofins Agrosience Services EcoChem GmbH, Niefern-Oeschelbronn, Germany GLP Unpublished	N	Nufarm

CA3301 / JOUST	Page 86 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

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
			Note to RMS: these are interim results, which will be updated when possible. The study was stopped, due to poor weather conditions and will recommence in 2022.		
KCP 10.3.2/01	Schmidt, T.	2021	250 EC Prothioconazole, NUL 3390 (CA3301) - Toxicity to the Predatory Mite <i>Typhlodromus pyri</i> (Acari, Phytoseiidae) under Worst-Case Conditions in the Laboratory Report no. 20190459 IES, Ltd., Witterswil, Switzerland GLP Unpublished	N	Nufarm
KCP 10.3.2/02	Schmidt, T.	2021	250 EC Prothioconazole, NUL 3390 (CA3301) - Acute Toxicity to Adults of the Parasitoid Wasp <i>Aphidius rhopalosiphi</i> (Hymenoptera: Braconidae) under Worst-Case Conditions in the Laboratory Report no. 20190458 IES, Ltd., Witterswil, Switzerland GLP Unpublished	N	Nufarm
KCP 10.3.2/03	Frainout, C.	2021	CA3301 – An extended laboratory study to determine the acute and sublethal effects on the non-target arthropod <i>Typhlodromus pyri</i> (Acari: Phytoseiidae). Report no. 029SRFR20C07 SynTech Research, Chapelle de Guinchay, France GLP Unpublished	N	Nufarm
KCP 10.3.2/04	Frainout, C.	2021	CA3301 – An extended laboratory study to determine the acute and sublethal effects on the non-target arthropod <i>Aphidius rhopalosiphi</i> (Hymenoptera: Braconidae). Report no. 029SRFR20C06 SynTech Research, Chapelle de Guinchay, France GLP Unpublished	N	Nufarm
KCP 10.3.2/05	Frainout, C.	2021	CA3301 – An extended laboratory study to determine the acute and sublethal effects on the non-target arthropod <i>Chrysoperla carnea</i> (Neuroptera: Chrysopidae). Report no. 029SRFR20C09 SynTech Research, Chapelle de Guinchay, France GLP Unpublished	N	Nufarm
KCP 10.3.2/06	Frainout, C.	2021	CA3301 – An extended laboratory study to determine the acute and sublethal effects on the	N	Nufarm

CA3301 / JOUST	Page 87 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

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
			non-target arthropod <i>Chrysoperla carnea</i> (Neuroptera: Chrysopidae) Report no. 029SRFR20C08 SynTech Research, Chapelle de Guinchay, France GLP Unpublished		
KCP 10.3.2/07	Fallowfield, L.	2021	CA3301– A Series of Aged-Residue Extended Laboratory Tests to Determine Effects on the Predatory Mite <i>Typhlodromus pyri</i> (Acari: Phytoseiidae) Report no. NUF-21-01 Mambo-Tox, Southampton, UK GLP Unpublished	N	Nufarm
KCP 10.3.2/08	Vaughan, R.	2021	CA3301 – A series of aged-residue extended laboratory tests to determine effects on the green lacewing, <i>Chrysoperla carnea</i> (Neuroptera, Chrysopidae) Report no. NUF-21-02 Mambo-Tox, Southampton, UK GLP Unpublished	N	Nufarm
KCP 10.4.1.1/01	Schmidt, T.	2021	250 EC Prothioconazole, NUL 3390 (CA3301) - Effects on Reproduction of <i>Eisenia fetida</i> (Annelida: Lumbricidae) in Artificial Soil Report no. 20190460 IES, Ltd., Witterswil, Switzerland GLP Unpublished	N	Nufarm
KCP 10.4.2.1/01	Frainout, C.	2021	CA3301 - A laboratory study to determine the acute and sublethal effects on the collembolan <i>Folsomia candida</i> (Arthropodea: Isotomidae) Report no. 029SRFR20C10 SynTech Research, Chapelle de Guinchay, France GLP Unpublished	N	Nufarm
KCP 10.4.2.1/02	Frainout, C.	2021	CA3301 - A laboratory study to determine the acute and sublethal effects on the predatory mite <i>Hypoaspis aculeifer</i> (Acari: Laelapidae) Report no. 029SRFR20C11 SynTech Research, Chapelle de Guinchay, France GLP Unpublished	N	Nufarm
KCP 10.6.2/01	Frainout, C.	2021	CA3301 - A study to determine the effects on the Vegetative Vigour of terrestrial plants Report no. 029SRFR20C13 SynTech Research, Chapelle de Guinchay, France GLP	N	Nufarm

CA3301 / JOUST	Page 88 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study (Y/N)	Owner
			Unpublished		
KCP 10.6.2/02	Frainout, C.	2021	CA3301 - A study to determine the effects on the Seedling Emergence and Growth of terrestrial plants Report no. 029SRFR20C12 SynTech Research, Chapelle de Guinchay, France GLP Unpublished	N	Nufarm

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
KCP XX	Author	YYYY	Title Company Report N Source GLP/non GLP/GEP/non GEP Published/Unpublished	Y/N	Owner

The following tables are to be completed by MS

CA3301 / JOUST	Page 89 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

List of data submitted by the applicant and not relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.2.1/04	Kuhl, K.	2018	<i>Desmodemus subspicatus</i> growth inhibition test with AE1194888 (BCS-AA53879) Report no. M-630874-01-1 Bayer AG, Crop Science Division, Monheim, Germany GLP Unpublished	N	Bayer
KCP 10.3.1.5/01	Hein, R.	2015	Assessment of side effects of prothioconazole EC 250 G on the honey bee (<i>Apis mellifera</i> L.) in the semi-field after one application on <i>Phacelia tanacetifolia</i> in Germany 2015 Report no. S15-02997 Eurofins Agroscience Services EcoChem GmbH, Niefern-Oeschelbronn, Germany GLP Unpublished	N	Bayer

List of data relied on not submitted by the applicant but necessary for evaluation

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP XX	Author	YYYY	Title Company Report N Source GLP/non GLP/GEP/non GEP Published/Unpublished	Y/N	Owner

CA3301 / JOUST	Page 90 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Appendix 2 Detailed evaluation of the new studies

A 2.1	KCP 10.1	Effects on birds and other terrestrial vertebrates
A 2.1.1	KCP 10.1.1	Effects on birds
A 2.1.1.1	KCP 10.1.1.1	Acute oral toxicity
A 2.1.1.2	KCP 10.1.1.2	Higher tier data on birds
A 2.1.2	KCP 10.1.2	Effects on terrestrial vertebrates other than birds
A 2.1.2.1	KCP 10.1.2.1	Acute oral toxicity to mammals
A 2.1.2.2	KCP 10.1.2.2	Higher tier data on mammals
A 2.1.3	KCP 10.1.3	Effects on other terrestrial vertebrate wildlife (reptiles and amphibians)
A 2.2	KCP 10.2	Effects on aquatic organisms
A 2.2.1	KCP 10.2.1	Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes

Study 1

Comments of zRMS:	<p>The study was conducted to OECD guideline 202 and according to the principles of GLP. No deviations to the guideline were noted. All validity criteria were met.</p> <p>The study is considered to be reliable and suitable for the risk assessment. Due to the decrease of the test item concentrations during the 24-hour renewal periods, the results refer to nominal and mean measured concentrations of formulation and of active substance.</p>
-------------------	---

Reference:	KCP 10.2.1/01
Report	<p>250 EC Prothioconazole (CA3301) – Acute Toxicity to <i>Daphnia magna</i> in a 48-Hour Immobilization Test</p> <p>Dupont, A., 2021, report no. 20190455</p>
Guideline(s):	Yes. OECD 202 (2004)
Deviations:	No
GLP:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive summary

CA3301 / JOUST	Page 91 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

The 48-h acute toxicity of the product CA3301 to *Daphnia magna* was studied under semi-static conditions, in accordance with OECD 202 (2004) test guideline. Daphnids were exposed to nominal concentrations of 0 (control), 0.46, 1.0, 2.2, 4.6, and 10 mg product/L, equivalent to 0, 0.118, 0.256, 0.56, 1.18, and 2.56 mg a.s./L (accounting for the analysed a.s. content of 25.57% w/w prothioconazole). Immobilisation was observed after 24 and 48 hours of exposure. Each treatment group was divided into four replicates, consisting of 5 daphnids each.

Since analytical verification of the test concentrations indicated that measured concentrations of the fresh and aged samples were between 34 to 85% (0 to 24 hours) and 44 to 95% (24 to 48 hours) and, thus, outside of the recommended range of 80-120%, biological endpoints are reported based on arithmetic mean-measured concentrations.

During the first 24 hours, the three highest product treatments caused between 70 to 100% immobility of the daphnids. Thus, the 24-hour NOEC and LOEC values, based on immobilization, were determined to be 0.66 and 1.8 mg product/L (equivalent to 0.17 and 0.46 mg a.s./L), whereas the 24- hour EC₅₀ value was calculated to be 1.6 mg product/L, equivalent to 0.4 mg a.s./L, based on mean-measured concentrations.

The 48- hour NOEC and LOEC values, based on immobilization, were determined to be 0.27 and 0.66 mg product/L (equivalent to 0.069 and 0.17 mg a.s./L), respectively, based on mean-measured concentrations, whereas the 48- hour EC₅₀ value was calculated to be 1.1 mg product/L, equivalent to 0.28 mg a.s./L, based on mean-measured concentrations.

Overall, the study satisfies the OECD 202 (2004) test-guideline requirements for acute toxicity to *D. magna* and is considered acceptable.

Materials and methods

Test materials

Test item

Name:	Prothioconazole 250 EC, NUL 3390 (CA3301)
Active substance:	Prothioconazole
Formulation type:	Emulsifiable concentrate (EC)
Product density:	0.9948 g/mL
Source and lot/batch no.:	A19045
Active-substance content:	250 g/L (nominal), 257.04 g/L (analysed)
Active substance density:	25.57% w/w
Appearance:	Clear, straw-coloured liquid
Expiry date of lot/batch:	17 June 2022
Storage conditions:	Store in the closed, original container in a cool, well-ventilated area. Do not store for prolonged periods in direct sunlight.

Test organism

Species:	<i>Daphnia magna</i> Straus
Age at study initiation:	≤24 hours
Source:	Bred at testing facility
Feeding during test:	No
Acclimation:	Not applicable

Test conditions

Water hardness:	250 mg CaCO ₃ /L
Test medium:	Reconstituted water (294 mg CaCl ₂ /L, 123 mg MgSO ₄ /L, 65 mg NaHCO ₃ /L, 5.8 mg KCl/L)
Test temperature:	21°C

CA3301 / JOUST	Page 92 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

pH:	7.6-8.0 (actual value: 7.8)
Dissolved oxygen:	8.2-8.8 mg O ₂ /L

The 48-hour semi-static test was performed in 100-mL glass beakers, filled with 50 mL of test medium, and loosely covered with glass sheets to reduce evaporative water loss. Water was renewed every 24 hours. The test consisted of a control and five product treatment groups of nominally 0 (test-water control), 0.46, 1.0, 2.2, 4.6, and 10 mg product/L, equivalent to 0, 0.118, 0.256, 0.56, 1.18, and 2.56 mg a.s./L (accounting for the analysed a.s. content of 25.57% w/w prothioconazole). Each treatment comprised four replicates containing five daphnids each.

A stock solution of nominal concentration of 100 mg product/L (nominally 25.704 mg a.s./L) was prepared by completely dissolving 49.23 and 48.32 mg of product in 500 mL of test water on days 0 and 1, respectively. Each product treatment was prepared just prior to the introduction of the daphnids.

For the quantification of the product concentrations, the concentration of the active substance, prothioconazole, was analytically determined. Duplicate samples were taken from all test concentrations and control at the start and end of the two 24-hour test medium renewal periods. For sampling from the aged test media, the contents of the respective replicates were combined prior to sampling. Immediately after sampling, acetonitrile (10 mL acetonitrile per 10 mL sample volume) was added to each sample to stabilize the latter during the storage period. Thereafter, all samples were frozen for storage (at about -20 °C). The concentrations of prothioconazole were analysed in one of the duplicate test medium samples from the control and all product concentrations, from the start and end of both test medium renewal periods. Additionally, the stock solution of 100 mg product/L was analysed at the start of the first renewal period.

After 24 and 48 hours of exposure, daphnids were visually inspected for immobility (daphnids failing to swim within 15 seconds of gentle agitation of the test vessel). The number of mobile and immobile daphnids was recorded, and the 24- and 48-hour EC₅₀ and 95% confidence limits (CLs) were calculated using a linear maximum likelihood regression, with a Weibull function. Statistical analysis was performed using ToxRat Professional®. The NOEC and EC₁₀₀ values were determined directly from the raw data.

Results

Analytical results

The HPLC-MS/MS analytical method for the determination of prothioconazole in test medium was validated with regards to specificity, linearity, accuracy and precision in accordance with guideline SANCO/3029/99, part. 4, rev. 4, 11/07/2000. Specificity was demonstrated by the absence of a significant peak at the characteristic retention time for prothioconazole in the control sample. The analytical calibration was shown to be linear ($R^2 = 0.9995$) over the range of 0.235 – 92.1 µg a.s./L (equivalent to 0.919 – 360 µg product/L). Accuracy was confirmed with recovery of spiked samples at relevant concentrations of the test item (0.00308 and 3.11 mg a.s./L, corresponding to 0.0120 mg and 12.2 mg product/L); mean recoveries were 108% and 109% (i.e., within 70-110%) at the 0.0120 and 12.2 mg product/L fortification levels, respectively. Precision was confirmed; the relative standard deviation (n = 5, per level) was 3.6% and 2.7% (i.e., within the guideline limit of ≤20%) at the 0.0120 and 12.2 mg product/L fortification levels, respectively. The limit of quantification (LOQ) was 0.00308 mg a.s./L (equivalent to 0.0120 mg product/L). The limit of detection (LOD) was 0.000470 mg a.s./L.

CA3301 / JOUST	Page 93 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Table CP 10.2.1/01-01. Nominal and measured concentrations of prothioconazole in test solutions in each replicate sample at the beginning and end of each 24-hour renewal period, throughout the 48-hour semi-static acute toxicity test

Time (day/hour)	Nominal conc.		PF	Measured conc. (µg a.s./L)	Determined conc.		
	mg product/L	mg a.s./L			mg a.s./L	mg product/L	% nominal
0/0, fresh	0 (control)	0	2	n.d.	<LOQ	<LOQ	-
	0.46	0.118	12	8.29	0.0995	0.389	85
	1	0.256	12	17.5	0.21	0.821	82
	2.2	0.56	22	24.4	0.536	2.1	95
	4.6	1.18	42	27.5	1.15	4.51	98
	10	2.56	102	25.4	2.59	10.1	101
	100*	25.6	802	32.5	26	102	102
1/24, aged	0 (control)	0	2	n.d.	<LOQ	<LOQ	-
	0.46	0.118	12	3.36	0.0403	0.158	34
	1	0.256	12	8.31	0.1	0.39	39
	2.2	0.563	22	14.5	0.319	1.25	57
	4.6	1.18	42	20.9	0.876	3.43	74
	10	2.56	102	21.3	2.17	8.48	85
1/0, fresh	0 (control)	0	2	n.d.	<LOQ	<LOQ	-
	0.46	0.118	12	9.22	0.111	0.433	94
	1	0.256	12	20.9	0.251	0.982	98
	2.2	0.563	22	26.2	0.576	2.25	102
	4.6	1.18	42	27.9	1.17	4.57	99
	10	2.56	102	25.5	2.6	10.2	102
2/48, aged	0 (control)	0	2	n.d.	<LOQ	<LOQ	-
	0.46	0.118	12	4.28	0.0513	0.201	44
	1	0.256	12	12	0.145	0.565	57
	2.2	0.563	22	19.7	0.433	1.69	77
	4.6	1.18	42	24.8	1.04	4.08	89
	10	2.56	102	23.8	2.43	9.5	95

The tabulated values of the samples represent rounded results obtained by calculation using the exact raw data. *Stock solution; limit of quantification, LOQ = 0.00308 mg a.s./L, corresponding to 0.0120 mg product/L; conc., concentration; n.d., not detected; PF, preparation factor of samples.

At the start of the two renewal periods the measured concentrations in the test media were between 82 and 102% of the nominal values. Thus, the correct dosage of the product could be verified. During the test renewal periods of 24 hours, the active ingredient concentrations in the test media decreased especially at the lower concentrations. At the end of the two renewal periods, the product concentrations (based on active substance) were in the range of 34 to 85% (0 to 24 hours) and 44 to 95% (24 to 48 hours) of the nominal values.

The mean-measured product concentrations over the test period of 48 hours were calculated as the arithmetic mean of the two geometric means, which were determined from the product concentrations measured at the start and end of each of the two test medium renewal periods, as demonstrated in the table below.

CA3301 / JOUST	Page 94 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Table CP 10.2.1/01-02. Summary of the nominal, measured and calculated arithmetic mean measured test item concentrations over the test period of 48 hours

Nominal concentration		Measured concentration (mg a.s./L)				Arithmetic mean-measured concentration	
		0 h	24 h		48 h		
mg product/L	mg a.s./L	Fresh	Aged	Fresh	Aged	mg a.s./L	mg product/L
Control	Control	<LOQ	<LOQ	<LOQ	<LOQ	n.a.	n.a.
0.46	0.118	0.0995	0.0403	0.111	0.0513	0.069	0.27
1	0.256	0.21	0.1	0.251	0.145	0.17	0.66
2.2	0.56	0.536	0.319	0.576	0.433	0.46	1.8
4.6	1.18	1.15	0.876	1.17	1.04	1.1	4.1
10	2.56	2.59	2.17	2.6	2.43	2.4	9.6

LOQ = 0.00308 mg a.s./L (equivalent to 0.0120 mg product/L)

Biological results

The effects of CA3301 on the immobility of *D. magna*, after 48 hours of exposure, is presented in the Table CP 10.2.1/01-03 below.

Table CP 10.2.1/01-03. The effects of CA3301 on immobilisation of *D. magna*, in a 48-hour semi-static acute toxicity test (N = 5 daphnids per replicate).

Nominal concentration		Mean-measured concentration		Replicate	Immobilized daphnids			
					24 h		48 h	
mg product/L	mg a.s./L	mg product/L	mg a.s./L		No.	(%)	No.	(%)
0 (control)	0	0		1	0	0.0	0	0.0
				2	0		0	
				3	0		0	
				4	0		0	
0.46	0.118	0.27	0.069	1	0	0.0	0	0.0
				2	0		0	
				3	0		0	
				4	0		0	
1	0.256	0.66	0.17	1	0	0.0	0	5.0
				2	0		1	
				3	0		0	
				4	0		0	
2.2	0.56	1.8	0.46	1	4	70.0	5	100.0
				2	3		5	
				3	3		5	
				4	4		5	
4.6	1.18	4.1	1.1	1	5	100.0	5	100.0
				2	5		5	
				3	5		5	
				4	5		5	
10	2.56	9.6	2.4	1	5	100.0	5	100.0
				2	5		5	
				3	5		5	
				4	5		5	

During the first 24 hours, the three highest product treatments caused between 70 to 100% immobility of the daphnids. Thus, the 24-hour NOEC and LOEC values were determined to be 0.66 and 1.8 mg product/L (equivalent to 0.17 and 0.46 mg a.s./L), respectively, based on mean-measured concentrations. The 24-hour EC₅₀ (95% CLs) and EC₁₀₀ values were calculated to be 1.6 (1.4-1.8), and 4.1 mg product/L, equivalent to 0.4 (0.35-0.46), and 1.1 mg a.s./L, respectively, based on mean-measured concentrations.

CA3301 / JOUST	Page 95 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

No assessments of swimming were made for the two highest product treatments, given they caused 100% immobility of exposed daphnids. Consequently, after 48 hours of exposure, the nominal concentration of 1.0 mg product/L caused 5% immobility. At the three-highest test concentrations, 100% immobilisation was observed. Thus, the 48-hour NOEC and LOEC values (based on immobility) were determined to be 0.27 and 0.66 mg product/L (equivalent to 0.069 and 0.17 mg a.s./L), respectively, based on mean-measured concentrations. The 48-hour EC₅₀ (95% CLs), and EC₁₀₀ values were calculated to be 1.1 (0.66-1.8) and 1.8 mg product/L, equivalent to 0.28 (0.17-0.45) and 0.46 mg a.s./L, respectively, based on mean-measured concentrations.

Validity

All validity criteria were met in accordance with the OECD 202 (2004) test guideline:

- Immobilisation in the control groups should be $\leq 10\%$ at test end (actual value: 0%).
- Dissolved oxygen concentration should be ≥ 3 mg/L in all test vessels at the end of the test (actual values ranged from 8.2-8.8 mg O₂/L throughout the test).
- Analytical measurement of test concentrations was included.

Conclusion

The 48-hour acute toxicity of CA3301 to *Daphnia magna* was studied under semi-static conditions, in accordance with the OECD 202 (2004) test guideline.

Analytical verification of the test concentrations found that measured concentrations of the fresh and aged samples were between 34 to 85% (0 to 24 hours) and 44 to 95% (24 to 48 hours). Biological endpoints are, therefore reported based on arithmetic mean-measured concentrations.

The 48-hour NOEC value was determined to be 0.27 mg product/L (equivalent to 0.069 mg a.s./L), based on mean-measured concentrations. The 48-hour EC₅₀ value was calculated to be 1.1 mg product/L, equivalent to 0.28 mg a.s./L, based on mean-measured concentrations.

This study is considered acceptable.

CA3301 / JOUST	Page 96 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Study 2

Comments of zRMS:	<p>The study was conducted to OECD guideline 221 and according to the principles of GLP. No deviations to the guideline were noted. All validity criteria were met.</p> <p>The study is considered to be reliable and suitable for the risk assessment. Due to the decrease of the test item concentrations during the renewal periods, the results refer to nominal and mean measured concentrations of formulation and of active substance.</p>
-------------------	---

Reference:	KCP 10.2.1/02
Report	<p>250 EC Prothioconazole (CA3301) – Effect on the Aquatic Higher Plant <i>Lemna gibba</i> in a 7-Day Growth Inhibition Test</p> <p>Dupont, A., 2021, report no. 20190456</p>
Guideline(s):	Yes. OECD 221 (2006)
Deviations:	No
GLP:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive summary

A 7-day acute toxicity test of the product CA3301 (analysed a.s. content of 25.57% w/w prothioconazole) with duckweed, *Lemna gibba*, was conducted as a dose-response study, under semi-static conditions, with six nominal concentrations of 0 (control), 0.0032, 0.016, 0.080, 0.40, and 2.0 mg product/L, equivalent to 0, 0.818, 4.09, 20.5, 102, and 511 µg a.s./L. Each treatment group was divided into three replicates, with an initial 12 fronds per three colonies per test unit. Analytical verification of test concentrations confirmed that measured concentrations of all fresh and 72-hour aged samples ranged between <LOQ and 106% of nominal concentrations, therefore, biological endpoints are reported based on time-weighted mean (TWM) measured concentrations of the product, CA3301 and the active substance, prothioconazole, throughout the 72-hour test period.

Treatment with the four-highest CA3301 concentrations significantly reduced frond growth rate and frond yield, at the end of the 7-day exposure. The 7-day $E_rC_{10/20/50}$ and $E_yC_{10/20/50}$ values for *Lemna gibba* (duckweed), based on frond number, were calculated to be 0.11, 1.1, and >1.7 mg product/L, equivalent to 29, 288, and >444 µg a.s./L, respectively, and 0.0088, 0.050, and 1.300 mg product/L, equivalent to 2.3, 13, and 342 µg a.s./L, respectively. The 7-day $E_rC_{10/20/50}$ values for *Lemna gibba*, based on dry weight, were all estimated to be >1.7 mg product/L, equivalent to >444 µg a.s./L, respectively. The 7-day $E_yC_{10/20/50}$ values, based on dry weight, were calculated to be 0.059, >1.7, and >1.7 mg product/L, equivalent to 15.0, >444, and >444 µg a.s./L, respectively. The 7-day NOEC and LOEC values for *Lemna gibba*, based on frond number, were determined to be 0.0014 and 0.0045 mg product/L, equivalent to 0.347 and 1.15 µg a.s./L, respectively. The 7-day NOEC and LOEC values for *Lemna gibba*, based on dry weight, were estimated to be ≥1.7 and >1.74 mg product/L, equivalent to ≥444 and >444 µg a.s./L, respectively. All results are based on mean measured concentrations.

Overall, the study satisfies the OECD 221 (2006) test-guideline requirements for a *Lemna sp.* Growth-inhibition study and is considered acceptable

CA3301 / JOUST	Page 97 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Materials and methods

Test materials

Test item

Name:	Prothioconazole 250 EC, NUL 3390 (CA3301)
Active substance:	Prothioconazole
Formulation type:	Emulsifiable concentrate (EC)
Product density:	0.9948 g/mL
Source and lot/batch no.:	A19045
Active-substance content:	250 g/L (nominal), 257.04 g/L (analysed)
Active substance density:	25.57% w/w
Appearance:	Clear, straw-coloured liquid
Expiry date of lot/batch:	17 June 2022
Storage conditions:	Store in the closed, original container in a cool, well-ventilated area. Do not store for prolonged periods in direct sunlight.

Reference item

Name:	3,5-dichlorophenol
-------	--------------------

Test organism

Species:	Duckweed, <i>Lemna gibba</i> (family Lemnaceae, Macrophyta)
Strain:	G3
Source:	The original culture was supplied by Noack Laboratorien GmbH (Sarstedt, Germany).
Acclimation:	The plants were cultivated for more than four weeks prior to the test and under standardised conditions, in the same nutrient medium as used in the test.

Test conditions

Test medium:	AAP growth medium
Test temperature:	20-21°C
Water hardness:	3.0 mmol CaCO ₃ /L (300 mg CaCO ₃ /L)
pH:	7.4-7.5 (new media), 8.6-9.2 (old media)
Photoperiod:	Continuous illumination
Light intensity:	87 to 91 µE m ⁻² s ⁻¹

A pre-culture of duckweed was maintained under test conditions for 7 days prior to the start of the test. Reconstituted test water (AAP) was prepared to culture the duckweed, by dissolving analytical-grade salts in sterile purified water. The 7-day semi-static test was performed in 250-mL glass dishes (9.5-cm diameter, 21-mm depth). The volume of test medium in each test dish was 150 mL (the test medium was renewed every 2-3 days). Each test dish was loosely covered with a glass lid, which allowed CO₂ exchange between the air and test medium.

The test was started with 12 fronds per three colonies per test dish. There were three replicates per treatment group. For the product treatment groups, a stock solution (nominally 100 mg product/L, equivalent to 25.57 mg a.s./L, when accounting for the product a.s. content of 25.57% w/w) was prepared by dissolving 51.08-52.38 mg product in 500 mL of test medium, using intense stirring for 10 minutes, at room temperature, in the dark. The stock solution was used to prepare the highest product treatment concentration and serially diluted was serially diluted to prepare the other product treatments. The six test concentration groups were nominally 0 (control), 0.0032, 0.016, 0.080, 0.40, and 2.0 mg product/L, equivalent to 0, 0.818, 4.09, 20.5, 102, and 511 µg a.s./L. The control group was prepared with untreated (unspiked) test medium.

CA3301 / JOUST	Page 98 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

On days 2, 5, and 7 (test end), the number of fronds and colonies of duckweed were counted. Fronds visibly projecting over the edge of the mother frond were counted as separate fronds. Additionally, the plants were inspected for changes in appearance (e.g., discoloration, sinking, root length, or other abnormalities). The dry weight of a sample of eleven fronds was determined at the start of the test. At the test termination, the dry weight of the plants of each test dish was determined. Inhibition of growth was determined by calculating specific growth rate and yield, which were calculated for each test dish.

For the quantification of the product concentrations, the concentration of the active substance, prothioconazole, was analytically determined. Duplicate samples were taken from the stock solution at the start of the first and third renewal, as well as from freshly prepared and aged of all treatment groups. For sampling of the aged test media, the test media of the three replicates per test concentration were pooled. Immediately after sampling, acetonitrile (10 mL acetonitrile per 10 mL sample volume) was added to each sample for freezer stabilization. The concentrations of the prothioconazole were determined in one of the duplicate test-medium samples from each treatment per sampling time.

Statistical analyses were performed using ToxRat Professional to calculate. EC_{10} , EC_{20} , and EC_{50} values for growth rate and yield, and their 95% confidence intervals (CIs), were calculated with a probit analysis, using a linear maximum likelihood regression. The NOEC and LOEC values for growth rate and yield were determined using either a William's or Dunnett's t-test.

For evaluation of the test system and experimental conditions, 3,5-dichlorophenol (3,5-DCP) was tested as a positive control to demonstrate satisfactory test conditions. The 7-day E_rC_{50} in the reference test was 6.3 mg 3,5-DCP/L, demonstrating the sensitivity of the test system was within the range recommended by the guideline (7-day E_rC_{50} for the growth rate 6.2-9.7 mg 3,5-DCP/L).

Results

Analytical results

The HPLC-MS/MS analytical method for the determination of prothioconazole in test medium was validated with regards to specificity, linearity, accuracy and precision in accordance with guideline SANCO/3029/99, part. 4, rev. 4, 11/07/2000. Specificity was demonstrated by the absence of a significant peak at the characteristic retention time for prothioconazole in the control sample. The analytical calibration was shown to be linear ($R^2 = 0.9998$) over the range of 0.0490 – 48.6 $\mu\text{g a.s./L}$ (equivalent to 0.192 - 190 $\mu\text{g product/L}$). Accuracy was confirmed with recovery of spiked samples at relevant concentrations of the test item (0.00100 and 3.03 mg product/L); mean recoveries were 109% and 105% (i.e. within 70-110%), at the 0.00100 and 3.03 mg product/L fortification levels, respectively. Precision was confirmed; the relative standard deviation ($n = 5$, per level) was 8.0% and 2.4% (i.e. within the guideline limit of $\leq 20\%$) at the 0.00100 and 3.03 mg product/L fortification levels, respectively. The limit of quantification (LOQ) was 0.257 mg a.s./L (equivalent to 0.00100 mg product/L). The limit of detection (LOD) was 0.0980 $\mu\text{g a.s./L}$.

The results obtained for the concentrations of test item in the test samples are presented in the Table CP 10.2.1/03-01, below.

CA3301 / JOUST	Page 99 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Table CP 10.2.1/03-01. Nominal and measured concentrations of prothioconazole in test solutions in each replicate sample, at each sampling point.

Time (day/hour)	Nominal conc.		PF	Measured conc. (µg a.s./L)	Determined conc.		
	mg product/L	µg a.s./L			µg a.s./L	mg product/L	% nominal
0/0, fresh	Control	Control	2	n.d.	<LOQ	<LOQ	-
	0.0032	0.818	2	0.435	0.869	0.0034	106
	0.016	4.09	2	2.06	4.12	0.0161	101
	0.08	20.5	2	9.62	19.2	0.0752	94
	0.4	102	5	21.3	107	0.417	104
	2.0	511	22	23.6	519	2.03	102
	100*	25570	1122	22.6	25377	99.2	99
2/48, aged	Control	Control	2	n.d.	<LOQ	<LOQ	-
	0.0032	0.818	2	n.d.	<LOQ	<LOQ	-
	0.016	4.09	2	0.0925	<LOQ	<LOQ	-
	0.08	20.5	2	1.98	3.95	0.0155	19
	0.4	102	5	13.2	65.9	0.258	64
	2.0	511	22	20.1	443	1.73	87
2/0, fresh	Control	Control	2	n.d.	<LOQ	<LOQ	-
	0.0032	0.818	2	0.346	0.691	0.00256	84
	0.016	4.09	2	1.81	3.61	0.0135	88
	0.08	20.5	2	9.47	18.9	0.0772	93
	0.4	102	5	20.1	100.4	0.372	98
	2.0	511	22	23	506	1.98	99
5/72, aged	Control	Control	2	n.d.	<LOQ	<LOQ	-
	0.0032	0.818	2	n.d.	<LOQ	<LOQ	-
	0.016	4.09	2	0.061	<LOQ	<LOQ	-
	0.08	20.5	2	0.742	1.48	0.0058	7
	0.4	102	5	7.56	37.8	0.148	37
	2.0	511	22	16.7	368	1.44	72
5/0, fresh	Control	Control	2	n.d.	<LOQ	<LOQ	-
	0.0032	0.818	2	0.328	0.656	0.00256	80
	0.016	4.09	2	1.72	3.44	0.0135	84
	0.08	20.5	2	9.87	19.7	0.0772	97
	0.4	102	5	19	95.1	0.372	93
	2.0	511	22	23.3	513	2	100
7/48, aged	100a	25570	1122	23.6	26436	103.4	103
	Control	Control	2	n.d.	<LOQ	<LOQ	-
	0.0032	0.818	2	n.d.	<LOQ	<LOQ	-
	0.016	4.09	2	0.158	0.317	0.00124	8
	0.08	20.5	2	2.81	5.61	0.0219	27
	0.4	102	5	11.7	58.5	0.229	57
	2.0	511	22	15.8	348	1.36	68

The tabulated values of the samples represent rounded results obtained by calculation using the exact raw data. *Stock solution; limit of quantification, LOQ = 0.257 µg a.s./L, corresponding to 0.00100 mg product/L; conc., concentration; n.d., not detected; PF, preparation factor of samples.

The concentrations as% nominal found in the fresh test samples (0 hours) ranged from 94% to 106% at day 0, from 84% to 99% at day 2 and from 80% to 100% at day 5. The concentrations as% nominal found in the aged test samples (48 hours) ranged from <LOQ to 87% at day 2, from <LOQ to 72% at day 5 and from <LOQ to 68% at day 7.

Due to the decrease of concentrations during the test period, the mean-measured concentrations were calculated as the time-weighted means (TWMs), as demonstrated in the table below. Thus, endpoints are based on mean-measured concentrations.

CA3301 / JOUST	Page 100 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Table CP 10.2.1/03-02. Summary of nominal, measured and calculated time-weighted mean measured test item concentrations over the test period of 72 hours

Nominal concentration		Measured concentration (µg a.s./L)						TWM-measured concentration	
		Fresh medium			Aged medium				
µg product/L	µg a.s./L	Day 0	Day 2	Day5	Day 2	Day 5	Day 7	µg a.s./L	µg product /L
3.2	0.818	0.869	0.691	0.656	<LOQ			0.347	1.4
16.2	4.09	4.12	3.61	3.44	<LOQ		0.317	1.15	4.5
80	20.5	19.2	18.9	19.7	3.95	1.48	5.61	8.89	35.0
400	102	107	100.4	95.1	65.9	37.8	58.5	73.2	286
2000	511	519	506	513	443	368	348	444	1740

LOQ, limit of quantification (LOQ = 0.257 µg active ingredient/L). *If the measured concentration was below the LOQ, half of the LOQ (½ LOQ = 0.129 µg a.s./L) was used to calculate the time-weighted mean (TWM)-measured concentration.

Biological results

Frond number

The effects of CA3301 on *Lemna gibba* frond number, after 7 days of exposure, are presented in the tables below.

Table CP 10.2.1/03-03. The effects of CA3301 on yield, based on frond number for *Lemna gibba*, after 7-days of exposure.

Concentration				Replicate	Number of fronds				7-day Yield		
Nominal		TWM-measured			Time (days)				7-day Yield		
mg prod/L	µg a.s./L	mg prod/L	µg a.s./L		0	2	5	7			
0 (control)		0		1	12	24	93	232	220	222 ± 6.24	0
				2	12	24	96	241	229		
				3	12	24	100	229	217		
0.0032 0.818		0.0014 0.347		1	12	20	100	227	215	222.7 ± 6.81	-0.3
				2	12	28	101	237	225		
				3	12	25	99	240	228		
0.016 4.09		0.0045 1.15		1	12	21	94	223	211	206.7 ± 4.51*	+6.9
				2	12	24	93	219	207		
				3	12	24	103	214	202		
0.08 20.5		0.035 8.89		1	12	22	98	206	194	188.7 ± 6.81*	+15
				2	12	27	80	203	191		
				3	12	26	104	193	181		
0.4 102		0.286 73.2		1	12	23	73	143	131	129.7 ± 5.13*	+41.6
				2	12	23	84	136	124		
				3	12	25	88	146	134		
2 511		1.74 444		1	12	23	76	123	111	114 ± 3.61*	+48.6
				2	12	20	66	130	118		
				3	12	22	68	125	113		

Statistically significant differences, relative to the control, revealed with either William's or Dunnett's t-tests, and are denoted with asterisks (*). Positive (+) and negative (-) signs indicate a decrease and increase in yield, relative to the control. Prod, product; TWM, time-weighted mean; SD, standard deviation; No., number.

CA3301 / JOUST	Page 101 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Table CP 10.2.1/03-04: Effects of CA3301 on growth rate, based on frond number, for *Lemna minor* after 7 days' exposure.

Concentration				Average growth rate (μ) and inhibition (I_r)					
Nominal		TWM-measured		Days 0-2		Days 0-5		Days 0-7	
mg prod/L	$\mu\text{g a.s./L}$	mg prod/L	$\mu\text{g a.s./L}$	μ (fronds/day)	I_r (%)	μ (fronds/day)	I_r (%)	μ (fronds/day)	I_r (%)
0 (control)		0		0.347	0.0	0.416	0.0	0.424	0.0
0.0032	0.818	0.0014	0.347	0.349	-0.6	0.424	-1.8	0.425	-0.1
0.016	4.09	0.0045	1.15	0.324	+6.4	0.417	-0.1	0.415*	+2.3
0.08	20.5	0.035	8.89	0.365	-5.3	0.41	+1.5	0.402*	+5.2
0.4	102	0.286	73.2	0.339	+2.1	0.383*	+8.1	0.353*	+16.9
2	511	1.74	444	0.295	+15	0.352*	15.4	0.336*	+20.8

Statistically significant differences, relative to the control, revealed with either William's or Dunnett's t-tests, and are denoted with asterisks (*). Positive (+) and negative (-) signs indicate a lower and higher inhibition, relative to the control. Prod, product; TWM, time-weighted mean.

After seven days' exposure, statistically significant effects were recorded in yield and growth rate based on frond number, at the four highest test concentrations, when compared to the control. The 7-day $E_rC_{10/20/50}$ values, based on frond number (with 95% CIs) were calculated to be 0.11 (0.062 to 0.17), 1.1 (0.78 to 1.8), and >1.7 mg product/L, equivalent to 29 (16 to 45), 288 (199 to >444), and >444 $\mu\text{g a.s./L}$, respectively (based on TWM-measured concentrations).

The 7-day $E_yC_{10/20/50}$ values based on frond number (with 95% CIs) were calculated to be 0.0088 (0.0034 to 0.017), 0.050 (0.027 to 0.078), and 1.3 mg product/L (0.88 to 2.3) mg product/L, equivalent to 2.3 (0.87 to 4.4), 13 (6.9 to 20), and 342 (224 to >444) $\mu\text{g a.s./L}$, respectively (based on TWM-measured concentrations).

The 7-day NOEC and LOEC values based on frond growth rate and frond yield, were determined to be 0.0014 and 0.0045 mg product/L, equivalent to 0.347 and 1.15 $\mu\text{g a.s./L}$ (based on TWM-measured concentrations).

Dry weight

The effects of CA3301 on yield and growth rate of *Lemna gibba*, based on dry weight after 7 days of exposure, are presented in the tables below.

Table CP 10.2.1/03-05: Effects of CA3301 on yield based on dry weight of *Lemna gibba*, after 7

Nominal concentration		Mean-measured concentration		Average growth rate (μ) and inhibition (I_r)		Yield (Y) and inhibition (I_y)	
$\mu\text{g product/L}$	$\mu\text{g a.s./L}$	$\mu\text{g product/L}$	$\mu\text{g a.s./L}$	μ (day^{-1})	I_r (%)	Y (mg)	I_y (%)
0 (control)	0	0	0	0.417	0	30.4	0
3.2	0.818	1.4	0.347	0.436	-4.4	34.9	-14.8
16.2	4.09	4.5	1.15	0.423	-1.2	31.5	-3.5
80.0000	20.5	35	8.89	0.421	-0.9	31.2	-2.6
400	102	286	73.2	0.4	4.1	26.6	12.5
2000	511	1740	444	0.399	4.3	26.5	13

Statistically significant differences in growth rate and yield, relative to the control, were analyzed with one-sided William's t-tests.

Treatment with CA3301 did not cause significant changes in biomass (dry weight) growth rate and biomass yield. The 7-day E_rC_{10} , E_rC_{20} , and E_rC_{50} (biomass) values were all determined to be >1740 $\mu\text{g product/L}$, equivalent to >444 $\mu\text{g a.s./L}$, respectively (based on TWM-measured concentrations). The 7-day E_yC_{10} , E_yC_{20} , and E_yC_{50} values (with 95% CIs), based on biomass, were calculated to be 59.0 (12.0 – 41.0), >1740, and >1740 $\mu\text{g product/L}$, equivalent to 15.0 (3.2 to 105), >444, and >444 $\mu\text{g a.s./L}$, respectively (based on mean-measured concentrations). The 7-day NOEC and LOEC (biomass growth rate and biomass yield) values were determined to be 1740 and >1740 $\mu\text{g product/L}$, equivalent 444 and >444 $\mu\text{g a.s./L}$, respectively

CA3301 / JOUST	Page 102 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

(based on TWM-measured concentrations).

Table CP 10.2.1/03-06. The effect of CA3301 on growth rate, based on dry weight of *Lemna gibba*, after 7 days of exposure.

Nominal concentration		Mean-measured concentration		Section-by-section growth rate (μ) and inhibition (I_r)					
				Days 0-2		Days 2-5		Days 5-7	
$\mu\text{g product/L}$	$\mu\text{g a.s./L}$	$\mu\text{g product/L}$	$\mu\text{g a.s./L}$	μ (day^{-1})	I_r (%)	μ (day^{-1})	I_r (%)	μ (day^{-1})	I_r (%)
0 (control)	0	0	0	0.347	0	0.463	0	0.444	0
3.2	0.818	1.4	0.347	0.349	-0.6	0.474	-2.4	0.426	3.9
16.2	4.09	4.5	1.15	0.324	6.4	0.479	-3.4	0.409	7.9
80.0000	20.5	35.0000	8.89	0.365	-5.3	0.441	4.8	0.382	13.9
400	102	286	73.2	0.339	2.1	0.412	11	0.277	37.6
2000	511	1740	444	0.295	15	0.391	15.6	0.295	33.6

There were no statistically significant differences in biomass growth rate and biomass yield, based on dry weight, at any of the concentrations tested, when compared to the control. The 7-day $E_rC_{10/20/50}$ biomass growth rate values were all determined to be >1.7 mg product/L, equivalent to >444 $\mu\text{g a.s./L}$, respectively (based on TWMean-measured concentrations). The 7-day $E_yC_{10/20/50}$ (biomass yield values (with 95% Confidence Intervals) were calculated to be 0.0590 mg product/L (0.012 – 0.41 mg product/L), >1740 mg product/L, and >1740 mg product/L, equivalent to 15.0 $\mu\text{g a.s./L}$ (3.2 to 105 $\mu\text{g a.s./L}$), >444 $\mu\text{g a.s./L}$ and >444 $\mu\text{g a.s./L}$, respectively (based on TWMean-measured concentrations).

The 7-day NOEC and LOEC (biomass growth rate and biomass yield) values were determined to be ≥ 1.7 and >1.7 mg product/L, equivalent ≥ 444 and >444 $\mu\text{g a.s./L}$ (based on TWMean-measured concentrations).

No abnormalities in appearance of the test plants were recorded in the control and the test item nominal concentrations of 0.0032 to 0.080 mg product/L. Based on visual assessment, at the concentrations of 0.40 to 2.0 mg product/L, the plants showed chlorosis. At the concentration of 2.0 mg product/L, the roots of the plants were shorter compared to the control.

Validity

The validity criterion was met, in accordance with the OECD 221 (2006) test guideline:

- Frond number doubling time in the control group was 0.275 fronds/day, or <2.5 days (actual value was 1.6 days).

Conclusion

In a 7-day growth inhibition study, the fresh-water floating aquatic vascular plant, duckweed (*Lemna gibba*) was exposed to CA3301 at nominal concentrations of 0 (control), 3.2, 16.0, 80.0, 400, and 2000 $\mu\text{g product/L}$, equivalent to 0, 0.818, 4.09, 20.5, 102, and 511 $\mu\text{g a.s./L}$, in accordance with the OECD 221 (2006) test guideline. Analytical verification of test concentrations confirmed that measured concentrations of all fresh and 72-hour aged samples ranged between $<\text{LOQ}$ and 106% of nominal concentrations, therefore, biological endpoints are reported based on time-weighted mean (TWM) measured concentrations of the product, CA3301 and the active substance, prothioconazole, throughout the 72-hour test period.

The 7-day $E_rC_{10/20/50}$ and $E_yC_{10/20/50}$ values for *Lemna gibba* (duckweed), based on frond number were calculated to be 0.11, 1.1, and >1.7 mg product/L, equivalent to 29, 288, and >444 $\mu\text{g a.s./L}$, respectively and 0.0088, 0.050, and 1.300 mg product/L, equivalent to 2.3, 13, and 342 $\mu\text{g a.s./L}$, respectively.

The 7-day $E_rC_{10/20/50}$ values for *Lemna gibba* based on dry weight were all estimated to be >1.7 mg product/L, equivalent to >444 $\mu\text{g a.s./L}$, respectively. The 7-day $E_yC_{10/20/50}$ values, based on dry weight were calculated to be 0.059, >1.7 , and >1.7 mg product/L, equivalent to 15.0, >444 , and >444 $\mu\text{g a.s./L}$, respectively.

CA3301 / JOUST	Page 103 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

The 7-day NOEC and LOEC values for *Lemna gibba* based on frond number were determined to be 0.0014 and 0.0045 mg product/L, equivalent to 0.347 and 1.15 µg a.s./L, respectively.

The 7-day NOEC and LOEC values for *Lemna gibba*, based on dry weight were estimated to be ≥ 1.7 and > 1.74 mg product/L, equivalent to ≥ 444 and > 444 µg a.s./L, respectively. All results are based on mean measured concentrations.

This study is considered acceptable.

Study 3

Comments of zRMS:	<p>The study was conducted to OECD guideline 201 and according to the principles of GLP. No deviations to the guideline were noted. All validity criteria were met.</p> <p>The study is considered to be reliable and suitable for the risk assessment. Due to the decrease of the test item concentrations during the test period, the results refer to nominal and mean measured concentrations of formulation and of active substance.</p>
-------------------	---

Reference:	KCP 10.2.1/03
Report	<p>250 EC Prothioconazole (CA3301) - Effect on <i>Skeletonema</i> sp. in a 72-Hour Algal Growth Inhibition Test</p> <p>Dupont, A., 2021, report no. 20190454</p>
Guideline(s):	Yes. OECD 201 (2006)
Deviations:	No
GLP:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive summary

A 72-h acute toxicity test of CA3301 (analysed a.s. content of 25.57% w/w prothioconazole) with the marine diatom *Skeletonema subcapitata* was conducted as a dose-response study, under static conditions, with seven nominal concentrations of 0 (control), 2.5, 8.0, 25.0, 80.0, 250, and 800 µg product/L, equivalent to 0, 0.639, 2.05, 6.39, 20.5, 63.9, 205 µg a.s./L. Each treatment group was divided into three replicates, six replicates for the control group, with an initial nominal algal cell density of 5000 cells/mL. Analytical verification of test concentrations confirmed that measured concentrations of all fresh and 72-hour aged samples ranged between 23 and 103% of nominal concentrations, therefore, biological endpoints are reported based on time-weighted mean (TWM) measured concentrations of prothioconazole throughout the 72-hour test period.

The 72-hour $E_rC_{10/20/50}$ values for the diatom, *Skeletonema subcapitata*, were calculated to be 104, 165 and 328 µg product/L, equivalent to 27, 42 and 84 µg a.s./L (mean measured), respectively. The 72-hour $E_yC_{10/20/50}$ values were calculated to be 1.1, 6.1 and 78 µg product/L, equivalent to 0.29, 1.6 and 20 µg (mean measured), respectively.

The 72-hour NOE_rC and LOE_rC values, based on growth rate, were estimated to be ≤ 0.93 and < 0.93 µg product/L, equivalent to ≤ 0.24 and < 0.24 µg a.s./L (TWM measured), respectively. The 72-hour NOE_yC and LOE_yC values, based on yield, were estimated to be ≤ 0.93 and < 0.93 µg product/L, equivalent to ≤ 0.24

CA3301 / JOUST	Page 104 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

and <0.24 µg a.s./L (TWM measured), respectively.

Overall, the study satisfies the OECD 201 (2011) test-guideline requirements for an algal growth-inhibition study and is considered acceptable.

Materials and methods

Test materials

Test item

Name:	Prothioconazole 250 EC, NUL 3390 (CA3301)
Active substance:	Prothioconazole
Formulation type:	Emulsifiable concentrate (EC)
Product density:	0.9948 g/mL
Source and lot/batch no.:	A19045
Active-substance content:	250 g/L (nominal), 257.04 g/L (analysed)
Active substance density:	25.57% w/w
Appearance:	Clear, straw-coloured liquid
Expiry date of lot/batch:	17 June 2022
Storage conditions:	Store in the closed, original container in a cool, well-ventilated area. Do not store for prolonged periods in direct sunlight.

Reference item

Name:	Potassium dichromate
-------	----------------------

Test organism

Species:	Marine diatom, <i>Skeletonema subcapitata</i>
Strain:	CCAP 1077/1C (formerly listed as <i>Skeletonema costatum</i>),
Source:	Culture Collection of Algae and Protozoa (CCAP, Scottish Marine Institute, Dunbeg, Oban, Argyll, Scotland).

Test conditions

Test medium:	Reconstituted test water (MAA), made with sterile seawater
Test temperature:	22°C
pH:	7.9-8.5
Photoperiod:	Continuous illumination
Light intensity:	67-72 µE/m ² s

The algae were cultivated at the test facility, under standardised conditions. 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.

For evaluation of the algal quality and experimental conditions, potassium dichromate (K₂Cr₂O₇) was tested as a positive control to demonstrate satisfactory test conditions. The 72-h E_rC₅₀ in the reference test was 1.6 mg K₂Cr₂O₇/L, demonstrating the sensitivity of the test system was within the range recommended by the guideline (96-h E_rC₅₀ for the growth rate 1.4-3.6 mg K₂Cr₂O₇/L).

Reconstituted test water (MAA) was prepared to culture the diatoms. Briefly, analytical-grade salts were dissolved in sterile purified seawater – 15 mL of the Metal Mix, 10 mL of the Minor Salt Mix, and 0.5 mL of the Vitamin Mix were added to 974.5 mL seawater.

The 72-h static test was performed in 125-mL Erlenmeyer flasks (incubation vessels). The volume of test solution in each test flask was 50 mL. Each test flask was loosely covered with a glass lid, which allowed CO₂ exchange between the air and test medium. The test was started using a nominal algal cell density of

CA3301 / JOUST	Page 105 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

5000 cells/mL. The test design included three replicates per product treatment group and six replicates for the control group. For the product treatment groups, a stock solution (nominally 100 mg product/L, equivalent to 25.57 mg a.s./L, when accounting for the product a.s. content of 25.57% w/w) was prepared by dissolving 99.92 mg product in 999.2 mL of test medium, using intense stirring for 10 minutes, at room temperature, in the dark. The stock solution was diluted with test medium to prepare a diluted stock solution of nominal 25 mg product/L (equivalent to 6.39 mg a.s./L). The diluted stock solution was serially diluted to prepare the nominal product treatments of 2.5, 8.0, 25.0, 80.0, 250, and 800 µg product/L, equivalent to 0.639, 2.05, 6.39, 20.5, 63.9, 205 µg a.s./L. The control group was prepared with untreated (unspiked) test medium.

Algal biomass was determined by fluorescence measurement (440-nm excitation, 675-nm emission), in duplicate, by taking a small volume (100 µL per sample) of the algal suspension from each test flask (replicate) daily. At the end of the test, a sample was taken from the control and two-highest product treatments (250 and 800 µg product/L), to determine a potential influence of the product on the algal morphology, using a microscope. Inhibition of algal growth was determined by calculating specific growth rate and yield, which were calculated for each test flask.

For the quantification of the product concentrations, the concentration of the active substance, prothioconazole, was analytically determined. Duplicate samples were taken from each treatment, the stock solution, and the diluted stock solution at the start of the test. After 24 and 48 h and at the end of the test (after 72 h), stability samples (containing algae) were taken in duplicate from all test concentrations and from the control. For sampling at the end of the test, the test medium of the treatment replicates was pooled. Immediately after sampling, acetonitrile (10 mL acetonitrile per 10 mL sample volume) was added to each sample for freezer stabilization. The concentrations of the prothioconazole were determined in one of the duplicate test-medium samples from each treatment per sampling time.

Statistical analyses were performed using ToxRat Professional to calculate. EC₁₀, EC₂₀, and EC₅₀ values for growth rate and yield, and their 95% confidence intervals (CIs), were calculated with a Weibull analysis, using a linear maximum likelihood regression. The NOEC and LOEC values for growth rate and yield were determined using either a William's, Welch, or step-down Jonckheere-Terpstra t-tests.

Results

Analytical results

The HPLC-MS/MS analytical method for the determination of prothioconazole in test samples was validated with regards to specificity, linearity, accuracy and precision in accordance with guideline SANCO/3029/99, part. 4, rev. 4, 11/07/2000. Specificity was demonstrated by the absence of a significant peak at the characteristic retention time for prothioconazole in the control samples. The analytical calibration was shown to be linear ($R^2 = 0.9993$) over the range of 0.0474 – 23.7 µg a.s./L (equivalent to 0.185 – 92.7 µg product/L). Accuracy was confirmed with recovery of spiked samples at relevant concentrations of the test item (0.00152 and 1.00 mg product/L); mean recoveries were 87% and 99% (i.e., within 70-110%) at the 0.00152 and 1.00 mg product/L fortification levels, respectively. Precision was confirmed; the relative standard deviation (n = 5, per level) was 12.5% and 4.2% (i.e., within the guideline limit of ≤20%) at the 0.00152 and 1.00 mg product/L fortification levels, respectively. The limit of quantification (LOQ) was 0.389 mg a.s./L (equivalent to 0.00152 mg product/L). The limit of detection (LOD) was 0.0948 µg a.s./L.

The results obtained for the concentrations of test item in the test samples are presented in Table CP 10.2.1/02-01.

CA3301 / JOUST	Page 106 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Table CP 10.2.1/02-01. Nominal and measured concentrations of prothioconazole in test solutions in each replicate sample, at each sampling point.

Time (day)	Nominal conc.		PF	Measured conc. (µg a.s./L)	Determined conc.		
	mg product/L	µg a.s./L			µg a.s./L	mg product/L	% nominal
0	Control	Control	2	n.d	<LOQ	<LOQ	-
	0.0025	0.639	2	0.316	0.632	0.00247	99
	0.008	2.05	2	0.952	1.9	0.00745	93
	0.025	6.39	2	3.29	6.58	0.0257	103
	0.08	20.5	2	8.36	16.7	0.0654	82
	0.25	63.9	6.67	8.61	57.4	0.225	90
	0.8	205	20	8.95	179	0.7	88
1	Control	Control	2	n.d	<LOQ	<LOQ	-
	0.0025	0.639	2	0.104	<LOQ	<LOQ	-
	0.008	2.05	2	0.324	0.647	0.00253	32
	0.025	6.39	2	1.73	3.47	0.0136	54
	0.08	20.5	2	6.34	12.7	0.0496	62
	0.25	63.9	6.7	7.37	49.2	0.192	77
	0.8	205	20	8.57	171	0.67	84
2	Control	Control	2	n.d	<LOQ	<LOQ	-
	0.0025	0.639	2	0.0906	<LOQ	<LOQ	-
	0.008	2.05	2	0.294	0.587	0.0023	29
	0.025	6.39	2	0.976	1.95	0.00763	31
	0.08	20.5	2	5.12	10.2	0.04	50
	0.25	63.9	6.7	6.12	40.8	0.16	64
	0.8	205	20	8.32	166	0.65	81
3	Control	Control	2	n.d	<LOQ	<LOQ	-
	0.0025	0.639	2	0.0939	<LOQ	<LOQ	-
	0.008	2.05	2	0.249	0.497	0.00194	24
	0.025	6.39	2	0.745	1.49	0.00583	23
	0.08	20.5	2	2.48	4.95	0.0194	24
	0.25	63.9	6.7	3.34	22.3	0.087	35
	0.8	205	20	5.95	119	0.465	58

LOQ, limit of quantification = 0.389 µg a.s./L, corresponding to 0.00152 mg product/L. n.d., not determined; conc., concentration; PF, preparation factor.

The measured concentrations of prothioconazole in the fresh test samples (0 hours) were between 82 and 103% at day 0, from 32% to 84% at 24 hours, from 29% to 81% at 48 hours and from 23% to 58% at 72 hours. Due to the decrease of product concentrations during the test period, the mean measured test item concentrations were calculated as the time-weighted means (TWMs), as demonstrated in the table below. Thus, endpoints are based on TWM-measured concentrations.

Table CP 10.2.1/03-02. Summary of nominal, measured and calculated time-weighted mean measured test item concentrations over the test period of 72 hours

Nominal concentration		Measured concentration (µg a.s./L)				TWM-measured concentration	
µg product/L	µg a.s./L	0 h	24 h	48 h	72 h	µg a.s./L	µg product/L
2.5	0.639	0.632	<LOQ	<LOQ	<LOQ	0.24*	0.93*
8.0	2.05	1.90	0.647	0.587	0.497	0.72	2.8
25.0	6.39	6.58	3.47	1.95	1.49	2.8	11
80.0	20.5	16.7	12.7	10.2	4.95	11	41
250	63.9	57.4	49.2	40.8	22.3	42	163
800	205	179	171	166	119	161	628

LOQ, limit of quantification (LOQ = 0.389 µg active ingredient/L). *If the measured concentration was below the LOQ, half of the LOQ (½ LOQ = 0.1945 µg a.s./L) was used to calculate the time-weighted mean-measured (TWM-measured) concentration.

CA3301 / JOUST	Page 107 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Biological results

The effects of CA3301 on biomass of *Skeletonema subcapitata*, after 72 h of exposure, is presented in the Table CP 10.2.1/03-03 below.

Table CP 10.2.1/03-03. The effects of CA3301 on *Skeletonema . subcapitata* biomass, during the 72-h exposure.

Nominal concentration		TWM-measured concentration		Replicate	Biomass of Algae*		
µg product/L	µg a.s./L	µg product/L	µg a.s./L		24 h	48 h	72 h
0 (control)	0	0	0	1	4.29	13.5	37.4
				2	3.18	12.8	36.1
				3	3.95	12.3	35.4
				4	3.76	12.4	42
				5	3.63	9.9	32
				6	3.94	13.3	34.1
				Mean	3.79	12.4	36.2
				SD	0.371	1.29	3.4
2.5	0.64	0.93	0.24	1	3.07	10.7	31.1
				2	2.84	12.9	31.9
				3	4.6	10.3	29.6
				Mean	3.5	11.3	30.9
				SD	0.955	1.382	1.16
8	2.05	2.8	0.72	1	3.32	11.7	30.6
				2	3.35	10.7	29.2
				3	2.86	12.6	27
				Mean	3.18	11.7	28.9
				SD	0.273	0.98	1.79
25	6.39	11	2.81	1	2.78	11.9	25
				2	3.28	10.7	26
				3	3.71	11.3	25.6
				Mean	3.25	11.3	25.5
				SD	0.462	0.57	0.53
80	20.46	41	10.48	1	3.54	10.2	24.9
				2	2.95	11.49	22.8
				3	3.31	10.92	23.4
				Mean	3.27	10.87	23.7
				SD	0.296	0.644	1.06
250	63.93	163	41.68	1	3.79	10.1	20.2
				2	2.79	8.87	18.8
				3	3.54	9.28	19.2
				Mean	3.37	9.4	19.4
				SD	0.519	0.606	0.713
800	204.56	628	160.58	1	1.33	1.47	1.47
				2	1.17	2.18	1.77
				3	1.17	1.48	1.21
				Mean	1.22	1.71	1.48
				SD	0.0896	0.406	0.282

TWM-measured, time-weighted mean-measured; SD, standard deviation. *Biomass was determined by fluorescence measurement (mean of duplicate measurements per replicate) and is given as relative fluorescence units (x 104). At the start of the test, the initial cell density was 5000 algal cells/mL, corresponding to 0.935 x 104 relative fluorescence units.

There was a clear dose-response effect on algal biomass, with an observed decrease in biomass with increasing product treatment concentration. Control group biomass increased 39-fold over 72 h.

Summaries of the effect of CA3301 on algal growth, yield, and section-by-section growth rate, during the 72-h exposure, are presented in the tables below.

CA3301 / JOUST	Page 108 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Table CP 10.2.1/03-04. The effects of CA3301 on *Skeletonema subcapitata* growth rate, during the 72-h exposure.

Nominal conc.	TWM-measured conc.		Average growth rate (μ) and inhibition (I_r)					
			24 h		48 h		72 h	
$\mu\text{g product/L}$	$\mu\text{g product/L}$	$\mu\text{g a.s./L}$	μ (day^{-1})	I_r (%)	μ (day^{-1})	I_r (%)	μ (day^{-1})	I_r (%) CV (%)
0 (control)	0	0	1.396	0	1.289	0	1.217	0
2.5	0.93	0.24	1.298	7	1.245	3.5	1.166*	4.2
8	2.8	0.72	1.221	12.5	1.26	2.3	1.143*	6.1
25	11	2.81	1.24	11.1	1.246	3.4	1.102*	9.4
80	41	10.48	1.249	10.5	1.226	4.9	1.077*	11.5
250	163	41.68	1.275	8.7	1.153*	10.5	1.010*	17
800	628	160.58	0.266*	80.9	0.293*	77.3	0.149*	87.8

Statistically significant differences in growth rate, relative to the control, were revealed with one-sided William's t-tests, when $P < 0.05$, and are denoted with an asterisk (*). Conc., concentration; CV, coefficient of variation.

Table CP 10.2.1/03-04. The effect of CA3301 (analysed a.s. content of 25.57% w/w) on *S. subcapitata* yield, during the 72-h exposure.

Nominal conc.	TWM-measured conc.		Yield (Y , $\times 10^4$) and inhibition (I_y)					
			24 h		48 h		72 h	
$\mu\text{g product/L}$	$\mu\text{g product/L}$	$\mu\text{g a.s./L}$	Y	I_y (%)	Y	I_y (%)	Y	I_y (%) CV (%)
0 (control)	0	0	2.9	0	11.5	0	35.2	0
2.5	0.93	0.24	2.6	10	10.4	9.2	30.0*	15
8	2.8	0.72	2.2	21.5	10.7	6.4	28.0*	20.6
25	11	2.81	2.3	18.8	10.4	9.5	24.6*	30.2
80	41	10.48	2.3	18.3	9.9*	13.3	22.8*	35.4
250	163	41.68	2.4	14.6	8.5*	26	18.4*	47.7
800	628	160.58	0.3*	89.9	0.8*	93.2	0.5*	98.5

Statistically significant differences in yield rate, relative to the control, when $P < 0.05$, are denoted with asterisks (*). Data were analyzed with one-sided Welch t-tests at 24 h, with one-sided William's t-tests at 48 h, and with one-sided, step-down Jonckheere-Terpstra t-tests at 72 h. Conc., concentration; CV, coefficient of variation.

Table CP 10.2.1/03-05. The effect of CA3301 (analysed a.s. content of 25.57% w/w) on *S. subcapitata* section-by-section growth rate, during the 72-h exposure.

Nominal conc.	TWM-measured conc.		Section-by-section growth rate (μ) and inhibition (I_r)					
			0-24 h		24-48 h		48-72 h	
$\mu\text{g product/L}$	$\mu\text{g product/L}$	$\mu\text{g a.s./L}$	μ (day^{-1})	I_r (%)	μ (day^{-1})	I_r (%)	μ (day^{-1})	I_r (%) CV (%)
0 (control)	0	0	1.396	0.0	1.183	0.0	1.073	0.0
2.5	0.93	0.24	1.298	7.0	1.192	-0.7	1.007	6.1
8	2.8	0.72	1.221	12.5	1.300	-9.9	0.910	15.2
25	11	2.81	1.240	11.1	1.251	-5.7	0.816	24.0
80	41	10.48	1.249	10.5	1.203	-1.7	0.780	27.3
250	163	41.68	1.275	8.7	1.032	12.7	0.724	32.5
800	628	160.58	0.266	80.9	0.319	73.1	-0.138	112.8

Statistically significant effects were recorded in algal growth rate and yield at all test concentrations, after 72 hours of exposure. No abnormalities in appearance of the algae at the start and at the end of the test were observed.

The 72-hour $E_4C_{10/20/50}$ values for *Skeletonema subcapitata* (with 95% Confidence Intervals), based on growth rate, were determined to be 104 (75 – 132) $\mu\text{g product/L}$, 165 (130 – 197) $\mu\text{g product/L}$, and 328 (287 – 370) $\mu\text{g product/L}$, equivalent to 27 (19 – 34) $\mu\text{g a.s./L}$, 42 (33 – 50) $\mu\text{g a.s./L}$, and 84 (73 – 95) $\mu\text{g a.s./L}$, respectively, based on time-weighted mean-measured concentrations.

The corresponding NOEC and LOEC values for *Skeletonema subcapitata*, based on growth rate, were

CA3301 / JOUST	Page 109 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

estimated to be ≤ 0.93 and < 0.93 $\mu\text{g product/L}$, equivalent to ≤ 0.24 and < 0.24 $\mu\text{g a.s./L}$, based on time-weighted mean-measured concentrations.

The 72-hour E_yC_{10} , E_yC_{20} , and E_yC_{50} values for *Skeletonema subcapitata* (with 95% Confidence Intervals), based on yield, were determined to be 1.1 (0.55 – 2.0) $\mu\text{g product/L}$, 6.1 (3.8 – 8.9) $\mu\text{g product/L}$, and 78 (62 – 97) $\mu\text{g product/L}$, equivalent to 0.29 (0.14 – 0.50) $\mu\text{g a.s./L}$, 1.6 (0.96 – 2.3) $\mu\text{g a.s./L}$, and 20 (16 – 25) $\mu\text{g a.s./L}$, respectively, based on time-weighted mean-measured concentrations.

The NOEC and LOEC values for *Skeletonema subcapitata*, based on yield, were estimated to be ≤ 0.93 and < 0.93 $\mu\text{g product/L}$, equivalent to ≤ 0.24 and < 0.24 $\mu\text{g a.s./L}$, based on time-weighted mean-measured concentrations.

Validity

All validity criteria were met in accordance with the OECD 201 (2011) test guideline:

- During the 72-hour test period, biomass in the control group increased by a factor of ≥ 16 at the end of the test (actual value: 39).
- The coefficient of variation of the average specific growth rates in the control replicates during the whole test period did not exceed 7% (actual value was 2.5%).
- The mean coefficient of variation for section-by-section specific growth rates in the control was $\leq 35\%$ (actual value was 16%)

Conclusion

In a 72-hour toxicity study, the marine diatom, *Skeletonema Subcapitata* was exposed to the product CA3301 (a.s. content of 25.57% w/w prothioconazole, analysed) at nominal concentrations of 0 (control), 2.5, 8.0, 25.0, 80.0, 250, and 800 $\mu\text{g product/L}$, equivalent to 0, 0.639, 2.05, 6.39, 20.5, 63.9, 205 $\mu\text{g a.s./L}$, under static conditions, in accordance with the OECD 201 (2011) test guideline. Analytical verification of test concentrations confirmed that measured concentrations of all fresh and 72-hour aged samples ranged between 23 and 103% of nominal concentrations, therefore, biological endpoints are reported based on time-weighted mean (TWM) measured concentrations of prothioconazole throughout the 72-hour test period

The 72-hour $E_rC_{10/20/50}$ values for *Skeletonema subcapitata* (with 95% Confidence Intervals), based on growth rate, were determined to be 104 (75 – 132) $\mu\text{g product/L}$, 165 (130 – 197) $\mu\text{g product/L}$, and 328 (287 – 370) $\mu\text{g product/L}$, equivalent to 27 (19 – 34) $\mu\text{g a.s./L}$, 42 (33 – 50) $\mu\text{g a.s./L}$, and 84 (73 – 95) $\mu\text{g a.s./L}$, respectively, based on time-weighted mean-measured concentrations. The corresponding NOEC and LOEC values for *Skeletonema subcapitata*, based on growth rate, were estimated to be ≤ 0.93 and < 0.93 $\mu\text{g product/L}$, equivalent to ≤ 0.24 and < 0.24 $\mu\text{g a.s./L}$, based on time-weighted mean-measured concentrations.

The 72-hour E_yC_{10} , E_yC_{20} , and E_yC_{50} values for *Skeletonema subcapitata* (with 95% Confidence Intervals), based on yield, were determined to be 1.1 (0.55 – 2.0) $\mu\text{g product/L}$, 6.1 (3.8 – 8.9) $\mu\text{g product/L}$, and 78 (62 – 97) $\mu\text{g product/L}$, equivalent to 0.29 (0.14 – 0.50) $\mu\text{g a.s./L}$, 1.6 (0.96 – 2.3) $\mu\text{g a.s./L}$, and 20 (16 – 25) $\mu\text{g a.s./L}$, respectively, based on time-weighted mean-measured concentrations. The NOEC and LOEC values for *Skeletonema subcapitata*, based on yield, were estimated to be ≤ 0.93 and < 0.93 $\mu\text{g product/L}$, equivalent to ≤ 0.24 and < 0.24 $\mu\text{g a.s./L}$, based on time-weighted mean-measured concentrations.

This study is considered acceptable.

CA3301 / JOUST	Page 110 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Study 4

Comments of zRMS:	Study not evaluated by zRMS as is not crucial for the risk assessment.
-------------------	--

Reference:	KCP 10.2.1/04
Report	<i>Desmodesmus subspicatus</i> growth inhibition test with AE1194888 (BCS-AA53879) Kuhl, K. 2018, report no. M-630874-01-1
Guideline(s):	Yes. OECD 201 (2006), OCSPP 850.4500 (2012)
Deviations:	No
GLP:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability:	Yes
Duplication (if vertebrate study)	No

Note to RMS: This study is protected, but the Owner (Bayer) has provided the Sponsor (Nufarm) access. However, since Nufarm does not have access to a copy of the final study report, this study summary is prepared using only the summary available in the 2018 RAR for prothioconazole.

Material and methods:

Test material:	JAU 6476-desthio, prothioconazole-desthio Code: BCS-AA53879, AE1194888 lot/batch: SES 10834-2-1 specification: not specified purity: 98.3%
Guideline(s) adaptation:	none
Test species:	<i>Desmodesmus subspicatus</i> Strain No. SAG 86.81 ESP Origin: Lab in-house culture
Culturing conditions:	400 µL of a 7-10 days old stock culture were transferred into a 300 mL cotton plugged Erlenmeyer flask containing about 100 mL of nutrient medium every 7-10 days. Stock cultures of algae were kept at 22 ± 2 °C with 24 hours light (4.50 – 7.00 klux). Culture vessels were shaken at 100 rpm on an orbital shaker table to prevent sedimentation of the cells. All operations were conducted under sterile conditions to handle an axenic algae culture, whereas axenic cultures are cultures of a single species. Pre-cultures were prepared from stock cultures 4 days before the start of the test using OECD medium.

CA3301 / JOUST	Page 111 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Test solutions:	<p>Test medium: OECD medium</p> <p>Nominal concentrations: 0.00596 – 0.0191 – 0.0610 – 0.195 – 0.625 – 2.00 mg p.m./L.</p> <p>Measured concentrations: all in the range 80-120% of nominal concentrations</p> <p>Controls: test medium</p> <p>Evidence of undissolved material: not observed, no remarkable observation</p>
Replication:	<p>No. of vessels per concentration (replicates):4</p> <p>No. of vessels per control (replicates): 4</p>
Initial cell density:	10 000 cells/mL
Exposure:	<p>Static conditions</p> <p>Total exposure duration: 96 h</p>
Test conditions:	<p>Temperature: 23.1 – 23.9 °C</p> <p>Photoperiod: continuous light</p> <p>Light intensity: 4.7 klux (mean), range 4.61 – 4.86 klux. The uniform overhead illumination was provided by a bank light containing cool fluorescent light.</p> <p>pH: 7.7-7.8 at test start and 7.8-8.3 at test end</p> <p>Water hardness: not reported</p>
Parameters Measured/Observations:	<p>Cell density on each observation time (24, 48, 72 and 96 h) was determined by spectrophotometric measurement.</p> <p>Morphological examination of cells using a microscope were made after 24, 48, 72 and 96 hours.</p>
Sampling for chemical analysis:	<p>Duplicate samples from the freshly prepared test media of all test concentrations and control were taken at the start of the test and duplicate samples of the pooled media of each concentrations and control at 72 and 96 h.</p>
Data analysis:	<p>With ToxRat 3.2.1</p> <p>Probit analysis for ECx determination and Welch t-test after Bonferroni-Holm or Williams multiple sequential t-test procedure for NOEC</p>

Results:

Table 2.3-1: Validity criteria

Validity criteria of OECD guideline after 72h	Required	Obtained
Cell density increase in control	Factor 16	49.8
Coefficient of variation of sectional (daily) growth rates in control cultures:	$\leq 35\%$	26.7
Coefficient of variation of average growth between control replicates:	$\leq 7\%$	1.4

CA3301 / JOUST	Page 112 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Analytical results:

The test substance was stable over the study duration, all measured concentration are in the range of 80-120% of nominal concentrations so the results are based on nominal concentrations.

Table 2.3-2: Analytical results

Nominal Concentration (mg p.m./L)	Day 0 Measured conc. (mg p.m./L)	Day 0 % of nominal	Day 3 Measured conc. (mg p.m./L)	Day 3 % of nominal	Day 4 Measured conc. (mg p.m./L)	Day 4 % of nominal
Control	< LOQ	--	< LOQ	--	< LOQ	--
0.00596	0.00517	87	0.00520	87	0.00509	85
0.0191	0.0166	87	0.0167	87	0.0165	86
0.0610	0.0560	92	0.0548	90	0.0535	88
0.195	0.180	92	0.174	89	0.173	89
0.625	0.562	90	0.543	87	0.551	88
2.00	1.76	88	1.74	87	1.75	88

LOQ = 0.000500 mg p.m./L

Concurrent validation

The analytical method 01387/M002 (M-526061-01-1) was validated concurrently within the present study. This original method was developed for the determination of AE1194888 (BCS-AA53879) in water via HPLC-MS. The method was validated successfully, and the validation report was already submitted within the prothioconazole dossier and is also part of the current RAR.

For the concurrent validation AE 1194888 (BCS-AA53879) standard injections were evaluated.

Table 2.3-3: Validation of method 01387/M002 for AE 119488 (BCS-AA53879)

AE 1194888 (BCS-AA53879) standard concentration (µg/L)	n	AE 1194888 (BCS-AA53879)			
		Peak area		Retention time	
		Mean value (area counts)	RSD (%)	Mean value (min)	RSD (%)
0.400	4	62204	2.6	2.72	< 0.1
1.00	6	150771	1.1	2.72	< 0.1
2.00	4	291914	1.2	2.72	< 0.1
5.00	4	733336	0.7	2.72	< 0.1
10.0	4	1474070	0.7	2.72	< 0.1

Conclusion

The concurrent validation shows that method 01387/M002 is applicable for this study (M-630874-01-1) and thus fit for purpose. The original method 01387/M002 (M-526061-01-1) is also fully valid with regard to SANCO guidelines 3029/99 rev.4 and 825/00 rev8.1.

CA3301 / JOUST	Page 113 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Biological results:

No morphological effects on algae from the control or test concentrations during the 96h test period.

Table 2.3-4: Biological results

Nominal concentrations (mg p.m/L)	Cell number after 72 h (means)	(0-72h)-average specific growth rates [days ⁻¹]	Inhibition of average specific growth rate (%) at 72 h	Cell number after 96 h (means)	(0-96h)-average specific growth rates [days ⁻¹]	Inhibition of average specific growth rate (%) at 96 h
control	498 000	1.302	--	1 247 000	1.206	--
0.00596	427 000	1.250	4.0*	1 158 000	1.187	1.6 [#]
0.0191	441 000	1.262	3.1*	1 120 000	1.179	2.3 [#]
0.0610	295 000	1.128	13.4*	792 000	1.093	9.4 [#]
0.195	136 000	0.869	33.3*	215 000	0.767	36.4 [#]
0.625	106 000	0.787	39.6*	128 000	0.637	47.2 [#]
2.00	86 000	0.717	44.9*	87 000	0.541	55.2 [#]

* significantly ($\alpha=0.05$, one-sided) reduced, based on Welch t-test after Bonferroni-Holm

[#] significantly ($\alpha=0.05$, one-sided) reduced, based on Williams multiple sequential t-test procedure

Conclusion:

The endpoints based on nominal concentrations are:

Results in mg p.m./L (95% CI)	Growth rate	Yield	Growth rate	Yield
	72 h		96 h	
EC ₅₀	2.03 (1.92 – 2.16)	0.0933 (0.0883 – 0.0986)	0.955 (0.923 -0.988)	0.0811 (0.0775 – 0.0850)
EC ₂₀	0.106 (0.101-0.112)	0.0171 (0.0157 – 0.0187)	0.100 (0.0959 – 0.105)	0.0318 (0.0295 – 0.0341)
EC ₁₀	0.0227 (0.0208 – 0.0247)	0.00707 (0.00625 – 0.00792)	0.0309 (0.0289 – 0.0329)	0.0195 (0.0176 – 0.0214)
NOEC	< 0.00596	< 0.00596	< 0.00596	0.00596

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

CA3301 / JOUST	Page 114 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Study 1

Comments of zRMS:	The study was conducted to OECD guidelines 213 and 214 and according to the principles of GLP. No deviations to the guideline were noted. The study is considered to be reliable and suitable for the risk assessment.
-------------------	--

Reference:	KCP 10.3.1.1/01
Report	CA3301 – Acute Oral and Contact Toxicity to the Honey Bee, <i>Apis mellifera</i> L. (Hymenoptera: Apidae) under Laboratory Conditions Couture, E., 2021, report no. 029SRFR20C01
Guideline(s):	Yes. OECD 213 and 214 (1998)
Deviations:	No
GLP:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive summary

In a 48-hour laboratory study, the acute oral and contact toxicity of the product, CA3301 (25.57% w/w prothioconazole, analysed), to the European honeybee, *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 bees were exposed to CA3301 at 0 (untreated sucrose solution control), 33.44, 73.48, 161.63, 355.5, and 782.2 µg product/bee, equivalent to 0, 8.55, 18.79, 41.33, 90.91, and 200 µg a.s./bee, respectively, plus three doses of a toxic reference (98% dimethoate technical). In the acute contact test, a topical solution was applied to the dorsal thorax of the bees (2 µL droplets), with treatments of 0 (water control), 0 (adjuvant control), 12.91, 28.31, 62.22, 136.9, and 301.1 µg product/bee, equivalent to 0, 3.3, 7.24, 15.91, 35, 77 µg a.s./bee, respectively, plus three doses of a toxic reference (dimethoate technical (98%)). Each treatment consisted of three replicates of 10 bees each.

The 48-h acute oral NOED and LOED values were 108.7 and 453.3 µg product/bee, equivalent to 27.79 and 115.9 µg a.s./bee (based actual consumption). The 24-h and 48-hour acute oral LD₅₀ values were calculated to be 458.1 and 427.7 µg product/bee, equivalent to 117.2 and 109.11 µg a.s./bee, respectively (based on actual consumption).

The 48-hour acute contact NOED and LOED values were determined to be 28.31 and 62.22 µg product/bee, equivalent to 7.24 and 15.91 µg a.s./bee. The 24-h and 48-h contact LD₅₀ values were calculated to be 89.5 and 80.1 µg product/bee, equivalent to 22.88 and 20.43 µg a.s./bee, respectively

For validation of the test system, treatment with 0.1, 0.2, and 0.35 µg dimethoate/bee (oral test) and 0.1, 0.2, and 0.3 µg dimethoate/bee (contact test) resulted in LD₅₀ values of 0.113 and 0.1811 µg dimethoate/bee.

The study is considered acceptable and satisfies the OECD 213 and 214 (1998) test guidelines.

CA3301 / JOUST	Page 115 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Materials and methods

Test materials

Test item

Name:	CA3301 (Prothioconazole 250 EC, NUL 3390)
Active substance:	Prothioconazole
Formulation type:	Emulsifiable concentrate (EC)
Source and lot/batch no.:	A19045
Active substance content:	250 g/L (nominal), 257.04 g/L or 25.57% w/w (analysed)
Appearance:	Clear, straw-coloured liquid
Expiry date of lot/batch:	17 June 2022
Storage conditions:	Stored between 15.9°C and 25.9°C, and protected from light

Reference item

Name:	Dimethoate
Formulation type:	Technical
Density:	1 g/mL
Batch no.:	NI19-313
Active-substance purity:	98 ±1% (analysed)
Appearance:	Solid

Adjuvant item

Name:	Triton X-100
Density:	1.06 g/mL (nominal)
Active substance:	p-tertiary-Octylphenoxy polyethyl alcohol
Batch no.:	STBJ4510
Active-substance purity:	≥90% (nominal)
Appearance:	Liquid

Test organism

Species:	European honeybee, <i>Apis mellifera</i>
Age at study initiation:	Young, adult workers of similar age
Source:	Obtained from adequately fed, healthy, disease-free and queen-right colonies with known history and physiological status
Feeding during test:	500 g/L sucrose solution
Acclimation:	Collected on the day before the experimental phase start and kept under test conditions

Test conditions

Test temperature:	24.7-25.5°C
Relative humidity:	49.3-62.1%
Photoperiod:	Complete darkness, except during collection and assessment (under red light)

Test units (for both oral and contact tests) comprised well-ventilated, stainless-steel cages, which measured 175 cm³, with a removable glass front side and a hole on top, into which the feeder syringe was inserted. Young 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 3 replicates per treatment group, and 10 bees per replicate.

Acute oral toxicity test

For the oral test, each bee was fed with 10 µL of untreated or treated 500 g/L sucrose solution (100 µL total

CA3301 / JOUST	Page 116 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

per replicate). There were nine treatment groups – an untreated control, five product treatments, and three toxic-reference treatments. The nominal product treatment groups were 33.44, 73.48, 161.63, 355.5, and 782.2 µg product/bee, equivalent to 8.55, 18.79, 41.33, 90.91, and 200 µg a.s./bee. The nominal toxic-reference treatments were 0.1, 0.2, and 0.35 µg dimethoate/bee. The product feeding solution was made by mixing 1.5642 g of product (equivalent to 0.40 g a.s., when accounting for the product a.s. content of 25.57%) into 20 mL of sucrose solution, for a target dose of 200 µg a.s./bee (the highest product treatment dose). The lower doses were made by diluting this feeding solution with the sucrose solution. The toxic-reference feeding solution was made by dilutions of a stock solution [0.0711 g of dimethoate technical (equivalent to 0.07 g dimethoate) dissolved in 20 mL of distilled water]; this stock solution was diluted in the sucrose solution to make the three toxic-reference doses. The syringes were weighed before and after introduction into the cages. After a maximum of 6 h, unconsumed treated sucrose solution was replaced with the untreated sucrose solution until the end of the test. Since the uptake of the treated sucrose solutions differed from the nominal 100 µg a.s./bee, the results are based on measured consumption.

Acute contact toxicity test

For the test item treatment doses, a stock solution was made by dissolving 3.0112 g product (equivalent to 0.77 g a.s.) in 20 mL distilled water; 10 mL of this mixture was then mixed with 0.1 mL of a 10% Triton X-100 solution (diluted in distilled water), for a target dose of 77 µg a.s./bee (the highest product treatment dose). The other four treatments were made by diluting the stock solution and mixing it with 10% Triton X-100 solution. The toxic-reference treatments were similarly made, but by making a stock solution of 0.0303 g dimethoate technical (equivalent to 0.0297 g pure dimethoate) dissolved in 20 mL distilled water. There were ten treatment groups – two controls (one of distilled water and one with adjuvant), five product treatments, and three toxic-reference treatments. The nominal product treatment groups were 12.91, 28.31, 62.22, 136.9, and 301.1 µg product/bee, equivalent to 3.3, 7.24, 15.91, 35, 77 µg a.s./bee. The nominal toxic-reference treatments were 0.1, 0.2, and 0.3 µg pure dimethoate/bee. The bees were anesthetized for 70 seconds maximum with carbon dioxide and individually treated by topical application of 2 µL of the test solution(s) 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). and has been validated with a non GLP pre-test.

Mortality and behaviour were assessed by visual counting of dead honeybees per test unit, after 4, 24, and 48 h of exposure. Mean mortality data were corrected for mortality in the control group, using a modified Abbott's formula. Mortality data were analysed with the statistical software R 3.3.2, and all statistical analyses were performed at 95% confidence level. Spearman's rank correlation sum tests confirmed the monotonicity of the oral and contact mortality data. Thus, significant differences in mortality between the control and product groups were revealed with Cochran-Armitage step-down procedures. 24-h and 48-h LD₅₀ (and 95% confidence intervals) values were calculated with a Bayesian inference, using a 3-parameter log-logistic binomial model. 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.

Results

Biological results

Mortality in the oral test

Mortality and behavioural abnormalities of honey bees exposed to CA3301 in the 48-hour oral toxicity test, are presented in table below.

CA3301 / JOUST	Page 117 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Table CP 10.3.1.1/01-01: The effects of exposure to CA3301 on mortality of *Apis mellifera* in a 48-hour toxicity test

Applied dose	Consumed	Rep.	Mortality (no.)			Behaviourally affected (no.)		
µg product/bee	µg product/bee		4 HAE	24 HAE	48 HAE	4 HAE	24 HAE	48 HAE
0 (control)	0	1	0	0	0	0	0	0
		2	0	1	2	0	0	0
		3	0	0	0	0	0	0
33.44	33.63	1	0	0	0	0	0	0
		2	0	0	0	0	0	0
		3	0	0	0	0	0	0
73.48	68.99	1	0	0	0	0	0	0
		2	0	0	0	0	0	0
		3	0	0	0	0	0	0
161.63	84.94	1	0	0	0	0	0	0
		2	0	0	0	0	0	0
		3	0	0	0	0	0	0
355.5	108.7	1	0	1	1	0	0	0
		2	0	3	4	0	0	0
		3	0	0	0	0	0	0
782.2	453.3	1	1	3	4	1	0	1
		2	0	8	8	1	0	2
		3	0	3	4	2	0	2
0.1 µg dimethoate/bee	-	1	0	3	9	0	4	1
		2	0	7	9	1	3	1
		3	0	4	10	2	6	-
0.2 µg dimethoate/bee	-	1	5	10	10	3	-	-
		2	0	8	10	4	2	-
		3	1	9	10	2	1	-
0.35 µg dimethoate/bee	-	1	5	10	10	2	-	-
		2	4	10	10	2	-	-
		3	7	10	10	3	-	-

Rep., replicate; HAE, hours after exposure; no., number.

Table CP 10.3.1.1/01-02. Mean mortality of *Apis mellifera* after 4, 24, and 48 hours of oral exposure to CA3301

Applied dose	Consumed	Mortality (%)						
		4 HAE		24 HAE		48 HAE		
µg product/bee		Mean	Corrected	Mean	Corrected	Mean ± SD	CV	Corrected
0 (control)	0	0	0	3.33	0	6.67 ± 11.6	173.2	0
33.44	33.63	0	0	0	-3.4	0.0 ± 0.0	0	-7.14
73.48	68.99	0	0	0	-3.4	0.0 ± 0.0	0	-7.14
161.63	84.94	0	0	0	-3.4	0.0 ± 0.0	0	-7.14
355.5	108.7	0	0	13.33	10.34	16.67 ± 20.8	124.9	10.71
782.2	453.3	3.33	3.33	46.67*	44.83	53.33 ± 23.1*	43.3	50
0.1 µg dimethoate/bee		0	-	46.67	-	-	-	-
0.2 µg dimethoate/bee		20	-	90	-	-	-	-
0.35 µg dimethoate/bee		53.33	-	100	-	-	-	-

Significant differences in mortality were revealed with a Cochran-Armitage step-down procedure, when $p < 0.05$, and are denoted with asterisks (*). Mortality was corrected using the Abbott formula; positive and negative values indicate an increase and decrease in mortality, respectively, relative to the control group. Rep., replicate; HAE, hours after exposure; CV, coefficient of variation.

A repellent effect was observed in the doses applied nominally at 161.63, 355.5, and 782.2 µg product/bee, equivalent to 41.33, 90.91, and 200.0 µg a.s./bee, respectively. Thus, endpoints are based on actual diet consumption. Treatment with CA3301 caused statistically significant increases in mortality after 24 and 48 hours of exposure, at the highest product treatment dose of 453.3 µg product/bee, equivalent to 115.9 µg a.s./bee (based on actual consumption), when compared to the control group. Thus, the 48-h NOED and LOED values were determined to be 108.7 and 453.3 µg product/bee, equivalent to 27.79 and 115.9 µg

CA3301 / JOUST	Page 118 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

a.s./bee (based actual consumption). The 24-h and 48-h LD₅₀ values were calculated to be 458.1 µg product/bee (95% CIs: 348.3-639.2 µg product/bee) and 427.7 µg product/bee (95% CIs: 314.6-562.7 µg product/bee), equivalent to 117.2 µg a.s./bee (95% CIs: 89.44-164 µg a.s./bee) and 109.11 µg a.s./bee (95% CIs: 80.82-145 µg a.s./bee), respectively (based on actual consumption). The LD₅₀ value for the toxic reference was 0.113 µg dimethoate/bee (95% confidence intervals: 0.0925 - 0.1388 µg dimethoate/bee).

Mortality in the contact test

Mortality and behavioural abnormalities of honey bees exposed to CA3301 in the contact toxicity test, after 48 h of exposure, are presented in the tables below.

Table CP 10.3.1.1/01-03. Effects of exposure to CA3301 on adult *Apis mellifera* mortality in a 48-hour contact toxicity test

Applied dose		Rep.	Mortality (no.)			Behaviourally affected (no.)		
µg product/bee	µg a.s./bee		4 HAE	24 HAE	48 HAE	4 HAE	24 HAE	48 HAE
0 (water control)	0	1	0	0	0	0	0	0
		2	0	0	0	0	0	0
		3	0	0	0	0	0	0
0 (adjuvant control)	0	1	0	0	0	0	0	0
		2	0	1	1	0	0	0
		3	0	0	0	0	0	0
12.91	3.3	1	0	1	1	0	0	0
		2	0	0	0	0	0	0
		3	0	0	0	0	0	0
28.31	7.24	1	0	0	0	0	0	0
		2	0	0	0	0	0	0
		3	0	0	0	0	1	1
62.22	15.91	1	0	0	0	0	0	0
		2	0	1	1	1	0	0
		3	0	9	9	2	1	1
136.9	35	1	0	10	10	3	-	-
		2	0	9	9	3	1	1
		3	0	8	9	5	1	0
301.1	77	1	0	8	9	9	1	0
		2	0	9	9	10	1	1
		3	0	8	10	10	2	0
0.1 µg dimethoate/bee		1	0	0	1	0	6	5
		2	0	2	4	0	2	0
		3	0	0	4	0	5	1
0.2 µg dimethoate/bee		1	0	8	10	2	1	0
		2	0	7	10	1	2	0
		3	1	5	7	1	3	3
0.3 µg dimethoate/bee		1	1	10	10	3	-	0
		2	0	9	10	4	0	0
		3	0	9	10	4	1	0

Rep., replicate; HAE, hours after exposure; 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 10.3.1.1/01-4: Mean mortality of *Apis mellifera* after 4, 24 and 48 hours of contact exposure to CA3301.

Applied dose		Mortality (%)						
		4 HAE		24 HAE		48 HAE		
µg product/bee	µg a.s./bee	Mean	Corrected	Mean	Corrected	Mean ± SD	CV	Corrected
0 (water control)		0	0	0	-3.45	0.0 ± 0.0	0	-3.45
0 (adjuvant control)		0	0	3.33	0	3.33 ± 5.8	173.2	0
12.91	3.3	0	0	3.33	0	3.33 ± 5.8	173.2	0
28.31	7.24	0	0	0	-3.45	0.0 ± 0.0	0	-3.45
62.22	15.91	0	0	33.33*	31.03	33.33 ± 49.3*	148	31.03
136.9	35	0	0	90.00*	89.66	93.33 ± 5.8*	6.2	93.1
301.1	77	0	0	83.33*	82.76	93.33 ± 5.8*	6.2	93.1
0.1 µg dimethoate/bee		0	-	6.67	-	-	-	-
0.2 µg dimethoate/bee		3.33	-	66.67	-	-	-	-
0.3 µg dimethoate/bee		3.33	-	93.33	-	-	-	-

Significant differences in mortality were revealed with a Cochran-Armitage step-down procedure, when $p < 0.05$, and are denoted with asterisks (*). Mortality was corrected using the Abbott formula; positive and negative values indicate an increase and decrease in mortality, respectively, relative to the control group. Rep., replicate; HAE, hours after exposure; CV, coefficient of variation.

In the contact toxicity test, statistically significant differences in mortality after 24 and 48 h of exposure, with the three-highest product treatment applications of 62.22, 136.9, and 301.100 µg product/bee, equivalent to 15.91, 35.0, and 77.0 µg a.s./bee, were observed, when compared with the control groups. The 48-h NOED and LOED values were determined to be 28.31 and 62.22 µg product/bee, equivalent to 7.24 and 15.91 µg a.s./bee. The 24-h and 48-h LD₅₀ values were calculated to be 89.5 µg product/bee (95% confidence intervals: 71.03-112.6 µg product/bee) and 80.1 µg product/bee (95% confidence intervals: 66.57-97.14 µg product/bee), equivalent to 22.88 µg a.s./bee (95% confidence intervals: 18.38-28.79 µg a.s./bee) and 20.43 µg a.s./bee (95% confidence intervals: 16.97-24.71 µg a.s./bee), respectively. The LD₅₀ value for the toxic reference was 0.1811 µg dimethoate/bee (95% confidence intervals: 0.1560 - 0.2051 µg dimethoate/bee).

Behaviour

In the oral test, adverse behavioural effects (bees categorized at affected or cramping) were observed in the dose applied at 200.0 µg a.s./bee, at 4 and 48 h after application. In the contact test, affected and cramping bees were found 4 h after application, with exposures to 15.91, 35.0, and 77.0 µg a.s./bee; 24 h after application, with exposures to 7.24, 15.91, 35.0, and 77.0 µg a.s./bee; and 48 h after application, with exposures to 7.24, 15.91, 35.0, and 77.0 µg a.s./bee.

Validity

All validity criteria were met in accordance with the OECD 213 and 214 (1998) test guidelines:

- Mean mortality in the control to be ≤10% at the end of both tests (actual values were 6.67%;0.0% (water control) and 3.33% (adjuvant control) in the oral and contact tests, respectively).
- 24-h LD₅₀ of the toxic reference to be between 0.10-0.35 and 0.10-0.30 µg dimethoate/bee in the oral and contact tests, respectively (actual values were 0.1130 and 0.1811 µg dimethoate/bee in the oral and contact tests, respectively).

Conclusion

The acute oral and contact toxicity of CA3301 to honey bees (*Apis mellifera*) was evaluated in accordance with the OECD 213 and 214 (1998) test guidelines.

The 48-h acute oral NOED and LOED values were determined to be 108.7 and 453.3 µg product/bee, equivalent to 27.79 and 115.9 µg a.s./bee (based actual consumption). The 48-h acute oral LD₅₀ value was calculated to be 427.7 µg product/bee, equivalent to 109.11 µg a.s./bee (based on actual consumption).

CA3301 / JOUST	Page 120 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

The 48-h contact NOED and LOED values were determined to be 28.31 and 62.22 µg product/bee, equivalent to 7.24 and 15.91 µg a.s./bee. The 48-h contact LD₅₀ value was calculated to be 80.1 µg product/bee, equivalent to 20.43 µg a.s./bee.

In the oral test, adverse behavioural effects (bees categorized at affected or cramping) were observed in the dose applied at 200.0 µg a.s./bee, at 4 and 48 h after application. In the contact test, affected and cramping bees were found 4 h after application, with exposures to 15.91, 35.0, and 77.0 µg a.s./bee; 24 h after application, with exposures to 7.24, 15.91, 35.0, and 77.0 µg a.s./bee; and 48 h after application, with exposures to 7.24, 15.91, 35.0, and 77.0 µg a.s./bee.

This study is considered acceptable.

Study 2

Comments of zRMS:	The study was conducted to the guidelines and according to the principles of GLP. The study is considered to be reliable and suitable for the risk assessment.
-------------------	--

Reference:	KCP 10.3.1.1/02
Report	CA3301 – A laboratory study to determine the acute oral and contact toxicity on the adult <i>Bombus terrestris</i> L. (Hymenoptera: Apidae) Couture, E., 2021, report no. 029SRFR20C02
Guideline(s):	Yes. OECD 246 (2017) and 247 (2017)
Deviations:	No
GLP:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive summary

In a 48-hour laboratory study, the acute oral and contact toxicity of the product, CA3301 (25.57% w/w prothioconazole, analysed), on the buff-tailed bumble bee, *Bombus terrestris*, was evaluated in a limit test, in accordance with OECD 246 and 247 (2017) test guidelines. In the acute oral test, adult worker bees were fed either untreated (control) or product-treated feeding solutions of 0 or 782.17 µg product/bee (equivalent to nominal 200 µg a.s./bee), respectively, and a toxic reference dose of 4.0 µg a.s./bee. In the acute contact test, a topical solution was applied to the dorsal thorax of the bees (2-µL droplets) of either 0 (untreated control) or 391.08 µg product/bee (equivalent to nominal 100 µg a.s./bee), and a toxic reference dose of 10 µg a.s./bee. Bees were exposed to the treatments in single-unit housing; each unit represented a replicate, with 50 replicates per treatment group. Since analytical recovery of the active substance, prothioconazole, was within ±20% of nominal concentrations, all endpoints are based on nominal concentrations.

Mortality was assessed 4, 24, and 48 hours after exposure. The 48-h acute oral NOED and LOED values were estimated to be <782.17 and 782.177 µg product/bee, equivalent to <200 and 200 µg a.s./bee, respectively. The 48-h acute oral LD₅₀ value was estimated to be >782.17 µg product/bee, equivalent to >200 µg a.s./bee.

The 48-h acute contact NOED and LOED values were estimated to be <391.08 and 391.08 µg product/bee, equivalent to <100 and 100 µg a.s./bee, respectively. The 48-hour acute contact LD₅₀ value was estimated to be >391.08 µg product/bee, equivalent to >100 µg a.s./bee.

CA3301 / JOUST	Page 121 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

For validation of the test system, treatments with 4 µg dimethoate/bee (acute oral) and 10 µg dimethoate/bee (acute contact) were used as the toxic reference and resulted in 100% and 93.33% mortality, respectively.

The study is considered acceptable and satisfies the OECD 246 (2017) and 247 (2017) test-guideline requirements.

Materials and methods

Test materials

Test item

Name:	CA3301 (Prothioconazole 250 EC, NUL 3390)
Active substance:	Prothioconazole
Formulation type:	Emulsifiable concentrate (EC)
Source and lot/batch no.:	A19045
Active substance content:	250 g/L (nominal), 257.04 g/L or 25.57% w/w (analysed)
Appearance:	Clear, straw-coloured liquid
Expiry date of lot/batch:	17 June 2022
Storage conditions:	Stored between 15.9°C and 26.4°C, and protected from light

Reference item

Name:	Dimethoate
Formulation type:	Technical
Density:	1 g/mL
Batch no.:	D088B200518
Active-substance purity:	98 ±1% (analysed)
Appearance:	Solid

Adjuvant item

Name:	Triton X-100
Density:	1.06 g/mL
Active substance:	p-tertiary-Octylphenoxy polyethyl alcohol
Batch no.:	STBJ4510
Active-substance purity:	≥90% (nominal)
Appearance:	Liquid

Test organism

Species:	Buff-tailed bumble bee, <i>Bombus terrestris</i>
Age at study initiation:	Adult workers
Source:	Three medium-sized bumblebee colonies, having brood at all stages of development and a laying queen (Bioline Agrosiences Ltd., Telstar Nursery, Little Clacton, UK)
Feeding during test:	50% w/v aqueous sucrose solution <i>ad libitum</i>
Acclimation:	Acclimatised to the test conditions (including single housing) for at least 8 hours before the start of the test. For oral test, bees were starved between 3 hours 13 minutes and 3 hours 36 minutes before the start of the exposure period.

Test conditions

Test temperature:	25.1 – 25.8°C
Relative humidity:	61.3 – 71.1%
Photoperiod:	Complete darkness, except during collection and assessment (under red light)

CA3301 / JOUST	Page 122 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Test units (for both oral and contact tests) comprised plastic cages, measuring 22 cm³, which were passively ventilated, and had a hole on top, into which the feeder syringe was inserted. Bumble bees from the three colonies were randomly assigned to the treatment groups, to avoid any colony effect within a treatment group, and were individually weighed before they were introduced to their single-housing test cages. Each test unit represented a replicate, with 50 replicates for the control and the product treatment groups and 30 replicates for the toxic-reference groups.

Acute oral toxicity test

For the oral test, there was a control group, a single product group, and a single toxic-reference group. The product feeding solution was made by mixing 0.3909 g of product (equivalent to 0.0100 g a.s.) into 20 mL of sucrose solution (500 g/L sucrose), for a target dose of 782.17 µg product/bee, equivalent to 200 µg a.s./bee. The reference-item feeding solution was made by mixing 0.0204 g of dimethoate technical into 20 mL of distilled water; 1 mL of this mixture was then mixed with 9 mL of sucrose solution, for a target dose of 4 µg dimethoate/bee. Each bee received 40 µL of control, product, or toxic-reference feeding solution. After a maximum of 4 h, unconsumed treated sucrose solution was replaced with the untreated sucrose solution until the end of the test.

Acute contact toxicity test

For the contact test, there was a control group, a single product group, and a single toxic-reference group. The product application solution was made by mixing 3.911 g of product (equivalent to 1.000 g a.s. into 20 mL of distilled water; 10 mL of this mixture was then mixed with 0.1 mL of a 10% Triton X-100 solution (diluted in distilled water), for a target dose of 391.08 µg product/bee, equivalent to 100 µg a.s./bee. The toxic-reference application solution was made by mixing 0.051 g of dimethoate technical into 10 mL of distilled water; 10 mL of this mixture was then mixed with 0.1 mL of a 10% Triton X-100 solution (diluted in distilled water), for a target dose of 10 µg dimethoate/bee (final Triton X-100 content of 0.1% v/v). The bees were anesthetized with carbon dioxide and individually treated by topical application of 2 µL of the test solution(s) onto the dorsal side of the thorax with control, product, or toxic-reference application solution.

Mortality and behaviour were assessed by visual counting of dead bumblebees per test unit, after 4, 24, and 48 h of exposure. Mean mortality data were corrected for mortality in the control group, using the Abbott formula. Mortality data were analyzed with the statistical software R 3.3.2, and all statistical analyses were performed at 95% confidence level. Significant differences in mortality between the control and product groups were revealed with a Fisher's exact test. Behaviour was quantitatively observed, according to the categories unaffected, affected (lacking coordination), and moribund (unable to walk, weakly response to stimuli). Behavioural data were not statistically analysed.

The concentration of the test solutions was confirmed by analytical verification. For the oral test, the final feeding solutions were sampled and, for the contact test, the stock solution without the Triton X-100 adjuvant was sampled. Prothioconazole was extracted from the homogenised water-treated solution and homogenised sucrose solution sample, using acetonitrile and acetonitrile/water as solvents, respectively; the final sample solutions were analysed in positive ionisation mode by Ultra High-Performance Liquid Chromatography, tandem High-Resolution Mass Spectrometry (UHPLC-TOF-MS/MS), following internal Renolab methods, which are described in the analytical final report (following Renolab method code MA RES 145 for sucrose solution as matrix and MA RES 147 for water as matrix).

Results

Analytical results

The UHPLC-TOF-MS/MS analytical method for the determination of prothioconazole in test medium was validated with regards to specificity, linearity, accuracy and precision in accordance with guideline

CA3301 / JOUST	Page 123 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

SANCO/3029/99, part. 4, rev. 4, 11/07/2000. Specificity for prothioconazole, in treated water and sucrose solutions, was demonstrated by the absence of significant interferences above 30% of the LOQ. For the matrix water, the analytical calibration was shown to be linear ($r \geq 0.995$) over the range of 50.05 to 2002 ng prothioconazole/mL. For the matrix sucrose solution, the analytical calibration was shown to be linear ($r \geq 0.995$) over two ranges of 1.001 to 50.05 ng prothioconazole/mL and 30.03 to 1001 ng prothioconazole/mL. Accuracy was confirmed with recovery of spiked samples at relevant concentrations of the test item in matrix water (10 and 100000 mg prothioconazole/L) and matrix sucrose solution (0.018 and 6006.5 mg prothioconazole/kg, corresponding to 0.022 and 7321.9 mg/L, respectively); recoveries for matrix water ranged from 76.7 – 91.9% and for matrix sucrose solution 84.1 – 93.6% (i.e., within 70-110%). Precision was confirmed; the relative standard deviation ($n = 4$, for each matrix) was 8.2 and 5.1% (i.e., within the guideline limit of $\leq 20\%$). The limit of quantification (LOQ) for prothioconazole in treated water was 10 mg/L and for treated sucrose solutions, 0.022 mg/L (corresponding to 0.018 mg/kg). The limit of detection (LOD) for prothioconazole in water was 2.5 mg/L and for treated sucrose solutions, 0.006 mg/L.

Table CP 10.3.1.1/02-01. Recovery of prothioconazole in the treated water and sucrose solution samples.

Sample solution	Nominal (mg a.s./L)	Measured		Recovery (% nominal)	Deviation (%)
		mg a.s./kg diet	mg a.s./L		
Oral feeding solution	50000	5020.1	50111 ^a	100.2	+0.2
Contact stock solution	5000	4063.4	4876.1 ^b	99.1	-0.9

^aCalculated considering the density of water at 20 °C (0.9982 g/mL). ^bCalculated considering the density of the sucrose solution at 20°C determined in study 20070-02R (1.219 g/mL)

The average recoveries of nominal concentrations were between 80% and 120% for all concentrations. Thus, nominal concentrations were used to calculate all endpoints.

Biological results

Mortality and behaviour in the oral test

Mortality and behavioural abnormalities of bumble bees exposed to CA3301 in the 48-hour oral toxicity test, are presented in the table below.

Table CP 10.3.1.1/02-02. The effects of exposure to CA3301 on mortality and behaviour of adult *Bombus terrestris* in a 48-hour toxicity test.

Nominal dose	Mortality (no.)								
µg product/bee µg a.s./bee	0 (control) 0			782.17 200.0			4 µg dimethoate/bee		
Replicate	Time (HAE)								
	4	24	48	4	24	48	4	24	48
1	0	0	0	0	0	1	0	0	1
2	0	0	0	0	0	1	0	1	1
3	0	0	0	0	0	1	0	0	1
4	0	0	0	0	1	1	0	1	1
5	0	0	0	0	0	1	0	0	1
6	0	0	0	0	0	1	0	0	1
7	0	0	0	0	0	1	0	0	1
8	0	0	0	0	0	1	0	0	1
9	0	0	0	0	0	1	0	0	1
10	0	0	1	0	0	1	0	0	1
11	0	0	0	0	0	1	0	0	1
12	0	0	0	0	0	1	0	0	1
13	0	0	0	0	0	1	0	0	1
14	0	0	0	0	1	1	0	1	1

CA3301 / JOUST	Page 124 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

15	0	0	0	0	0	1	0	0	1
16	0	0	0	0	0	1	0	0	1
17	0	0	0	0	0	1	0	0	1
18	0	0	1	0	0	1	0	0	1
19	0	0	0	0	0	1	0	0	1
20	0	0	0	0	0	1	0	0	1
21	0	0	0	0	0	1	0	0	1
22	0	0	0	0	0	1	0	0	1
23	0	0	0	0	0	1	0	0	1
24	0	0	0	0	0	1	0	0	1
25	0	0	0	0	0	1	0	0	1
26	0	0	0	0	0	1	0	0	1
27	0	0	0	0	0	1	0	0	1
28	0	0	0	0	0	1	0	0	1
29	0	0	0	0	0	1	0	0	1
30	0	0	1	0	0	1	0	0	1
31	0	0		0	0		0	0	
32	0	0		0	1		0	1	
33	0	0		0	1		0	1	
34	0	0		0	0		0	0	
35	0	0		0	0		0	0	
36	0	0		0	0		0	0	
37	0	0		0	0		0	0	
38	0	0		1	0		1	0	
39	0	0		0	0		0	0	
40	0	0		0	1		0	1	
41	0	0		0	0		0	0	
42	0	0		0	1		0	1	
43	0	0		0	0		0	0	
44	0	0		0	0		0	0	
45	0	0		0	0		0	0	
46	0	0		0	0		0	0	
47	0	0		0	0		0	0	
48	0	0		0	0		0	0	
49	0	0		0	0		0	0	
50	0	0		0	0		0	0	
Nominal dose	Behaviourally affected bees (no.)								
µg product/bee	0 (control)			782.17			4 µg dimethoate/bee		
µg a.s./bee	0			200.0					
Replicate	Time (HAE)								
	4	24	48	4	24	48	4	24	48
1	0	0	1	0	1	-	0	1	-
2	0	0	1	0	0	-	0	-	-
3	0	0	1	0	0	-	0	0	-
4	0	0	1	0	-	-	0	-	-
5	0	0	1	0	0	-	0	0	-
6	0	0	1	0	0	-	0	0	-
7	0	0	0	0	0	-	0	0	-
8	0	0	1	0	0	-	0	0	-
9	0	0	1	0	0	-	0	0	-
10	0	0	-	0	0	-	0	0	-
11	0	0	1	0	0	-	0	0	-
12	0	0	1	0	0	-	0	0	-
13	0	0	0	0	0	-	0	0	-
14	0	0	1	0	-	-	0	-	-
15	0	0	1	0	0	-	0	0	-
16	0	0	1	0	0	-	0	0	-

CA3301 / JOUST	Page 125 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

17	0	0	1	0	0	-	0	0	-
18	0	0	-	0	0	-	0	0	-
19	0	0	1	0	0	-	0	0	-
20	0	0	1	0	0	-	0	0	-
21	0	0	1	0	0	-	0	0	-
22	0	0	1	0	0	-	0	0	-
23	0	0	1	0	0	-	0	0	-
24	0	0	1	0	0	-	0	0	-
25	0	0	1	0	0	-	0	0	-
26	0	0	1	0	0	-	0	0	-
27	0	0	1	0	0	-	0	0	-
28	0	0	1	0	0	-	0	0	-
29	0	0	0	0	0	-	0	0	-
30	0	0	-	0	0	-	0	0	-
31	0	0		0	0		0	0	
32	0	0		0	-		0	-	
33	0	0		0	-		0	-	
34	0	0		0	0		0	0	
35	0	0		0	0		0	0	
36	0	0		0	0		0	0	
37	0	0		0	0		0	0	
38	0	0		-	0		-	0	
39	0	0		0	0		0	0	
40	0	0		0	-		0	-	
41	0	0		0	0		0	0	
42	0	0		0	-		0	-	
43	0	0		0	0		0	0	
44	0	0		0	0		0	0	
45	0	0		0	0		0	0	
46	0	0		0	0		0	0	
47	0	0		0	0		0	0	
48	0	0		0	0		0	0	
49	0	0		0	0		0	0	
50	0	0		0	0		0	0	

HAE, hours after exposure; no., number.

Table CP 10.3.1.1/02-03: Mean mortality of *Bombus terrestris* after 4, 24 and 48 hours of oral exposure to CA3301

Nominal dose		No. of bees		Mortality		
		Total	Excluding non-feeders	No.	Mean (%)	Corrected (%)
µg product/bee	µg a.s./bee	4 HAE				
0 (control)	0.0	50	50	0	0.0	0.0
782.17	200.0	50	30	0	0.0	0.0
4 µg dimethoate/bee		30	25	3	12.0	+12.0
µg product/bee	µg a.s./bee	24 HAE				
0 (control)	0.0	50	50	1	2	0.0
782.17	200.0	50	30	6	20.0*	+18.37
4 µg dimethoate/bee		30	25	25	100.0	+100.0
µg product/bee	µg a.s./bee	48 HAE				
0 (control)	0.0	50	50	1	2.0	0.0
782.17	200.0	50	30	7	23.33*	+21.77
4 µg dimethoate/bee		30	25	25	100.0	+100.0

Significant differences in mortality were revealed with a Fisher's exact test, when $p < 0.05$, and are denoted with asterisks (*). Mortality was corrected using the Abbott formula; positive values indicate an increase in mortality, relative to the control group. No., number, HAE, hours after exposure.

CA3301 / JOUST	Page 126 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Treatment with CA3301 caused statistically significant differences in mortality after 24 and 48 hours of exposure, when compared to the control group. The 48-h NOED and LOED (mortality) values were estimated to be <782.17 and 782.17 µg product/bee, respectively (based on nominal concentrations). Since treatment with CA3301 did not affect mortality by ≥50%, the 48-h LD₅₀ value was estimated to be >782.17 µg product/bee (based on nominal concentrations). These values are equivalent to NOEC, LOED, and LD₅₀ (mortality) values of <200, 200, and >200 µg a.s./bee, respectively (based on nominal concentrations).

Mortality and behaviour in the contact test

Mortality and behavioural abnormalities of bumble bees exposed to CA3301 in the contact toxicity test, after 48 h of exposure, are presented in the tables below.

Table CP 10.3.1.1/02-04: The effects of exposure to CA3301 on adult *Bombus terrestris* mortality in a 48-hour contact toxicity test.

Nominal dose	Mortality (no.)								
µg product/bee µg a.s./bee	0 (control) 0			391.08 100			10 µg dimethoate/bee		
Replicate	Time (HAE)								
	4	24	48	4	24	48	4	24	48
1	0	0	0	0	1	1	0	1	1
2	0	0	0	0	0	1	0	0	1
3	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	1	0	1	1
5	0	0	0	0	0	1	0	0	1
6	0	0	1	0	0	1	0	0	1
7	0	0	0	0	0	1	0	0	1
8	0	0	0	0	0	1	0	0	1
9	0	1	1	0	1	1	0	1	1
10	0	0	0	0	0	1	0	0	1
11	0	0	1	0	0	1	0	0	1
12	0	0	1	0	0	1	0	0	1
13	0	0	0	0	0	1	0	0	1
14	0	0	0	0	0	1	0	0	1
15	0	0	0	0	0	0	0	0	1
16	0	0	0	0	0	1	0	0	1
17	0	0	0	0	1	1	0	1	1
18	0	0	0	0	0	1	0	0	1
19	0	0	0	0	0	0	0	0	1
20	0	0	0	0	0	1	0	1	1
21	0	0	1	0	0	1	0	0	1
22	0	0	0	0	0	1	0	0	1
23	0	0	0	0	0	1	0	0	1
24	0	0	0	0	0	1	0	0	1
25	0	0	1	0	0	1	0	0	1
26	0	0	1	0	0	1	0	0	1
27	0	0	0	0	0	1	0	0	1
28	0	0	0	0	1	1	0	1	1
29	0	0	0	0	0	1	0	0	1
30	0	0	0	0	0	0	0	0	0
31	0	0		0	0		0	0	
32	0	0		0	0		0	0	
33	0	0		0	0		0	0	
34	0	0		0	0		0	0	
35	0	0		0	0		0	0	
36	0	0		0	0		0	0	
37	0	0		0	0		0	0	
38	0	0		0	0		0	0	

CA3301 / JOUST	Page 127 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

39	0	0		0	1		0	1	
40	0	0		0	0		0	0	
41	0	0		0	0		0	0	
42	0	0		0	0		0	0	
43	0	0		0	0		0	0	
44	0	0		0	0		0	0	
45	0	0		0	0		0	0	
46	0	0		0	0		0	0	
47	0	1		0	1		0	1	
48	0	0		0	0		0	0	
49	0	0		0	0		0	0	
50	0	0		0	0		0	0	
Nominal dose	Behaviourally affected bees (no.)								
µg product/bee	0 (control)			391.08			10 µg dimethoate/bee		
µg a.s./bee	0			100					
Replicate	Time (HAE)								
	4	24	48	4	24	48	4	24	48
1	0	0	0	0	-	-	0	-	-
2	0	0	0	0	0	-	0	0	-
3	0	0	0	0	0	0	0	0	1
4	0	0	1	0	0	-	0	-	-
5	0	0	0	0	0	-	0	0	-
6	0	0	-	0	0	-	0	0	-
7	0	0	0	0	0	-	0	0	-
8	0	0	1	0	0	-	0	0	-
9	0	-	-	0	-	-	0	-	-
10	0	0	0	0	0	-	0	0	-
11	0	0	-	0	0	-	0	0	-
12	0	0	-	0	0	-	0	0	-
13	0	0	0	0	0	-	0	0	-
14	0	0	0	0	0	-	0	0	-
15	0	0	0	0	0	1	0	0	-
16	0	0	1	0	0	-	0	0	-
17	0	0	0	0	-	-	0	-	-
18	0	0	1	0	0	-	0	0	-
19	0	0	0	0	0	0	0	0	-
20	0	0	0	0	0	-	0	-	-
21	0	0	-	0	0	-	0	0	-
22	0	0	0	0	0	-	0	0	-
23	0	0	1	0	0	-	0	0	-
24	0	0	0	0	0	-	0	0	-
25	0	0	-	0	0	-	0	0	-
26	0	0	-	0	0	-	0	0	-
27	0	0	0	0	0	-	0	0	-
28	0	0	0	0	-	-	0	-	-
29	0	0	0	0	0	-	0	0	-
30	0	0	0	0	0	1	0	0	1
31	0	0		0	0		0	0	
32	0	0		0	0		0	0	
33	0	0		0	0		0	0	
34	0	0		0	0		0	0	
35	0	0		0	0		0	0	
36	0	0		0	0		0	0	
37	0	0		0	0		0	0	
38	0	0		0	0		0	0	
39	0	0		0	-		0	-	
40	0	0		0	0		0	0	

CA3301 / JOUST	Page 128 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

41	0	0	0	0	0	0
42	0	0	0	0	0	0
43	0	0	0	0	0	0
44	0	0	0	0	0	0
45	0	0	0	0	0	0
46	0	0	0	0	0	0
47	0	-	0	-	0	-
48	0	0	0	0	0	0
49	0	0	0	0	0	0
50	0	0	0	0	0	0

HAE, hours after exposure; no., number.

Table CP 10.3.1.1/02-05: Mean mortality of *Bombus terrestris* after 4, 24 and 48 hours of contact exposure to CA3301

Nominal dose		Total no. of bees	Mortality		
			No.	Mean (%)	Corrected (%)
$\mu\text{g product/bee}$	$\mu\text{g a.s./bee}$		4 HAE		
0 (control)	0	50	0	0.0	0.0
391.08	100	50	2	4.0	4.0
10 $\mu\text{g dimethoate/bee}$		30	7	23.33	23.33
$\mu\text{g product/bee}$	$\mu\text{g a.s./bee}$		24 HAE		
0 (control)	0	50	0	0.0	0.0
391.08	100	50	6	12.0*	12.0
10 $\mu\text{g dimethoate/bee}$		30	26	86.67	86.67
$\mu\text{g product/bee}$	$\mu\text{g a.s./bee}$		48 HAE		
0 (control)	0	50	0	0.0	0.0
391.08	100	50	8	16.0*	16.0
10 $\mu\text{g dimethoate/bee}$		30	28	93.33	93.33

Significant differences in mortality were revealed with a Fisher's exact test, when $p < 0.05$, and are denoted with asterisks (*). Mortality was corrected using the Abbott formula; positive values indicate an increased in mortality, relative to the control group. No., number, HAE, hours after exposure.

Treatment with CA3301 caused statistically significant differences in mortality after 24 and 48 hours of exposure, when compared to the control group. The 48-hour NOED and LOED (mortality) values were estimated to be <391.08 and $391.08 \mu\text{g product/bee}$ (based nominal concentrations). Since treatment with CA3301 did not affected mortality by $\geq 50\%$, the 48-h LD_{50} value was estimated to be $>391.08 \mu\text{g a.s./bee}$ (based on nominal concentrations). These values are equivalent to NOEC, LOED, and LD_{50} (mortality) values of <100 , 100, and $>100 \mu\text{g a.s./bee}$, respectively (based nominal concentrations).

Behaviour

In the oral test, one moribund bee was observed 24 and 48 h after exposure, in the product treatment group. However, as only one bee of the 50 replicates tested was observed to be moribund, it is unlikely this adverse behavioural effect was related to the product. No adverse behavioural effects were observed for the acute contact test.

Validity

All validity criteria were met in accordance with the OECD 246 and 247 (2017) test guidelines:

- Mean mortality in the control was $\leq 10\%$ at the end of both tests (actual values were 2% and 0% in the oral and contact tests, respectively).
- Mean mortality in the toxic-reference group was $> 50\%$ at the end of both tests (actual values were 100% and 93.33% in the oral and contact tests, respectively).

CA3301 / JOUST	Page 129 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Conclusion

The acute oral and contact toxicity of CA3301 to bumble bees, *Bombus terrestris*, was evaluated, in accordance with the OECD 246 and 247 (2017) test guidelines.

The 48-h acute oral NOED value was estimated to be <782.17 µg product/bee, equivalent to <200 µg a.s./bee. The 48-hour acute oral LOED value was determined to be 782.177 µg product/bee, equivalent to 200 µg a.s./bee. The 48-h acute oral LD₅₀ value was estimated to be >782.17 µg product/bee, equivalent to >200µg a.s./bee.

The 48-h acute contact NOED value was estimated to be <391.08 µg product/bee, equivalent to <100 µg a.s./bee. The 48-hour acute contact LOED value was determined to be 391.08 µg a.s./bee, equivalent to 100 µg a.s./bee. The 48-hour acute contact LD₅₀ value was estimated to be >391.08 µg product/bee, equivalent to >100µg a.s./bee.

In the oral test, one moribund bee was observed 24 and 48 h after exposure, in the product treatment group. However, as only one bee of the 50 replicates tested was observed to be moribund, it is unlikely this adverse behavioural effect was related to the product. No adverse behavioural effects were observed for the acute contact test.

This study is considered acceptable.

Study 3

Comments of zRMS:	The study was conducted to the guideline and according to the principles of GLP. The study is considered to be valid.
-------------------	---

Reference:	KCP 10.3.1.1/03
Report	CA3301 – A laboratory study to determine the acute contact toxicity on the adult <i>Osmia bicornis</i> (Hymenoptera: Megachilidae) Couture, E., 2021, report no. 029SRFR20C03
Guideline(s):	Yes. OECD 214 (1998) and 246 (2017)
Deviations:	No
GLP:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive summary

In a 96-hour laboratory study, the acute contact toxicity of the product, CA3301 (25.57% w/w prothioconazole, analysed), on the mason bee, *Osmia bicornis*, was evaluated in a dose-response test, in accordance with the OECD 214 (1998) and 246 (2017) test guidelines. Unmated, adult female bees were exposed to CA3301 as a topical solution, applied to the dorsal side of the thorax (2-µL droplets), in six treatment groups of 0 (control), 22.72, 49.98, 109.89, 241.77, and 531.87 µg product/bee, equivalent to 0, 5.81, 12.78, 28.10, 61.82, 136 µg a.s./bee, respectively, plus one dose of a toxic reference (98% dimethoate. There were six replicates for each treatment group, 5 bees per replicate.

The 96-hour acute contact NOED and LOED values were estimated to be <22.72 and 22.72 µg product/bee, equivalent to <5.81 and 5.81 µg a.s./bee, respectively. The acute contact 96-hour LD₅₀ value was

CA3301 / JOUST	Page 130 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

determined to be 45.21 µg product/bee (95% confidence intervals: 23.90 – 82.88 µg product/bee), equivalent to 11.63 µg a.s./bee (95% confidence intervals: 6.082 – 21.51 µg a.s./bee). All endpoints are based on nominal concentrations, since analytical recovery of the active substance, prothioconazole, was within ±20% of nominal concentrations.

For validation of the test system, treatment with 1.5 µg dimethoate/bee, as the toxic reference, resulted in 96.67% mortality.

The study is considered acceptable and satisfies the OECD 214 (1998) and 246 (2017) test-guideline requirements.

Materials and methods

Test materials

Test item

Name:	CA3301
Active substance:	Prothioconazole
Formulation type:	Emulsifiable concentrate (EC)
Source and lot/batch no.:	A19045
Active substance content:	250 g/L (nominal), 257.04 g/L or 25.57% w/w (analysed)
Appearance:	Clear, straw-coloured liquid
Expiry date of lot/batch:	17 June 2022
Storage conditions:	Stored between 15.9°C and 26.4°C, and protected from light

Reference item

Name:	Dimethoate
Formulation type:	Technical
Density:	1 g/mL
Batch no.:	D088B200518
Active-substance purity:	98 ±1%
Appearance:	Solid

Adjuvant item

Name:	Triton X-100
Density:	1.06 g/mL
Active substance:	p-tertiary-Octylphenoxy polyethyl alcohol
Batch no.:	STBJ4510
Active-substance purity:	≥90%
Appearance:	Liquid

Test organism

Species:	Mason bee, <i>Osmia bicornis</i> (Hymenoptera: Megachilidae)
Age at study initiation:	<1-week-old adults
Sex:	Unmated females
Source:	Cocoons, placed in an emergence box at ambient temperature room. Obtained from WAB – Mauerbienenzucht (Konstanz, Germany)
Feeding during test:	500 g/L sucrose solution given <i>ad libitum</i>
Acclimation:	None.

Test conditions

Test temperature:	21.4°C – 23.8°C
Relative humidity:	49.3 – 73.8%
Photoperiod:	16 h light:8 h dark

CA3301 / JOUST	Page 131 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Test units consisted of a cage, which measured 350 cm³ and which were passively ventilated. Before and after exposure (until the end of the test), a pierced Eppendorf tube, filled with dental roll and sucrose solution (50% w/v), allowed access to food *ad libitum*., Emerging bees were collected and randomly assigned to the treatment groups and stored at 5°C ± 2°C, until a sufficient number of healthy females was reached. On the day of application, the anesthetised bees were individually weighed to determine the average weight (87.17 ± 14.7 mg body weight) before they were introduced to their test cages, five bees per cage. Each test unit represented a replicate, with six replicates for each treatment and toxic-reference group.

There were a control group, five product groups, and a single toxic-reference group. A stock solution (S6) was made by dissolving 9.3078 g of product (equivalent to 2.38 g a.s., when accounting for the product a.s. content of 25.57%) in 35 mL of distilled water; this solution was then mixed with 0.1 mL of 10% Triton X-100 solution (diluted with distilled water) to produce the highest product application solution. The other four product application solutions were produced by taking an aliquot of the stock solution and mixing it with an appropriate amount of distilled water (to produce solutions S5, S4, S3, S2) and adding 0.1 mL of the 10% Triton X-100 solution. The target topical application doses were 5.81, 12.78, 28.10, 61.82, 136 µg a.s./bee. Droplets of 2 µL of each topical solution was applied to the dorsal thorax of each anesthetised bee with a Burkard hand-operated precision micro-applicator.

The toxic-reference application solution was made by dissolving 0.0306 dimethoate technical in 20 mL of distilled water; 5 mL of this solution was then diluted with 5 mL of distilled water; a 10-mL aliquot of this dilution was then mixed with 0.1 mL of the 10% Triton X-100 solution. The target dose was 1.5µg dimethoate/bee.

Mortality and behaviour were assessed by visual counting of dead mason bees per test unit, after 4, 24, 48, 72, and 96 hours of exposure. Mean mortality data were corrected for mortality in the control group, using the Abbott formula. Mortality data were analysed with the statistical software R 3.3.2, and all statistical analyses were performed at 95% confidence level. A Spearman's rank correlation sum test confirmed that the mortality data were monotonic. Thus, a step-down Cochran-Armitage procedure was used to determine significant differences in mortality between the control and product treatment groups and the controls group. When there were no significant differences in mortality, the results were further confirmed with a Fisher's exact test, with Bonferroni-Holm corrections. LD₅₀ value and its 95% confidence intervals (CIs) were calculated with a Bayesian inference, using a 3-parameter log-logistic binomial model. Behaviour was quantitatively observed, according to the categories unaffected, affected (lacking coordination), cramping (uncontrolled contraction of the abdomen or entire body), and moribund (unable to walk, weakly response to stimuli). Behavioural data were not statistically analysed.

The concentration of the product was confirmed by analytical verification of all the treated solutions (without adjuvant). Prothioconazole was extracted from the homogenised water-treated solution and homogenised sucrose solution sample, using acetonitrile and acetonitrile/water as solvents, respectively; the final sample solutions were analysed in positive ionisation mode by Ultra High-Performance Liquid Chromatography, tandem High-Resolution Mass Spectrometry (UHPLC-TOF-MS/MS), following internal Renolab methods, which are described in the analytical final report (following Renolab method code MA RES 145 for sucrose solution as matrix and MA RES 147 for water as matrix).

Results

Analytical results

The UHPLC-TOF-MS/MS analytical method for the determination of prothioconazole in test medium was validated with regards to specificity, linearity, accuracy and precision in accordance with guideline SANCO/3029/99, part. 4, rev. 4, 11/07/2000. Specificity for prothioconazole, in treated water and sucrose solutions, was demonstrated by the absence of significant interferences above 30% of the LOQ. The analytical calibration was shown to be linear ($r \geq 0.998$) over the range of 50 to 2000 ng prothioconazole/mL.

CA3301 / JOUST	Page 132 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Accuracy was confirmed with recovery of spiked samples at relevant concentrations of the test item in matrix water (10 and 100000 mg prothioconazole/L); recoveries ranged from 94.4 – 99.4% (i.e., within 70-110%). Precision was confirmed; the relative standard deviation (n = 4) was 2.4% (i.e., within the guideline limit of $\leq 20\%$). The limit of quantification (LOQ) for prothioconazole in treated water was 10 mg/L. The limit of detection (LOD) for prothioconazole in water was 2.5 mg/L.

Table CP 10.3.1.1/03-01. Recovery of prothioconazole in treated water solution samples.

Sample solution [#]	Nominal (mg a.s./L)	Measured		Recovery (% nominal)	Deviation (%)
		mg a.s./Kg	mg a.s./L*		
S2	2905	2870.0	2864.9	98.62	-1.4
S3	6390	6259.9	6248.6	97.79	-2.2
S4	14050	13216	13192	93.89	-6.1
S5	30910	31411	31355	101.44	+1.4
S6	68000	63306	63192	92.93	-7.1

[#]Sample solutions indicate a product treatment application solution, prior to its dilution with Triton X-100. *Calculated considering the density of water at 20 °C (0.9982 g/mL)

The recoveries of nominal concentrations were between 92.93% and 101.44% (i.e., between 80% and 120%) for all test concentrations. Thus, nominal concentrations were used to calculate all biological endpoints.

Biological results

Mortality

Mortality and behavioural abnormalities of mason bees exposed to CA3301 in the 96-hour contact toxicity test, are presented in the tables below.

CA3301 / JOUST	Page 133 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Table CP 10.3.1.1/03-02. The effects of exposure to CA3301 on mortality of adult *Osmia bicornis* in a 96-hour toxicity test

Applied dose		Rep.	Mortality (no.)					Behaviourally affected (no.)				
µg product/bee	µg a.s./bee		Time (HAE)									
			4	24	48	72	96	4	24	48	72	96
0 (control)	0	1	0	0	1	1	1	0	0	0	0	0
		2	0	1	1	1	1	0	0	0	0	0
		3	0	1	1	1	1	0	0	0	0	0
		4	0	0	0	1	1	0	0	0	0	0
		5	0	0	0	0	0	0	0	0	0	0
		6	0	0	0	1	1	0	0	0	0	0
22.72	5.81	1	0	0	0	1	1	0	0	0	0	0
		2	0	1	2	3	3	0	0	0	0	0
		3	0	2	2	2	2	0	0	0	0	0
		4	0	1	1	1	1	0	0	0	0	0
		5	0	0	1	4	4	0	1	1	0	0
		6	0	0	1	3	3	0	0	0	0	0
49.98	12.78	1	0	0	1	4	4	1	1	0	0	0
		2	0	1	2	4	4	1	0	0	0	0
		3	0	1	2	2	2	1	0	0	0	0
		4	0	0	1	2	2	0	0	1	0	0
		5	0	0	1	4	4	0	0	0	0	0
		6	0	2	3	3	3	0	0	0	0	0
109.89	28.1	1	0	1	1	5	5	0	1	1	-	-
		2	0	1	2	5	5	1	1	0	-	-
		3	0	1	1	3	3	0	1	1	0	0
		4	0	1	1	3	3	2	1	1	0	0
		5	0	0	1	3	3	0	1	0	0	0
		6	0	1	2	5	5	0	0	0	-	-
241.77	61.82	1	0	1	2	5	5	1	1	2	-	-
		2	0	1	3	4	4	2	1	0	0	0
		3	0	0	1	5	5	1	1	2	-	-
		4	0	0	2	5	5	0	0	2	-	-
		5	0	0	0	3	3	2	1	1	0	0
		6	1	2	3	5	5	3	1	1	-	-
531.87	136	1	1	4	5	5	5	3	0	-	-	-
		2	1	3	3	4	4	2	0	1	0	0
		3	0	1	2	4	4	1	3	1	1	1
		4	0	2	3	5	5	1	2	0	-	-
		5	1	1	1	5	5	1	2	2	-	-
		6	1	2	3	5	5	1	2	0	-	-
1.5 µg dimethoate/bee		1	1	4	5	5	5	1	0	-	-	-
		2	0	3	5	5	5	2	1	-	-	-
		3	0	2	4	4	4	2	0	0	0	0
		4	0	2	5	5	5	1	2	-	-	-
		5	0	3	4	5	5	1	1	1	-	-
		6	0	2	4	5	5	3	1	1	-	-

Rep., replicate; HAE, hours after exposure; no., number.

Table CP 10.3.1.1/02-03: Mean mortality of *Osmia bicornis* after 24 and 48 hours of oral exposure to CA3301

Applied dose		Mortality (%)					
		4 HAE		24 HAE		48 HAE	
µg product/bee	µg a.s./bee	Mean	Corrected	Mean	Corrected	Mean	Corrected
0 (control)	0	0.0	0.0	6.67	0.0	10.00	0.0
22.72	5.81	0.0	0.0	13.33	7.14	23.33	14.81
49.98	12.78	0.0	0.0	13.33	7.14	33.33	25.93
109.89	28.1	0.0	0.0	16.67	10.71	26.67	18.52
241.77	61.82	3.33	3.33	13.33	7.14	36.67*	29.63
531.87	136	13.33	13.33	43.33*	39.29	56.67*	51.85
1.5 µg dimethoate/bee		3.33	-	53.33	-	90.00	-

Applied dose		72 HAE		96 HAE		
µg product/bee	µg a.s./bee	Mean	Corrected	Mean ± SD	CV	Corrected
0 (control)	0	16.67	0.0	16.67 ± 0.41	48.99	0.0
22.72	5.81	46.67*	36.0	46.67 ± 1.21*	51.90	36.0
49.98	12.78	63.33*	56.0	63.33 ± 0.98*	31.05	56.0
109.89	28.1	80.0*	76.0	80.0 ± 1.1*	27.39	76.0
241.77	61.82	90.0*	88.0	90.0 ± 0.84*	18.59	88.0
531.87	136	93.33*	92.0	93.33 ± 0.52*	11.07	92.0
1.5 µg dimethoate/bee		96.67	-	96.67 ± 0.0	-	-

Significant differences in mortality were revealed with a step-down Cochran-Armitage procedure, when $p < 0.05$, and are denoted with asterisks (*). Mortality was corrected using the Abbott formula; positive values indicate an increased in mortality, relative to the control group. HAE, hours after application; SD, standard deviation; CV, coefficient of variation.

After 72 hours of exposure to CA3301, statistically significant differences in mortality were recorded at all test doses, compared to the control group. However, mortality did not change between 72 and 96 hours, therefore, the 96-hour acute contact NOED value was estimated to be <22.72 µg product/bee, equivalent to <5.81 µg a.s./bee. The acute contact LOED value was determined to be 22.72 µg product/bee, equivalent to 5.81 µg a.s./bee.

The acute contact 96-hour LD₅₀ value was determined to be 45.21 µg product/bee (95% confidence Intervals: 23.90 – 82.88 µg product/bee), equivalent to 11.63 µg a.s./bee (95% Confidence Intervals: 6.082 – 21.51 µg a.s./bee). All endpoints are based on nominal concentrations, since analytical recovery of the active substance, prothioconazole, was within ±20% of nominal concentrations.

Behaviour

Table CP 10.3.1.1/03-04. The effects of CA3301 on adult *Osmia bicornis* behaviour, after 96 h of exposure (N = 30 bees per treatment group).

Nominal application dose		Number of behaviourally affected bees			
µg product/bee	µg a.s./bee	24 HAE	48 HAE	72 HAE	96 HAE
0 (control)	0	0	0	0	0
22.72	5.81	1	1	0	0
49.98	12.78	1	1	0	0
109.89	28.1	5	3	0*	0*
241.77	61.82	5	8	1*	0*
531.87	136	9	4	0*	1*

*Several replicates exhibited 100% mortality, resulting in fewer behaviourally affected bees, compared to 48 h. HAE, hours after exposure.

Adverse behavioural effects were observed:

- 4 hours after exposure, in doses at 49.98, 109.89, 241.77, and 531.87 µg product/bee, equivalent to 12.78, 28.10, 61.82, and 136 µg a.s./mason bee (cramps).
- 24 hours after exposure, in all the tested doses (cramps).

CA3301 / JOUST	Page 135 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

- 48 hours after exposure, in all the tested doses (cramps), and in the dose at 241.77 µg product/bee (affected).
- 72 hours after exposure, in the doses at 531.87 µg product/mason bee (cramps).
- 96 hours after exposure, in the doses at 531.87 µg product/mason bee (cramps).

Validity

All validity criteria were met in accordance with the OECD 214 (1998) and 246 (2017) test guidelines:

- Mean mortality in the control to be ≤20% at the end of the test (actual value was 16.67%).
- Mean mortality in the toxic-reference group to be >50% at the end of the test (actual value was 96.67%).

Conclusion

The acute contact toxicity of CA3301 to *Osmia bicornis* was evaluated, in accordance with the OECD 214 (1998) and 246 (2017) test guidelines.

The 96-hour acute contact NOED value was estimated to be <22.72 µg product/bee, equivalent to <5.81 µg a.s./bee. The acute contact LOED value was determined to be 22.72 µg product/bee, equivalent to 5.81 µg a.s./bee.

The acute contact 96-hour LD₅₀ value was determined to be 45.21 µg product/bee (95% confidence Intervals: 23.90 – 82.88 µg product/bee), equivalent to 11.63 µg a.s./bee (95% Confidence Intervals: 6.082 – 21.51 µg a.s./bee). All endpoints are based on nominal concentrations.

Adverse behavioural effects were observed:

- 4 hours after exposure, in doses at 49.98, 109.89, 241.77, and 531.87 µg product/bee, equivalent to 12.78, 28.10, 61.82, and 136 µg a.s./mason bee (cramps).
- 24 hours after exposure, in all the tested doses (cramps).
- 48 hours after exposure, in all the tested doses (cramps), and in the dose at 241.77 µg product/bee (affected).
- 72 hours after exposure, in the doses at 531.87 µg product/mason bee (cramps).
- 96 hours after exposure, in the doses at 531.87 µg product/mason bee (cramps).

This study is considered acceptable.

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, 2, and 3

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, 2, and 3

A 2.3.1.2 KCP 10.3.1.2 Chronic toxicity to bees

CA3301 / JOUST	Page 136 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Study 1

Comments of zRMS:	The study was conducted to the guideline and according to the principles of GLP. All validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.
-------------------	---

Reference:	KCP 10.3.1.2/01
Report	CA3301 (Prothioconazole 250 EC, NUL 3390): Honey Bee (<i>Apis mellifera</i> L.) Chronic Oral Toxicity Test (10-Day Feeding) under Laboratory Conditions Ansaloni, T., 2021, report no. S20-09402
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 toxicity of the product CA3301 (analyzed a.s. content of 25.57% w/w prothioconazole) on the adult European honeybees (*Apis mellifera*) was conducted as a dose-response test. The study comprised two control treatment groups, five product treatment groups, and one reference-item treatment group. Each treatment group consisted of 50 bees, divided into five replicates of 10 bees each. Bees were fed a sucrose solution treated with either distilled water, CA3301, or reference item.

The concentrations of feeding solutions were 0 (control) 673.31, 1615.93, 3878.24, 9307.78, and 22338.68 mg product/L diet, equivalent to 0, 565.8, 1357.93, 3259.03, 7821.67, and 18772 mg product/kg diet (when accounting for the diet density of 1.19 g/mL). These concentrations corresponded to 0, 172.16, 413.19, 991.67, 2380, and 5712 mg a.s./L diet, equivalent to 0, 144.68, 347.22, 833.33, 2000, and 4800 mg a.s./kg diet. The reference item was provided at a concentration of 1.07 mg a.s./L diet, equivalent to 0.90 mg a.s./kg diet.

Mortality, feed consumption, and behavioural abnormalities were recorded every daily before each application (start of feeding). Mean daily consumption was 0 µg a.s./bee in both control groups, ranged between 2.15 and 38.08 µg a.s./bee in the product treatment groups, and was 0.018 µg dimethoate/bee/day in the reference-item group. Treatment with CA3301 began to affect bees after 1 day of exposure in the three-highest product treatment groups that lasted the duration of the study. Behaviour was not affected in the control groups or in the two-lowest product treatment groups.

Treatment with CA3301 significantly increased mortality in the three-highest product treatment groups. Thus, the 10-day NOEC and LOEC (mortality) values were determined to be 1357.93 and 3259.03 mg product/kg diet, equivalent to 347.22 and 833.33 mg a.s./kg diet, respectively (based on nominal concentrations). The corresponding 10-day NOEDD and LOEDD values were determined to be 20.1 and 42.82 µg product/bee/day, equivalent to 5.14 and 10.96 µg a.s./bee/day, respectively (based on nominal concentrations). The calculated 10-day LC₁₀, LC₂₀, and LC₅₀ (mortality) values were 2428.71, 3048.26, and 4707.94 mg product/kg diet, equivalent to 621.02, 779.44, and 1203.82 mg a.s./kg diet, respectively (based on nominal concentrations). The calculated 10-day LDD₁₀, LDD₂₀, and LDD₅₀ (mortality) values 8.79, 10.27, and 13.82 µg a.s./bee/day, equivalent to 34.38, 40.16, and 54.05 µg product/bee/day, respectively (based on nominal concentrations).

CA3301 / JOUST	Page 137 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Overall, the study satisfies the OECD 245 (2017) test-guideline requirements for acute oral and contact tests, respectively, with *Apis mellifera* and is considered acceptable.

Materials and methods

Test materials

Test item

Name:	CA3301
Active substance:	Prothioconazole
Formulation type:	Emulsifiable concentrate (EC)
Source and lot/batch no.:	A19045
Active-substance content:	250 g/L (nominal), 257.04 g/L or 25.57% w/w (analyzed)
Appearance:	Light, yellow liquid
Expiry date of lot/batch:	17 June 2022
Storage conditions:	Stored between 11.8 – 17.9 °C, in original container, and protected from light

Reference item

Name:	BAS 152 65 I
Formulation type:	Emulsifiable concentrate (EC)
Density:	1.062 g/mL
Active substance:	Dimethoate
Active-substance content:	400 g/L (nominal), 409 g/L (analysed)
Batch no.:	10248664A
Appearance:	Orange liquid
Expiry date:	16 Feb 2022
Storage conditions:	Kept in fridge, protected from moisture and light.

Test organism

Species:	European honeybee, <i>Apis mellifera</i> L. (Hymenoptera, Apidae),
Age at study initiation:	Newly hatched, young adult worker bees, 1-to-2-days old
Source:	Obtained from healthy beehives located in a commercial apiary near Trialcamp facilities. Bees were foraging on wildflowers
Feeding during test:	50% w/v sucrose solution <i>ad libitum</i>
Acclimation:	On day prior to start of study, under test conditions.

Test conditions

Test temperature:	29.5-33.9°C
Relative humidity:	48.8-65.2%
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 two control treatment groups, five product treatment groups, and one reference-item treatment 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.

Solution for the highest product concentration was prepared daily by mixing a defined amount of the

CA3301 / JOUST	Page 138 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

product with a defined amount of 50% (w/v) sucrose solution + 0.1% xanthan, and this solution was used as a stock solution (18772 mg product/kg diet) for the preparation of the other product treatment solutions. Aliquots of the stock product solution were mixed daily with 50% (w/v) sucrose solution + 0.1% xanthan up to a defined volume to obtain the treatment solutions. A feeding volume of 1 mL per replicate was offered daily to the bees *ad libitum*. The bees in one cage shared the feeding solution, receiving similar doses via trophallaxis.

The concentrations of feeding solutions were 0 (control) 673.31, 1615.93, 3878.24, 9307.78, and 22338.68 mg product/L diet, equivalent to 0, 565.8, 1357.93, 3259.03, 7821.67, and 18772 mg product/kg diet (when accounting for the diet density of 1.19 g/mL). These concentrations corresponded to 0, 172.16, 413.19, 991.67, 2380, and 5712 mg a.s./L diet, equivalent to 0, 144.68, 347.22, 833.33, 2000, and 4800 mg a.s./kg diet. The reference item was provided at a concentration of 1.07 mg dimethoate/L diet, equivalent to 0.90 mg dimethoate/kg diet.

Mortality and behavioural abnormalities were recorded every daily before each application (start of feeding). 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. 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.

Statistical calculations were made with the statistical program ToxRatPro Version 3.3.0. The LDD_x/LC_x values were calculated by a probit analysis, using a linear maximum likelihood regression.

For the estimation of the NOEDD/NOEC values, mortality observed for each product concentration was compared to mortality of the control by means of a one-sided, step-down Rao-Scott-Cochran-Armitage test procedure ($\alpha = 0.050$).

Samples of each product concentration feeding solution and of the thickener 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 and the thickener control solution collected on day 9 were analyzed.

Results

Analytical results

Table CP 10.3.1.2/01-01. Recovery of prothioconazole from analysed samples

Treatment concentration	Prothioconazole concentration (mg/L)		Recovery (% nominal)
	Nominal	Measured	
Control	0	<LOD	--
Lowest	144.68	116	80
Highest	4800.00	4270	89

LOD = 20.0 mg prothioconazole/kg (30% of the LOQ).

The measured concentrations in all analysed samples (feeding solutions used to prepare the highest and lowest diet concentrations) were 80-89% of nominal values and, therefore, were within $\pm 20\%$ of nominal concentrations. Accordingly, endpoints are based on nominal concentrations.

Biological results

Feed consumption

A summary of the effect of CA3301 on food consumption, during the 10-day exposure, are presented in

CA3301 / JOUST	Page 139 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Tables CP 10.3.1.2/01-02 and 01-03 below.

Table CP 10.3.1.2/01-02. The effect of CA3301 (analysed a.s. content of 25.57% w/w) on daily feed consumption, during the 10-day exposure (N = 50 larvae per treatment group).

Nominal feed concentration		Time (day)									
mg product/kg diet	mg a.s./kg diet	1	2	3	4	5	6	7	8	9	10
0 (control)	0	20.3	25.5	18.4	21.8	15.5	24.5	19.1	25.5	19.6	28.1
0 (control)	0	15.2	20.4	21.3	10.8	19.6	16.2	18.9	24.9	14.5	31.2
565.8	144.68	10.2	16.8	13.3	13.7	16.1	11.1	17	13.9	16.1	20.6
1357.93	347.22	14.8	20.1	11.7	12.5	11.7	18.2	13.1	14.3	16.4	15.3
3259.03	833.33	13.7	15.6	13.5	8.2	15.7	8.4	15.5	12.2	15.4	13.4
7821.67	2000	9.7	12.1	8.9	12.3	10.7	9.4	8.3	13	6.7	5.3
18772	4800	12.8	7.6	1.2	4.2	6.3	13.7	4.3	30.4	n.s.	n.s.
0.90 mg dimethoate/kg diet		15.3	15.9	11.2	18.1	22	26.8	22.5	26.1	12.9	67.6

Food consumption could not be analyzed for treatment groups with no surviving (n.s.) individuals. Ref., toxic reference

Table CP 10.3.1.2/01-03. The effect of CA3301 (analysed a.s. content of 25.57% w/w) on mean feed consumption, during the 10-day exposure (N = 50 larvae per treatment group).

Nominal feed concentration		Consumed diet (mg a.s./bee/day)		Mean consumed dose (µg a.s./bee)	
mg product/kg diet	mg a.s./kg diet	Mean ± SD	Standard error	Daily	Cumulative
0 (control)	0	21.8 ± 8.1	1.1	0	0
0 (control)	0	19.3 ± 11.4	1.6	0	0
565.80	144.68	14.9 ± 6	0.9	2.15	21.51
1357.93	347.22	14.8 ± 6.4	0.9	5.14	51.4
3259.03	833.33	13.1 ± 5.7	0.8	10.96	109.56
7821.67	2000.00	9.6 ± 7.4	1.1	19.26	192.55
18772.00	4800.00	7.9 ± 8.9	1.7	38.08	198.01*
0.90 mg dimethoate/kg diet		20 ± 13.5	2.0	0.018	0.166

*Over 8 days' exposure, given 100% after this day. SD, standard deviation.

The overall mean daily consumption of feeding solutions was 19.3 to 21.8 mg a.s./bee/day in both control groups, ranged between 7.9 and 14.9 mg a.s./bee/day in the product treatment groups, and was 20 mg a.s./bee/day. The corresponding mean daily consumption was 0 µg a.s./bee in both control groups, ranged between 2.15 and 38.08 µg a.s./bee in the product treatment groups, and 0.018 µg dimethoate/bee/day in the reference-item group.

Mortality and behaviour

The effect of CA3301 on mortality and behaviour, during the 10-day exposure, are presented in tables below.

CA3301 / JOUST	Page 140 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Table CP 10.3.1.2/01-05. The effect of CA3301 on mortality, after 10 days of exposure (N = 10 larvae per replicate).

Nominal feed concentration		Actual consumption	Rep.	Mortality (no.)									
mg product/kg diet mg a.s./kg diet		µg a.s./bee/day		Time (day)									
				1	2	3	4	5	6	7	8	9	10
0 (control)	0	0	1	0	0	0	2	0	0	0	0	0	0
			2	0	0	0	0	0	0	0	0	0	0
			3	0	0	0	0	0	0	0	0	0	0
			4	0	0	0	0	0	0	0	0	0	0
			5	0	0	0	0	0	0	0	0	0	0
0 (control)	0	0	1	0	0	0	0	0	0	0	0	0	0
			2	0	0	0	0	0	0	0	0	0	0
			3	0	0	1	0	0	0	0	0	0	0
			4	0	0	0	0	0	0	0	0	0	0
			5	0	0	0	0	0	1	0	0	0	0
565.8	144.68	2.15	1	0	0	0	0	0	0	0	0	0	0
			2	0	0	0	0	0	0	0	0	0	0
			3	0	0	0	0	0	0	0	0	0	0
			4	0	0	0	0	0	1	0	0	0	0
			5	0	0	0	0	0	0	0	0	0	0
1357.93	347.22	5.14	1	0	0	0	0	0	0	0	0	0	0
			2	0	0	0	0	0	0	0	0	0	0
			3	0	0	0	0	0	0	0	0	0	0
			4	0	0	0	0	0	0	0	0	0	0
			5	0	0	0	0	0	0	0	0	0	0
3259.03	833.33	10.96	1	0	1	0	0	0	0	0	0	0	0
			2	0	0	1	0	0	1	0	1	1	0
			3	0	1	0	0	1	0	0	2	1	0
			4	0	0	0	0	0	0	1	0	2	1
			5	0	0	0	0	1	0	0	0	0	0
7821.67	2000	19.26	1	1	1	1	1	0	0	1	0	1	2
			2	0	3	4	0	0	0	1	0	0	1
			3	0	3	0	0	2	0	2	0	1	2
			4	0	1	1	2	2	0	0	1	0	0
			5	0	5	0	0	1	0	0	0	1	0
18772	4800	38.08	1	5	1	1	2	1	-	-	-	-	-
			2	2	7	1	-	-	-	-	-	-	-
			3	8	1	1	-	-	-	-	-	-	-
			4	8	1	0	0	0	0	1	-	-	-
			5	8	0	0	0	1	0	0	1	-	-
0.90 mg dimethoate/kg diet		0.018	1	0	0	0	0	0	0	4	4	1	0
			2	0	0	0	0	1	0	2	6	1	-
			3	0	0	0	0	1	1	4	3	1	-
			4	0	0	0	0	0	1	1	5	3	-
			5	0	0	0	0	1	0	3	3	3	-

Rep., replicate; no., number; Dashes (-) indicate no surviving individuals.

CA3301 / JOUST	Page 141 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Table CP 10.3.1.2/01-05. The effect of CA3301 on cumulative mortality, after 10 days of exposure (N = 50 bees per treatment group).

Nominal feed concentration		Actual consumption	Cumulative mortality		
mg product/kg diet	mg a.s./kg diet	µg a.s./bee/day	Total (no.)	Mean ± SE (%)	Corrected (%)
0 (control)	0	0	2	4.0 ± 4.0	0.0
0 (control)	0	0	2	4.0 ± 2.45	0.0
565.8	144.68	2.15	1	2.0 ± 2.0	-2.08
1357.93	347.22	5.14	0	0.0 ± 0.0	-4.17
3259.03	833.33	10.96	15	30.0 ± 8.37.0*	+27.08
7821.67	2000	19.26	41	82.0 ± 5.83*	+81.25
18772	4800	38.08	50	100.0 ± 0.0*	+100.0
0.90 mg dimethoate/kg diet		0.018	49	98.0 ± 2.0	+97.92

Statistically significant differences in mortality, relative to the control, were revealed with a one-sided, step-down Rao-Scott-Cochran-Armitage test procedure ($\alpha = 0.050$) and are denoted with asterisks (*). Mortality for the reference-item group was not statistically analyzed. Mortality data corrected for control mortality, using a modified Abbott's formula. Positive and negative signs denote an increase and decrease in mortality, relative to the control, respectively. No., number; SE, standard error.

Mortality in both control groups was 0.0% (0.0% corrected), ranged between 0.0 and 100.0% (-4.17 and 100.0% corrected) in the product treatment groups, and was 98.0% in the reference-item group (97.92% corrected). Treatment with CA3301 significantly increased mortality in the three-highest product treatment groups. Thus, the 10-day NOEC and LOEC (mortality) values were determined to be 347.22 and 833.33 mg a.s./kg diet, equivalent to 1357.93 and 3259.03 mg product/kg diet, respectively (based on nominal concentrations). The corresponding 10-day NOEDD and LOEDD values were determined to be 5.14 and 10.96 µg a.s./bee/day, equivalent to 20.1 and 42.82 µg product/bee/day, respectively (based on nominal concentrations).

The calculated 10-day LC₁₀, LC₂₀, and LC₅₀ (mortality) values were 621.02, 779.44, and 1203.82 mg a.s./kg diet, equivalent to 2428.71, 3048.26, and 4707.94 mg product/kg diet, respectively (based on nominal concentrations). The calculated 10-day LDD₁₀, LDD₂₀, and LDD₅₀ (mortality) values 8.79, 10.27, and 13.82 µg a.s./bee/day, equivalent to 34.38, 40.16, and 54.05 µg product/bee/day, respectively (based on nominal concentrations).

Table CP 10.3.1.2/01-06. The effect of CA3301 (analysed a.s. content of 25.57% w/w) on behaviour, during 10 days of exposure (N = 50 bees per treatment group).

Nominal diet concentration	Consumption	Behaviourally affected bees (%)										
		Time (days)										
mg a.s./kg diet	µg a.s./bee/day	1	2	3	4	5	6	7	8	9	10	
0	0	0	0	0	0	0	0	0	0	0	0	
0	0	0	0	0	0	0	0	0	0	0	0	
144.68	2.15	0	0	0	0	0	0	0	0	0	0	
347.22	5.14	0	0	0	2	2	0	0	0	0	0	
833.33	10.96	20	33.3	100	100	37.8	11.4	7	22.5	22.2	8.6	
2000.00	19.26	20.4	63.9	100	100	59.1	45.5	33.3	47.1	64.3	22.2	
4800.00	38.08	84.2	100	100	100	100	100	100	n.s.	n.s.	n.s.	

Behaviour could not be observed for treatment groups with no surviving (n.s.) individuals.

Treatment with CA3301 began to affect bees after 1 day of exposure in the three-highest product treatment groups that lasted the duration of the study. Behaviour was not affected in the control groups or in the two-lowest product treatment groups.

Validity

All validity criteria were met in accordance with the OECD 245 (2017) test guideline:

- Mean mortality to be ≤15%, at the end of the study (actual values range from 4.0% for both control groups).
- Mean mortality to be ≥50% on day 8, at the end of the study (actual values ranged from 98.0%).

CA3301 / JOUST	Page 142 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Conclusion

The toxicity of CA3301 to adult *Apis mellifera* during a 10-day test was studied in accordance with the OECD 245 (2017) test guideline.

The 10-day NOEC and LOEC (mortality) values were determined to be 347.22 and 833.33 mg a.s./kg diet, equivalent to 1357.93 and 3259.03 mg product/kg diet, respectively (based on nominal concentrations).

The 10-day NOEDD and LOEDD (mortality) values were determined to be 5.14 and 10.96 µg a.s./bee/day, equivalent to 20.1 and 42.82 µg product/bee/day, respectively (based on nominal concentrations).

The calculated 10-day LC₁₀, LC₂₀, and LC₅₀ (mortality) values were 621.02, 779.44, and 1203.82 mg a.s./kg diet, equivalent to 2428.71, 3048.26, and 4707.94 mg product/kg diet, respectively (based on nominal concentrations).

The calculated 10-day LDD₁₀, LDD₂₀, and LDD₅₀ (mortality) values 8.79, 10.27, and 13.82 µg a.s./bee/day, equivalent to 34.38, 40.16, and 54.05 µg product/bee/day, respectively (based on nominal concentrations).

This study is considered acceptable.

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:	The study was conducted to the guideline and according to the principles of GLP. All validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.
-------------------	---

Reference:	KCP 10.3.1.3/01
Report	CA3301 (Prothioconazole 250 EC, NUL 3390): Honey Bee (<i>Apis mellifera</i> L.) Larval Toxicity Test following Repeated Exposure under laboratory conditions Ansaloni, T., 2021, report no. S20-09403
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

A 22-day repeated-exposure toxicity test of the product CA3301 (analysed a.s. content of 25.57% w/w prothioconazole) on the European honeybee (*Apis mellifera* L.) was conducted as a dose-response study. It comprised of one control group and five product groups of five different concentrations: 3.14, 9.41, 28.22, 84.65, and 253.95 mg product/kg diet, equivalent to 0.80, 2.41, 7.22, 21.65, and 64.94 mg a.s./kg diet (when accounting for the product a.s. content of 25.57% w/w). Based on the cumulative application volume of 140 µL/larva, the corresponding doses were 0.48, 1.45, 4.35, 13.04, and 39.11 µg a.s./larva, equivalent to

CA3301 / JOUST	Page 143 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

0.123, 0.370, 1.111, 3.333, and 10.0 µg a.s./larva. One reference-item group with 48 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 larvae, divided into three replicates of 16 larvae.

Larval mortality was assessed, before feeding on days 4, 5, and 6 before feeding 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. Analytical verification revealed that application solutions contained prothioconazole within $\pm 20\%$ of nominal concentrations, thus, endpoints were based on nominal values.

Treatment with CA3301 did not cause significant decreases in emergence between the control and product treatment groups. Thus, the 22-day NOEC (emergence) value was 253.95 mg product/kg diet, equivalent to 64.94 mg a.s./kg diet, and the 22-day NOED (emergence) value was 10.0 µg a.s./larva/developmental period, equivalent to 39.11 µg product/larva (based on nominal concentrations). Since treatment with CA3301 did not cause an effect on emergence $\geq 10\%$, relative to the control, the 22-day ED₁₀, ED₂₀, and ED₅₀ values were all estimated to be >10.0 µg a.s./larva/developmental period, equivalent to >39.11 µg product/larva/developmental period (based on nominal concentrations). Likewise, the EC₁₀, EC₂₀, and EC₅₀ values were all estimated to be >253.95 mg product/kg diet, equivalent to >64.94 mg a.s./kg diet (based on nominal concentrations).

Overall, the study satisfies the OECD test guidance document no. 239 (2016) requirements for a repeated-exposure larval toxicity test and is considered acceptable.

Materials and methods

Test materials

Test item

Name:	CA3301
Active substance:	Prothioconazole
Formulation type:	Emulsifiable concentrate (EC)
Source and lot/batch no.:	A19045
Active-substance content:	250 g/L (nominal), 257.04 g/L or 25.57% w/w (analyzed)
Appearance:	Light, yellow liquid
Expiry date of lot/batch:	17 June 2022
Storage conditions:	Stored between 11.8 – 17.9 °C, in original container, and protected from light

Reference item

Name:	BAS 152 I
Active substance:	Dimethoate
Purity:	98.2% w/w
Batch no.:	COD-002332
Appearance:	White-to-grey crystalline flakes
Expiry date:	23 Jan 2022
Storage conditions:	≤ 25 °C; dark and dry

Test organism

Species:	European honeybee, <i>Apis mellifera</i> L. (Hymenoptera, Apidae),
Age at study initiation:	First instar larvae (L1)
Source:	Collected from three different bee hives sited in a commercial apiary from the in-house test facility stock near Eurofins Trialcamp facilities
Feeding during test:	

Test conditions

CA3301 / JOUST	Page 144 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Test temperature: 32.3 – 34.7°C
Relative humidity: 59.9 – 98.1%
Photoperiod: Continuous darkness, except during feeding and assessments

Several queen bees were confined to their own colonies (with excluder cages) four days before grafting (D-3), to ensure the production of larvae. Three days prior to grafting (D-2), and a maximum 30 h after caging, queens were released from the excluder. The combs containing the newly laid eggs were left in the excluder during the incubation period until hatching on day 1 (D1). On D1, three excluded combs with the highest number of synchronised eggs were selected and transferred to the laboratory, which were used for grafting. All larvae used in the study were alive and of similar size.

The study was conducted as a 21-day dose-response test (from D1 to the final assessment on D22). It comprised of one control group and five product groups of five different concentrations: 3.14, 9.41, 28.22, 84.65, and 253.95 mg product/kg diet, equivalent to 0.80, 2.41, 7.22, 21.65, and 64.94 mg a.s./kg diet (when accounting for the product a.s. content of 25.57% w/w). Based on the cumulative application volume of 140 µL/larva, the corresponding doses were 0.48, 1.45, 4.35, 13.04, and 39.11 µg a.s./larva, equivalent to 0.123, 0.370, 1.111, 3.333, and 10.0 µg a.s./larva. One reference-item group with 48 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 larvae, from three different hives were tested. Each hive corresponded to one replicate; 16 larvae from each replicate were used.

The larval diets were prepared from applications solutions (which were made by diluting stock solutions of 279.35 mg a.s./L or nominal 2800 mg dimethoate/L). Larvae were fed on days D1, D3, and D4 to D6, with diets A, B, and C, respectively. Each diet was composed of 50% royal jelly + 50% weight of aqueous solutions containing: 2% weight of yeast extract, 12% weight of glucose, and 12% weight of fructose (diet A), 3% weight of yeast extract, 15% weight of glucose and 15% weight of fructose (diet B), and 4% weight of yeast extract, 18% weight of glucose and 18% weight of fructose (diet C). The administered volumes of diets A, B, and C 20, 20, and 30 µL/larva.

On day 1 (D1), 20 µL of diet A was dropped into each grafting cell of the well plate and larvae were kept transferred to crystal polystyrene grafting cells, with a 9-mm diameter. Each cell was placed into a 48-well culture plate, left uncovered, and placed into hermetically sealed desiccators. On day 15, each plate was transferred to an emergence box (18 x 13 x 7 cm³) in an incubator. Larvae in the emergence box had access to untreated aqueous sucrose solution *ad libitum*.

Larval mortality was assessed, before feeding on days 4, 5, and 6 before feeding 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 daily 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. Samples of the application solutions used to prepare the highest and lowest diet concentrations from days 3 to 6 and the control application solution taken on day 6 were analyzed. All other samples were maintained in storage, at ≤-18°C.

Statistical calculations were made with MS Excel 2016 and the statistical program ToxRatPro® Version 3.3.0. A χ^2 2x2 test, with Bonferroni corrections ($\alpha = 0.05$, one sided greater), was used to determine the NOEC/NOED and the LOEC/LOED values. Since no dose-response curve was obtained, no regression analysis, for the estimation of the EC_x/ED_x values and 95% confidence limits, was performed. Cumulative larval mortality was calculated as the number of dead larvae from days 3 to 8. Cumulative pupal mortality was calculated as the number of larvae not transforming into pupae on day 15 and the number of pupae that did not emerge on day 22. Adult emergence was calculated as the number of bees emerged on day 22. Mortality was corrected for control mortality, using a modified Abbott's formula.

CA3301 / JOUST	Page 145 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Results

Analytical results

Table CP 10.3.1.3/01-01. Recovery of prothioconazole from analysed samples.

Sampling day	Prothioconazole concentration (mg/L)		Recovery (% nominal)	
	Nominal	Measured	Sample value	Mean
6	0 (water control)	<LOD	0	0
1	8.82	9.20	104	108
2		9.80	111	
3		9.70	110	
4		9.25	105	
1	714.29	735	103	102
2		760	106	
3		755	106	
4		660	92	

The measured concentrations in all analysed samples (application solutions used to prepare the highest and lowest diet concentrations) were 92–111% of nominal values and, therefore, were within $\pm 20\%$ of nominal concentrations. Accordingly, endpoints are based on nominal concentrations.

Biological results

Larval mortality

The effect of CA3301 on larval mortality, after 21 days of exposure, is presented in tables below.

Table CP 10.3.1.3/01-03. The effect of CA3301 on larval mortality, after 21 days of exposure (N = 16 larvae per replicate).

Nominal concentration		Consumed	Rep.	Mortality (no.)						
				Time (day)						
mg product/kg diet	mg a.s./kg diet	µg a.s./larva		4	5	6	7	8	5	22
0 (control)	0	0	1	0	0	1	1	1	2	2
			2	1	1	1	1	1	1	2
			3	0	0	0	0	0	1	1
3.14	0.8	0.123	1	0	0	0	0	0	2	2
			2	0	0	0	0	0	1	1
			3	0	0	0	0	0	0	1
9.41	2.41	0.37	1	0	0	0	0	0	0	0
			2	0	0	0	0	0	2	2
			3	0	0	0	0	0	0	0
28.22	7.22	1.111	1	1	1	1	1	1	1	1
			2	0	0	0	0	0	0	0
			3	1	1	1	1	1	1	1
84.65	21.65	3.333	1	0	0	0	0	0	1	1
			2	1	1	1	1	1	1	1
			3	0	0	0	0	0	0	0
253.95	64.94	10	1	0	0	0	0	1	1	1
			2	0	0	0	0	0	0	0
			3	0	0	0	0	0	0	0
48 mg dimethoate/kg diet (7.39 µg dimethoate/larva)			1	5	8	11	14	15	16	16
			2	3	11	13	14	15	15	16
			3	0	4	11	11	12	15	16

Rep., replicate; no., number.

CA3301 / JOUST	Page 146 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Table CP 10.3.1.3/01-03. The effect of CA3301 on mean cumulative larval mortality, after 21 days of exposure (N = 48 larvae per treatment group).

Nominal concentration	Consumed	Mean cumulative mortality (%)						
		Time (days)						
mg product/kg diet	µg a.s./larva	4	5	6	7	8	15	22
0 (control)	0	2.08	2.08	4.17	4.17	4.17	8.33	10.42
3.14	0.123	0.00	0.00	0.00	0.00	0.00	6.25	8.33
9.41	0.37	0.00	0.00	0.00	0.00	0.00	4.17	4.17
28.22	1.111	4.17	4.17	4.17	4.17	4.17	4.17	4.17
84.65	3.333	2.08	2.08	2.08	2.08	2.08	4.17	4.17
253.95	10.0	0.00	0.00	0.00	0.00	2.08	2.08	2.08
48 mg dimethoate/kg diet (7.39 µg dimethoate/larva)		16.67	47.92	72.92	81.25	87.50	95.83	100.0

Table CP 10.3.1.3/01-04 The effect of CA3301 on corrected cumulative larval mortality, after 21 days of exposure (N = 48 larvae per treatment group).

Nominal concentration	Consumed	Corrected cumulative mortality (%)*						
		Time (days)						
mg product/kg diet	µg a.s./larva	4	5	6	7	8	15	22
0 (control)	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3.14	0.123	-2.13	-2.13	-4.35	-4.35	-4.35	-2.27	-2.33
9.41	0.37	-2.13	-2.13	-4.35	-4.35	-4.35	-4.55	-6.98
28.22	1.111	2.13	2.13	0.00	0.00	0.00	-4.55	-6.98
84.65	3.333	0.00	0.00	-2.17	-2.17	-2.17	-4.55	-6.98
253.95	10.0	-2.13	-2.13	-4.35	-4.35	-2.17	-6.82	-9.30

*Mortality corrected for control mortality, using a modified Abbott's formula.

Mean cumulative larval mortality on day 8 was 4.17% (0.00% corrected) in the control group, ranged between 0.00% and 4.17% (-4.35% and 0.00% corrected) in the product treatment groups, and 87.5% in the reference-item group. Mean cumulative larval mortality on day 15 was 8.33% (0.00% corrected) in the control group, ranged between 2.08% and 6.25% (-6.82% and -2.27% corrected) in the product treatment groups, and 95.83% in the reference-item group. Mean cumulative larval mortality on day 22 was 10.42% (0.00% corrected) in the control group, ranged between 2.08% and 8.33% (-9.3% and -2.33% corrected) in the product treatment groups, and 100% in the reference-item group. There were no statistically significant differences in mortality between the control and product treatment groups. Moreover, on day 8, no uneaten food was observed.

Pupal mortality and emergence

A summary of the effect of CA3301 on pupal mortality and emergence, after 15 and 21 days of exposure, respectively, is presented in table below.

CA3301 / JOUST	Page 147 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Table CP 10.3.1.3/01-05. The effect of CA3301 on pupal mortality and emergence (N = 16 larvae per replicate).

Nominal concentration mg product/kg diet	Consumed µg a.s./larva	Rep.	Pupal mortality (%)			Individuals emerged	
			Time (days)			Day 22	
			8-15	15-22	8-22	No.	Mean (%)
0 (control)	0	1	4.35	2.27	6.52	14	89.58
		2				14	
		3				15	
3.14	0.123	1	6.25	2.22	8.33	14	91.67
		2				15	
		3				15	
9.41	0.37	1	4.17	0	4.17	16	95.83
		2				14	
		3				16	
28.22	1.111	1	0	0	0	15	95.83
		2				16	
		3				15	
84.65	3.333	1	2.13	0	2.13	15	95.83
		2				15	
		3				16	
253.95	10	1	0	0	0	15	97.92
		2				16	
		3				16	
48 mg dimethoate/kg diet (7.39 µg dimethoate/larva)		1	0	0	0	0	0
		2				0	
		3				0	

Rep., replicate; no., number.

Cumulative pupal mortality on day 22 was 6.52% in the control and range between 0.00% and 8.33% in the product treatment groups. There were no statistically significant differences in mortality between the control and product treatment groups. Emergence on day 22 was 89.58% in the control and range between 91.67% and 97.92% in the product treatment groups. There were no statistically significant differences in emergence between the control and product treatment groups. Thus, the 22-day NOEC (emergence) value was 253.95 mg product/kg diet, equivalent to 64.94 mg a.s./kg diet, and the 22-day NOED (emergence) value was 39.11 µg product/larva, equivalent to 10.0 µg a.s./larva/developmental period (based on nominal concentrations). Since treatment with CA3301 did not cause an effect on emergence $\geq 10\%$, relative to the control, the 22-day ED₁₀, ED₂₀, and ED₅₀ values were all estimated to be >39.11 µg product/larva/developmental period, equivalent to >10.0 µg a.s./larva/developmental period (based on nominal concentrations). Likewise, the EC₁₀, EC₂₀, and EC₅₀ values were all estimated to be >253.95 mg product/kg diet, equivalent to >64.94 mg a.s./kg diet (based on nominal concentrations).

At the end of the test (day 22), no emerged bees were observed to be visually or morphologically affected by treatment with CA3301.

Validity

All validity criteria were met in accordance with the OECD test guidance document no. 239 (2016):

- Cumulative larval mortality to be $\leq 15\%$, from day 3 to day 8, across all replicates (actual values range from 0 to 6.25%).
- Emergence on day 22 to be $\geq 70\%$, across all replicates (actual values range from 87.5 to 93.75%).
- Cumulative larval mortality to be $\geq 50\%$ on day 8, across all replicates (actual values ranged from 75 to 93.75%).

CA3301 / JOUST	Page 148 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Conclusion

The repeated exposure of CA3301 to honeybee (*Apis mellifera* L.) larvae was tested under laboratory conditions over a period of 22 days and in accordance with the OECD test guidance document no. 239 (2016).

The 22-day NOEC (emergence) value was 253.95 mg product/kg diet, equivalent to 64.94 mg a.s./kg diet, and the 22-day NOED (emergence) value was 39.11 µg product/larva/developmental period, equivalent to 10.0 µg a.s./larva/developmental period (based on nominal concentrations).

The 22-day ED₁₀, ED₂₀, and ED₅₀ (emergence) values were all estimated to be >39.11 µg product/larva/developmental period, equivalent to >10.0 µg a.s./larva/developmental period (based on nominal concentrations).

The 22-day EC₁₀, EC₂₀, and EC₅₀ (emergence) values were all estimated to be >253.95 mg product/kg diet, equivalent to >64.94 mg a.s./kg diet (based on nominal concentrations).

This study is considered acceptable.

A 2.3.1.4 KCP 10.3.1.4 Sub-lethal effects

A 2.3.1.5 KCP 10.3.1.5 Cage and tunnel tests

Study 1

Comments of zRMS:	Study not evaluated by zRMS. Please refer to RAR for prothioconazole.
-------------------	---

Reference:	KCP 10.3.1.5/01
Report	Assessment of side effects of prothioconazole EC 250 G on the honey bee (<i>Apis mellifera</i> L.) in the semi-field after one application on <i>Phacelia tanacetifolia</i> in Germany 2015 Hein, R., 2015, report no. S15-02997, Bayer report No: M-532419-01-1
Guideline(s):	Yes. OECD No.75 (2007), AG Bienenschutz (Pistorius et al., 2012), and OEPP/EPPO 170(4) (2010), US EPA OCSP Guideline No. 850.SUPP
Deviations:	Yes, but acceptable
GLP:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability:	Yes
Duplication (if vertebrate study)	No

Note to RMS: This study is protected, but the Owner (Bayer) has provided the Sponsor (Nufarm) access. However, since Nufarm does not have access to a copy of the final study report, this study summary is prepared using only the summary available in the 2018 RAR for prothioconazole.

Materials and methods

Test substance: Prothioconazole EC 250 G; analyzed a.s. content: 246.9 g/L; Batch No. ECE2101729; Specification No.: 102000008022, Density: 1.005 g/cm³ (at 20°C).

The crop used was full-flowering *Phacelia tanacetifolia* (Stala variety) and the study was conducted in Stutensee in Baden-Württemberg, Germany (49°04'51.82" N, 08°29'08.78" E).

CA3301 / JOUST	Page 149 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

The study included three treatment groups with four replicates (tunnels) each: one tap-water treated control group (C), one test-item group (T) and one reference item group (R).

Figure 2.2-1 depicts the tunnel arrangement and size and Figure 2.2-2 provides a detailed representation of a single tunnel.

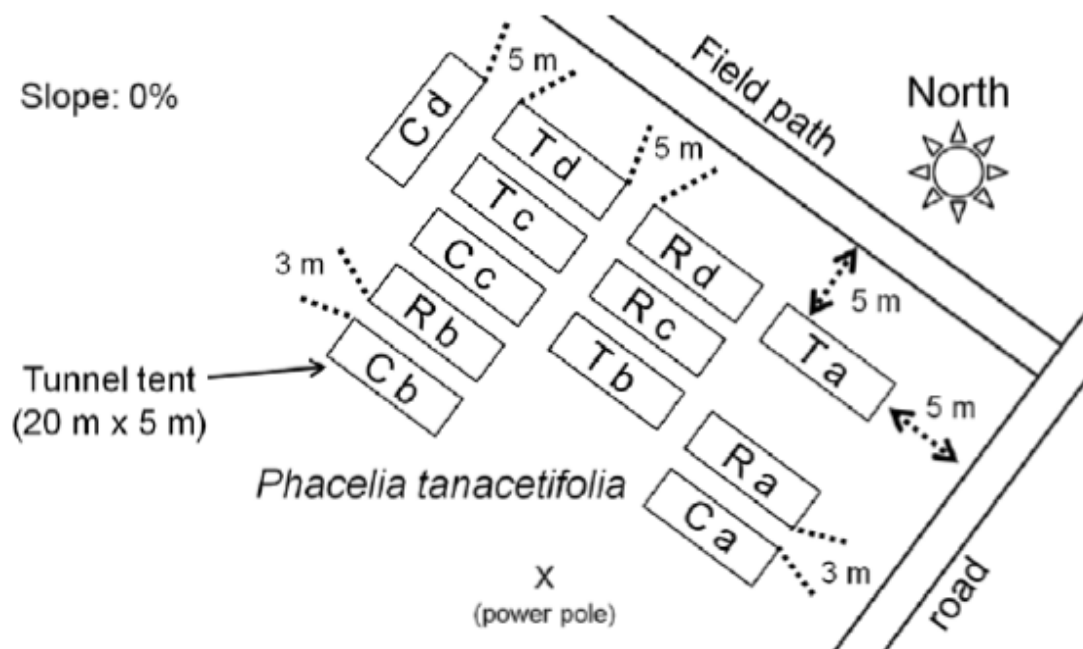


Figure 2.2-1: Schematic representation of the arrangements of the tunnels within the field site.

CA3301 / JOUST	Page 150 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

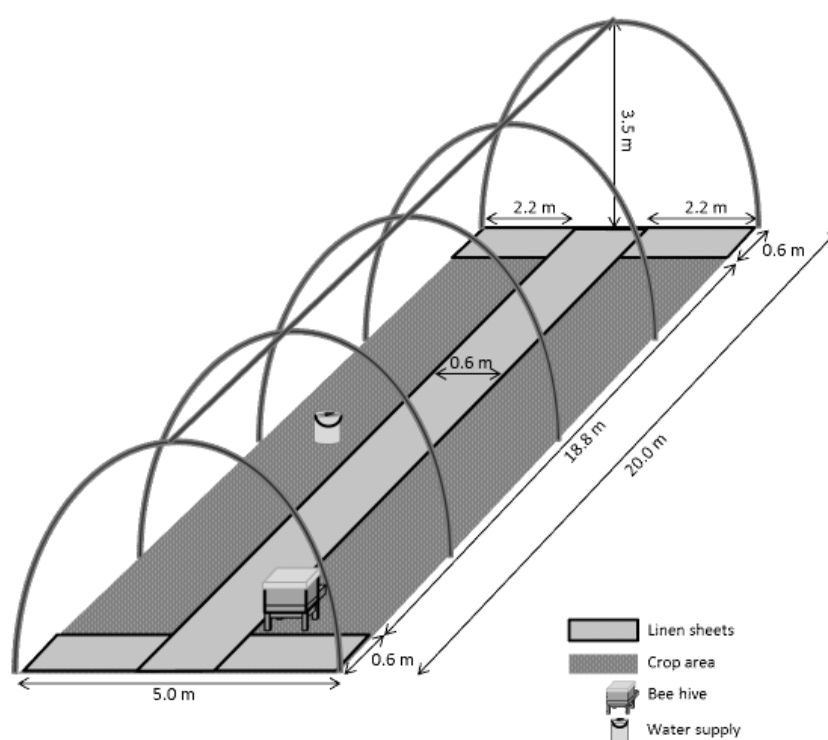


Figure 2.2-2: design of tunnels

Applications were made at full-flowering (BBCH 64 – 65) with honey bees actively foraging on the crop. The target application rate of the test substance Prothioconazole EC 250 G was 187.5 g a.s./ha (actual mean rate applied 199.2 g a.s./ha). Tap water was applied in the control group and Insegar was applied at a target rate of 1200 g product/ha in the reference item group (corresponding to 300 g fenoxycarb/ha). The spray volume was 400 L/ha in all treatment groups. The application was carried out with a portable boom sprayer simulating a commercial application. The amount of test substance, control or reference item solution actually applied was determined by measuring the prepared and the remaining spray solution.

The following conditions during the applications were met:

- Crop at full flowering during application (BBCH 64 – 65)
- Bees were actively foraging during the applications in C, T and R (≥ 10 bees/m² per treatment group shortly before application)
- Wind speed did not exceed 2.0 m/s during all applications except for one tunnel (Ra) where a maximum of 2.3 m/s was measured
- Air temperature did not exceed 29.6 °C
- The accepted spray tolerance of 0 to +10% per treatment group was met in all treatment groups
- There was no rainfall within at least two hours after the applications

The initial mean colony sizes per treatment group were in the range of 8109 to 8759 bees. The honey bees remained in the tunnels for 12 days and colonies were assessed twice during the confined phase and four times afterwards.

After the end of the confined period, the colonies were relocated to a forestal monitoring site without flowering main crops attractive to honey bees within 3 km.

Table 2.2-1 indicates the critical dates for the study.

CA3301 / JOUST	Page 151 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Table 2.2-1: Critical dates of the test

Date	Timing	Activity
09 Jun 2015	5DBA	Set-up of the colonies in the tunnels in the late evening (BBCH 61 – 63)
10 Jun 2015	4DBA	Start of mortality, flight intensity and behaviour assessments under confined conditions
13 Jun 2015	1DBA / BFD	1st colony assessment and brood area fixing day
14 Jun 2015	0DAA	Application (BBCH 64 – 65)
19 Jun 2015	5DAA / BFD + 6 days	2nd colony assessment and assessment of brood development
21 Jun 2015	7DAA	Last assessments of mortality, flight intensity and behaviour under confined conditions. Relocation of the colonies from the tunnels to the monitoring location in the evening (BBCH 66)
22 Jun 2015	8DAA	First assessment of mortality and behaviour at the monitoring location
24 Jun 2015	10DAA / BFD + 11 days	Third colony assessment and assessment of brood development
29 Jun 2015	15DAA / BFD + 16 days	Fourth colony assessment and assessment of brood development
06 Jul 2015	22DAA / BFD + 23 days	Fifth colony assessment and assessment of brood development
10 Jul 2015	26DAA	Sixth colony assessment, last assessment of mortality at the monitoring site

DBA: Days before application; DAA: days after application; BFD: Brood area fixing day

The following endpoints were assessed:

- Total and mean number of dead bees on the linen sheets in tunnels, in the dead bee traps and in the dead bee bottoms before as well as after the start of exposure in T and the application in C and R, respectively. Dead bees were differentiated into worker bees, pupae and larvae. Dead male bees and male brood stages were also recorded but are not reported and evaluated.
- Flight intensity (mean number of forager bees/m² *Phacelia tanacetifolia*) before as well as after the start of exposure in T and the application in C and R, respectively.
- Behaviour of the bees in the crop and around the hive.
- Condition of the colonies (colony strength and area of the different brood stages and food storage per colony and assessment date).
- Development of the bee brood assessed in individual brood cells. For this particular assessment, between 206 and 385 individually marked cells per colony were selected.

Meteorological Data

For the exposure period with the bees inside the tunnels (5DBA to 7DAA) the data logger and a rain gauge was placed in one of the tunnels (GLP record).

During the post exposure phase (outside the tunnels: 8DAA to 26DAA), meteorological data (non-GLP) were provided from two EAS weather stations in Niefern-Öschelbronn and Mühlacker-Enzberg which were closest to the monitoring site (distance of approx. 2.5 km and 7.0 km).

Data were also recurred during applications.

The following data were recorded in each phase:

CA3301 / JOUST	Page 152 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

- Air temperature (daily minimum/maximum)
- Relative air humidity (daily minimum/maximum)
- Daily precipitation

Various statistical analyses were conducted using SAS release Version 9.3.

Findings

Biological results

Mortality

Throughout the study (before and following exposure) worker bee mortality was similar across all treatments, indicating no effect of the test substance. Statistically significant higher values in T were found on 7DAA and 23DAA but these were only minor in nature and not related to the treatment.

The pupal mortality in T and C was on a very low level throughout the study. There were no statistically significant differences between C and T on any individual day of the pre- or post-application period.

The mean value for the entire confinement period (0DAA to 7DAA) was on a very low level in T (0.5 dead pupae/day). Although this value was statistically significantly different from the control (0.2 dead pupae/day), it was within the range of natural variability, only slightly higher than the pre-application mortality in T (0.2 dead pupae/day) and even lower than the pre-application pupal mortality in the untreated R (0.9 dead pupae/day recorded from 4DBA to 0DBA) and it is therefore not considered as biologically relevant and treatment related.

For the whole post-application period (0DAA to 26DAA), no statistically significant difference between C (0.4 dead pupae/day) and T (0.3 dead pupae/day) was observed.

In contrast, a clear and statistically significant effect of the reference item treatment R on pupal mortality was observed for the periods from 0DAA to 7DAA (0.8 dead pupae/day) and from 0DAA to 26DAA (35.3 dead pupae/day). Moreover, dead malformed pupae with white eyes or sickle shaped (rimmed) eyes were observed in R on most days from 9DAA to 26DAA. Effects on pupae of the reference substance are a well-known effect.

Table 2.2-2: Mortality

Assessment timing	Control (C)	Test substance (T)	Reference Item (R)
	Daily mean mortality (dead worker bees/colony) ± STD		
4DBA – 0DBA	49.0 ± 19.6	56.4 ± 20.9	55.5 ± 18.8
0DAA	30.0 ± 6.6	38.0 ± 13.0	16.5 ± 4.4
0DAA – 7DAA	40.9 ± 5.2	52.0 ± 13.8	24.7 ± 5.5
08DBA2 – 26DAA	23.8 ± 8.2	24.6 ± 6.4	17.8 ± 3.3
Assessment timing	Daily mean mortality (dead larvae+pupae/ colony) ± STD		
4DBA – 0DBA	0.3 ± 0.4	0.2 ± 0.2	0.9 ± 1.0
0DAA	0.0 ± 0.0	1.3 ± 1.5	0.0 ± 0.0
0DAA – 7DAA	0.2 ± 0.2	0.5 ± 0.2*	0.8 ± 0.4*
08DBA2 – 26DAA	0.4 ± 0.3	0.3 ± 0.3	35.3 ± 20.9*

DAA: days after application; DBA: days before application; STD: standard deviation

*statistically significantly higher than control group

Flight intensity

During the pre-application period (4DBA to 0DBA), flight activity in T was slightly though statistically significantly lower than the control (Tukey's test, two-sided, $\alpha = 0.05$). Since T was still untreated at this time, this difference is not related to the test substance. Pre-application flight activity in T was on the same level as R which may be used for comparison since it was also still untreated at this time (not significantly different; Tukey's test, two-sided, $\alpha = 0.05$). Therefore, pre-application flight activity in T was on a normal

CA3301 / JOUST	Page 153 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

level for this kind of crop and within the natural range of variability.

After the application until the end of the confinement period (0DAA to 7DAA), foraging rates in the test substance treatment were slightly lower but not statistically significantly different from the control. Actually, flight activity in T was higher after the application than before, and no repellence effect could be discerned.

On the day of the application (0DAA), the mean daily flight intensity, assessed over a period of 6 hours, accounted to 26.2, 21.3 and 25.2 forager bees/m², for C, T, and R, respectively (no statistically significant differences; Student's t-test, method pooled, one-sided, $\alpha = 0.05$). The slight difference of flight activity in T compared to the control has no biological relevance and was on a normal level (higher than before application at 0DBA) in T throughout this day.

Overall, none of these slight differences between C and T is considered as biologically relevant or treatment-related.

Table 2.2-3: Flight intensity

Assessment timing	Control (C)	Test substance (T)	Reference Item (R)
	Daily mean flight intensity (bees/m ²) \pm STD		
4DBA – 0DBA [#]	13.3 \pm 3.3	8.2 \pm 1.6*	9.4 \pm 2.0
0DAA	26.2 \pm 4.4	21.3 \pm 3.0	25.2 \pm 4.6
0DAA – 7DAA [#]	19.0 \pm 3.1	15.6 \pm 2.0	19.8 \pm 1.0

DAA: days after application; DBA: days before application; STD: standard deviation

*statistically significantly lower than control group

[#]Data on 4DAA and 6DAA were excluded from the calculation of mean values and STD because there was hardly any flight activity in any treatment due to bad weather

Behaviour of the Bees

Small numbers of bees displaying unusual behaviour were observed in T on several days after the application (0DAA, 1DAA, 3DAA, 7DAA, 11DAA, 16DAA, 18DAA, 21DAA, 26DAA), but similar observations were also made in the control during this period, and in few cases also in the untreated C and T before application (4DBA to 0DBA). Therefore, no test substance related adverse effect on honey bee behaviour was discerned.

Development of Honey bee Brood in Individual Cells

In the control group C, successful development was observed in the majority of the marked brood cells, indicating a healthy development of brood. The mean termination rate at the end of the observation period (BFD+23) was acceptable at 30.57%.

In the reference item treatment group R, the post treatment mean values of the brood and compensation indices were clearly lower than those observed in the control, indicating a strong adverse effect.

The mean brood and compensation indices as well as the mean termination rates in R were statistically significantly different from the respective values in the control for all post treatment assessments (Student's t-Test, method pooled, one-sided, $\alpha = 0.05$). The mean termination rate at the end of the observation period (BFD+23) was 97.54%, indicating that the majority of the initially marked eggs had not completed its development.

In the test substance treatment group T the brood and compensation indices were slightly lower and mean termination rates were slightly higher than in the control on all assessment dates after BFD 0. The mean termination rate at the end of the observation period (BFD+23) was at 46.63%. No statistically significant differences between control and test substance were found.

Table 2.2-4: Brood and compensation indices and termination rates

Replicate	Brood index / Compensation index at x days after brood area fixing day (BFD)					Termination rate (BFD +23)
	0	+6	+11	+16	+23	
Control	1.00/1.00	2.48/2.51	2.97/3.01	2.93/3.03	3.47/3.98	30.57
STD	0.00/0.00	0.65/0.63	0.71/0.68	0.72/0.64	0.98/0.73	19.61
Test substance T	1.00/1.00	1.88/1.96	2.32/2.38	2.30/2.61	2.67/3.79	46.63
STD	0.00/0.00	0.14/0.14	0.17/0.15	0.20/0.27	0.33/0.34	6.47
Reference item R	1.00/1.00	0.12*/0.18*	0.12*/0.23*	0.10*/1.09*	0.12*/2.57*	97.54*
STD	0.00/0.00	0.11/0.15	0.11/0.17	0.12/0.47	0.14/0.70	2.75

BFD: Brood area fixing day; STD: Standard deviation

*Statistically significantly lower (brood and compensation indices) or higher (termination rate) compared to the control

Strength of the Colonies

The overall development of colony strength of all treatment groups showed fluctuations in a typical and normal range. The colony strength values of the test substance group were on approximately the same level during the entire study than the corresponding values of the control group, except colony Td where a slight decrease of the colony size from 1DBA to 5DAA was observed while all other colonies were growing. No similar observation was made in the other colonies of treatment T and colony Td developed normal on all following assessments. Therefore, no test-item related adverse effects on colony strength were observed.

Development of the Brood Area

The mean amount of brood in the colonies (sum of cells containing eggs, larvae, and pupae) was assessed. Overall, on the level of whole colonies, honey bee brood development in the test substance treatment group T was not affected when compared to the control.

Development of the Food Storage Area

The mean amount of food stores in the colonies (sum of cells containing nectar and pollen) was assessed. All colonies were well provided during the course of the study and there was no lack of pollen or nectar in any colony at any assessment date. No test-item related adverse effects on the development of the food storage area were observed.

Conclusion

Prothioconazole EC 250 G was applied at a target rate corresponding to 187.5 g a.s./ha (actual mean rate applied 199.2 g a.s./ha) at full-flowering *Phacelia tanacetifolia* during honey bee foraging activity. The effects on honey bee colonies under confined conditions considering mortality, flight intensity, behaviour, colony strength, amount of brood and brood cell development were evaluated.

No test-item related adverse effects on mortality of adult worker bees, flight intensity and behaviour were observed. No biologically relevant effect in pupae mortality was observed over the entire test period.

The quantitative assessments of brood development in individually marked cells containing eggs did not result in statistically significant differences on honey bee brood development.

No test-item related adverse effects on colony strength (mean number of bees per colony), amount of brood (mean number of cells covered with the different types of brood) or on the development of the food storage area were observed.

CA3301 / JOUST	Page 155 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Study 2

Comments of zRMS:	<p>The objective of the study was to determine the potential effects of CA3301 and CA3642 on the honey bee (<i>Apis mellifera</i> L.) after two foliar applications, once before and once during early flowering to <i>Phacelia tanacetifolia</i> in Germany 2021 in a semi-field study. The study consists of four treatment groups: two test item groups T1 (CA3301) and T2 (CA3642) a toxic reference item group R and a control group C with four replicates each. For the test item treatment groups T1 and T2 one additional tunnel was installed for sampling (T1s and T2s). Potential effects were evaluated against those observed in honey bees treated with tap water (control) and a toxic reference item. This interim report includes data of mortality, flight and behaviour assessments generated for trial S21-00461-02. According to the information in the study report, the test was discontinued due to not fulfilled the validity criteria for single cel observations of honey bee brood (the mean brood termination rate averaged over the four biological replicates of the control group exceeded 50% on BFD11). Thus, Trial S21-00461-02 was considered invalid for assessment of effects on honey bee colony brood and no data were generated after 15DAA2.</p> <p>Results</p> <p>No statistically significant increased mean worker honey bee mortality values were observed during the exposure phase inside the tunnels (0DAA2 to 7DAA2) and outside the tunnels (8DAA2 to 15DAA2). The overall mean honey bee mortality during the monitoring period outside the tunnels clearly decreased in the control group C and in both treatment groups. No test item related effects on honey bee foraging activity were observed following exposure to CA3301 or CA3642 with the exception of on the day of the application 9during bee flight). Numbers of honey bees with abnormal behaviour were within the range of the normal background level.</p> <p>As validity criteria for single cel observations of honey bee brood were not fulfilled, this interim report should be considered as supplementary data, only.</p>
-------------------	--

Reference:	KCP 10.3.1.5/02
Report	<p>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 <i>Phacelia tanacetifolia</i> in Germany 2021</p> <p>Kaiser, F., 2021, report no. S21-00461</p>
Guideline(s):	Yes. OECD 75 (2007), OEPP/EPPO 170(4) (2010), EU Guideline 7029/V1/95 rev.4 (1997), EFSA Journal 2013; 11(7): 3295
Deviations:	-
GLP:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability:	Yes, acceptable
Duplication (if vertebrate study)	No

Note to RMS: these are interim results, which will be updated when possible. The study was stopped, due to poor weather conditions and will recommence in 2022.

CA3301 / JOUST	Page 156 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Executive summary

In a semi-field study, the toxicity of the product CA3301 (analyzed a.s. content of 25.57% w/w prothioconazole) on the European honeybee (*Apis mellifera*) was conducted as a dose-response test. The study comprised two treatment groups of 0 (water control) and 806.8 L product/ha, equivalent to 0 and 199.2 g a.s./ha, respectively, and a toxic-reference group of 1200 g Insegar/ha, equivalent to 300 g fenoxycarb/ha.

The study was conducted under semi-field conditions, in tunnels containing full-flowering lacy phacelia (*Phacelia tanacetifolia*), at BBCH growth stages 64-65, onto which the control, product, or toxic-reference treatments were applied. The study used actively foraging honeybees, which were confined to the tunnels for 12 days. During these 12 days, brood development; colony quality; and adult mortality, flight, and behaviour were assessed. After this confinement period, the colonies were relocated to a forestal monitoring site, without flowering main crops, for a further 14 days, during which brood development, colony quality, and mortality (adults, pupae, and larvae) were assessed.

No product-related adverse effects on mortality of adult worker bees, flight intensity, and behaviour were observed. No biologically relevant effect in pupae mortality was observed over the entire test period.

The quantitative assessments of brood development, in individually marked cells containing eggs, did not result in statistically significant differences on honeybee brood development in the product treatment group, when compared to the control group. No product-related adverse effects on colony strength (mean number of bees per colony), amount of brood (mean number of cells covered with the different types of broods), or on the development of the food storage area were observed.

Overall, the study satisfies the OECD 75 (2007) test-guideline requirements for a semi-field brood study with *Apis mellifera* and is considered acceptable.

Materials and methods

Test materials

Test item

Name:	CA3301
Density:	0.992 g/mL (nominal), 0.995 g/mL (analysed)
Active substance:	Prothioconazole
Formulation type:	Emulsifiable concentrate (EC)
Source and lot/batch no.:	A21001
Active substance content:	250 g/L (nominal), 250.5 g/L or 25.18% w/w (analyzed)
Appearance:	Clear, straw-coloured liquid
Expiry:	15 Jan 2023

Reference item

Name:	Insegar
Active substance:	Fenoxycarb
Formulation type:	WG
Source and lot/batch no.:	SSP8B004
Active substance content:	25-30% w/w (nominal), 24.9% w/w (analyzed)
Appearance:	Grey-to-brown solid
Expiry:	12 May 2022

Test crop

CA3301 / JOUST	Page 157 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Name:	Lacy phacelia, <i>Phacelia tanacetifolia</i> (Stala variety)
Age:	Full-flowering plant (BBCH 63-65)

Test organism

Species:	European honeybee, <i>Apis mellifera</i>
----------	--

The first application (A1) comprised a tap-water control group, a test-item group, and a toxic-reference group, which were applied 12DBA2 on lacy phacelia before flowering. The target application rate in the test-item treatment group was 200 g a.s./ha, corresponding to 798.4 ml product/ha (actual mean rate applied was 196.6 g a.s./ha). The target application rate in the toxic-reference group was 300 g fenoxycarb/ha, corresponding to 1200 g Insegar/ha (actual mean rate applied was 302.0 g fenoxycarb/ha).

The second application (A2) comprised a tap-water control group, a test-item group, and a toxic-reference group, which were applied on lacy phacelia during flowering and during daily bee flight (BBCH 63-65). The target application rate in the test-item treatment group was 200 g a.s./ha, corresponding to 798.4 ml product/ha (actual mean rate applied was 196.6 g a.s./ha). The target application rate in the toxic-reference group was 300 g fenoxycarb/ha, corresponding to 1200 g Insegar/ha (actual mean rate applied was 302.0 g fenoxycarb/ha).

All applications were carried out with a spray volume of 300 L/ha.

The initial colony sizes per hive for the replicates for biological assessments were in the range of 6435 to 13130 honey bees per hive. The honey bees remained in the tunnels for 11 days and the colony condition was assessed twice during and once after the end of the confined period.

The following endpoints were assessed:

Mortality on the linen sheets, in the dead bee traps and the bottom drawer (mean number of dead bees);

Flight intensity (mean number of forager bees/m²/10-15 sec);

Behaviour of the honey bees in the crop and around the hive;

Condition of the colonies: Number of honey bees (colony strength) and development of the bee brood and food storage area; (results not included in this interim report)

Brood index as an indicator for the bee brood development; (results not included in this interim report)

Compensation index as an indicator for recovery the colony; (results not included in this interim report)

Brood termination rate of the initially marked eggs; (results not included in this interim report)

Mortality of the honey bees was recorded by counting the number of dead honey bees in the dead bee traps in front of the hives, on the bottom drawer inside the hive, and on the linen sheets which were spread out in the tunnels. During the monitoring phase only the dead honey bees in the dead bee traps and on the bottom drawer were counted. The dead bees found were differentiated into adult worker bees, pupae, and larvae during each assessment. Dead male bees (drones) and male brood were recorded, but not evaluated (due to the high variability of drones present in bee colonies and the potential onset of drone killing at the end of the season, which is not test-item related).

The mortality of the honey bees was carried out according to the assessment schedule given in Table CP 10.3.1.5/01-01 below.

CA3301 / JOUST	Page 158 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Table CP 10.3.1.5/01-01. Assessment schedule of mortality.

Time of the test	DBA/DAA	Evaluations of number of dead honey bees
Pre-exposure A2 period (inside tunnels)*	3DBA2 to 1DBA2	Once a day, if possible at about the same time of day (linen sheets, dead bee trap, bottom drawer)
Day of application A2 during bee-flight	0DBA2	Shortly before application in the respective tunnels (linen sheets, dead bee trap and bottom drawer)
	0DAA2	2 h after application 4 h after application (linen sheets and dead bee trap) 6 h after application
		In the evening after daily flight activity of the honey bees (linen sheets, dead bee trap, and bottom drawer)
Exposure period (inside tunnels)	1DAA2 up to 7DAA2	Once a day, if possible at about the same time of day (linen sheets, dead bee trap, bottom drawer)
Monitoring period (outside tunnels)	8DAA2 to 15DAA2	Once a day, if possible at about the same time of day (dead bee trap, bottom drawer)

*One pre-flowering application A1 was already performed. DBA/DAA, days before/after the application; h, hours; A2, second application.

During the presence of the colonies in the tunnels, the flight activity of the honey bees was recorded daily during the pre-exposure period (3DBA2 to 1DBA2), six times on the day of application (0DBA2/0DAA2), three times on the day after application (1DAA2), and daily from 2DAA2 to 7DAA2. The flight activity of honey bees was assessed in three randomly chosen flight observation areas per tunnel (1 m² per observation area). At each assessment, the number of bees that were both foraging on flowers and flying over the crop were counted for 10 to 15 seconds in each observation area. The observations were carried out according to the assessment schedule given in Table CP 10.3.1.5/01-02 below.

Table CP 10.3.1.5/01-02. Assessment schedule of honey-bee flight activity

Time of the test	DBA/DAA	Evaluations of number of forager honey bees
Pre-exposure A2 period (inside tunnels)*	3DBA2 to 1DBA2	Once a day during flight activity of the honey bees
Day of application during bee-flight	0DBA2	Once shortly before application in all tunnels
	0DAA2	Twice during the first hour after application 2 h after application 4 h after application 6 h after application
Exposure period (inside tunnels)	1DAA2	Three times a day during flight activity of the honey bees (morning, noon and afternoon)
	2DAA2 to 7DAA2	Once a day during flight activity of the honey bees

*One pre-flowering application A1 was already performed. DBA/DAA, days before/after the application; h, hours; A2, second application.

During the assessments of mortality and flight activity, the behaviour of the honey bees in the crop and around the hive was observed with respect to the following behavioural categories:

- Intensive Cleaning,
- Trembling,
- Cramping,
- Locomotion problems,
- Inactive bees,
- Bees hanging on the crop,
- Filtering bees (Guard bees attacking and/or preventing returning bees from entering the hive),
- Clustering of large numbers of bees at the hive entrance.

During the monitoring period after confinement, behaviour was only assessed around the hives.

CA3301 / JOUST	Page 159 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

The possible influence of the test item was evaluated by comparing the results in the test-item group and toxic-reference group to the control group, under consideration of the results of:

- Mortality on the linen sheets, in the bottom drawer and in the dead bee traps (mean number of dead bees);
- Flight activity (mean number of forager bees/m²/10-15 sec);
- Behaviour of the bees in the crop and around the hive

For statistical analysis of mortality and flight-activity results, data were tested for normality and homoscedasticity using the Shapiro-Wilk's test ($p > 0.05$) and folded F-Test ($p > 0.05$; for the toxic-reference group), respectively. Data were statistically compared using pooled, one-sided Student's t-tests ($p \leq 0.05$) in case of normality and homoscedasticity. In case of non-homoscedastic but non-normal data, one-sided Satterthwaite t-tests were conducted. In case of non-normal and non-homoscedastic data, one-sided Mann-Whitney exact tests ($p \leq 0.05$) were used. Data were log-transformed to achieve normality and homoscedasticity. For the post-application period, right-sided tests were used for mortality and left-sided tests were used for flight intensity. For the pre-application period before application A2, two-sided tests were used. All statistical analysis was conducted using SAS version 9.4.

Results

Biological results

Mortality

A summary of the effect of prothioconazole on mortality is presented in the tables below.

Table CP 10.3.1.5/01-03. Mortality of honeybees in the control group.

Timing	Mortality (number of dead worker honey bees and pupae)									
	Replicate A		Replicate B		Replicate C		Replicate D		Mean*	STD*
	LS	TD	LS	TD	LS	TD	LS	TD		
3DBA2	15	4	87	14	80	27	39	20	71.5	41
2DBA2	53	0	548	4	591	34	231	14	368.8	267.2
1DBA2	65	0	298	2	267	26+2P	148	10	204.5	113.9
0DBA2	35	8	221	15	166	22+1P	100	12	145	85.1
Mean 3-to-0DBA2 (inside tunnels)	45		297.3		304		143.5		197.5	125.8
STD	19.5		189		227.4		78.8		---	---
0DAA2 (+2h)	20	1	57	2	107	3	43	3	59	37.5
0DAA2 (+4h)	4	0	18	1	25	2	14	2	16.5	9.5
0DAA2 (+6h)	6	0	6	3	7	2	3	0	6.8	2.9
0DAA2 (evening)	4	1	10	3	6	11	1	3	9.8	6.3
Sum 0DAA2	34	2	91	9	145	18	61	8	92	54.1
1DAA2	31	1	49	6	75	8	26	2	49.5	25.3
2DAA2	65	0+1P	130	16	121	17+11P	75	7	110.8	43
3DAA2	57	1+1P	236	11	222	46+21P	88	8	172.8	112.3
4DAA2	39	3	180	3	149	34+33P	111	9	140.3	76.7
5DAA2	44	4	177	13	173	23+17P	75	7	133.3	80.6
6DAA2	21	4	163	12	76(1P)	15+7P	70	16	96.3	61.6
7DAA2	31	2	145	18	66	17+17P	69	8	93.3	54.2
Mean 0-to-7DAA2 (exposure period)	42.6		157.4		164		80		111	59.4
STD	14.2		58.6		71.6		26		---	---
8DAA2	8		17		14(18P)		13		17.5	10.3
9DAA2	5		18		11(5P)		1		10	8.3
10DAA2	7		5		26(3P)		7		12	11.4
11DAA2	3		18		40(6P)		14(1P)		20.5	18.2
12DAA2	2		17(1P)		23(11P)		13		16.8	13.3
13DAA2	3(2P)		5		11(5P)		21(1P)		12	8.4
14DAA2	4		12		12		15		10.8	4.7
15DAA2	15		18		12		21		16.5	3.9
Mean 8-to-15DAA2 (Monitoring period, outside tunnels)	6.1		13.9		24.6		13.4		14.5	7.6
STD	4.1		5.8		12.5		6.9		---	---
Mean 0-to-15DAA2 (Post-application A2 period)	24.4		85.6		94.3		46.7		62.8	32.9
STD	21.4		84.2		87.4		39		---	---

*For calculation of means and STD (standard deviation), the numbers of dead worker bees and on the linen were added to the bees assessed in bee trap and bottom drawer, and counted as one value. DBA2/DAA2, days before/after application A2; LS, number of dead bees in trap and bottom drawer; TD, sum of dead bees in trap and bottom drawer; ---, not applicable; P, pupae.

CA3301 / JOUST	Page 161 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Table CP 10.3.1.5/01-04. Mortality of honeybees in the CA3301 test-item group.

Timing	Mortality (number of dead worker honey bees and pupae)									
	Replicate A		Replicate B		Replicate C		Replicate D		Mean*	STD*
	LS	TD	LS	TD	LS	TD	LS	TD		
3DBA2	85	9+3P	18	2	126	23	41	40	86.8	53.1
2DBA2	63	7+1P	165	2	684	26	216	14	294.5	284.6
1DBA2	133	10	217	3	237	14+2P	262	4	219.3	56.6
0DBA2	69	16+1P	67	4	212	42	207	14	158.0	93.0
Mean 3-to-0DBA2 (inside tunnels)	98.5		119.5		341.5		199.5		189.8	110.1
STD	26.6		90.6		250.6		81.4		---	---
0DAA2 (+2h)	32	1	40	1	98	4	116	2	73.5	42.8
0DAA2 (+4h)	7	1	7	0	19	1	18	4	14.3	7.8
0DAA2 (+6h)	5	1	2	0	5	1	4	3	5.3	2.2
0DAA2 (evening)	6	1	1	2	8	3	9	5	8.8	4.8
Sum 0DAA2	50	4	50	3	130	9	147	14	101.8	56.4
1DAA2	35	5	19	1	83	5	105	5+2P	65.0	42.4
2DAA2	69	2	94	1	121	7	130	12+4P	110.0	33.5
3DAA2	123	4+6P	87	5	182	16	172	10+5P	152.5	49.3
4DAA2	74	5+1P	90	0	157	5	257	13	150.5	87.6
5DAA2	101	10+3P	110	6	139	6	288	20	170.8	92.6
6DAA2	67	10+4P	73	12	80	18	76	25	91.3	9.7
7DAA2	40	15+5P	65	10+1P	147	14	115 +1P	24+1P	109.5	49.0
Mean 0-to-7DAA2 (exposure period)	79.1		78.4		139.9		178.3		118.9	49.0
STD	31.0		29.6		35.7		74.1		---	---
8DAA2	24+6P		17		20+1P		21		22.3	5.5
9DAA2	14+7P		5+1P		8+1P		23+3P		15.5	9.5
10DAA2	21+7P		1		10		15+1P		13.8	11.3
11DAA2	26		7		20		11		16.0	8.6
12DAA2	21+4P		4		16		40		21.3	15.2
13DAA2	3+4P		7		7		20		10.3	6.5
14DAA2	16+8P		2		20		39		21.3	15.2
15DAA2	28+1P		16		16		15+2P		19.5	6.4
Mean 8-to-15DAA2 (Monitoring period, outside tunnels)	23.9		7.5		14.9		23.8		17.5	7.9
STD	7.4		6.0		5.5		10.6		---	---
Mean 0-to-15DAA2 (Post-application A2 period)	51.4		42.9		77.4		101.0		68.2	26.3
STD	35.9		42.0		69.1		94.8		---	---

*For calculation of means and STD (standard deviation), the numbers of dead worker bees and on the linen were added to the bees assessed in bee trap and bottom drawer, and counted as one value. DBA2/DAA2, days before/after application A2; LS, number of dead bees in trap and bottom drawer; TD, sum of dead bees in trap and bottom drawer; ---, not applicable; P, pupae.

Table CP 10.3.1.5/01-05. Mortality of honeybees in the toxic-reference group.

Timing	Mortality (number of dead worker honey bees and pupae)									
	Replicate A		Replicate B		Replicate C		Replicate D		Mean*	STD*
	LS	TD	LS	TD	LS	TD	LS	TD		
3DBA2	13	1+1P	121	5	222	17	26	4+55P	116.3	93.8
2DBA2	111	5	96	1	289	14	204 +3P	6+65P	198.5	107.0
1DBA2	144	1	137	2+1P	183	8+1P	129	13+18P	159.3	23.4
0DBA2	76	9	57	3+1P	213	21+1P	123	9+28P	135.3	78.7
Mean 3-to-0DBA2 (inside tunnels)	90.3		106.0		242.3		170.8		152.4	69.4
STD	55.8		34.9		45.7		79.8		---	---
0DAA2 (+2h)	52	2	43	5	78	1	89	8	69.5	22.7
0DAA2 (+4h)	9	1	7	0	10	0	8	0	8.8	1.5
0DAA2 (+6h)	8	0	2	1	4	2	6	1	6.0	2.2
0DAA2 (evening)	4	0	0	0	6	4	2	0+6P	5.5	4.4
Sum 0DAA2	73	3	52	6	98	7	105	9+6P	89.8	28.0
1DAA2	28	3+2P	10	6+1P	42	2+6P	75	2+11P	47.0	30.5
2DAA2	104	2+1P	114	5+1P	89	9+9P	100	3+9P	111.5	6.1
3DAA2	122	8	94	1	101	13+11P	172	7+2P	132.8	35.7
4DAA2	84	3	61	0	79	7+10P	141	6+5P	99.0	38.3
5DAA2	75	9+1P	98	1+1P	109	6+25P	113	4+7P	112.3	24.5
6DAA2	68	7	59	8	75	13+22P	82	7+6P	86.8	19.5
7DAA2	75	11	31	3+2P	97	8+51P	97	7+11P	98.3	50.5
Mean 0-to-7DAA2 (exposure period)	84.9		69.3		111.1		123.4		97.2	24.6
STD	27.8		34.3		31.8		30.3		---	---
8DAA2	7+3P		2		10+94P		10+23P		37.3	46.4
9DAA2	19+38P		1+3P		17+64P		10+25P		44.3	32.8
10DAA2	16+91P		4+41P		7+96P		9+47P		77.8[#]	31.8
11DAA2	21+144P		18+84P		20+175P		11+90P		140.8[#]	46.9
12DAA2	12+140P		5+122P		22+181P		15+85P		145.5[#]	43.8
13DAA2	20+107P		8+69P		4+145P		14+59P		106.5[#]	37.5
14DAA2	32+97P		7+149P		13+161P		8+39P		126.5[#]	56.1
15DAA2	48+54P		7+15P		18+25P		29+33P		57.3[#]	34.0
Mean 8-to-15DAA2 (Monitoring period, outside tunnels)	106.1		66.9		131.5		63.4		92.0[#]	32.7
STD	51.0		58.0		57.6		26.5		---	---
Mean 0-to-15DAA2 (Post-application A2 period)	95.5		68.1		121.3		93.4		94.6	21.7
STD	41.2		46.0		46.1		41.4		---	---

*For calculation of means and STD (standard deviation), the numbers of dead worker bees and on the linen were added to the bees assessed in bee trap and bottom drawer, and counted as one value. Values in **bold** are significantly different than the control (pooled Student's or Satterthwaite t-test; right-sided; $p \leq 0.05$) and are indicated by pound signs (#). DBA2/DAA2, days before/after application A2; LS, number of dead bees in trap and bottom drawer; TD, sum of dead bees in trap and bottom drawer; ---, not applicable; P, pupae.

The mean daily mortality during the pre-exposure period before application A2 (3DBA2 to 0DBA2) increased inside the tunnels to 197.5 ± 125.8 dead honey bees/day in the control, 189.8 ± 110.1 dead honey bees/day in test-item group, 188.0 ± 47.9 , and 152.4 ± 69.4 dead honey bees/day in the toxic-reference

CA3301 / JOUST	Page 163 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

group. No statistically significant differences were recorded between treatments during the pre-exposure period before application A2 at the monitoring site or inside the tunnels.

Honey bee mortality from 0DAA2 to 7DAA2 (exposure phase inside the tunnels after application A2) showed no effects of the test-item treatment, compared to the control. The mean honey bee mortality from 0DAA2 to 7DAA2 was on a similar level, resulting in the following mean values: 111.0 ± 59.4 and 118.9 ± 49.0 dead bees/colony/day in the control and test-item groups, respectively. Mortality in toxic-reference group was 97.2 ± 24.6 bees/colony/day for the same period. No statistically significant increased mean worker bee mortality values were observed during the exposure phase.

During the monitoring period outside the tunnels, from 8DAA2 to 15DAA2, no statistically significant differences were detected for the test-item group, compared to the control. In comparison to the exposure phase inside the tunnels, the overall mean honey bee mortality during the monitoring period outside the tunnels clearly decreased in the control group and test-item group, corresponding to 14.5 ± 7.6 and 17.5 ± 7.9 dead bees/colony/day, respectively. For the toxic-reference group, statistically significant increased mean bee mortality, compared to the control, was observed from 10DAA2 to 15DAA2 and for the entire mean monitoring period (8DAA2 to 15DAA2), with an overall mean mortality during the monitoring period of 92.0 ± 32.7 dead bees/colony/day.

Overall, there was no test-item-related effect on honey bee mortality.

Flight intensity

A summary of the effect of prothioconazole on flight intensity is presented in the tables below.

CA3301 / JOUST	Page 164 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Table CP 10.3.1.5/01-06. Honeybee flight intensity in the control group.

Timing	Flight activity (number of forager bees/m ² /10-15s)					
	Replicate A*	Replicate B*	Replicate C*	Replicate D*	Mean	STD
3DBA2	5.3	15	14.7	14	12.3	4.7
2DBA2	5	11.7	13.7	13.7	11	4.1
1DBA2	10.7	10.3	13.7	14.3	12.3	2
0DBA2	10.7	12.3	16	12.3	12.8	2.2
Mean 3DBA2 to 0DBA2	7.9	12.3	14.5	13.6	12.1	2.9
STD	3.2	2	1.1	0.9	---	---
0DAA2 (+0.5 h)	15.3	21.7	16.7	18.3	18	2.8
0DAA2 (+1 h)	10.7	11.7	12.7	7.7	10.7	2.2
0DAA2 (+2 h)	0	0	0	0	0	0
0DAA2 (+4 h)	6.3	8	7.3	6.7	7.1	0.7
0DAA2 (+6 h)	4.7	1	1	2.3	2.3	1.7
Mean 0DAA2	7.4	8.5	7.5	7	7.6	0.6
1DAA2 (morning)	15.7	18.3	13.7	15.7	15.9	1.9
1DAA2 (noon)	2.7	2.7	0.7	0.7	1.7	1.2
1DAA2 (afternoon)	3.3	9	7.3	4.3	6	2.6
Mean 1DAA2	7.2	10	7.2	6.9	7.8	1.5
2DAA2	4	5.7	6.3	4.3	5.1	1.1
3DAA2	13	19	13	16.3	15.3	2.9
4DAA2	20.7	22.3	21.3	18.3	20.7	1.7
5DAA2	18	26.3	20.7	11.3	19.1	6.2
6DAA2	10	12	12.7	9	10.9	1.7
7DAA2	4.7	0	0	1	1.4	2.2
Mean 0DAA2 to 7DAA2	10.6	13	11.1	9.3	11	1.5
STD	6.1	8.9	7.3	5.8	---	---

*Mean value of three random flight observation areas of 1 m² per tunnel. DBA2/DAA2, days before/after application A2; STD, standard deviation; ---, not applicable.

CA3301 / JOUST	Page 165 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Table CP 10.3.1.5/01-07. Honeybee flight intensity in the CA3301 test-item group.

Timing	Flight activity (number of forager bees/m ² /10-15s)					
	Replicate A*	Replicate B*	Replicate C*	Replicate D*	Mean	STD
3DBA2	5.0	8.7	19.7	7.7	10.3	6.5
2DBA2	8.0	14.0	17.0	14.0	13.3	3.8
1DBA2	10.3	12.0	12.7	14.0	12.3	1.5
0DBA2	11.0	12.3	17.0	11.7	13.0	2.7
Mean 3DBA2 to 0DBA2	8.6	11.8	16.6	11.9	12.2	3.3
STD	2.7	2.2	2.9	3.0	---	---
0DAA2 (+0.5 h)	15.3	16.3	18.3	12.3	15.6	2.5
0DAA2 (+1 h)	11.7	9.0	8.3	5.3	8.6	2.6
0DAA2 (+2 h)	4.7	7.7	10.0	6.7	7.3	2.2
0DAA2 (+4 h)	3.0	3.3	4.3	3.0	3.4[#]	0.6
0DAA2 (+6 h)	2.0	3.7	4.3	4.0	3.5	1.0
Mean 0DAA2	7.3	8.0	9.0	6.3	7.7	1.1
1DAA2 (morning)	14.7	17.3	16.7	11.0	14.9	2.8
1DAA2 (noon)	0.3	1.0	0.7	1.0	0.8	0.3
1DAA2 (afternoon)	8.0	5.7	11.0	7.7	8.1	2.2
Mean 1DAA2	7.7	8	9.5	6.6	8.0	1.2
2DAA2	3.0	6.0	14.3	7.0	7.6	4.8
3DAA2	12.7	15.3	18.7	13.0	14.9	2.8
4DAA2	21.3	26.7	35.3	19.7	25.8	7.0
5DAA2	20.3	21.0	29.3	19.0	22.4	4.7
6DAA2	10.0	12.7	14.3	10.3	11.8	2.0
7DAA2	2.0	1.3	0.3	1.3	1.2	0.7
Mean 0DAA2 to 7DAA2	10.5	12.4	16.3	10.4	12.4	2.8
STD	7.2	8.4	11.3	6.5	---	---

*Mean value of three random flight observation areas of 1 m² per tunnel. Values in **bold** are significantly different than the control (pooled Dunnett's t-test; left-sided; p≤0.05) and are indicated by pound signs (#). DBA2/DAA2, days before/after application A2; STD, standard deviation; ---, not applicable.

Table CP 10.3.1.5/01-08. Honeybee flight intensity in the CA3301 toxic-reference group.

Timing	Flight activity (number of forager bees/m ² /10-15s)					
	Replicate A*	Replicate B*	Replicate C*	Replicate D*	Mean	STD
3DBA2	9.3	7.0	7.0	6.0	7.3	1.4
2DBA2	9.0	8.7	14.7	19.7	13.0	5.2
1DBA2	14.3	9.7	18.7	16.0	14.7	3.8
0DBA2	12.7	10.0	16.7	11.0	12.6	3.0
Mean 3DBA2 to 0DBA2	11.3	8.9	14.3	13.2	11.9	2.4
STD	2.6	1.4	5.1	6.0	---	---
0DAA2 (+0.5 h)	11.3	4.7	4.3	4.0	6.1[#]	3.5
0DAA2 (+1 h)	0.3	0.7	0.7	0.3	0.5[#]	0.2
0DAA2 (+2 h)	8.0	8.7	10.0	12.0	9.6	1.9
0DAA2 (+4 h)	3.0	2.0	6.3	6.0	4.3[#]	2.2
0DAA2 (+6 h)	1.0	0.0	0.0	0.7	0.4[#]	0.5
Mean 0DAA2	4.7	3.2	4.3	4.6	4.2[#]	0.7
1DAA2 (morning)	18.0	12.0	12.0	14.0	14.0	2.8
1DAA2 (noon)	1.7	1.7	1.0	0.3	1.2	0.7
1DAA2 (afternoon)	8.0	8.7	3.3	5.3	6.3	2.5
Mean 1DAA2	9.2	7.5	5.4	6.5	7.2	1.6
2DAA2	5.7	3.0	5.3	3.0	4.3	1.5
3DAA2	15.3	12.0	18.0	18.0	15.8	2.9
4DAA2	20.3	19.0	17.0	19.3	18.9	1.4
5DAA2	24.0	23.7	10.3	11.3	17.3	7.5
6DAA2	10.7	12.3	12.0	8.7	10.9	1.6
7DAA2	3.0	0.0	0.0	1.7	1.2	1.5
Mean 0DAA2 to 7DAA2	11.6	10.1	9.0	9.1	10.0	1.2
STD	7.6	8.3	6.4	6.6	---	---

*Mean value of three random flight observation areas of 1 m² per tunnel. Values in **bold** are significantly different than the control (pooled Student's t-test; left-sided; p≤0.05) and are indicated by pound signs (#). DBA2/DAA2, days before/after application A2; STD, standard deviation; ---, not applicable.

The overall daily mean honey-bee flight activity during the pre-exposure period before application A2 inside the tunnels (3DBA2 to 0DBA2) was 12.1 ± 2.9 , 12.2 ± 3.3 , and 11.9 ± 2.4 forager honey bees/m²/10-15 sec in the control, test-item, and toxic-reference groups, respectively. No statistically significant differences in flight activity were determined.

On the day of application A2, during honey-bee flight (0DAA2), after the application, flight activity slightly decreased in all treatment groups and was statistically significantly lower in the test-item group, compared to the control, at the 4-hour assessment. The overall mean daily flight activity on 0DAA2 was similar in the control to the test-item group, with 7.6 ± 0.6 and 7.7 ± 1.1 forager bees/m²/10-15 sec, respectively. For the toxic-reference group, the mean daily flight activity on 0DAA2 was statistically significantly lower, compared to the control, with 4.2 forager honey bees/m²/10-15 sec.

On the day following the application (1DAA2), the mean honey bee flight activity slightly increased to 7.8 ± 1.5 , 7.3 ± 1.6 , and 7.2 ± 1.6 forager honey bees/m²/10-15 sec in the control, test-item, and toxic-reference groups, respectively, with no statistically significant differences.

During the post-application period (0DAA2 to 7DAA2), the mean daily honey bee flight activity was 11.0 ± 1.5 , 12.0 ± 0.9 , and 10.0 ± 1.2 forager honey bees/m²/10-15 sec in the control, test-item, and toxic-reference groups, respectively, with no statistically significant differences.

Overall, no test-item-related effects on honey bee foraging activity were observed following exposure to CA3301.

CA3301 / JOUST	Page 167 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Behaviour

A summary of the effect of prothioconazole on behaviour is presented in the tables below.

Table CP 10.3.1.5/01-09. Honeybee behaviour in the control group

Timing	Observation categories and observed number of bees								
	LP	IA	Cr	Tr	ICL	HA	Filt	FwL	Clu
3DBA2	---	---	---	---	---	---	---	---	---
2DBA2	R4: 1	R2: 1 R3: 5	R3: 4 R4: 1	---	---	---	---	---	---
1DBA2	R2: 2	R3: 7 R4: 1	R3: 2	R3: 3	---	---	---	---	---
0DBA2	-	R4: 27		R3: 1	---	---	---	---	---
Sum 3-to-0DBA2 (inside tunnels)	3	41	7	4	0	0	0	0	0
0DAA2 (+0.5 h)	---	---	---	---	---	---	---	---	---
0DAA2 (+1 h)	---	---	---	---	---	---	---	---	---
0DAA2 (+2 h)	---	---	R2: 1	---	---	---	---	---	---
0DAA2 (+4 h)	R2: 1	---	---	---	---	---	---	---	---
0DAA2 (+6 h)	---	---	---	---	---	---	---	---	---
0DAA2 (evening)	---	---	---	---	---	---	---	---	---
Sum 0DAA2	1	0	1	0	0	0	0	0	0
1DAA2 (morning)	R4: 1	---	---	---	---	---	---	---	---
1DAA2 (noon)	---	---	---	---	---	---	---	---	---
1DAA2 (afternoon)	---	---	---	---	---	---	---	---	---
Sum 1DAA2	1	0	0	0	0	0	0	0	0
2DAA2	---	---	---	---	---	---	---	---	---
3DAA2	R2: 2 R3: 2	R2: 3 R3: 2	R2: 2 R3: 2	---	---	---	---	---	---
4DAA2	---	---	---	---	---	---	---	---	---
5DAA2	R4: 2	---	R4: 2	---	---	---	---	---	---
6DAA2	---	R1: 1	---	---	---	---	---	---	---
7DAA2	R4: 3	---	---	---	---	---	---	---	---
Sum 0-to-7DAA2 (exposure Period inside tunnels)	11	6	7	0	0	0	0	0	0
8DAA2	---	---	---	---	---	---	---	---	---
9DAA2	---	---	R4: 1	---	---	---	---	---	---
10DAA2	---	---	---	---	---	---	---	---	---
11DAA2	R3: 2	---	R2: 1	R4: 1	---	---	---	---	---
12DAA2	---	---	R2: 3 R3: 1	---	---	---	---	---	---
13DAA2	---	---	R2: 1 R4: 1	R3: 3	---	---	---	---	---

CA3301 / JOUST	Page 168 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

14DAA2	---	---	---	---	---	---	---	---	---
15DAA2	---	---	---	---	---	---	---	---	---
Sum 8-to-15DAA2 (Monitoring period)	2	0	8	4	0	0	0	0	0
Mean 0-to-15DAA2 (Post- application period)	13	6	15	4	0	0	0	0	0

R1 to R4, replicates 1 to 4; DBA2, days before application A2; DAA2, days after application A2; IA, Inactive; LP, Locomotor problems; Cr, Cramping; Tr, Trembling; ICL, Intensive cleaning; HA, Hanging bees; Clu, Clustering; Fwl, Flying without landing on crop; FilIt, Filtering at entrance to other bees; ---, Not observed.

Table CP 10.3.1.5/01-10. Honeybee behaviour in the test-item group

Timing	Observation categories and observed number of bees								
	LP	IA	Cr	Tr	ICL	HA	Filt	FwL	Clu
3DBA2	---	---	R1: 1 R4: 3	---	---	---	---	---	---
2DBA2	---	R3: 5	R3: 5	---	---	---	---	---	---
1DBA2	R1: 5 R3: 1	R1: 9 R2: 5 R3: 5	R1: 1 R3: 2	---	---	---	---	---	---
0DBA2	R4: 1	---	R4: 2	---	---	---	---	---	---
Sum 3-to-0DBA2 (inside tunnels)	7	24	14	0	0	0	0	0	0
0DAA2 (+0.5 h)	---	---	---	---	---	---	---	---	---
0DAA2 (+1 h)	---	---	---	---	---	---	---	---	---
0DAA2 (+2 h)	---	---	---	---	---	---	---	---	---
0DAA2 (+4 h)	R1: 1	R3: 3	R1: 2 R3: 2 R4: 3	R3: 1	---	R2: 2 R3: 2	---	---	---
0DAA2 (+6 h)	R1: 1	---	---	---	---	---	---	---	---
0DAA2 (evening)	---	---	---	---	---	---	---	---	---
Sum 0DAA2	2	3	7	1	0	4	0	0	0
1DAA2 (morning)	---	R4: 2	R4: 4	---	---	---	---	---	---
1DAA2 (noon)	---	---	---	---	---	---	---	---	---
1DAA2 (afternoon)	---	---	---	---	---	---	---	---	---
2DAA2	---	---	---	---	---	---	---	---	---
3DAA2	R3: 3 R4: 2	R1: 2 R4: 2	R3: 3	---	---	---	---	---	---
4DAA2	---	---	---	---	---	---	---	---	---
5DAA2	---	---	---	---	---	---	---	---	---
6DAA2	R3: 5	R1: 3 R3: 10	R3: 1	---	---	---	---	---	---
7DAA2	---	R1: 1	---	---	---	R3: 5	---	---	---
Sum 0-to-7DAA2 (exposure Period inside tunnels)	12	23	15	1	0	9	0	0	0
8DAA2	---	---	---	---	---	---	---	---	---
9DAA2	R1: 11	---	---	---	---	---	---	---	---
10DAA2	---	---	---	---	---	---	---	---	---
11DAA2	R3: 1	---	R1: 2	---	---	---	---	---	---
12DAA2	---	R1: 1 R3: 1	---	---	---	---	---	---	---
13DAA2	---	---	---	---	---	---	---	---	---
14DAA2	---	---	---	---	---	---	---	---	---
15DAA2	---	---	---	---	---	---	---	---	---
Sum 8-to-15DAA2 (Monitoring period)	12	2	2	0	0	0	0	0	0
Sum 0-to-15DAA2 (Post application A2 period)	24	25	17	1	0	9	0	0	0

R1 to R4, replicates 1 to 4; DBA2, days before application A2; DAA2, days after application A2; IA, Inactive; LP, Locomotor problems; Cr, Cramping; Tr, Trembling; ICL, Intensive cleaning; HA, Hanging bees; Clu, Clustering; FwL, Flying without landing on crop; Filt, Filtering at entrance to other bees; ---, Not observed.

Table CP 10.3.1.5/01-11. Honeybee behaviour in the toxic-reference group

Timing	Observation categories and observed number of bees								
	LP	IA	Cr	Tr	ICL	HA	Filt	FwL	Clu
3DBA2	R3: 13	R2: 2 R3: 6	R2: 1 R3: 2	R3: 2	R3: 1	---	---	---	---
2DBA2	R3: 3 R4: 5	R4: 3	R2: 1	---	---	---	R3: 1	---	---
1DBA2	R4: 3	R2: 42	---	---	---	---	---	---	---
0DBA2	---	R4: 17	---	---	---	---	---	---	---
Sum 3-to-0DBA2 (inside tunnels)	24	70	4	2	1	0	1	0	0
0DAA2 (+0.5 h)	---	---	---	---	---	---	---	---	---
0DAA2 (+1 h)	---	---	---	---	---	---	---	---	---
0DAA2 (+3 h) ¹⁾	---	---	---	---	---	---	---	---	---
0DAA2 (+4 h)	---	---	---	---	---	---	---	---	---
0DAA2 (+6 h)	---	---	R4: 2	---	---	---	---	---	---
0DAA2 (evening)	---	---	---	---	---	---	---	---	---
Sum 0DAA2	0	0	2	0	0	0	0	0	0
1DAA2 (morning)	---	---	R3: 1 R4: 2	---	---	---	---	---	---
1DAA2 (noon)	---	---	---	---	---	---	---	---	---
1DAA2 (afternoon)	---	---	---	---	---	---	---	---	---
Sum 1DAA2	0	0	3	0	0	0	0	0	0
2DAA2	---	---	R3: 2	---	---	---	---	---	---
3DAA2	---	---	R1: 2 R2: 1	---	---	---	---	---	---
4DAA2	---	---	---	---	---	---	---	---	---
5DAA2	---	---	---	---	---	---	---	---	---
6DAA2	---	---	---	---	---	---	---	---	---
7DAA2	---	R3: 2	R3: 1 R4: 1	---	---	---	---	---	---
Sum 0-to-7DAA2 (exposure Period inside tunnels)	0	2	12	0	0	0	0	0	0
8DAA2	---	---	---	---	---	---	---	---	---
9DAA2	---	---	---	---	---	---	---	---	---
10DAA2	---	---	---	---	---	---	---	---	---
11DAA2	---	---	R3: 1	---	---	---	---	---	---
12DAA2	---	---	---	---	---	---	---	---	---
13DAA2	---	---	---	---	---	---	---	---	---
14DAA2	---	---	---	---	---	---	---	---	---
15DAA2	R4: 1	R1: 3	---	---	---	---	---	---	---
Sum 8-to-15DAA2 (Monitoring period)	1	3	1	0	0	0	0	0	0
Sum 0-to-15DAA2 (Post application A2 period)	1	5	13	0	0	0	0	0	0

R1 to R4, replicates 1 to 4; DBA2, days before application A2; DAA2, days after application A2; IA, Inactive; LP, Locomotor problems; Cr, Cramping; Tr, Trembling; ICL, Intensive cleaning; HA, Hanging bees; Clu, Clustering; FwL, Flying without landing on crop; Filt, Filtering at entrance to other bees; ---, Not observed.

Before application A2 inside the tunnels (3DBA2 to 0DBA2), 3 bees with locomotor problems, 41 inactive bees, 7 cramping bees, and 4 trembling bees were observed in the control group; 7 bees with locomotor problems, 24 inactive bees, and 14 cramping bees were observed in the test-item group; and 24 bees with locomotor problems, 70 inactive bees, 4 cramping bees, 2 trembling bees, 1 intensive cleaning bee, and 1 filtering bees were observed in toxic-reference group. Increased abnormal behaviour were observed in all treatment groups during the pre-exposure period can be regarded as a consequence of the confinement, the reorientation, and the resulting stress of the honey bees during the first days inside the tunnel tents. On the day of application (0DAA2), only single honey bees with unusual behaviour were observed:

CA3301 / JOUST	Page 171 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

- Control group: 1 bee with locomotor problems and 1 cramping bee
- Test-item group: 2 bees with locomotion problems, 3 inactive bees, 7 cramping bees, 1 trembling bee, and 4 hanging bees
- Toxic-reference group: 2 cramping bees

During the entire exposure period inside the tunnels from 0DAA2 to 7DAA2, unusual behaviour for single honey bees was observed in low numbers across all treatment groups (11 bees with locomotor problems, 6 inactive bees, and 7 cramping bees in the control; 12 bees with locomotor problems, 23 inactive bees, 15 cramping bees, 1 trembling bee, and 9 hanging bees in the test-item group; and 2 inactive bees and 12 cramping bees in toxic-reference group).

During the monitoring period (8DAA2 to 15DAA2; outside the tunnels), unusual behaviour of single honey bees was observed in low numbers across all treatment groups (2 bees with locomotion problems, 8 cramping bees and 4 trembling bees in the control; 12 bees with locomotor problems, 2 inactive bees, and 2 cramping bees in the test-item group; and 1 bee with locomotor problems, 3 inactive bees, and 1 cramping bee in the toxic-reference group).

The number of honey bees showing abnormal behaviour in the test-item treatment group was low and on par with the control group C. Overall, the observed numbers of honey bees with abnormal behaviour are within the range of the normal background level, therefore, no test-item-related effects on honey bee behaviour were observed following exposure to CA3301.

Conclusion

CA3301 was applied at a target rate corresponding to 200 g a.s./ha, equivalent to 798.4 ml product/ha, on full-flowering *Phacelia tanacetifolia*, during honeybee foraging activity. The effects on honeybee (*Apis mellifera*) colonies, under confined conditions, considering mortality, flight intensity, and behaviour, were evaluated, in accordance with the OECD 75 (2007) test guideline.

No product-related adverse effects on mortality of adult worker bees, flight intensity, and behaviour were observed. No biologically relevant effect in pupae mortality was observed over the entire test period.

Overall, there were no test-item-related effects on honey-bee mortality, flight intensity, and behaviour following exposure to CA3301

This study is considered acceptable.

A 2.3.1.6 KCP 10.3.1.6 Field tests with honey bees

A 2.3.1.7 KCP 10.3.2 Effects on non-target arthropods other than bees

CA3301 / JOUST	Page 172 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Study 1

Comments of zRMS:	<p>The study was conducted to the guideline and according to the principles of GLP. All validity criteria were met. One minor deviation was noted without negative impact on the overall outcome of the study:</p> <ul style="list-style-type: none"> - relative humidity was in the range of 42.8 and 61.4 % with a mean of 56.0 % (recommended 60-90%). <p>The study is considered to be reliable and suitable for the risk assessment.</p>
-------------------	--

Reference:	CP 10.3.2/01
Report	<p>250 EC Prothioconazole, NUL 3390 (CA3301) - Toxicity to the Predatory Mite <i>Typhlodromus pyri</i> (Acari, Phytoseiidae) under Worst-Case Conditions in the Laboratory</p> <p>Schmidt, T., 2021, report no. 20190459</p>
Guideline(s):	Yes. IOBC, Blümel, S. 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

A 14-day reproductive study of a predatory mite, *Typhlodromus pyri*, with CA3301 was conducted on glass plates, with six treatment groups of 0 (water control), 37.5, 75, 150, 300, and 600 mL product/ha, equivalent to 9.4, 18.8, 37.5, 75, and 150 g a.s./ha (accounting for the nominal product a.s. content of 250 g/L).

Each treatment group was comprised of four replicates, with twenty protonymphs per replicate. Adult mortality was assessed after 3 and 7 days' exposure and reproduction after 10, 13, and 14 days' exposure.

Treatment with CA3301 significantly increased mortality in all but the lowest product treatment group. Thus, the 7-day NOER and LOER values for mortality were determined to be 37.5 and 75 mL product/ha, equivalent to 9.4 and 18.8 g a.s./ha, respectively. The LR₁₀, LR₂₀, and LR₅₀ values were calculated to be 97.3, 127, and 209 mL product/ha, equivalent to 24.3, 31.8, and 52.3 g a.s./ha, respectively.

The two highest product groups resulted in >50% mortality, thus, reproductive assessments were not made for these treatments. The 14-day NOER and LOER values for reproduction were determined to be 150 and >150 mL product/ha, equivalent to 37.5 and >37.5 g a.s./ha, respectively. Given the lack of a rate-response relationship, ER_x values could not be calculated, but the ER₅₀ value was estimated to >150 mL product/ha, equivalent to >37.5 g a.s./ha.

For validation of the test system, treatment with Perfekthion (6 g dimethoate/ha), as the reference item, which resulted in 68.8% corrected mortality.

Overall, the study satisfies the IOBC Blümel (2000) test-guideline requirements for chronic toxicity to *Typhlodromus pyri* and is considered acceptable.

Materials and methods

CA3301 / JOUST	Page 173 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Test materials

Test item

Name:	CA3301
Active substance:	Prothioconazole
Formulation type:	Emulsifiable concentrate (EC)
Source and lot/batch no.:	A19045
Active substance content:	250 g/L (nominal), 257.04 g/L or 25.57% w/w (analyzed)
Appearance:	Clear, straw-coloured liquid
Expiry date of lot/batch:	17 June 2022
Storage conditions:	Stored in closed, original container in a cool, well-ventilated area, protected from light

Reference item

Name:	Perfekthion
Density:	1.04 – 1.10 g/cm ³
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 ³)
Appearance:	Blue liquid
Expiry date:	31 January 2026

Test organism

Species:	<i>Typhlodromus pyri</i> Scheuten (Acari, Phytoseiidae)
Age at study initiation:	<24-hours-old protonymphs
Source:	Katz Biotech AG, An der Birkenpfuhlheide 10, 15837 Baruth, Germany.
Feeding during test:	apple pollen (<i>Malus vulgaris</i>) on day 0, 3, 7, 10, and 13 after application

Test conditions

Test temperature:	23.1 to 25.9°C, mean of 24.7°C
Relative humidity:	42.8 to 61.4%, mean of 56.0%*
Light intensity:	1237 and 1375 lux

*The majority of single humidity measurements were below the range of 60-90% recommended by the test guideline, due to unexpected, insufficient humidity control of the climate cabinet. These deviations had no impact on the study, because the validity criteria of the test organisms in the control and the reference-item treatment were fulfilled.

Exposure test units were built according to the “open method.” Test arenas were placed within plastic petri dishes (approx. 9-cm diameter, 1.6-cm height). The test arena consisted of two glass cover slides (approx. 24 × 50 mm²), each adjoined lengthwise by clear adhesive tape (the gap between the two slides prevented mites from escaping, but large enough to ensure availability of drinking water). The mites were confined to an area of approximately 10-13 cm² 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 that was position on the bottom of the test unit. The test units were placed in plastic petri dishes, which were partly filled with water. Perforations 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.

Treatments with an average mortality-escape rate of 50% or more on day 7 were excluded from the

CA3301 / JOUST	Page 174 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

reproduction phase.

Prior to application, a stock solution was made, by mixing 1.5 mL product in 500 mL of ultrapure water (equivalent to 750 mg a.s./L, when accounting for the nominal product purity of 250 g a.s./L). The application rates used in the study were 37.5, 75, 150, 300, and 600 mL product/ha, equivalent to 9.4, 18.8, 37.5, 75, and 150 g a.s./ha (accounting for the nominal product a.s. content of 250 g/L). 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 15 mL Perfekthion/ha (equivalent to 6 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 18.75 µL Perfekthion in 250 mL of ultrapure water (equivalent to 30 µg dimethoate/L).

After application and after the spray deposits on the glass plates had dried, 20 protonymphs per replicate were transferred onto the glass-plate surfaces. The transfer of the mites from the holding vessels to all test units was completed within 75 minutes after the application. The time interval between spraying and transfer of the mites was similar for all treatments. Cumulative mortality was assessed on days 3 and 7 after application and cumulative reproduction per female was assessed on days 7 through 14.

Test organisms were considered dead when motionless, even after prodding. Mortality was calculated as the sum of dead and escaped mites (mites unaccounted for in the test units). 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, but not for the reference-item group. 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 color. Eggs laid prior to the observation on day 7 were removed from the test units and not included in the reproduction assessment. Treatments with an average mortality escape rate of $\geq 50\%$ on day 7 (i.e., 300 and 600 mL/ha) were excluded from the reproduction phase.

On days 7, 10, 13, and 14, the number of females and males was recorded: On days 10, 13, and 14, the number of larvae and eggs was recorded. Larvae and eggs were removed afterwards. The number of eggs per female during the reproduction period was calculated for each replicate separately. The result for each treatment was the mean cumulative number of eggs per female. In all treatments up to and including 150 mL/ha, the sex ratio was at least 1 male:5 females at day 7 (the start of the reproduction phase). Therefore, transfer of males from one replicate to another was not necessary.

Differences in mortality between the product treatments and the control were statistically evaluated using a one-sided, multiple sequentially rejective Welch t-test, with Bonferroni corrections ($\alpha = 0.05$). The NOER and LOER for mortality were determined according to the results of the statistics. A probit analysis, with a linear maximum likelihood regression, was used to calculate LR_{10} , LR_{20} , and LR_{50} values [including 95% confidence intervals (CIs)]. Two probit were performed: one run with all replicates of each treatment and the other with replicates 3 and 4 from the 37.5 mL product/ha group excluded from the statistical analysis (due to a high number of not found test organisms not found on days 3 and 7; thus, not related to a treatment effect, but caused by unexpectedly leaky test systems). The reliability of the two probit analysis runs was assessed by comparing the determination coefficient (r^2 , as measure for goodness of fit of the rate-response relationship) and the distance between the lower and upper 95% CIs, giving preference to probit analysis 2 ($r^2 = 0.802$) over probit analysis 1 ($r^2 = 0.399$).

Differences in mean reproduction values between the product treatments and the control were statistically evaluated by means of a one-sided William's t-test ($\alpha = 0.05$). The NOER and LOER values for reproduction were determined according to the results of the statistics. The ER_{10} , ER_{20} , and ER_{50} values (including 95% CIs) could not be calculated, due to a missing rate-response relationship, up to and including 150 mL/ha. No reproduction was determined for the treatments with 300 and 600 mL product/ha, since mortality was $>50\%$ in the preceding mortality phase. The ER_{50} value was determined directly from the

CA3301 / JOUST	Page 175 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

raw data.

Statistical analysis was performed using ToxRat Professional.

Results

Biological results

Mortality

A summary of the effect of CA3301 on adult mortality from the definitive test, after 7 days of exposure, is presented in the Table CP 10.3.2/01-01 below.

Table CP 10.3.2/01-01. The effect of CA3301 (nominal product a.s. content of 250 g/L) on adult *T. pyri* mean mortality, after 7 days of exposure (N = 20 mites per replicate).

Application rate		Replicate	Mortality		
mL product/ha	g a.s./ha		Number	Mean \pm SD (%)	Mean corrected (%)
0 (control)	0	1	0	0 ± 0	0
		2	0		
		3	0		
		4	0		
37.5	9.4	1	0	32.5 ± 32	32.5
		2	2		
		3	12		
		4	12		
75	18.8	1	3	$12.5 \pm 5^*$	12.5
		2	3		
		3	3		
		4	1		
150	37.5	1	3	$25 \pm 10.8^*$	25.0
		2	8		
		3	5		
		4	4		
300	75	1	13	$73.8 \pm 10.3^*$	73.8
		2	17		
		3	16		
		4	13		
600	150	1	20	$98.8 \pm 2.5^*$	98.8
		2	20		
		3	20		
		4	19		
15 mL Perfekthion/ha 6 g dimethoate/ha		1	16	$68.8 \pm 16^*$	68.8
		2	11		
		3	11		
		4	17		

Statistically significant differences, between the product or reference-item groups and the control, are denoted with asterisks (*) and were revealed with one-sided Welch t-tests (for non-equal variances, $\alpha = 0.05$, with all replicates included) or one-sided William's t-tests (when excluding replicates 3 and 4 from 37.5 mL/ha, $\alpha = 0.05$). Mortality corrected with a modified Abbott formula. SD, standard deviation.

After seven days of exposure, mean mortality in the control and in the reference-item treatment groups was 0.0 and 68.8%, respectively. Mean corrected mortality in the product treatments ranged from 12.5 to 98.8% and exhibited a rate-response relationship. When including all replicates, overall mortality was statistically significantly increased at all product rates, including the lowest test rate of 37.5 mL product/ha compared to the control (Welch t-test, one-sided smaller, $\alpha = 0.05$). When excluding replicates 3 and 4 from 37.5

CA3301 / JOUST	Page 176 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

mL/ha, overall mortality at 37.5 mL/ha did not differ statistically significantly from the control (Williams t-test, one-sided smaller, $\alpha = 0.05$).

Excluding replicates 3 and 4 from 37.5 mL/ha, the calculated LR₁₀, LR₂₀, and LR₅₀ values 97.3, 127, and 209 mL product/ha, equivalent to 24.3, 31.8, and 52.3 g a.s./ha, respectively. The 7-day NOER and LOER values were determined to be 37.5 and 75 mL product/ha, equivalent to 9.4 and 18.8 g a.s./ha, respectively.

Reproduction

A summary of the effect of CA3301 on reproduction from the definitive test, after 14 days of exposure, is presented in the tables below.

Table CP 10.3.2/01-03. The effect of CA3301 on *T. pyri* reproduction (fecundity), after 14 days of exposure (N = 20 mites per replicate).

Application rate		Rep.	No. females				No. eggs			No. larvae		
							Time (days)					
mL product/ha	g a.s./ha		7	10	13	14	7-10	10-13	13-14	7-10	10-13	13-14
0 (control)	0	1	8	8	8	8	22	11	21	5	1	1
		2	10	10	10	10	27	21	45	4	0	1
		3	14	14	14	14	37	31	17	11	2	2
		4	9	9	9	8	32	23	1	2	2	0
37.5	9.4	1	14	14	14	13	40	36	17	8	3	1
		2	9	9	9	9	27	21	13	3	4	4
		3	6	6	6	6	18	16	4	1	1	1
		4	6	5	5	5	13	10	3	3	3	1
75	18.8	1	11	11	10	10	22	24	13	2	1	1
		2	9	9	9	9	25	20	18	0	0	0
		3	10	10	9	9	22	20	13	0	4	1
		4	7	4	4	4	7	10	10	2	3	0
150	37.5	1	10	10	10	10	21	26	13	1	2	0
		2	8	8	8	8	20	29	9	0	4	0
		3	7	7	7	7	24	25	2	1	4	2
		4	8	8	8	8	26	9	0	1	5	5
300	75	1										
		2										
		3										
		4										
600	150	1										
		2										
		3										
		4										

Rep., replicate; no., number.

CA3301 / JOUST	Page 177 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Table CP 10.3.2/01-04. The effect of CA3301 on *T. pyri* cumulative reproduction (fecundity), after 14 days of exposure (N = 20 mites per replicate).

Application rate		Rep.	Cumulative reproduction (eggs/female)		Reduction in reproduction (% control)
mL product/ha	g a.s./ha		Rep. mean	Treatment mean \pm SD	
0 (control)	0	1	7.6	7.8 \pm 1.4	0
		2	9.8		
		3	7.1		
		4	6.7		
37.5	9.4	1	7.6	7.1 \pm 0.8	8
		2	8		
		3	6.8		
		4	6.6		
75	18.8	1	6.1	6.6 \pm 0.6	16
		2	7		
		3	6.4		
		4	8		
150	37.5	1	6.3	7.0 \pm 1.2	10
		2	7.8		
		3	8.3		
		4	5.8		
300	75	1	n.d.	n.d.	n.d.
		2			
		3			
		4			
600	150	1	n.d.	n.d.	n.d.
		2			
		3			
		4			

No statistically significant differences, relative to the control, were revealed with a one-sided William's t-test ($\alpha = 0.05$). Due to 100% mortality after 7 days' exposure, reproduction could not be determined (n.d.). Rep., replicate; SD, standard deviation.

Mean egg production from day 7 to day 14 in the control was 7.8 eggs per female. The mean egg production of females in the product treatments was 8-16% lower than the control, without a rate-response relationship, and there were no significant differences in reproduction, relative to the control. Due to high mortality in the 300 and 600 mL product/ha groups, reproduction was not assessed. The 14-day NOER and LOER values were determined to be 150 and >150 mL product/ha, equivalent to 37.5 and >37.5 g a.s./ha, respectively.

Given the lack of a rate-response relationship, ER_x values could not be calculated and, therefore, the ER_{50} value was estimated to >150 mL product/ha, equivalent to >37.5 g a.s./ha.

Validity

All validity criteria were met in accordance with IOBC Blümel (2000) test guideline:

- Mean mortality in the control to be $\leq 20\%$, after 7 days' exposure (actual value was 0%).
- Mean corrected mortality in the reference-item group to be between 50-100%, after 7 days' exposure (actual value 68.8%)
- Cumulative mean number of eggs per female in the control to be ≥ 4 (actual value was 7.8).

Conclusion

The 14-day chronic toxicity of CA3301 to *Typhlodromus pyri* was studied in accordance with the IOBC Blümel (2000) test guideline.

CA3301 / JOUST	Page 178 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

The 7-day NOER and LOER values for mortality were determined to be 37.5 and 75 mL product/ha, equivalent to 9.4 and 18.8 g a.s./ha, respectively. The LR₁₀, LR₂₀, and LR₅₀ values were calculated to be 97.3, 127, and 209 mL product/ha, equivalent to 24.3, 31.8, and 52.3 g a.s./ha, respectively.

The 14-day NOER and LOER values for reproduction were determined to be 150 and >150 mL product/ha, equivalent to 37.5 and >37.5 g a.s./ha, respectively. Given the lack of a rate-response relationship, ER_x values could not be calculated, but the ER₅₀ value was estimated to >150 mL product/ha, equivalent to >37.5 g a.s./ha.

This study is considered acceptable.

Study 2

Comments of zRMS:	<p>The study was conducted to the guideline and according to the principles of GLP. All validity criteria were met. One minor deviation was noted without negative impact on the overall outcome of the study:</p> <ul style="list-style-type: none"> - the number of reproduction test units with individual female wasps was 13 and 12 in the control and the treatment with 7.5 mL/ha, respectively (recommended 15). <p>The study is considered to be reliable and suitable for the risk assessment.</p>
-------------------	---

Reference:	KCP 10.3.2/02
Report	<p>250 EC Prothioconazole, NUL 3390 (CA3301) - Acute Toxicity to Adults of the Parasitoid Wasp <i>Aphidius rhopalosiphii</i> (Hymenoptera: Braconidae) under Worst-Case Conditions in the Laboratory</p> <p>Schmidt, T., 2021, report no. 20190458</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

A 48-day mortality and reproduction study of a parasitoid wasp, *Aphidius rhopalosiphii*, with CA3301 was conducted on glass plates, with six treatment groups of 0 (water control), 7.5, 15, 30, 60, and 120 mL product/ha, equivalent to 0, 1.9, 3.8, 7.5, 15, and 30 g a.s./ha (accounting for the nominal product a.s. content of 250 g/L).

Each treatment group was comprised of four replicates, with ten wasps per replicate (≤48-h-old and at least five females per replicate). Mortality was assessed after 48 h of exposure. Reproduction was then assessed by introducing surviving female wasps to units containing barley seeds infested with aphids (12-15 replicates per treatment, one adult female wasp per replicate). Females were left parasitize aphids for 24 and the number of mummies (parasitized aphids) was counted after 11 days.

Treatment with CA3301 significantly decreased mortality in the two highest product treatments (60 and 120 mLg product/ha). Therefore, the 48-h NOER and LOER (mortality) values were determined to be 30 and 60 mL product/ha (equivalent to 7.5 and 15 g a.s./ha), respectively. The calculated 48-h LR₁₀, LR₂₀,

CA3301 / JOUST	Page 179 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

and LR₅₀ values were calculated to be 13.7, 22.3, and 56.6 mL product/ha (equivalent to 3.4, 5.6, and 14.2 g a.s./ha), respectively.

Treatment with CA3301 caused significant reduction in mummy production in the 30 and 60 mL product/ha treatment groups, while reproductive effects could not be determined in the highest product treatment, due to 100% adult mortality. Therefore, the NOER and LOER (reproduction) values were determined to be 15 and 30 mL product/ha (equivalent to 7.5 and 15 g a.s./ha), respectively. The calculated reproductive ER₁₀, ER₂₀, and ER₅₀ values were 19.4, 25.5, and 43.1 mL product/ha (equivalent to 4.9, 6.4, and 10.8 g a.s./ha), respectively.

For validation of the test system, treatment with Perfekthion (0.12 g dimethoate/ha), as the reference item, which resulted in 100% mortality.

Overall, the study satisfies the IOBC Mead-Briggs et al. (2000) test-guideline requirements for toxicity to *Aphidius rhopalosiphi* and is considered acceptable.

Materials and methods

Test materials

Test item

Name:	CA3301
Active substance:	Prothioconazole
Formulation type:	Emulsifiable concentrate (EC)
Source and lot/batch no.:	A19045
Active substance content:	250 g/L (nominal), 257.04 g/L or 25.57% w/w (analyzed)
Appearance:	Clear, straw-coloured liquid
Expiry date of lot/batch:	17 June 2022
Storage conditions:	Stored in closed, original container in a cool, well-ventilated area, protected from light

Reference item

Name:	Perfekthion
Density:	1.04 – 1.10 g/cm ³
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 ³)
Appearance:	Blue liquid
Expiry date:	31 January 2026

Test organism

Species:	<i>Aphidius rhopalosiphi</i> De Stefani-Perez (Hymenoptera: Braconidae)
Age at study initiation:	≤48 h
Sex:	Mixed, with at least 5 wasps per replicate
Source:	Pupal wasps, obtained from Katz Biotech AG, An der Birkenpfehlheide 10, 15837 Baruth, Germany.
Feeding during test:	1:3 v/v solution of honey-in-water

Test conditions

Test temperature:	20.3-21.5°C, mean of 21.0°C
Relative humidity:	64.9- 72.3%, mean of 68.7%
Photoperiod:	16 h light:8 h dark
Light intensity:	769-1696 lux (exposure phase), 4320-6770 lux (reproductive phase)

Exposure test units were built with the following components. Two glass plates (each 12 x 12 cm²) were

CA3301 / JOUST	Page 180 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

held 2 cm apart by a stainless-steel square frame (9.7×9.7 cm², 2-cm tall). There were four holes (0.7-cm diameter) on each side of the frame, to provide ventilation, which were covered by a fine-gauge mesh to prevent wasps from escaping, except for one hole on the bottom. 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 by plastic tubing from one of the mesh-covered, using a small pump.

Untreated pots of aphid-infested barley seedlings (*Hordeum vulgare*) were covered with cylinders (9-cm diameter, 20-cm high), made of clear acryl acetate. 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 test units were kept in a temperature-controlled room set at 20 ± 2 °C with 60-90% relative humidity and a 16-h light:8-h dark photoperiod during the test.

The study consisted of seven treatment groups: a control, five product treatments, and a reference treatment. During the exposure phase, each treatment consisted of four replicates, with ten \leq 48-h-old wasps per replicate (at least five females per replicate), for a total of 40 wasps per treatment. The parasitoids were randomized transferred from the emergence boxes to the test units with an aspirator. During the reproductive phase, 15 surviving females were transferred individually from the exposure test units to the reproduction units and were allowed to parasitize for 24 hours. Note that some female wasps unintentionally escaped during the transfer from the exposure test units into the reproduction test units, thus, only 13 and 12 reproductions units in the control and the 7.5 mL product/ha treatment, respectively, could be filled with individual female wasps. However, this did not impact the outcome of the study.

Prior to application, a stock solution was made, by mixing 0.3 mL product in 500 mL of ultrapure water (equivalent to 150 mg a.s./L, when accounting for the nominal product a.s. content of 250 g/L). The application rates used in the study were 7.5, 15, 30, 60, and 120 mL product/ha, equivalent to 1.9, 3.8, 7.5, 15, and 30 g a.s./ha (accounting for the nominal product a.s. content of 250 g/L). 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 0.3 mL Perfekthion/ha (equivalent to 0.12 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 48 h of exposure. The stock solution for the reference item was made by mixing 37.5 μ L Perfekthion in 500 mL of ultrapure water (equivalent to 30 mg dimethoate/L).

After application and after the spray deposits on the glass plates had dried, 10 wasps per replicate were transferred onto the glass-plate surfaces. The transfer of the wasps from the holding vessels to all test units was completed within 90 minutes after the application. The exposure phase lasted 48 h.

Differences in mortality between the product treatments and the control were statistically evaluated using a one-sided, multiple sequentially rejective Welsh t-test, with Bonferroni corrections ($\alpha = 0.05$). The NOER and LOER for mortality were determined according to the results of the statistics. A probit analysis, with a linear maximum likelihood regression, was used to calculate LR₁₀, LR₂₀, and LR₅₀ values [including 95% confidence intervals (CIs)].

Differences in mean reproduction values between the product treatments and the control were statistically evaluated by means of a one-sided William's t-test ($\alpha = 0.05$). The NOER and LOER values for reproduction were determined according to the results of the statistics. A non-linear regression analysis (3-parametric cumulative distribution function using downhill-simplex as optimization method) was used to calculate ER₁₀, ER₂₀, and ER₅₀ values (including 95% CIs).

Statistical analysis was performed using ToxRat Professional.

Results

Biological results

Mortality

A summary of the effect of CA3301 on adult mortality from the definitive test, after 48 h of exposure, is presented in the Table CP 10.3.2/02-01 below.

Table CP 10.3.2/02-01. The effect of CA3301 on adult *A. rhopalosiphi* behaviour and mortality, after 48 h of exposure (N = 10 wasps per replicate).

Application rate		Rep.	Behaviourally affected (no.)	Mean mortality		
mL product/ha	g a.s./ha			Number	Mean \pm SD (%)	Corrected (%)
0 (control)	0	1	0	0	10 \pm 8.2	0.0
		2	0	1		
		3	0	1		
		4	0	2		
7.5	1.9	1	0	1	12.5 \pm 9.6	2.8
		2	0	2		
		3	0	0		
		4	0	2		
15	3.8	1	0	0	12.5 \pm 25	2.8
		2	0	0		
		3	0	0		
		4	0	5		
30	7.5	1	0	0	7.5 \pm 5.0	-2.8
		2	0	1		
		3	0	1		
		4	0	1		
60	15	1	4	6	55 \pm 5.8*	50
		2	5	5		
		3	5	5		
		4	4	6		
120	30	1	0	10	100 \pm 0.0*	100.0
		2	0	10		
		3	0	10		
		4	0	10		
0.3 mL Perfekthion/ha	0.12 g dimethoate/ha	1	0	10	100 \pm 0.0	100.0
		2	0	10		
		3	0	10		
		4	0	10		

Mortality corrected with a modified Abbott formula; Statistically significant differences, relative to the control, were revealed with a one-sided Welch t-test, with Bonferroni-Holm corrections ($\alpha = 0.05$), and are denoted with asterisks (*). SD, standard deviation; Rep., replicate.

The 48-h mean mortality of the control and reference-item treatment groups was 10 and 100% (0 and 100% corrected), respectively. Mean mortality in the product treatments ranged from 7.5 to 100% (-2.8 and 100% corrected), and exhibited a rate-response relationship. There were significant differences in mortality between the two highest product treatments (60 and 120 mLg product/ha) and the control group. The 48-h NOER and LOER (mortality) values were determined to 30 and 60 mL product/ha (equivalent to 7.5 and 15 g a.s./ha), respectively. The calculated 48-h LR₁₀, LR₂₀, and LR₅₀ values were calculated to be 13.7, 22.3, and 56.6 mL product/ha (equivalent to 3.4, 5.6, and 14.2 g a.s./ha), respectively.

CA3301 / JOUST	Page 182 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Reproduction

A summary of the effect of CA3301 on reproduction from the definitive test, after 24 h of parasitism, is presented in the Table CP 10.3.2/02-02 below.

Table CP 10.3.2/02-02. The effect of CA3301 (nominal product a.s. content of 250 g/L) on *A. rhopalosiphi* reproduction (fecundity), after 24 h of parasitism.

Application rate						
mL product/ha g a.s./ha	0 (control)	7.5	15	30	60	120
	0	1.9	3.8	7.5	15	30
Replicate	Reproduction (mummies per female)					
1	38	37	33	7	0	n.d.
2	36	112	40	19	8	n.d.
3	51	59	52	53	19	n.d.
4	50	41	61	34	19	n.d.
5	42	39	27	36	0	n.d.
6	33	66	49	23	25	n.d.
7	36	65	51	26	-	n.d.
8	42	30	37	20	-	n.d.
9	63	27	34	49	-	n.d.
10	65	33	59	76	-	n.d.
11	33	36	28	38	-	n.d.
12	91	55	21	21	-	n.d.
13	41	-	50	51	-	n.d.
14	-	-	41	32	-	n.d.
15	-	-	41	56	-	n.d.
Mean ± SD	47.8 ± 16.7	50 ± 23.8	41.6 ± 11.9	36.1 ± 18.1*	11.8 ± 10.7*	
CV	34.9	47.5	28.6	50.2	90.3	n.d.
Output (% control)	100	105	87	76	25	
Inhibition (% control)	0	-5	13	24	75	

Difference in reproduction with the highest product treatment were not determined (n.d.), since mortality was 100% in this group. Statistically significant differences, relative to the control, revealed with a one-sided William's t-test ($\alpha = 0.05$) and denoted with asterisks (*). Negative and positive signs indicate an increase or decrease in reproduction, respectively, relative to the control. Dashes (-) indicate non-reproducing females. SD, standard deviation; CV, coefficient of variation.

Mean mummy production after 24 h of parasitism in the control was 47.8 mummies per female and ranged between 11.8 and 50.0 in the 60 and 7.5 mL product/ha treatment groups. Reproduction could not be assessed for 120 mL product/ha treatment group, due to 100% mortality of adult wasps. There were significant reductions in reproduction (mummy production) in the 30 and 60 mL product/ha treatment groups. Therefore, the NOER and LOER (reproduction) values were determined to be 15 and 30 mL product/ha (equivalent to 7.5 and 15 g a.s./ha), respectively. The calculated reproductive ER_{10} , ER_{20} , and ER_{50} values were 19.4, 25.5, and 43.1 mL product/ha (equivalent to 4.9, 6.4, and 10.8 g a.s./ha), respectively.

Validity

All validity criteria were met in accordance with IOBC Mead-Briggs et al. (2000) test guideline:

- Mean mortality in the control to be $\leq 13\%$, after 48 h of exposure (actual value was 10%).
- Mean mortality in the reference-item group to be between $\geq 50\%$ after 48 h of exposure (actual value 100%)
- Mean mummy production per female in the control group to be ≥ 5 (actual values ranged between 33 to 91).
- No more than two wasps in the control to produce zero mummies (actual value: all wasps produced at least one wasp).

CA3301 / JOUST	Page 183 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Conclusion

The toxicity of CA3301 to *Aphidius rhopalosiphii* was studied in accordance with the IOBC Mead-Briggs et al. (2000) test guideline.

The NOER and LOER (mortality) values were determined to 30 and 60 mL product/ha (equivalent to 7.5 and 15 g a.s./ha), respectively. The calculated LR₁₀, LR₂₀, and LR₅₀ values were calculated to be 13.7, 22.3, and 56.6 mL product/ha (equivalent to 3.4, 5.6, and 14.2 g a.s./ha), respectively.

The reproductive NOER and LOER values were determined to be 15 and 30 mL product/ha (equivalent to 7.5 and 15 g a.s./ha), respectively. The calculated reproductive ER₁₀, ER₂₀, and ER₅₀ values were 19.4, 25.5, and 43.1 mL product/ha (equivalent to 4.9, 6.4, and 10.8 g a.s./ha), respectively.

This study is considered acceptable.

Study 3

Comments of zRMS:	The study was conducted to the guideline and according to the principles of GLP. All validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.
-------------------	---

Reference:	KCP 10.3.2/03
Report	CA3301 – An extended laboratory study to determine the acute and sublethal effects on the non-target arthropod <i>Typhlodromus pyri</i> (Acari: Phytoseiidae). Fraimout, C., 2021, report no. 029SRFR20C07
Guideline(s):	Yes. IOBC, Blümel, S. et al. (2000)
Deviations:	No
GLP:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive summary

In a 14-day extended laboratory study, the acute and reproductive effects of the product CA3301 on *Typhlodromus pyri* were assessed in a rate-response test, in accordance with the Blümel *et al.* (2000) IOBC guideline.

CA3301 was evaluated at six nominal treatment rates of 0 (distilled water), 0.53, 0.85, 1.36, 2.18, 3.48 L product/ha, equivalent to 0, 134.82, 216.21, 345.94, 554.53, and 885.21 g a.s./ha, plus a toxic-reference treatment (10 g dimethoate/ha).

T. pyri protonymphs were introduced to test units containing treated pepper-plant leaves (*Capsicum annum*). Mortality was assessed on days 3 and 7 after exposure and reproduction was assessed on days 10, 12, and 14. Each test unit was considered a replicate, with six replicates per test treatment, each containing 20 protonymphs of *T. pyri*.

Treatment with all product rates caused significant increases in mortality. Thus, the 7-day NOER and LOER (mortality) values were determined to be <0.53 and 0.53 L product/ha (equivalent to <134.82 and 134.82 g a.s./ha), respectively. The 7-day LR₅₀ value was calculated to be 0.862 L product/ha, equivalent to 219.14

CA3301 / JOUST	Page 184 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

g a.s./ha.

Treatment with the second-highest product treatment (0.85 L product/ha) caused a significant decrease in reproduction, relative to the control. Since mean mortality after 7 days exceeded 50% for the product rates of 1.36, 2.18, and 3.48 L product/ha, the effects on reproductive performance were not evaluated for these treatments and the ER₅₀ could not be calculated. Therefore, the reproductive NOER and LOER values were determined to be 0.53 and 0.85 L product/ha (equivalent to 134.82 and 216.21 g a.s./ha), respectively. The reproductive ER₅₀ was estimated to be >0.85 L product/ha, equivalent to 216.21 g a.s./ha.

For validation of the test system, treatment with 10 g dimethoate/ha, as the reference item, resulted in 81.48% corrected mortality.

Overall, the study satisfies the IOBC Blümel (2000) test-guideline requirements for an extended-laboratory study on *Typhlodromus pyri* and is considered acceptable.

Materials and methods

Test materials

Product

Name:	CA3301
Active substance:	Prothioconazole
Formulation type:	Emulsifiable concentrate (EC)
Product density:	0.9948 g/mL
Source and lot/batch no.:	A19045
Active substance content:	250 g/L (nominal), 257.04 g/L or 25.57% w/w (analyzed)
Expiry date of lot/batch:	17 June 2022
Storage conditions:	Stored between 13.5°C and 25.5°C, protected from light

Reference item

Name:	Dimethoate
Purity:	98% (CoA)
Density:	1g/mL
Batch no.:	D088B191112
Appearance:	Solid
Expiry date of lot/batch:	12 November 2022
Storage conditions:	Stored between 2.2°C and 7.7°C, protected from light

Test organism

Species:	<i>Typhlodromus pyri</i>
Age at study initiation:	Protonymphs (approx. 24-hours-old post-moulting)
Source:	BiasLabs Ltd., Kirkcaldy, Fife, UK
Feeding during test:	Two portions of apple pollen (<i>Typha spp.</i>) and one portion of 70:30 bean:nut pollen, added into each test unit just before exposure; pollen was renewed at least every 3 days.

Test conditions

Test temperature:	23.5 – 26.5°C, mean of 24.6°C
Relative humidity:	52.7 – 86.1%, mean of 79.6%*
Photoperiod:	16 h light:8 h dark
Light intensity:	Mean of 561 lux

*Short-term (less than 2 hours) deviation which is not implying a deviation to the study plan.

Spray applications were made onto untreated pepper plants (*Capsicum annuum*) leaves, on which the test

CA3301 / JOUST	Page 185 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

organisms were exposed to the freshly dried residues. The test units used in the study were an adaptation of the “open method,” and consisted of a treated leaf placed onto a piece of wet cotton wool, laid over a sponge, inside an opened plastic container filled with mineral water. A treated area of about (15 mm x 80 mm) was delimited by strips of wet filter paper, forming a water barrier for the mites, to prevent them from escaping and to provide drinking water.

Each treatment comprised six replicates (1 test unit per replicate, 20 protonymph *T. pyri* per replicate) and there were seven treatment groups: a distilled water control, five product rates, and one reference item. The five product treatment groups were 0.53, 0.85, 1.36, 2.18, 3.48 L product/ha, equivalent to 134.82, 216.21, 345.94, 554.53, and 885.21 g a.s./ha. The reference item was applied at 10 g dimethoate/ha. Prior to application, stock solutions were made, by mixing 8.654 mL of product in 500 mL of distilled water (equivalent to 2.164 g a.s./L, when accounting for the nominal product a.s. content of 250 g/L), and by mixing 0.0253 g dimethoate in 500 mL of distilled water (50.6 mg dimethoate/L).

Stock solutions were made on the day of the applications. Leaves were sprayed using a laboratory spraying apparatus (DeVries Spray Booth Generation III, nozzles Teejet 8002E), at rate of 200 L/ha and left to dry for approximately 1.5 h. As soon as the leaves dried, the test units were assembled, and two small portions of pollen were added as food. Then, twenty *T. pyri* protonymphs were randomly assigned to the test units into the centre of each treated area, using a clean brush for each treatment.

The environmental conditions during the laboratory phase were recorded for temperature, humidity, and light (at regular intervals or continuously). During the test, relative humidity was outside the guideline-recommended ranges for short periods (less than two hours), which were not expected to have an adverse effect on results and were not reported as deviations from the study plan.

Mortality was assessed 3 (± 1) and 7 days after introduction of the protonymphs to the test units. Test units were observed beneath a microscope and the nymphs were recorded as dead (sum of dead, missing, and motionless protonymphs) or alive. The mortality data were corrected for control mortality, using the Schneider-Orelli-modified Abbott's formula.

After 10, 12, and 14 days of exposure, reproductive output of surviving mites was evaluated for only the control and product treatment groups, except in the product treatment groups in which corrected mortality exceeded 50% (corrected from the distilled water control). From days 7 to 14, the number of males, females, juveniles, and eggs was counted (eggs laid on or before day 7 were removed from the test units and not counted). All eggs and juveniles were removed after counting. The reproductive data were corrected for control reproduction.

All data were analysed with the statistical software R (version 3.3.2) and performed at $\alpha = 0.05$.

The 7-day mortality data were analysed to determine any significant differences between control and product treatment groups and to estimate NOER/LOER values. One replicate in the lowest product treatment group was found to be an outlier, based on residuals analysis. The mortality data were found to be monotonic (Spearman's correlation test). Thus, a step-down Cochran-Armitage test was used to determine statistical differences between the treatment groups. LR_X and 95% confidence intervals (CIs) were calculated with a 3-parameter Bayesian inference.

The reproductive data were analysed to determine any significant differences between control and product treatment groups and to estimate NOER/LOER values. One replicate in the control was found to be an outlier, based on residuals analysis. The reproductive data were found to be monotonic (Spearman's correlation test). Thus, a step-down Jonckheere-Terpstra exact test was used to determine statistical differences between the treatment groups. Since the product treatments did not produce effects exceeding 50%, the 14-day ER_{50} value could only be estimated.

CA3301 / JOUST	Page 186 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Results

Biological results

Mortality

The effect of CA3301 on mortality, after 7 days of exposure, is presented in Table CP 10.3.2/03-01 below.

Table CP 10.3.2/03-01. The effect of CA3301 on *T. pyri* mortality, after 7 days of exposure.

Application rate		Rep.	Mortality			
L product/ha	g a.s./ha		No.	%	Mean (%)*	Corrected (%)#
0 (control)	0	1	0	0.0	10.0	0.0
		2	5	25.0		
		3	0	0.0		
		4	5	25.0		
		5	0	0.0		
		6	2	10.0		
0.53	134.82	1	14	70.0	28.0 (sig.)	20.0
		2	5	25.0		
		3	5	25.0		
		4	9	45.0		
		5	1	5.0		
		6	8	40.0		
0.85	216.21	1	13	65.0	54.17 (sig.)	49.07
		2	11	55.0		
		3	7	35.0		
		4	7	35.0		
		5	12	60.0		
		6	15	75.0		
1.36	345.94	1	15	75.0	82.50 (sig.)	80.56
		2	17	85.0		
		3	20	100.0		
		4	18	90.0		
		5	12	60.0		
		6	17	85.0		
2.18	554.53	1	19	95.0	95.83 (sig.)	95.37
		2	18	90.0		
		3	19	95.0		
		4	20	100.0		
		5	19	95.0		
		6	20	100.0		
3.48	885.21	1	20	100.0	99.17 (sig.)	99.07
		2	20	100.0		
		3	20	100.0		
		4	20	100.0		
		5	19	95.0		
		6	20	100.0		
10 g dimethoate/ha		1	18	90.0	90.0	81.48
		2	14	70.0		
		3	15	75.0		
		4	15	75.0		
		5	19	95.0		
		6	19	95.0		

*Significant (sig.) differences in mortality, relative to the control, were revealed with a step-down Cochran-Armitage test. #Data were corrected for control mortality with a modified Abbott's formula. Positive (+) and negative (-) signs indicate higher and lower mortality, respectively, relative to the control. Rep., replicate; No., number.

CA3301 / JOUST	Page 187 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Treatment with all product treatment rates caused significant increases in morality. Thus, the 7-day NOER and LOER (mortality) values were determined to be <0.53 and 0.53 L product/ha (equivalent to <134.82 and 134.82 g a.s./ha), respectively. The 7-day LR₅₀ value was calculated to be 0.862 L product/ha (95% CIs: 0.776 – 0.959 L product/ha), equivalent to 219.14 g a.s./ha (95% CIs: 196.9 – 244.2 g a.s./ha).

Reproduction

The effects on the reproduction capacity of *T. pyri* after 14 days of exposure to the product CA3301 are presented in Table 8.3.2.2/03.

Table CP 10.3.2/03-02. The effect of CA3301 on *T. pyri* reproduction (fecundity), 14 days after exposure.

Application rate		Rep.	Reproduction			
L product/ha	g a.s./ha		Eggs (No.)	Mean (eggs/female)	Mean (eggs/female)*	Change (% control)#
0 (control)	0	1	42	4.85	5.13	0
		2	57	5.05		
		3	79	5.79		
		4	50	6.25		
		5	103	8.58		
		6	26	3.71		
0.53	134.82	1	24	8.0	6.16 (not sig.)	19.99
		2	39	5.57		
		3	41	6.67		
		4	39	6.50		
		5	56	5.47		
		6	27	4.72		
0.85	216.21	1	21	4.20	3.10 (sig.)	-39.53
		2	14	4.50		
		3	26	3.36		
		4	27	3.32		
		5	15	2.58		
		6	1	0.67		
1.36	345.94	1	Not evaluated			
		2				
		3				
		4				
		5				
		6				
2.18	554.53	1	Not evaluated			
		2				
		3				
		4				
		5				
		6				
3.48	885.21	1	Not evaluated			
		2				
		3				
		4				
		5				
		6				

*Significant (sig.) differences in reproduction, relative to the control, were revealed with a step-down Jonckheere-Terpstra exact test. #Data were corrected for control reproduction. Positive (+) and negative (-) signs indicate higher and lower reproductive effects, respectively, relative to the control. Rep., replicate; No., number.

Since the mean mortality after 7 days exceeded 50% for the product rates 1.36, 2.18, and 3.48 L product/ha, the effects on reproductive performance were not evaluated for these treatments and the ER₅₀ could not be

CA3301 / JOUST	Page 188 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

calculated. However, treatment with the second-highest product treatment caused a significant decrease in reproduction. There were no reproductive effects $\geq 50\%$, compared to the control, at the highest rate tested for reproductive performance (0.85 L formulated product/ha). Therefore, reproductive NOER and LOER values were determined to be 0.53 and 0.85 L product/ha (equivalent to 134.82 and 216.21 g a.s./ha), respectively. The reproductive ER_{50} could not be calculated and is estimated to be >0.85 L product/ha, equivalent to >216.21 g a.s./ha.

Validity

All validity criteria were met in accordance with IOBC Blümel (2000) test guideline:

- Mean mortality in the control to be $\leq 20\%$, after 7 days' exposure (actual value: 10%).
- Mean corrected mortality in the reference-item group to be $\geq 50\%$, after 7 days' exposure (actual value: 81.48%)
- Cumulative mean number of eggs per female in the control group (from day 7 to day 14) to be ≥ 4 eggs/female (actual value: 5.13).

Conclusion

The 14-day extended laboratory study of CA3301 on *Typhlodromus pyri* was studied in accordance with the IOBC Blümel (2000) test guideline.

The 7-day NOER and LOER values for mortality were determined to be <0.53 and 0.53 L product/ha (equivalent to <134.82 and 134.82 g a.s./ha), respectively. The calculated 7-day LR_{50} value was determined to be 0.862 L product/ha, equivalent to 219.14 g a.s./ha.

The 14-day NOER and LOER values for reproduction were determined to be 0.53 and 0.85 L product/ha (equivalent to 134.82 and 216.21 g a.s./ha), respectively. The 14-day reproductive ER_{50} was estimated to be >0.85 L product/ha, equivalent to 216.21 g a.s./ha.

This study is considered acceptable.

CA3301 / JOUST	Page 189 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Study 4

Comments of zRMS:	<p>The study was conducted to the guideline and according to the principles of GLP. All validity criteria were met. One minor deviation was noted without negative impact on the overall outcome of the study:</p> <ul style="list-style-type: none"> - slight and short-lived variations in temperature and variations of the relative humidity due to the air-conditioning system were observed. <p>The study is considered to be reliable and suitable for the risk assessment.</p>
-------------------	---

Reference:	KCP 10.3.2/04
Report	<p>CA3301 – An extended laboratory study to determine the acute and sublethal effects on the non-target arthropod <i>Aphidius rhopalosiphii</i> (Hymenoptera: Braconidae).</p> <p>Frainout, C., 2021, report no. 029SRFR20C06</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

A 48-h extended-laboratory study of the acute and reproductive effects of the product, CA3301, on the parasitoid wasp, *Aphidius rhopalosiphii*, was conducted. The study consisted of six treatment rates of 0 (water control), 0.09, 0.22, 0.55, 1.36, and 3.4 L product/ha, equivalent to 0, 22.9, 55.9, 139.9, 345.9, and 864.9 g a.s./ha, sprayed onto a three-dimensional substrate, barley seedlings (*Hordeum vulgare*).

Mortality was assessed by introducing adult wasps (≤ 48 -h-old, minimum of 5 females per replicate) to exposure test units consisting of treated barley seedlings, with 6 replicates per treatment. Using these test units, the potential repellent effects of CA3301 were determined during the initial 3 h, then at 24, and 48 h after exposure, and mortality was determined at 48 h. Afterwards, a subsample of 15 surviving females from each treatment group were transferred to reproductive test units consisting of pots of aphid-infested, untreated barley seedlings. There were 15 reproductive test units (replicates) containing one female wasp each; the wasps were left in the test units for 24 h to assess the effects of CA3301 on the ability of the wasps to parasitize (mummify) aphids. After 24 h, the wasps were removed, and aphid parasitism was assessed after 10 to 12 days.

Treatment with CA3301 did not cause any repellent effects and did not cause significant changes in adult mortality or reproduction. The NOER values for mortality and reproduction were determined to be 3.4 L product/ha (equivalent to 864.9 g a.s./ha) and the LOER values for mortality and reproduction were estimated to be >3.4 L product/ha (equivalent to >864.9 g a.s./ha), respectively. Given that no product treatment had an effect greater than 50% on mortality and reproduction, the corresponding LR₅₀ and ER₅₀ values could only be estimated to be >3.4 L product/ha, equivalent to >864.9 g a.s./ha.

For validation of the test system, treatment with 4 g dimethoate/ha, as the reference item, resulted in 75.86% mortality.

Overall, the study satisfies the IOBC Mead-Briggs et al. (2000) test-guideline requirements for an extended-

CA3301 / JOUST	Page 190 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

laboratory study with *Aphidius rhopalosiphi* and is considered acceptable.

Materials and methods

Test materials

Test item

Name:	CA3301
Active substance:	Prothioconazole
Formulation type:	Emulsifiable concentrate (EC)
Source and lot/batch no.:	A19045
Product density:	0.9948 g/mL
Active substance content:	250 g/L (nominal), 257.04 g/L or 25.57% w/w (analyzed)
Appearance:	Clear, straw-coloured liquid
Expiry date of lot/batch:	17 June 2022
Storage conditions:	Stored between 13.5°C and 25.5°C, and protected from light

Reference item

Name:	Dimethoate
Formulation type:	Analytical standard
Density:	1 g/mL
Batch no.:	D088B191112
Active-substance purity:	98%
Appearance:	Solid

Test organism

Species:	<i>Aphidius rhopalosiphi</i>
Age at study initiation:	≤48 h
Sex:	Adult females (<48-h-old), 5 wasps per replicate
Source:	Katz Biotech AG, An der Birkenpfuhlheide 10, 15837 Baruth, Germany.
Feeding during test:	10% w/w solution of sugar

Test conditions

Test temperature:	18.2-22.9°C (exposure phase), 20.4-21.8°C (parasitism phase), and 17.8-22.2°C (reproductive phase)
Relative humidity:	63.2-99% (exposure phase)
Photoperiod:	16 h light:8 h dark
Light intensity:	Mean values of 1100 (exposure phase), 2130 lux (parasitism phase), and 2211 (reproductive phase) lux

Mortality phase

The test units for the mortality phase consisted of pots of treated barley (approximately 8-10 seedlings at the 2nd leaf growth stage, sown 8 days prior to the application), enclosed within clear, plastic cylinders (11-cm diameter, 20-cm high), and enclosed with wasp-proof netting. The seedlings were trimmed to a uniform height of 10-12 cm, prior to being sprayed. Around 1-2 h before treatment application, the seedlings were lightly sprayed using a sprayer with a 10% w/w solution of sugar in distilled water (sucrose, to provide both food and a foraging stimulus). After the sugar solution was applied, the plants were left to dry and then the soil in the pots was covered with dry, light-coloured sand to create a uniform surface before the plants were treated. There were 6 replicate arenas (test units) per treatment and 5 female wasps in each test unit.

The study consisted of seven treatment groups: a control, five product treatments, and a reference treatment. The application rates used in the study were 0.09, 0.22, 0.55, 1.36, and 3.4 L product/ha, equivalent to 22.9,

CA3301 / JOUST	Page 191 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

55.9, 139.9, 345.9, and 864.9 g a.s./ha. The product concentrations in the application solutions were based on the spray volume of 400 L/ha, applied with a laboratory sprayer (DeVries Spray Booth Generation III, nozzles Teejet 8002E). The study also included a control of ultrapure water. The reference item treatment was applied at a rate of 4 g dimethoate/ha.

During the exposure phase, wasps were assessed for mortality and signs of toxicity at 2, 24, and 48 h after their introduction to the test units. To determine whether the freshly dried product residues were potentially repellent, assessments of the position of the individual organisms within the test arenas were carried out during the initial 3 hours (5 separate sets of observations, at 30 ± 15 -min intervals, starting approximately 30 min after the introduction of all the wasps in a treatment). In each replicate of the product treatment and the distilled water control, wasps were recorded as settled on the plant, cylinder, or sand. Because <30% of wasps in the control and product treatment groups settled on the plants, an additional time-point (48 h) was added to assess mortality.

Fecundity phase

The reproductive test units consisted of pots of untreated barley (10-40 seedlings, approximately 10-days-old), infested with at least 100 cereal aphids (*Rhopalosiphum padi*), enclosed within a clear plastic cylinder (11-cm diameter, 20-cm high), and enclosed with wasp-proof netting. Each reproductive test unit contained one surviving adult female wasp.

Reproduction of surviving wasps was evaluated for the distilled water control and product treatments, except in product groups where corrected mortality exceeded 50%, after 48 h exposure. A sample of 15 surviving adult female wasps from each treatment group was collected for the reproduction assessments and transferred to individual reproductive test units. Wasps were allowed to parasitize the aphids for 24 h before being removed (72 h after exposure). The reproduction assessments were carried out 10 to 12 days after (13-15 days after exposure to treatments in mortality test units) and the number of mummies (parasitized aphids) was recorded.

Data were analysed with the statistical software R (version 3.3.2). All statistical analyses were performed at $\alpha = 0.05$.

Mortality data were initially analyzed for monotonicity, using Spearman's correlation, which revealed non-monotonic data. Therefore, differences in mortality between the product treatments and the control were statistically evaluated using a unilateral Fisher's exact pairwise test, with Bonferroni-Holm correction. The NOER and LOER for mortality were determined according to the results of the statistics.

Repellency data was analyzed by first transforming the data (arcsine square-root transformation) and then comparing the data with a unilateral pairwise Wilcoxon-Mann-Whitney test, with Bonferroni-Holm corrections.

Reproductive data was checked for normal distribution (Shapiro-Wilk's test) and homoscedasticity (Bartlett's test). Because the data were not normally distributed, differences in mean reproduction values between the product treatments and the control were evaluated using the non-parametric, unilateral pairwise Wilcoxon-Mann-Whitney test, with Bonferroni-Holm corrections. The NOER and LOER values for reproduction were determined according to the results of the statistics.

Statistical analysis was performed using ToxRat Professional.

Results

Biological results

Mortality

CA3301 / JOUST	Page 192 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

A summary of the effect of CA3301 on adult mortality from the definitive test, after 48 h of exposure, is presented in the Table CP 10.3.2/04-01 below.

Table CP 10.3.2/04-01. The effect of CA3301 on adult *A. rhopalosiphi* mean mortality, after 48 h of exposure (N = 5 wasps per replicate).

Application rate		Mean mortality		
L product/ha	g a.s./ha	%	P-value*	Corrected mortality (%) [#]
0 (control)	0	3.33	-	0
0.09	22.9	8.00	Not sig.	+4.83
0.22	55.9	0.00	Not sig.	-3.44
0.55	139.9	23.33	Not sig.	+20.69
1.36	345.9	4.00	Not sig.	+0.69
3.4	864.9	26.67	Not sig.	+24.14
4.0 g dimethoate/ha		76.67	-	+75.86

*Based on the mean number of dead, moribund, and unobserved wasps. *Significant difference in mortality, relative to the control, were revealed with a unilateral Fisher's exact pairwise test, with Bonferroni-Holm corrections. Not sig., Not significant. [#]Data corrected for control mortality using the Abbott and Schneider-Orelli formula. Positive (+) and negative (-) signs indicate an increase and decrease in mortality, relative to the control, respectively.

The 48-h mean mortality of the control group was 3.33% (0% corrected), whereas mean mortality in the product treatments ranged from 0 to 26.67% (-3.44 and 24.14% corrected). Despite these differences, there were no significant differences in mortality between the control and product treatments. Thus, the 48-h NOER (mortality) and LOER (mortality) values were determined to 3.4 and >3.4 L product/ha (equivalent to 864.9 and >864.9 g a.s./ha), respectively. The 48-h LR₅₀ value was estimated to be >3.4 L product/ha (equivalent >864.9 g a.s./ha), given that no product treatment had an effect greater than 50% on reproduction.

Repellency

A summary of the repellency effects of CA3301 on adult *A. rhopalosiphi* at 0, 24, and 48 hours after exposure, is presented in the Table CP 10.3.2/04-02 below.

Table CP 10.3.2/04-02. The repellency effects of CA3301 on adult *A. rhopalosiphi* at 0, 24, and 48 hours after exposure.

Application rate		Wasps observed settling on the treated plants (%) [*]		
L product/ha	g a.s./ha	0 h	24 h	48 h
0 (control)	0	23.78	18.89	10.83
0.09	22.9	40.00 (not sig.)	7.50 (not sig.)	8.33 (not sig.)
0.22	55.9	17.94 (not sig.)	6.67 (not sig.)	4.17 (not sig.)
0.55	139.9	21.67 (not sig.)	5.56 (not sig.)	12.22 (not sig.)
1.36	345.9	13.50 (not sig.)	16.00 (not sig.)	4.00 (not sig.)
3.4	864.9	26.11 (not sig.)	20.83 (not sig.)	5.56 (not sig.)

*Significant difference in repellency, relative to the control, were revealed with a unilateral pairwise Wilcoxon-Mann-Whitney test, with Bonferroni-Holm corrections. not sig., not significant.

There were no significant differences in settling between the control and product treatment groups, thus, no repellent effects of CA3301 to *A. rhopalosiphi* were demonstrated.

Reproduction

The effect of CA3301 on the reproductive capacity of *A. rhopalosiphi*, after 24 h of parasitism, is presented in the Table CP 10.3.2/04-03 below.

CA3301 / JOUST	Page 193 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Table CP 10.3.2/04-03. The effect of CA330 on *A. rhopalosiphi* reproductive capacity (fecundity), after 24 h of parasitism.

Application rate		Rep.	Fecundity		
L product/ha	g a.s./ha		Mummies (no.)	Mean (mummies/female)	Change in fecundity (%) [#]
0 (control)	0	1	28	16.7	0
		2	0		
		3	30		
		4	8		
		5	11		
		6	19		
		7	15		
		8	27		
		9	21		
		10	8		
		11	31		
		12	17		
		13	2		
		14	8		
		15	10		
0.09	22.9	1	20	17.5 (not sig.)	4.26
		2	18		
		3	13		
		4	19		
		5	16		
		6	0		
		7	21		
		8	14		
		9	28		
		10	7		
		11	25		
		12	12		
		13	24		
		14	5		
		15	23		
0.22	55.9	1	34	23.38 (not sig.)	39.25
		2	38		
		3	21		
		4	12		
		5	36		
		6	17		
		7	0		
		8	26		
		9	16		
		10	20		
		11	29		
		12	0		
		13	0		
		14	31		
		15	24		
0.55	139.9	1	16	22.93 (not sig.)	36.6
		2	0		
		3	44		
		4	0		
		5	4		
		6	40		
		7	12		
		8	53		

CA3301 / JOUST	Page 194 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

		9	19		
		10	16		
		11	37		
		12	1		
		13	16		
		14	26		
		15	37		
1.36	345.9	1	20	13.6 (not sig.)	-18.98
		2	31		
		3	9		
		4	0		
		5	1		
		6	13		
		7	37		
		8	3		
		9	24		
		10	29		
		11	13		
		12	4		
		13	8		
		14	12		
		15	0		
3.4	864.9	1	18	10.5 (not sig.)	-37.45
		2	2		
		3	0		
		4	0		
		5	15		
		6	3		
		7	35		
		8	2		
		9	1		
		10	0		
		11	20		
		12	19		
		13	16		
		14	2		
		15	14		

Significant (sig.) or not significant (not sig.) difference in reproduction, relative to the control, were revealed with a pairwise Wilcoxon-Mann-Whitney test, with Bonferroni-Holm corrections. Positive (+) and negative (-) signs indicate an increase and decrease in reproduction, relative to the control, respectively. Rep., replicate; no., number.

Mean mummy production after 24 h of parasitism in the control was 16.7 mummies per female and ranged between 10.5 and 23.38 in the product treatment groups. Despite these differences, there were no significant reductions in reproductive capacity of *A. rhopalosiphi* in the product treatment groups, compared to the control group. The reproductive NOEC and LOEC (reproduction) values were determined to be 3.4 and >3.4 L product/ha (equivalent to 864.9 and >864.9 g a.s./ha), respectively. The reproductive ER₅₀ value was estimated to be >3.4 L product/ha (equivalent >864.9 g a.s./ha), given that no product treatment had an effect greater than 50% on reproduction.

Validity

All validity criteria were met in accordance with IOBC Mead-Briggs et al. (2000) test guideline:

- Mean mortality in the control to be ≤13 %, after 48 h of exposure (actual value was 3.33%).
- Mean mortality in the reference-item group to be between ≥50% after 48 h of exposure (actual value 75.86%)
- Mean mummy production per female in the control group to be ≥5 (actual value was a mean of 16.79).

CA3301 / JOUST	Page 195 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

- No more than two wasps in the control to produce zero mummies (actual value: all wasps produced at least one mummy).

Conclusion

The toxicity of CA3301 to *Aphidius rhopalosiphi* in an extended laboratory test was studied in accordance with the IOBC Mead-Briggs et al. (2000) test guideline.

The 48-h NOER and LOER (mortality) values were determined to 3.4 and >3.4 L product/ha (equivalent to 864.9 and >864.9 g a.s./ha), respectively. The 48-h LR₅₀ value was estimated to be >3.4 L product/ha (equivalent >864.9 g a.s./ha).

The reproductive 48-h NOER and LOER values were determined to be 3.4 and >3.4 L product/ha (equivalent to 864.9 and >864.9 g a.s./ha), respectively. The reproductive 48-h ER₅₀ value was estimated to be >3.4 L product/ha (equivalent >864.9 g a.s./ha).

This study is considered acceptable.

Study 5

Comments of zRMS:	<p>The study was conducted to the guideline and according to the principles of GLP. All validity criteria were met. One minor deviation was noted without negative impact on the overall outcome of the study:</p> <ul style="list-style-type: none"> - on 05, 06 and from 25 to 26 December 2020, temperatures were outside the required range of 25±2°C. <p>The study is considered to be reliable and suitable for the risk assessment.</p>
-------------------	---

Reference:	KCP 10.3.2/05
Report	<p>CA3301 – An extended laboratory study to determine the acute and sublethal effects on the non-target arthropod <i>Chrysoperla carnea</i> (Neuroptera: Chrysopidae).</p> <p>Fraimout, C., 2021, report no. 029SRFR20C09</p>
Guideline(s):	Yes. IOBC, Vogt et al. (2000)
Deviations:	No
GLP:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive summary

In an extended-laboratory study, the acute and reproductive effects of the product CA3301 on the green lacewing, *Chrysoperla carnea*, were assessed in a rate-response test, in accordance with the IOBC Vogt et al. (2000) test guideline.

CA3301 was evaluated at six treatment rates of 0 (distilled water), 0.22, 0.55, 1.36, 3.4, and 8.5 L product/ha, equivalent to 0, 55.96, 139.9, 345.94, 864.86, and 2162.15 g a.s./ha, plus a toxic reference treatment of dimethoate (27 g dimethoate/ha), sprayed onto the leaves of pepper plants (*Capsicum annuum*).

C. carnea larvae were introduced to test units containing treated pepper plant leaves, with one larva per test

CA3301 / JOUST	Page 196 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

unit (replicate), and 40 replicates per treatment. Mortality was assessed and viable cocoons were transferred to breeding test units, in which reproductive and fertility assessments were made.

Treatment with the three-highest product rates caused significant increases in morality. Thus, the NOER and LOER (mortality) values were determined to be 0.55 and 1.36 L product/ha (equivalent to 139.9 and 345.94 g a.s./ha), respectively. The LR₅₀ value for mortality was calculated to be 1.6 L product/ha, corresponding to 406.98 g a.s./ha.

Results for fecundity and fertility data were judged solely on whether they met or exceeded the criteria of the (Vogt et al., (2000) test guideline. The mean number of eggs laid per female per day in the control treatment was 36.17 (≥ 15) and the percentage of hatched (fertile) eggs in the control was 85.55% ($\geq 70\%$), which satisfy the validity criteria of Vogt et al. (2000) test guideline. The reproductive ER₅₀ was estimated to be >8.5 L product/ha, equivalent to >2162.15 g a.s./ha.

For validation of the test system, treatment with dimethoate resulted in 94.74% pre-imaginal mortality ($\geq 50\%$).

Overall, the study satisfies the IOBC Vogt et al. (2000) test-guideline requirements for an extended-laboratory study on *C. carnea* and is considered acceptable.

Materials and methods

Test materials

Test item

Name:	CA3301
Active substance:	Prothioconazole
Formulation type:	Emulsifiable concentrate (EC)
Product density:	0.9948 g/mL
Source and lot/batch no.:	A19045
Active substance content:	250 g/L (nominal), 257.04 g/L or 25.57% w/w (analysed)
Expiry date of lot/batch:	17 June 2022
Storage conditions:	Stored between 9.2°C and 25.5°C, protected from light

Reference item

Name:	Dimethoate
Purity:	98% (CoA)
Density:	1 g/mL
Batch no.:	D088B191112
Appearance:	Solid
Expiry date of lot/batch:	12 November 2022
Storage conditions:	Stored between 2.4°C and 7.7°C, protected from light

Test organism

Species:	Green lacewing (<i>Chrysoperla carnea</i>)
Age at study initiation:	2-day-old larvae
Source:	BiasLabs Ltd., Kirkcaldy, Fife, UK
Acclimation:	Kept in breeding room, under study conditions (25°C, 60-90% relative humidity)
Feeding during test:	Larvae fed with UV-sterilized mill moth (<i>Ephestia kuehniella</i>) eggs, immediately after exposure and renewed 3x weekly. Adults fed with 15 mL condensed milk, 1 egg, 1 egg yolk, 30 g honey, 20 g fructose, 30 g dried brewer's yeast, 50 g wheat germ and 45 mL distilled water, 3x weekly, along with continuous access to distilled drinking water.

CA3301 / JOUST	Page 197 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Test conditions

Test temperature:	21.9 – 27.8°C
Relative humidity:	46.3– 88.8%
Photoperiod:	16 h light:8 h dark
Light intensity:	Mean of 1086.65 lux

Mortality test units consisted of a glass plate, on which sat a cotton-wool pad. Pepper plant leaves (*Capsicum annuum*) were placed on the pad and were secured with elastic to a plastic ring (diameter 4.3 cm, height 5 cm). Rings were treated with talcum to prevent larvae from climbing. Reproductive test units consisted of a rearing plastic container (1500 to 2500 cm³) covered with a nylon gauze. The bottom of the container was covered up with folded filter paper to encourage eggs laying.

Each treatment comprised 40 replicates (1 test unit per replicate, one lacewing larva per replicate) and there were seven treatment groups: a distilled water control, five product concentrations, and one reference item. The five product groups were 0.22, 0.55, 1.36, 3.4, and 8.5 L product/ha, equivalent to 55.96, 139.9, 345.94, 864.86, and 2162.15 g a.s./ha. The reference item was applied at 27 g dimethoate/ha. Prior to application, stock solutions were made, by mixing 10.57 mL of product in 250 mL of distilled water (equivalent to 2.643 g a.s./L, when accounting for the nominal product a.s. content of 250 g/L), and by mixing 0.0338 g dimethoate in 250 mL of distilled water (135.2 mg dimethoate/L).

Stock solutions were made on the day of the applications. Leaves were sprayed with a laboratory sprayer (DeVries Spray Booth Generation III, nozzles Teejet 8002E) at rate of 200 L/ha and left to dry for approximately 1 h. As soon as the leaves dried, the test units were assembled; then one green lacewing larva was randomly assigned to each test unit. Lacewing larvae were fed with *Ephestia kuehniella* UV-sterilized eggs (source: Crisop and Biobest), sprinkled in a small quantity in each test unit, immediately after exposure and renewed at least 3 times a week, to ensure freshness of eggs.

Cumulative mortality was assessed at least 3x weekly, by counting the number of dead larvae pupae, and dead emerged adults, and non-moulted pupae. Behavioral and morphological abnormalities were also recorded. Mortality data were corrected for control mortality, using a modified Abbott's formula.

Cocoons were collected before adult emergence, but not earlier than 5 days after pupation, to avoid damaging the young pupae. All cocoons from a treatment were split between 2 rearing containers, to avoid overcrowding. Reproductive output was assessed on successfully emerged adult green lacewings from only the control and product treatment groups. There were at least 3 females and 2 males from each treatment group to perform the reproductive assessment. Adults began laying eggs six days after emerging, during which mill moth eggs were added to the test units to avoid cannibalism by emerging green lacewing larvae. Twelve days after the first egg laying was observed, reproduction was assessed, by comparing the number of eggs laid and eggs hatched per viable female. Adults were fed with an artificial diet at least three times a week and distilled water was offered continuously on a cotton plug.

All data were analysed with the statistical software R (version 3.3.2) and performed at $\alpha = 0.05$.

The mortality data were analysed to determine any significant differences between control and product treatments and estimate NOER/LOER values. The mortality data were not monotonic (Spearman's correlation test). Thus, a Fisher's exact pairwise test, with Bonferroni-Holm corrections, was used to determine statistical differences between the treatment groups. The LR₅₀ value and its 95% confidence intervals (CIs) were calculated with the Bayesian inference model MORSE (log-logistic binomial model with 3 parameters).

Fecundity and fertility data were not statistically analysed and were assessed solely on whether they met or exceeded the criteria outlined by the IOBC (Vogt et al., 2000) test guideline (mean number of eggs/female/day ≥ 15 , mean hatching rate $\geq 70\%$).

CA3301 / JOUST	Page 198 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Results

Biological results

Mortality

Mortality of *C. carnea*, 21 days after exposure to the product CA3301, is presented in table CP 10.3.2/05-01, below.

Table CP 10.3.2/05-01. The effect of CA3301 on green lacewing *C. carnea* mortality.

Application rate		DAE	Cumulative mortality	
L product/ha	g a.s./ha		Mean (%)	Corrected (%) [#]
0 (control)	0	1	2.63	0.00
		2	2.63	0.00
		5	2.78	0.00
		7	2.78	0.00
		9	2.78	0.00
		12	2.78	0.00
		13	2.78	0.00
		15	2.78	0.00
		21	8.13	0.00
0.22	55.96	1	2.50	-0.14
		2	2.50	-0.14
		5	2.56	-0.22
		7	2.56	-0.22
		9	2.56	-0.22
		12	2.56	-0.22
		13	2.56	-0.22
		15	2.56	-0.22
		21	2.56	-6.29
0.55	139.9	1	10.26	7.83
		2	10.26	7.83
		5	13.16	10.68
		7	13.16	10.68
		9	13.16	10.68
		12	13.16	10.68
		13	13.16	10.68
		15	13.16	10.68
		21	18.42	11.00
1.36	345.94	1	35.00	33.24
		2	60.53	59.46
		5	72.97	72.20
		7	75.68	74.98
		9	78.38	77.76
		12	78.38	77.76
		13	81.08	80.54
		15	81.08	80.54
		21	81.08*	79.36
3.4	864.86	1	27.78	25.83
		2	30.56	28.68
		5	44.44	42.86
		7	47.22	45.71
		9	47.22	45.71
		12	50.00	48.57
		13	50.00	48.57
		15	52.78	51.43
		21	52.78*	48.48

CA3301 / JOUST	Page 199 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

8.5	2162.15	1	72.97	72.24
		2	86.49	86.12
		5	91.89	91.66
		7	91.89	91.66
		9	91.89	91.66
		12	91.89	91.66
		13	91.89	91.66
		15	91.89	91.66
		21	91.89*	91.15
27 g dimethoate/ha		1	92.11	91.89
		2	92.11	91.89
		5	92.11	91.88
		7	92.11	91.88
		9	94.74	94.59
		12	94.74	94.59
		13	94.74	94.59
		15	94.74	94.59
		21	94.74*\$	94.74

Significant differences in mortality at 21 DAE (days after application), relative to the control, were revealed with a Fisher's exact pairwise test, with Bonferroni-Holm corrections, and denoted with asterisks (*). #Data were corrected for control mortality with a modified Abbott's formula. Positive (+) and negative (-) signs indicate higher and lower mortality, respectively, relative to the control. \$Based on pre-imaginal mortality – the number of dead larvae and pupae – rather than cumulative mortality.

Mean mortality was 8.13% in the control and ranged from 91.15 to -6.29% in the product treatment groups. Treatment with three highest product rates caused significant increases in mortality. Thus, the NOER and LOER (mortality) values were determined to be 0.55 and 1.36 L product/ha (equivalent to 139.9 and 345.94 g a.s./ha), respectively. The LR₅₀ value for mortality was calculated to be 1.6 L product/ha, corresponding to 406.98 g a.s./ha (CI: 297.60 – 582.4 g a.s./ha).

Reproduction

The effects on the reproduction capacity of *C. carnea* after exposure to the product CA3301 are presented in Table CP 10.3.2/05-2 below.

CA3301 / JOUST	Page 200 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Table CP 10.3.2/05-02. The effect of CA3301 on green lacewing (*C. carnea*) reproduction (fecundity).

Application rate		DAE	No. of live		Reproduction		
L product/ha	g a.s./ha		Males	Females	Eggs (no.)	Mean \pm SD (eggs/female/day)*	Change (% control)#
0 (control)	0	33	13	15			
		34	10	15	521		
		35	8	13	489	36.17 \pm 2.04	0.00
0.22	55.96	33	14	16			
		34	14	15	379		
		35	12	13	623	36.59 \pm 16.02	+1.16
0.55	139.9	33	8	7			
		34	8	7	169		
		35	8	7	162	23.64 \pm 0.71	-34.64
1.36	345.94	33	1	5		Not evaluated ^{\$}	
		34	1	5			
		35	1	5			
3.4	864.86	33	5	8			
		34	4	8	216		
		35	3	7	231	30 \pm 4.24	-17.07
8.5	2162.15	33	2	1			
		34	2	1	54		
		35	2	1	53	53.5 \pm 0.71	+47.89
27 g dimethoate/ha		33				Not evaluated ^{\$}	
		34					
		35					

*Based on the mean number of eggs laid per female per day. #Data were corrected for control reproduction. Positive (+) and negative (-) signs indicate higher and lower reproductive effects, respectively, relative to the control. \$Data not evaluated, given that there were insufficient numbers of males and females to assess reproductive output. DAE, days after exposure.

The mean number of eggs laid per female per day in the control was 36.17. As the fecundity data in all the product groups were above the criterion outlined by Vogt *et al.* (2000) – mean number of eggs/female/day ³ 15 – fecundity was not considered affected by any product treatment. The reproductive ER₅₀ was estimated to be >8.5 L product/ha, equivalent to >2162.15 g a.s./ha.

Fertility

A summary of the effects of CA3301 on fertility of the green lacewing (*C. carnea*) is presented in the Table CP 10.3.2/05-03 below.

Table CP 10.3.2/05-03. The effect of CA3301 on green lacewing, (*C. carnea*) fertility, 40 days after exposure.

Application rate		Fertility (hatched eggs/female/day)	
L product/ha	g a.s./ha	%*	Change (% control)#
0 (control)	0	85.55	NA
0.22	55.96	79.58	-6.97
0.55	139.90	80.08	-6.40
1.36	345.94	NA	
3.40	864.86	78.93	-7.74
8.50	2162.15	NA	

*Based on the mean number of eggs hatched per female per day. #Data were corrected for control reproduction. Positive (+) and negative (-) signs indicate higher and lower fertility, respectively, relative to the control. NA, not applicable, given that there were insufficient numbers of males and females to assess reproductive output.

The mean percentage of fertile eggs per female per day in the control was 85.55%. As the fertility data in

CA3301 / JOUST	Page 201 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

all the product group were above the criterion outlined by Vogt et al. (2000) – mean egg hatching rate \geq 70% – fertility was not considered affected by any product treatment.

Validity

All validity criteria were met in accordance with IOBC Vogt et al. (2000) test guideline:

- Cumulative mortality in the control to be \leq 20% (actual value was 8.33%).
- Cumulative pre-imaginal mortality for the toxic reference item to be \geq 50% (actual value: 94.59%).
- Mean of eggs per female per day in the control to be \geq 15 (actual value 36.17).
- Mean egg hatching in the control to be \geq 70% (actual value was 85.55%).

Conclusion

The extended laboratory study of CA3301 on *Chrysoperla carnea* was studied in accordance with the IOBC Vogt et al. (2000) test guideline.

The 7-day NOER and LOER values for mortality were determined to be 0.55 and 1.36 L product/ha (equivalent to 139.9 and 345.94 g a.s./ha), respectively. The LR₅₀ value for mortality was calculated to be 1.6 L product/ha, corresponding to 406.98 g a.s./ha.

The mean number of eggs laid per female per day in the control treatment was 36.17 and the percentage of hatched (fertile) eggs in the control was 85.55%, which satisfy the validity criteria of the IOBC Vogt et al., (2000) test guideline. The reproductive ER₅₀ was estimated to be >8.5 L product/ha, equivalent to >2162.15 g a.s./ha.

This study is considered acceptable.

CA3301 / JOUST	Page 202 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Study 6

Comments of zRMS:	<p>The study was conducted to the guideline and according to the principles of GLP. All validity criteria were met. One minor deviation was noted without negative impact on the overall outcome of the study:</p> <ul style="list-style-type: none"> - following technical issues, there were several deviations for relative humidity during the test (actual min. value 40.4%). <p>The study is considered to be reliable and suitable for the risk assessment.</p>
-------------------	---

Reference:	KCP 10.3.2/06
Report	<p>CA3301 – An extended laboratory study to evaluate potential adverse effects on the rove beetles <i>Aleochara bilineata</i> (Coleoptera: Staphylinidae)</p> <p>Frainout, C., 2021, report no. 029SRFR20C08</p>
Guideline(s):	Yes. IOBC, Grimm et al., 2000
Deviations:	No
GLP:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive summary

In an extended-laboratory study, the reproductive effects of the product CA3301 on the rove beetle, *Aleochara bilineata*, was evaluated. The study consisted of six treatment rates of 0 (water control), 0.09, 0.22, 0.55, 1.36, and 3.4 L product/ha, equivalent to 0, 22.9, 55.9, 139.9, 345.9, and 864.9 g a.s./ha, applied to the soil of exposure test units. Each test unit represented a replicate and there were four replicates per treatment, consisting of 10 male and 10 female adult beetles per replicate. 7, 14, and 21 days after adult beetles were exposed, onion fly (*Delia antiqua*) pupae were mixed into the soil, to act as host organisms for the beetle larvae. Adult beetle mortality was assessed after 28 days, and the parasitized pupae were transferred to hatching test units to assess beetle emergence (offspring production) from 77 to 91 days after exposure.

Exposure to CA3301 resulted in a significant increase in mortality only with the third-highest product treatment (345.0 g a.s./ha). Mean mortality of the control group was 6.67% (0% corrected), whereas mean mortality in the product treatments ranged from 0.00 to 18.75% (-6.67 to 13.33% corrected). Thus, the NOER and LOER (mortality) values were determined to 0.22 and 0.55 L product/ha (equivalent to 55.9 and 139.9 g a.s./ha), respectively. Given that no product treatment caused >50% mortality, the LR₅₀ value was estimated to be >3.4 L product/ha (equivalent >864.9 g a.s./ha), the highest rate tested.

Treatment with CA3301 did not cause significant changes in emergence (reproduction/fecundity), 91 days after exposure. The mean number of offspring per beetle was 23.53 in the control group and ranged between 22.75 and 24.81 in the product treatment groups. Given the lack of significant differences in reproduction and that no product treatment caused >50% change in reproduction, the NOER, LOER, and ER₅₀ (reproduction) values were all estimated to be >3.4 L product/ha (equivalent to >864.9 g a.s./ha), the highest rate tested.

Overall, the study satisfies the IOBC Grimm et al. (2000) test-guideline requirements for an extended-laboratory study with *Aleochara bilineata* and is considered acceptable.

CA3301 / JOUST	Page 203 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Materials and methods

Test materials

Test item

Name:	CA3301
Active substance:	Prothioconazole
Formulation type:	Emulsifiable concentrate (EC)
Source and lot/batch no.:	A19045
Product density:	0.9948 g/mL (nominal)
Active substance content:	250 g/L (nominal), 257.04 g/L or 25.57% w/w (analysed)
Appearance:	Clear, straw-coloured liquid
Expiry date of lot/batch:	17 June 2022
Storage conditions:	Stored between 8.5°C and 25.5°C, and protected from light

Reference item

Name:	Dimethoate
Formulation type:	Analytical standard
Density:	1 g/mL
Batch no.:	D088B191112
Active-substance purity:	98% (analysed)
Appearance:	Solid

Test organism

Species:	Rove beetle, <i>Aleochara bilineata</i> (source: De Groene Vlieg).
Age at study initiation:	Adults, 1-to-3-days-old
Sex:	Mixed sex
Feeding during test:	Adult rove beetles were supplied with red mosquito larvae, starting 1 hour after exposure to the end of the exposure phase, every 1 to 3 days, placed on the surface of the substrate.

Test substrate

Soil type:	Standard sandy soil
Source:	Natural soil LUFA 2.1
Batch:	Sp 2.1 1420
Maximum WHC:	35 ± 5% (actual WHC value: 35%)

Test conditions

Test temperature:	13.6 – 23.0°C*
Relative humidity:	40.4 – 100.0%
Photoperiod:	16 h light:8 h dark
Light intensity:	1422.7 lux (mean)

*Test temperature deviated from the guideline recommendation of 20 ± 2°C for less than 2 h and did not affect the outcome of the study.

The test units used in the exposure phase consisted of a plastic container (>150cm² and >600 cm³), filled with a 4-cm layer of moistened soil. The soil placed in the test units was standard sandy soil and moistened to 35 ± 5% of its maximum water-holding capacity (WHC). Each test unit was enclosed in nylon gauze to allow ventilation. Each test unit represented a replicate and there were four replicates per treatment, consisting of 10 male and 10 female beetles per replicate.

The test units used in the hatching phase consisted of a vessel with a sieve bottom (mesh size approximately 2 mm) and a second vessel below (as a Berlese device without additional light). Pupae were placed onto the sieve, and emerging rove beetles fell through the mesh and were collected in the bottom vessel. Each

CA3301 / JOUST	Page 204 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

hatching test unit also represented a replicated and contained all the pupae recovered from an exposure test unit, so that there were 4 replicates per treatment.

The study consisted of seven treatment groups: a distilled water control, five product treatments, and a toxic reference treatment (dimethoate). The application rates used in the study were 0 (control), 0.09, 0.22, 0.55, 1.36, and 3.4 L product/ha, equivalent to 0, 22.9, 55.9, 139.9, 345.9, and 864.9 g a.s./ha. The product spray solutions were made by diluting a stock solution; the stock solution was made by dissolving 2.35 g of product in 300 mL of distilled water. The reference-item treatment was applied at a rate of 440 g dimethoate/ha. The stock reference-item solution was made by dissolving 109.8 mg in 100 mL of distilled water. The concentrations in all application solutions were based on the spray volume of 400 L/ha, applied with a laboratory sprayer (DeVries Spray Booth Generation III, nozzles Teejet 8002E).

For the exposure phase, adult beetles were added to the test units for a 28-day exposure, during which time they mated to produce parasitic larval offspring. After 7, 14, and 21 day of exposure, onion fly (*Delia antiqua*) pupae were mixed into the soil, to act as host organisms for the beetle larvae. After 28 days, the surviving adult beetles were removed, and their mortality assessed. The parasitized fly pupae were left in the soil for an additional week, during which the soil was allowed to dry. The hatching phase started by removing parasitized pupae from the dried soil with a sieve and placing them in the hatching test units. Emergence of beetles from the pupae was assessed 3x weekly from 77 to 91 days after exposure.

Data was analysed with the statistical software R (version 3.3.1). All statistical analyses were performed at $\alpha = 0.05$.

Mortality data were corrected for the mortality in the control group, using a modified Abbott's formula. The data were initially analyzed for monotonicity, using Spearman's correlation, which revealed non-monotonic data. Therefore, differences in mortality between the product treatments and the control were statistically evaluated using a unilateral Fisher's exact pairwise test, with Bonferroni-Holm correction. The NOER and LOER for mortality were determined according to the results of the statistics.

The mean number of beetles emerging from the fly pupae per test unit was calculated for each treatment. Moreover, the average number of beetles emerging from the fly pupae per adult beetle initially introduced in the test units was calculated for each treatment. The results in the product treatments were compared with results of the control data. Reproductive data were normally distributed (Shapiro-Wilk's test) and had equal variances (Bartlett's test), however, a Spearman's correlation test revealed that the reproductive data were not monotonic. Thus, data were analyzed for statistical significance using a single-step test ANOVA, with Dunnett's test corrections. The NOER and LOER values for reproduction were determined according to the results of the statistics.

Results

Biological results

Mortality

The effects of CA3301 on adult mortality, after 28 days of exposure, are presented in the Table CP 10.3.2/06-01 below.

CA3301 / JOUST	Page 205 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Table CP 10.3.2/06-01. The effect of CA3301 on adult *A. bilineata* mean mortality, after 28 days of exposure (N = 10 male and 10 female adult beetles per replicate).

Application rate		Rep.	Mortality			
L product/ha	g a.s./ha		No.	%	Mean (%)	Corrected (%) [#]
0 (control)	0	1	2	10	6.25	0
		2	0	0		
		3	0	0		
		4	0	0		
0.09	22.9	1	3	15	12.5	+6.67
		2	1	5		
		3	0	0		
		4	6	30		
0.22	55.9	1	0	0	0	-6.67
		2	0	0		
		3	0	0		
		4	0	0		
0.55	139.9	1	0	0	18.75*	+13.33
		2	5	25		
		3	2	10		
		4	8	40		
1.36	345.9	1	2	10	12.5	+6.67
		2	2	10		
		3	2	10		
		4	4	20		
3.4	864.9	1	3	15	11.25	+5.33
		2	2	10		
		3	2	10		
		4	2	10		
440 g dimethoate/ha		1	18	90	98.75	+98.67
		2	19	95		
		3	20	100		
		4	20	100		

Significant differences in mortality, relative to the control, were revealed with a Fisher's exact pairwise test, with Bonferroni-Holm corrections and are denoted with an asterisk (*). [#]Data corrected for control mortality using the Abbott formula. Positive (+) and negative (-) signs indicate an increase and decrease in mortality, respectively, relative to the control. Rep., replicate; No., number.

Exposure to CA3301 resulted in a significant increase in mortality only with the third-highest product treatment (345.0 g a.s./ha). Mean mortality of the control group was 6.67% (0% corrected), whereas mean mortality in the product treatments ranged from 0.00 to 18.75% (-6.67 to 13.33% corrected). Thus, the 28-day NOER and LOER (mortality) values were determined to 0.22 and 0.55 L product/ha (equivalent to 55.9 and 139.9 g a.s./ha), respectively. Given that no product treatment caused >50% mortality, the LR₅₀ value was estimated to be >3.4 L product/ha (equivalent >864.9 g a.s./ha), the highest rate tested.

Reproduction

The effects of CA3301 on the reproductive capacity of *A. bilinata*, 91 days after exposure, are presented in the Table CP 10.3.2/06-02 below.

CA3301 / JOUST	Page 206 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Table CP 10.3.2/06-02. The effect of CA3301 on rove beetle, *A. Bilineata*, reproductive capacity (fecundity), 91 days after exposure (N = 10 male and 10 female adult beetles per replicate).

Application rate		Rep.	Fecundity (number of offspring)			
L product/ha	g a.s./ha		No.	Mean	Mean number/beetle*	Change in fecundity (%) [#]
0 (control)	0	1	494	470.5	23.53	0
		2	341			
		3	393			
		4	654			
0.09	22.9	1	551	455	22.75	-3.29
		2	411			
		3	322			
		4	536			
0.22	55.9	1	451	452.25	22.61	-3.88
		2	402			
		3	531			
		4	425			
0.55	139.9	1	591	453.75	22.69	-3.56
		2	482			
		3	332			
		4	410			
1.36	345.9	1	636	496.25	24.81	+5.47
		2	419			
		3	601			
		4	329			
3.4	864.9	1	432	456	22.8	-3.08
		2	515			
		3	409			
		4	468			
440 g dimethodate/ha		1	21	7.25	0.36	-98.46
		2	0			
		3	0			
		4	8			

*No significant difference in reproduction were revealed with an ANOVA. [#]Positive (+) and negative (-) signs indicate an increase and decrease in fecundity, relative to the control, respectively. Rep., replicate; No., number.

After 91 days after exposure, treatment with CA3301 did not cause significant changes in reproductive capacity. The mean number of offspring per beetle was 23.53 in the control group and ranged between 22.75 and 24.81 in the product treatment groups. Given the lack of significant differences in reproduction and that no product treatment caused >50% change in reproduction, the NOER, LOER, and ER₅₀ (reproduction) values were all estimated to be >3.4 L product/ha (equivalent to >864.9 g a.s./ha), the highest concentration rate tested.

Validity

All validity criteria were met in accordance with IOBC Grimm et al. (2000) test guideline:

- Mean offspring production (emergence) in the control group should be ≥ 400 (actual value: 470.5).
- The reference-item treatment should reduce reproductive capacity (emergence) by $\geq 50\%$ corrected (actual value: 98.46%)

Conclusion

The toxicity of CA3301 to *Aleochara bilineata* in an extended laboratory test was evaluated in accordance with the IOBC (Grimm *et al.*, (2000)) test guideline.

CA3301 / JOUST	Page 207 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

The NOER and LOER (mortality) values were determined to 0.22 and 0.55 L product/ha (equivalent to 55.9 and 139.9 g a.s./ha), respectively. The LR₅₀ value was estimated to be >3.4 L product/ha (equivalent >864.9 g a.s./ha), the highest rate tested.

The NOER, LOER, and ER₅₀ (reproduction) values were all estimated to be >3.4 L product/ha (equivalent to >864.9 g a.s./ha).

This study is considered acceptable.

Study 7

Comments of zRMS:	The study was conducted to the guideline and according to the principles of GLP. All validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.
-------------------	---

Reference:	KCP 10.3.2/07
Report	CA3301– A Series of Aged-Residue Extended Laboratory Tests to Determine Effects on the Predatory Mite <i>Typhlodromus pyri</i> (Acari: Phytoseiidae) Fallowfield, L., 2021, report no. NUF-21-01
Guideline(s):	Yes. IOBC, Blümel, S. et al. (2000)
Deviations:	No
GLP:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive summary

In a 14-day age-residue, extended-laboratory study, the effects of the product CA3301 on *Typhlodromus pyri* mortality and reproduction were assessed in a limit test, in accordance with the Blümel *et al.* (2000) IOBC guideline. The study comprised a bioassay with freshly dried residues of CA3301 and a bioassay with 7-day field-aged residues of CA3301.

CA3301 was evaluated at a single application rate, intended to be equivalent to 800 mL test item/ha. However, the dilution of the test item was calculated using the nominal density and, thus, the actual application rate was 741.3 mL test item/ha, equivalent to 191.3 g a.s./ha (0.922 g test item dissolved in 500 mL of purified water). The toxic-reference treatment was applied at 60 mL product/ha, equivalent to 24 g dimethoate/ha.

T. pyri protonymphs were introduced to test units containing treated French bean leaves (*Phaseolus vulgaris* L.). Mortality was assessed on days 1 and 7 after exposure and reproduction was assessed on days 10, 13, and 14. Each test unit was considered a replicate, with five replicates per test treatment, each containing 20 protonymphs of *T. pyri* (<24-hours old).

When applied to dwarf French bean plants, at a rate of 741.3 mL test item/ha (equivalent to 191.3 g a.s./ha), freshly dried residues and 7-day field-aged residues resulted in no unacceptable effects on either the survival or the subsequent reproductive capacity of the mites, (i.e., <50% corrected mortality and <50% reduction in reproduction, relative to the control).

CA3301 / JOUST	Page 208 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Overall, the study satisfies the IOBC Blümel (2000) test-guideline requirements for an age-residue, extended-laboratory study on *Typhlodromus pyri* and is considered acceptable.

Materials and methods

Test materials

Product

Name:	CA3301
Active substance:	Prothioconazole
Formulation type:	Emulsifiable concentrate (EC)
Product density:	0.922 g/mL (nominal), 0.995 g/mL (analysed)
Source and lot/batch no.:	A21001
Active substance content:	250 g/L (nominal), 258 g/L or 25.93% w/w (analyzed)
Expiry date of lot/batch:	January 2023
Appearance:	Clear straw/yellow-coloured liquid
Storage conditions:	Stored at ambient laboratory conditions

Reference item

Name:	BAS 152 65 I
Active substance:	Dimethoate
Formulation type:	Emulsifiable concentrate (EC)
Active substance content:	400 g/L (nominal)
Batch no.:	FRE-001793
Expiry date of lot/batch:	31 March 2022

Test organism

Species:	<i>Typhlodromus pyri</i>
Age at study initiation:	Protonymphs (approx. 24-hours-old post-hatch)
Source:	Obtained from a culture maintained at the Test Facility
Feeding during test:	1:1 v/v mixture of almond (<i>Prunus</i> sp. var. a mix of Aldrich, Nonpareil and Wood Colony) and apple (<i>Malus</i> sp. var. Red Delicious) pollen

Test conditions

<i>0-DAT Bioassay</i>	
Test temperature:	24.4-26.1°C
Relative humidity:	61-78%
Photoperiod:	16 h light:8 h dark
Light intensity:	400-1350 lux

<i>7-DAT Bioassay</i>	
Test temperature:	24.4-26.2°C
Relative humidity:	61-74%
Photoperiod:	16 h light:8 h dark
Light intensity:	800-1350 lux

The test substrate comprised excised leaves of the dwarf French bean plant (*Phaseolus vulgaris* L., var. “The Prince”). For use in each bioassay, discs were cut from flattened sections of treated bean leaves, (approximate diameter of 5 cm), onto which spray applications were made.

The test arenas comprised plastic Petri dish bases (9 cm in diameter), lined with water-saturated cotton wool. The treated leaf discs were laid on the cotton wool, with their sprayed, adaxial surfaces facing upwards. The edges of each disc were gently pushed down to ensure contact with the cotton wool. One end of a 5-mm-wide strip of ‘Benchkote’ (Whatman International Ltd., Maidstone, England) was laid onto the

CA3301 / JOUST	Page 209 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

leaf and the other end was extended onto the cotton wool, to provide the mites with a source of drinking water. A barrier of sticky gel, placed on the edge of a leaf disc, prevented the dispersal of the mites (approximately 4 cm in diameter, enclosing an area of ca. 12.5 cm²). Following the placement of the mites, a 5-cm high, clear plastic collar was placed around each arena to reduce airflow.

For the reproduction assessments [made 7-14 days after initiation (DAI)], the mites were left *in situ* on the test arenas described above.

In each bioassay, there were 5 replicate arenas per treatment, each containing 20 mites (total of 100 mites per treatment).

Treatments were applied to the bean plants using a laboratory track-sprayer (Chr. Schachtner, Ludwigsburg, Germany). The spray pressure was set to approximately 3 bar and a flat-fan nozzle (Teejet 8004 EVS) was used. The sprayer was calibrated in advance of the test applications using purified water, to confirm a deposition rate equivalent to 400 L/ha (i.e., 4 mg deposit/cm², with an actual range of $\pm 10\%$ of the target rate and a mean range of $\pm 5\%$ of the target rate).

Treatments were applied in the order of the control, test item, and, finally, the toxic reference (T2 application only). For each bioassay, leaf discs were cut from the treated first true leaves and used for the preparation of the test arenas. For the 0-DAT₂ bioassay, the leaves were used once residues had dried on the freshly sprayed plants (within 1 h of treatment).

CA3301 was evaluated at a single application rate, intended to be equivalent to 800 mL test item/ha. However, the dilution of the test item was calculated using the nominal density and, thus, the actual application rate was 741.3 mL test item/ha, equivalent to 191.3 g a.s./ha (0.922 g test item dissolved in 500 mL of purified water). The toxic-reference treatment was applied at 60 mL product/ha, equivalent to 24 g dimethoate/ha.

For all treatments, the numbers of male and female mites in each replicate were recorded at 7 DAI (females appear significantly larger and more rounded than males). Where necessary male mites were moved between replicates within treatments to ensure a male to female ratio of at least one male per five females. Any eggs that were produced prior to 7 DAI were discarded. Untreated fruit tree pollen (ca. 1 mg per arena) was added as food and was replenished every day during the following week.

Over 7 days, the total egg production (number of eggs plus live and dead juvenile stages) were determined for each arena and the mean egg production per female was calculated. Three assessments of oviposition activity were carried out during the assessment period. On each assessment date, any eggs and larvae/nymphs present were recorded separately and then removed. In addition, the condition and number of live female and male mites in each arena were assessed.

The condition of the mites was assessed with the aid of a binocular microscope at 1 and 7 DAI. They were recorded as being:

- Alive: still moving
- Dead: no sign of movement
- Stuck: embedded in the sticky barrier
- Drowned: dead on the water supply

The numbers of any drowned, stuck, or missing mites were added to the number of dead mites found in each treatment to derive the overall mortality. For each bioassay, mean mortality after 7 days was calculated for each treatment and then corrected for any losses in the control treatment using Abbott's formula.

For the reproduction data in each bioassay, the mean cumulative number of eggs per surviving female was determined for the period 7-14 DAI. To calculate this value, the total number of eggs laid in each replicate between each assessment date was divided by half of the sum of the numbers of female mites recorded as alive at the start and end of each assessment period (see formula below). Any progeny recorded as larvae/nymphs (i.e., eggs that had presumably been missed in a previous assessment and had subsequently

CA3301 / JOUST	Page 210 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

hatched) were added to the egg totals from the previous assessment period (as per Blümel et al., 2000).

Data were analysed using ToxRatPro® (ToxRat Solutions GmbH, 2018). ToxRat output is provided in Appendix X. Mortality in each treatment was compared to the respective control using a one-sided χ^2 2x2 table test ($\alpha = 0.05$). The reproduction data (mean number of eggs per female in each replicate) were checked for normality using the Shapiro-Wilk test and for homogeneity of variance using Levene's test. The test-item treatment was then compared to the respective control using a one-sided Student's t-test for equal variances ($\alpha = 0.05$).

Results

Biological results

The effect of CA3301 on mortality in the 0- and 7-DAT₂ bioassays, after 7 days of exposure, is presented in Tables CP 10.3.2/07-01 and CP 10.3.2/07-02 below.

Mortality in the 0-DAT Bioassay

Table CP 10.3.2/07-01. The effect of CA3301 on *T. pyri* mortality in the 0-DAT₂ bioassay, after 7 days of exposure.

Treatment rate		Replicate	1 DAI		7 DAI		7-day mortality	
mL/ha	g a.s./ha		Live	Dead	Live	Dead	Mean (%)	Corrected (%) [#]
0.0	0.0	1	20	0	20	0	2.0	0.0
		2	20	0	20	0		
		3	20	0	20	0		
		4	20	0	20	0		
		5	20	0	18	2		
		Total	100	0	98	2		
741.3	191.3	1	19	1	19	1	11.0*	+9.2
		2	17	3	17	3		
		3	19	1	19	1		
		4	16	4	16	4		
		5	20	0	18	2		
		Total	91	9	89	11		
60.0	24.0	1	0	20	0	20	100.0*	+100.0
		2	0	20	0	20		
		3	0	20	0	20		
		4	2	18	0	20		
		5	1	19	0	20		
		Total	3	97	0	100		

Significant differences in mortality, relative to the control, were revealed with a one-sided χ^2 2x2 table test ($\alpha = 0.05$) and are denoted with asterisks (*). [#]Mortality corrected by control mortality, using Abbott's formula; positive values indicate an increase in mortality, relative to the control. DAI, days after initiation.

In the bioassay initiated at 0 DAT₂, there was 2% mortality in the control treatment at 7 days, compared with 11% mortality in the 741.3 mL/ha treatment rate of CA3301. When taking into account the deaths in the control, the corrected mortality was 9.2% in the test-item treatment. Statistically, this result differed significantly from the control (one-sided χ^2 2x2 table test). In the toxic reference treatment, 100% mortality (100% corrected mortality) was recorded at 7 days, which met the validity criterion imposed for this treatment.

Mortality in the 7-DAT Bioassay

The effect of CA3301 on mortality, after 7 days of exposure, is presented in Table CP 10.3.2/07-01 below.

CA3301 / JOUST	Page 211 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Table CP 10.3.2/07-02. The effect of CA3301 on *T. pyri* mortality in the 7-DAT₂ bioassay, after 7 days of exposure.

Treatment rate		Replicate	1 DAI		7 DAI		7-day mortality	
mL/ha	g a.s./ha		Live	Dead	Live	Dead	Mean (%)	Corrected (%) [#]
0.0	0.0	1	20	0	19	1	7.0	0.0
		2	20	0	20	0		
		3	20	0	16	4		
		4	20	0	20	0		
		5	20	0	18	2		
		Total	100	0	93	7		
741.3	191.3	1	20	0	18	2	6.0	-1.1
		2	20	0	19	1		
		3	20	0	20	0		
		4	20	0	20	0		
		5	20	0	17	3		
		Total	100	0	94	6		

[#]Mortality corrected by control mortality, using Abbott's formula; positive values indicate an increase in mortality, relative to the control. DAI, days after initiation.

In the bioassay initiated at 7 DAT, there was 7% mortality in the control treatment at 7 days, compared with 6% mortality in the 741.3 mL/ha treatment rate of CA3301. When taking into account mortality in the control, the corrected mortality was - 1.1% in the test-item treatment. Statistically, this result did not differ significantly from the control (one-sided χ^2 2x2 table test).

Reproduction

The effect of CA3301 on reproduction in the 0- and 7-DAT₂ bioassays, after 14 days of exposure, is presented in Tables CP 10.3.2/07-03 and CP 10.3.2/07-04 below.

Reproduction in the 0-DAT Bioassay

Table CP 10.3.2/07-03. The effect of CA3301 on *T. pyri* reproduction (fecundity) in the 0-DAT₂ bioassay, after 14 days of exposure.

Test-item rate		Rep.	7 DAI		14 DAI				14-day mean reproduction	
mL/ha	g a.s./ha		♀	♂	♀	♂	Eggs	Ny.	eggs/female	Inhibition (% control) [#]
0.0	0.0	1	17	4	17	4	23	2	9.8	0.0
		2	15	5	13	5	15	2		
		3 ^s	14	5	12	5	15	0		
		4	16	4	15	4	12	3		
		5	14	4	14	4	11	7		
		Total	76	22	71	22	76	14		
741.3	191.3	1	14	5	13	5	15	6	8.7*	+11.9
		2	12	5	11	4	19	2		
		3	12	7	10	7	9	3		
		4	12	4	11	3	9	1		
		5	13	5	13	5	13	4		
		Total	63	26	58	24	65	16		

Significant differences in reproduction, relative to the control, were revealed with a one-sided Student's t-test ($\alpha = 0.05$) and are denoted with asterisks (*). [#]Positive values indicate a decrease in mean reproduction, relative to the control. ^sMale mites were moved at 7 days after treatment from replicate 3 to replicate 1, to achieve a 1 male: 5 female ratio. DAI, days after initiation; Rep., replicate; ♀, female; ♂, male; Ny., larvae/nymphs.

In the bioassay initiated at 0 DAT, the mean number of eggs per female was 9.8 in the control, compared with 8.7 in the 741.3 mL/ha treatment rate of CA3301. The reduction in reproduction was equivalent to 11.9%, compared to the control. Statistically, this result differed significantly when compared with the control (one-sided Student's t-test).

Reproduction in the 7-DAT Bioassay

Table CP 10.3.2/07-04. The effect of CA3301 on *T. pyri* reproduction (fecundity) in the 7-DAT₂ bioassay, after 14 days of exposure.

Test-item rate		Rep.	7 DAI		14 DAI				14-day mean reproduction	
mL/ha	g a.s./ha		♀	♂	♀	♂	Eggs	Ny.	eggs/female	Inhibition (% control)
0.0	0.0	1	9	10	8	10	4	5	8.8	0.0
		2	11	9	9	9	13	12		
		3	10	6	7	6	6	2		
		4	11	9	10	9	5	11		
		5	10	8	8	5	8	5		
		Total	51	42	42	39	36	35		
741.3	191.3	1	8	10	6	10	11	3	8.1	+7.5
		2	8	11	7	11	9	5		
		3	8	12	5	12	8	5		
		4	7	13	6	12	4	2		
		5	10	7	9	6	16	0		
		Total	41	53	33.0	51	48	15		

Significant differences in reproduction, relative to the control, were revealed with a one-sided Student's t-test ($\alpha = 0.05$) and are denoted with asterisks (*). *Positive values indicate a decrease in mean reproduction, relative to the control. DAI, days after initiation; Rep., replicate; ♀, female; ♂, male; Ny., larvae/nymphs.

In the bioassay initiated at 7 DAT₂, the mean number of eggs per female was 8.8 in the control, compared with 8.1 in the 741.3 mL/ha treatment rate of CA3301. The reduction in reproduction was equivalent to 7.5%, compared to the control. Statistically, this result did not differ significantly when compared with the control (one-sided Student's t-test).

Validity

All validity criteria were met in accordance with IOBC Blümel (2000) test guideline:

- Mean mortality in the control to be $\leq 20\%$, after 7 days' exposure, in each bioassay (actual values: 2.0% and 7.0%, in the 0- and 7-DAT bioassays, respectively).
- Mean corrected mortality in the toxic-reference group to be $\geq 50\%$, after 7 days' exposure (actual value: 100.0%)
- Cumulative mean number of eggs per female in the control group (from day 7 to day 14) to be ≥ 4 eggs/female, in each bioassay (actual values: 9.8 and 8.8, in the 0- and 7-DAT bioassays, respectively).

Conclusion

The effects of freshly dried and field-aged foliar residues of CA3301 on the predatory mite *Typhlodromus pyri* were evaluated in a series of extended laboratory tests. When applied to dwarf French bean plants at a rate of 741.3 mL test item/ha (equivalent to 191.3 g a.s./ha), freshly dried residues and 7-day field-aged residues resulted in no unacceptable effects on either the survival or the subsequent reproductive capacity of the mites, (i.e., $<50\%$ corrected mortality and $<50\%$ reduction in reproduction, relative to the control).

This study is considered acceptable.

CA3301 / JOUST	Page 213 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Study 8

Comments of zRMS:	The study was conducted to the guideline and according to the principles of GLP. All validity criteria were met. One minor deviation was noted without negative impact on the overall outcome of the study: The study is considered to be reliable and suitable for the risk assessment.
-------------------	---

Reference:	KCP 10.3.2/08
Report	CA3301 – A Series of Aged-Residue Extended Laboratory Tests to Determine Effects on the Green Lacewing, <i>Chrysoperla carnea</i> (Neuroptera, Chrysopidae) Vaughan, R., 2021, report no. NUF-21-02
Guideline(s):	Yes. IOBC, Vogt. et al. (2000)
Deviations:	No
GLP:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive summary

In a 14-day age-residue, extended-laboratory study, the effects of the product CA3301 on *Chrysoperla carnea* mortality and reproduction were assessed in a limit test, in accordance with the Blümel *et al.* (2000) IOBC guideline. The study comprised a bioassay with freshly dried residues of CA3301 and a bioassay with 7-day field-aged residues of CA3301.

CA3301 was evaluated at a single application rate, intended to be equivalent to 800 mL test item/ha. However, the dilution of the test item was calculated using the nominal density and, thus, the actual application rate was 741.3 mL test item/ha, equivalent to 191.3 g a.s./ha (0.922 g test item dissolved in 500 mL of purified water). The toxic-reference treatment was applied at 60 mL product/ha, equivalent to 24 g dimethoate/ha. Treatments were applied twice, with a 14-day interval in between.

The bioassay commenced after the second application and continued until residues no longer resulted in unacceptable effects (i.e., where corrected mortality was < 50% and that certain fecundity and fertility criteria were met).

When applied to dwarf French bean plants on two occasions, with a 14-day interval, at a rate of 800 mL test item/ha, equivalent to 206.4 g a.s./ha, fresh (0-day-old) and 7-day-old aged-residues showed no unacceptable effects on lacewing survival, or on the subsequent reproductive capacity of adult lacewings.

Overall, the study satisfies the IOBC Vog et al. (2000) test-guideline requirements for an age-residue, extended-laboratory study on *Chrysoperla carnea* and is considered acceptable.

Materials and methods

Test materials

Product

Name: CA3301
Active substance: Prothioconazole

CA3301 / JOUST	Page 214 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Formulation type: Emulsifiable concentrate (EC)
 Product density: 0.992 g/mL (nominal), 0.995 g/mL (analysed)
 Source and lot/batch no.: A21001
 Active substance content: 250 g/L (nominal), 258 g/L or 25.93% w/w (analyzed)
 Expiry date of lot/batch: January 2023
 Appearance: Clear straw/yellow-coloured liquid
 Storage conditions: Stored at ambient laboratory conditions

Reference item

Name: BAS 152 65 I
 Active substance: Dimethoate
 Formulation type: Emulsifiable concentrate (EC)
 Active substance content: 400 g/L (nominal)
 Batch no.: FRE-001793
 Expiry date of lot/batch: 31 March 2022

Test organism

Species: *Chrysoperla carnea*
 Age at study initiation: 2-to-3-day-old larvae
 Source: Obtained from a culture maintained at the Test Facility
 Feeding during test: Every 2-3 days, with UV-light-killed eggs of the grain moth (*Sitotroga cerealella*)

Test conditions

0-DAT Bioassay

Test temperature: 23.8–25.7°C
 Relative humidity: 70-84%
 Photoperiod: 16 h light:8 h dark
 Light intensity: 2400-4600 lux

7-DAT Bioassay

Test temperature: 23.8–25.7°C
 Relative humidity: 70-84%
 Photoperiod: 16 h light:8 h dark
 Light intensity: 2400-4000 lux
 DAT, days after treatment

The test substrate comprised excised leaves of the dwarf French bean plant (*Phaseolus vulgaris* L., var. “The Prince”). For use in each bioassay, discs were cut from flattened sections of treated bean leaves, (approximate diameter of 5 cm), onto which spray applications were made.

Test units to assess mortality

For the 0-DAT bioassay, leaves with freshly dried residues, and for subsequent bioassays, with field-aged residues, were used to line the floor of simple test arenas. Each comprised a square glass plate (7.5 cm x 7.5 cm), a Perspex[®] supporting plate of similar size, with a 5-cm-diameter hole cut through it, and an acrylic cylinder of (5-cm outer diameter, 4.4-cm internal diameter, 2.5-cm tall). The treated leaf was laid on the glass plate with its adaxial (upper) treated surface facing upwards and the Perspex[®] sheet was placed on top. This was held firmly in place using elastic bands. The petiole of the leaf was wrapped in wet cotton wool, which was draped into a water trough. Separate water troughs were used for each treatment. The acrylic cylinder was then fitted into the hole in the Perspex[®] sheet. The inside wall of the cylinder was treated with an aqueous suspension of polytetrafluoroethylene (Fluon[®]; manufacturer Whitford Ltd) to prevent larvae from climbing away from the treated leaf. A ventilated lid, covered with 0.5-mm x 0.5-mm mesh nylon netting, was placed over each cylinder to prevent larvae from escaping.

CA3301 / JOUST	Page 215 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Test units to assess pupal development, reproduction, and egg viability

As the lacewing pupae developed, they were transferred to large, translucent, plastic storage boxes (27 cm x 27 cm x 14 cm). Treatments were kept in separate boxes and food and water was provided for the emerging adult lacewings.

As the adult lacewings emerged, they were transferred to clear polystyrene boxes (15 cm x 27 cm x 10 cm), with close-fitting lids. A sheet of fibrous tissue (a proprietary brand of nappy liner – Mioliner – Bambino Mio Ltd.) was placed under the lid of each box, to serve as an oviposition site. Treatments were kept in separate boxes and food and water were provided.

The egg-bearing fibrous tissue previously used to line the oviposition boxes were transferred to additional clear polystyrene boxes (15 cm x 27 cm x 10 cm). The emerging larvae were provided with UV-killed eggs of *S. cerealella*, to limit any cannibalism of the eggs of *C. carnea*.

In each bioassay, there were 40 individually confined larvae per treatment. For the reproduction assessments, adults from each treatment were grouped in 1 or 2 boxes, but these were not considered replicates for statistical purposes.

Treatment application

For both bioassays, treatments were applied to the bean plants using a hand-held-boom sprayer powered by compressed air (Azo Ltd., Ede, the Netherlands). The spray pressure was set to approximately 3 bar and a flat-fan nozzle (XR Teejet 11002VS) was used. The sprayer was calibrated in advance of the test applications using purified water, to confirm a deposition rate equivalent to 400 L/ha, with an actual mean range of $\pm 5\%$ of the target rate).

Treatments were applied in the order of the control, test item, and, finally, the toxic reference (T2 application only). CA3301 was evaluated at a single application rate of 800 mL test item/ha, equivalent to 206.4 g a.s./ha (1.984 g test item dissolved in 100 mL of purified water). The toxic-reference treatment was applied at 100 mL product/ha, equivalent to 40 g dimethoate/ha.

Assessments

Over 7 days, the total egg production (number of eggs plus live and dead juvenile stages) were determined for each arena and the mean egg production per female was calculated. Three assessments of oviposition activity were carried out during the assessment period. On each assessment date, any eggs and larvae/nymphs present were recorded separately and then removed. In addition, the condition and number of live female and male mites in each arena were assessed.

The condition of the larvae was assessed every 1-3 days, until they pupated. They were categorised as follows:

- Alive: apparently healthy and unaffected.
- Abnormal pupa: larva pupating without spinning a cocoon or appearing different from the norm.
- Dead: no longer moving.
- Pupated: larvae having pupated.

Any larvae that escaped or were accidentally killed were noted and excluded from any data analyses. As pupae developed, they were collected before emerging, but were not removed from the surface to which they were attached (typically the sidewall of the arena). Treatments were kept in separate boxes and food and water (changed three times per week) were provided for the emerging adults. The number of lacewings that had emerged successfully was also recorded every 2-3 days. The second subsequent bioassay initiated at 7 DAT₂ was performed in the same manner.

The adults in the individual treatments used for the assessments emerged within 7 days. For the reproduction

CA3301 / JOUST	Page 216 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

assessments they were transferred to the polystyrene oviposition boxes. The sex of the adult lacewings was determined by eye, based on the shape of the end of their abdomen (Vogt *et al.*, 2000). When the oviposition assessments were complete, the adults were killed in a freezer and then examined using a binocular microscope to confirm their sex.

In the 0 DAT₂ bioassay, assessments commenced 10 days after the majority (>75%) of the adult lacewings had emerged, which was 7 days after egg laying was first been noted in the individual boxes. In the 7 DAT₂ bioassay, assessments commenced 9 days after the majority (>75%) of the adult lacewings had emerged, which was 7 days after egg laying was first been noted in the individual boxes. Eggs were sampled from the reproduction boxes by removing and replacing the fibrous sheet used to line the lids.

The assessments made were as follows:

- i. The number of eggs laid in each box (on the fibrous tissue and walls) were recorded for two 24-h periods within one week.
- ii. The viability of the eggs laid on the fibrous tissue was determined. Having first counted the numbers of eggs on the sheets, they were laid in individual boxes. Once the larvae started to hatch, the sheets were removed once each day and shaken to remove those larvae. After at least 6 days, the number of unhatched eggs remaining was recorded, so that the percentage viability could be calculated.

The pre-imaginal mortality of insects was calculated for each treatment. Mortality was defined as the number of dead larvae and pupae failing to develop into adult insects, combined. The corrected percentage pre-imaginal mortality was calculated using Abbott's formula.

Data were analysed using ToxRatPro® (ToxRat Solutions GmbH, 2018). ToxRat output is provided. Mortality in each treatment was compared to the respective control, using a one-sided Fisher's exact binomial test ($\alpha = 0.05$). Effects on lacewing reproduction in the test-item treatment group were assessed qualitatively, on the basis of triggers, as specified in the guideline of Vogt *et al.* (2000). Namely, there should be ≥ 15 eggs produced per female per day and that the egg hatching rate should be $\geq 70\%$, if a treatment is to be deemed harmless.

Results

Biological results

The effect of CA3301 on pre-imaginal mortality in the 0- and 7-DAT₂ bioassays, after 7 days of exposure, is presented in Tables CP 10.3.2/08-01 and CP 10.3.2/08-02 below.

Mortality in the 0-DAT bioassay

CA3301 / JOUST	Page 217 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Table CP 10.3.2/08-01. The effect of CA3301 on pre-imaginal *C. carnea* mortality in the 0-DAT₂ bioassay, after 7 days of exposure.

Treatment rate	0 mL test item/ha 0 g a.s./ha (control)					800 mL test item/ha 206.4 g a.s./ha					100 mL BAS 152 65 I/ha 40 g dimethoate/ha				
Days after initiation	L	P	A	D	E	L	P	A	D	E	L	P	A	D	E
2	37	0	0	3	0	38	0	0	2	0	3	0	0	37	0
5	32	0	0	8	0	33	0	0	7	0	2	0	0	38	0
7	31	1	0	8	0	26	6	0	8	0	2	0	0	38	0
9	9	23	0	8	0	8	24	0	8	0	1	1	0	38	0
12	1	31	0	8	0	0	31	0	9	0	0	2	0	38	0
14	0	32	0	8	0	-	-	-	-	-	-	-	-	-	-
Pre-imaginal mortality (%)	20.0					25.0					95.0*				
Corrected mortality (%) [#]	0.0					+6.3					+93.8				

Significant differences in mortality, relative to the control, were revealed with a one-sided Fisher's exact binomial test ($\alpha = 0.05$) and are denoted with asterisks (*). [#]Mortality corrected by control mortality, using Abbott's formula; positive values indicate an increase in mortality, relative to the control. L, larvae; P, pupae; A, abnormal pupae; D, dead; E, escaped. Dashes (-) indicate that no larvae were remaining, thus, no assessment necessary.

In the bioassay initiated at 0 DAT, control treatment pre-imaginal mortality was observed to be 20.0%, compared with 25.0% (6.3% corrected mortality) pre-imaginal mortality in the 800 mL/ha treatment rate of CA3301. The test-item treatment did not differ significantly from the control. Mortality in the toxic reference treatment was 95.0% (corrected mortality 93.8%).

Mortality in the 7-DAT bioassay

The effect of CA3301 on mortality, after 7 days of exposure, is presented in Table CP 10.3.2/08-01 below.

Table CP 10.3.2/08-02. The effect of CA3301 on *C. carnea* mortality in the 7-DAT₂ bioassay, after 7 days of exposure.

Treatment rate	0 mL test item/ha (control) 0 g a.s./ha					800 mL test item/ha 206.4 g a.s./ha				
Days after initiation	L	P	A	D	E	L	P	A	D	E
2	40	0	0	0	0	38	0	0	2	0
5	39	0	0	1	0	33	0	0	7	0
7	35	0	0	5	0	32	0	0	8	0
9	30	5	0	5	0	31	1	0	8	0
12	3	32	0	5	0	7	25	0	8	0
14	0	35	0	5	0	0	31	0	9	0
Pre-imaginal mortality (%)	15.0					25.0				
Corrected mortality (%)	0.0					+11.8				

Significant differences in mortality, relative to the control, were revealed with a one-sided Fisher's exact binomial test ($\alpha = 0.05$) and are denoted with asterisks (*). [#]Mortality corrected by control mortality, using Abbott's formula; positive values indicate an increase in mortality, relative to the control. L, larvae; P, pupae; A, abnormal pupae; D, dead; E, escaped.

In the 7 DAT bioassay, 15.0% pre-imaginal mortality was observed in the control treatment, compared to 25.0% (11.8% corrected mortality) in the 800 mL CA3301/ha treatment. The test-item treatment did not differ significantly from the control.

The effect of CA3301 on pupal development in the 0- and 7-DAT₂ bioassays, after 7 days of exposure, is presented in Tables CP 10.3.2/08-03 and CP 10.3.2/08-04 below.

Pupal development in the 0-DAT bioassay

CA3301 / JOUST	Page 218 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Table CP 10.3.2/08-03. The effect of CA3301 on *C. carnea* pupal development in the 0-DAT bioassay, after 14 days of exposure.

Treatment rate	DAI	P	P _{total}	A	D	F	E
0 mL test item/ha 0 g a.s./ha (control)	16	3	32	0	0	0	32
	19	25					
	21	28					
	23	32					
800 mL test item/ha 206.4 g a.s./ha	16	6	31	0	1	0	30
	19	27					
	21	30					
	23	30					
100 mL BAS 152 65 I/ha 40 g dimethoate/ha	16	0	2	0	0	0	2
	19	2					
	21	2					
	23	2					

DAI, days after initiation; P, larvae pupating successfully; A, pupae with abnormal cocoons; D, pupae dying during emergence; F, pupae failing to develop; E, number of adults successfully emerged.

Table CP 10.3.2/08-04. The effect of CA3301 on *C. carnea* pupal development in the 7-DAT bioassay.

Treatment rate	DAI	P	P _{total}	A	D	F	E
0 mL test item/ha 0 g a.s./ha (control)	16	3	35	0	0	1	34
	19	33					
	21	34					
	23	34					
800 mL test item/ha 206.4 g a.s./ha	16	1	31	0	1	0	30
	19	28					
	21	30					
	23	30					

DAI, days after initiation; P, larvae pupating successfully; A, pupae with abnormal cocoons; D, pupae dying during emergence; F, pupae failing to develop; E, number of adults successfully emerged.

Reproduction in the 0-DAT Bioassay

Table CP 10.3.2/08-05. The effect of CA3301 on *C. carnea* reproduction in the 0-DAT bioassay.

Test-item rate	0 mL test item/ha 0 g a.s./ha				800 mL test item/ha 206.4 g a.s./ha			
	A		B		A		B	
Box								
DAI	33-34	34-35	33-34	34-35	33-34	34-35	33-34	34-35
No. of adults	10	10	10	10	9	9	7	7
Total produced	388	458	256	277	165	260	81	163
Eggs/female/day	38.8	45.8	25.6	27.7	18.3	28.9	11.6	23.3
Hatched	247	378	110	159	103	158	30	97
Not hatched	5	7	7	6	4	6	2	8
Egg viability (%)	98	98.1	93.6	96.2	96.1	96.2	93.3	91.8
Mean eggs/female/day	34.5				20.9			
Mean egg viability (%)	97.2				94.8			
Mean viable eggs/female/day	33.5				19.8			
Mean reduction in reproduction (%)*	0.0				+40.8			

*Positive values indicate a decrease in mean reproduction, relative to the control. DAI, days after initiation.

CA3301 / JOUST	Page 219 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Table CP 10.3.2/08-06. The effect of CA3301 on *C. carnea* reproduction in the 7-DAT bioassay.

Test-item rate	0 mL test item/ha 0 g a.s./ha				800 mL test item/ha 206.4 g a.s./ha			
	A		B		A		B	
Box								
DAI	28-29	29-30	28-29	29-30	28-29	29-30	28-29	29-30
No. of adults	10	10	10	10	7	7	8	8
Total produced	308	404	209	286	231	320	211	301
Eggs/female/day	30.8	40.4	20.9	28.6	33	45.7	26.4	37.6
Hatched	246	315	164	236	210	286	179	274
Not hatched	6	8	5	6	13	4	6	7
Egg viability (%)	97.6	97.5	97	97.5	93.8	98.6	96.6	97.4
Mean eggs/female/day	30.2				35.4			
Mean egg viability (%)	97.4				96.8			
Mean viable eggs/female/day	29.4				34.3			
Mean reduction in reproduction (%)*	0.0				-16.8			

*Negative values indicate an increase in mean reproduction, relative to the control. DAI, days after initiation.

In the 0-DAT₂ bioassay, the mean number of viable eggs per female per day was 33.5 in the control, compared with 19.8 in the 800 mL CA3301/ha treatment rate. In the 7-DAT bioassay, the control treatment showed 29.4 viable eggs per female per day, compared to 34.3, in the test item treatment.

Validity

All validity criteria were met in accordance with IOBC Vogt et al. (2000) test guideline:

- Pre-imaginal mortality in the control treatment should be ≤20% in each bioassay (actual values: 20% and 15%, in the 0- and 7-DAT bioassays, respectively).
- Mean corrected mortality in the toxic-reference group to be ≥50% (actual value: 93.8%)
- For the reproduction assessments, the mean egg production in the control should be ≥15 eggs per female per day and mean viability of the eggs should be ≥70%, in each bioassay (actual values: 34.5 eggs/female/day and 97.2% in the 0-DAT bioassay and 20.9 egg/female/day and 94.8% in the 7-DAT bioassay).

Conclusion

The effects of both fresh and aged foliar residues of the test item CA3301 on the green lacewing, *Chrysoperla carnea*, were evaluated under extended-laboratory conditions. When applied to dwarf French bean plants on two occasions, with a 14-day interval, at a rate of 800 mL test item/ha, equivalent to 206.4 g a.s./ha, fresh (0-day-old) and 7- day- old aged-residues showed no unacceptable effects on lacewing survival, or on the subsequent reproductive capacity of adult lacewings.

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

CA3301 / JOUST	Page 220 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Study 1

Comments of zRMS:	<p>The study was conducted to the OECD guideline 222 and according to the principles of GLP. All validity criteria were met. One minor deviation was noted without negative impact on the overall outcome of the study:</p> <ul style="list-style-type: none">- one measurement of temperature was 23.3 ° C and was therefore above the upper limit of 22 ° C. <p>The study is considered to be reliable and suitable for the risk assessment.</p> <p>The endpoints expressed in the test were based on analyzed content of Prothioconazole instead nominal value used by Applicant in this summary. According study report following endpoints were derived:</p> <p>The NOEC, LC₁₀, LC₂₀ and LC₅₀ values based on adult mortality were all estimated to be ≥ 1000 µL/kg dry soil, corresponding to 257 mg Prothioconazole/kg dry soil (based on an active ingredient content of 257.04 g/L). The NOEC, LC₁₀, LC₂₀ and LC₅₀ values based on adult body weight were all estimated to be ≥ 1000 µL/kg dry soil, corresponding to 257 mg Prothioconazole/kg dry soil (based on an active ingredient content of 257.04 g/L).</p> <p>The NOEC and LOEC values for reproduction were determined to be 556 and 1000 µL/kg dry soil, corresponding to 143 and 257 mg Prothioconazole/kg dry soil, respectively (based on an active ingredient content of 257.04 g/L).</p> <p>The values of EC₁₀, EC₂₀ and EC₅₀ for reproduction presented in the study report were calculated to be 277, 434 and > 1000 µL/kg dry soil, corresponding to 71.2, 112 and > 257 mg Prothioconazole/kg dry soil, respectively. The reliability of EC₁₀- and EC₂₀-calculation was estimated to be “bad” and “poor” according to EFSA (2019):</p> <table><tr><th>Estimate</th><th>EC₁₀</th><th>EC₂₀</th><th>EC₅₀</th><th>Estimate</th><th>EC₁₀</th><th>EC₂₀</th><th>EC₅₀</th></tr><tr><td colspan="8">µL/kg dry soil</td></tr><tr><td>Median</td><td>70.7</td><td>177</td><td>1017</td><td>Median</td><td>277</td><td>434</td><td>1024</td></tr><tr><td>lower 95 % CI</td><td>26.1</td><td>101</td><td>584</td><td>lower 95 % CI</td><td>126</td><td>257</td><td>840</td></tr><tr><td>upper 95 % CI</td><td>240</td><td>482</td><td>2517</td><td>upper 95 % CI</td><td>746</td><td>930</td><td>1493</td></tr><tr><td>NW</td><td>3.0</td><td>2.2</td><td>1.9</td><td>NW</td><td>2.2</td><td>1.6</td><td>0.64</td></tr><tr><td>NW-based classification^a</td><td>bad</td><td>bad</td><td>poor</td><td>NW-based classification^a</td><td>bad</td><td>poor</td><td>fair</td></tr></table> <p>CI: Confidence Interval NW: Normalized Width as ratio of the difference between the upper and lower CI and the EC_x a: NW-based classification according to point 4.1 of the appendix E of EFSA Supporting publication 2019:EN-1673 [1]. Note: values of < 1, < 2 and ≥ 2 for the ratio between the difference of both 95 % CIs and the corresponding EC_x are defined as “fair”, “poor” and “bad”, respectively.</p> <p>Taking to consideration the low reliability of EC_{10/20} values presented in the study report, zRMS agree with the endpoints derived by the Applicant. The following values were accepted by zRMS: the EC₁₀, EC₂₀, and EC₅₀ values for reproduction were calculated to be 94.6, 216, and 1041 µL product/kg sdw (equivalent to 23.7, 54.0, and 260.3 mg a.s./kg sdw, when accounting for nominal product a.s. content of 250 g/L, and corresponding to 24.3, 55.5 and 267.6 mg Prothioconazole/kg dry soil, respectively based on an analyzed active ingredient content of 257.04 g/L), respectively.</p> <p>The EC₁₀ value of 24.3 mg a.s./kg sdw (94.6 µL product/kg sdw) will be used in the risk assessment.</p>	Estimate	EC ₁₀	EC ₂₀	EC ₅₀	Estimate	EC ₁₀	EC ₂₀	EC ₅₀	µL/kg dry soil								Median	70.7	177	1017	Median	277	434	1024	lower 95 % CI	26.1	101	584	lower 95 % CI	126	257	840	upper 95 % CI	240	482	2517	upper 95 % CI	746	930	1493	NW	3.0	2.2	1.9	NW	2.2	1.6	0.64	NW-based classification ^a	bad	bad	poor	NW-based classification ^a	bad	poor	fair
Estimate	EC ₁₀	EC ₂₀	EC ₅₀	Estimate	EC ₁₀	EC ₂₀	EC ₅₀																																																		
µL/kg dry soil																																																									
Median	70.7	177	1017	Median	277	434	1024																																																		
lower 95 % CI	26.1	101	584	lower 95 % CI	126	257	840																																																		
upper 95 % CI	240	482	2517	upper 95 % CI	746	930	1493																																																		
NW	3.0	2.2	1.9	NW	2.2	1.6	0.64																																																		
NW-based classification ^a	bad	bad	poor	NW-based classification ^a	bad	poor	fair																																																		

CA3301 / JOUST	Page 221 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Reference:	KCP 10.4.1.1/01
Report	250 EC Prothioconazole, NUL 3390 (CA3301) - Effects on Reproduction of <i>Eisenia fetida</i> (Annelida: Lumbricidae) in Artificial Soil Schmidt, T., 2021, report no. 20190460
Guideline(s):	Yes. OECD Test Guideline 222 (2016)
Deviations:	No
GLP:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive summary

A 56-day reproductive study of *Eisenia fetida* with CA3301 (nominally 250 g prothioconazole/L) was conducted in artificial soil, with ten treatment groups of 0 (water control), 9.07, 16.3, 29.4, 52.9, 95.3, 171, 309, 556, and 1000 µL product/kg dry soil, equivalent to 0, 2.3, 4.1, 7.4, 13.2, 23.8, 42.8, 77.3, 139, and 250 mg a.s./kg dry soil (accounting for the nominal product a.s. content of 250 g/L).

The control group was comprised of eight replicates and the product treatment groups were comprised of four replicates each, with ten adult earthworms per replicate. After 28 days' exposure, adult worms were removed from their test units, to assess biomass and mortality. Treatment with CA3301 did not significantly affect biomass or mortality. Accordingly, the 28-day NOEC and LOEC values (adult biomass and adult mortality) were determined to be 1000 and >1000 µL product/kg sdw, equivalent to 250 and >250 g a.s./kg sdw, respectively, and the 28-day EC₁₀, EC₂₀, and EC₅₀ values (adult biomass and adult mortality) were all estimated to be >1000 µL product/kg sdw, equivalent to >250 g a.s./kg sdw.

After the adults were removed, the test units were incubated for a further 28 days, to assess reproduction (fecundity). Treatment with CA3301 at the highest concentration (1000 µL product/kg sdw, equivalent to 250 mg a.s./kg sdw) significantly decreased the number of juveniles by 59% (relative to the control group). The 56-day NOEC and LOEC values for reproduction were determined to be 556 and 1000 µL product/kg sdw (equivalent to 139 and 250 mg a.s./kg sdw.), respectively. The EC₁₀, EC₂₀, and EC₅₀ values for reproduction were calculated to be 94.6, 216, and 1041 µL product/kg sdw (equivalent to 23.7, 54.0, and 260.3 mg a.s./kg sdw), respectively.

For validation of the test system, treatment with boric acid, as the reference item, was assessed separately, also in a 56-day test.

Overall, the study satisfies the OECD 222 (2016) test-guideline requirements for chronic toxicity to earthworms and is considered acceptable.

Materials and methods

Test materials

Test item

Name:	Prothioconazole 250 EC, NUL 3390 (CA3301)
Active substance:	Prothioconazole
Formulation type:	Emulsifiable concentrate (EC)
Source and lot/batch no.:	A19045
Active substance content:	250 g/L (nominal), 257.04 g/L (analyzed)

CA3301 / JOUST	Page 222 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Active substance density: 25.57% w/w
Appearance: Clear, straw-coloured liquid
Expiry date of lot/batch: 17 June 2022
Storage conditions: Store in the closed, original container in a cool, well-ventilated area. Do not store for prolonged periods in direct sunlight.

Reference item

Name: Boric acid, was assessed separately, also in a 56-day test

Test organism

Species: *Eisenia fetida* (Annelida: Lumbricidae)
Age at study initiation: 10 months, with developed clitellum
Weight at study initiation: 300-600 g
Source: Stock culture, maintained at IES Ltd
Feeding during test: 2-6 g horse manure, one day after test initiation and once weekly (during first 4 weeks of study), then 6 g (with 12 mL ultrapure water) on day 28, when adults removed from test units.
Acclimation: 2 d in artificial test soil

Test medium:

Soil type: Artificial soil
5.0 kg sphagnum peat
10.0 kg kaolin clay (kaolinite content > 30%),
34.5 kg fine quartz-sand,
180 g CaCO₃, to obtain optimal pH of 6.0 ± 0.5.
pH: 5.8-6.1 (test initiation), 6.0-6.1 (test termination)
Water-holding capacity: 52.5-55.8% (mean of 57.94%)

Test conditions

Test temperature: 18.7-23.2°C
Photoperiod: 16 h light:8 h dark
Light intensity: 400-800 lux

510 g of dry artificial soil (equivalent to 670 g wet soil) were placed into each plastic test unit (17 × 12 × 11.8 cm³) that were covered with lids that enabled exchange of air and minimized evaporation from artificial soil. The artificial soil was prepared with a laboratory mixer. The maximum water-holding capacity (MWHC) was determined to be 57.94%. The final water content was 679.92 mL purified water corresponding to 31.33% water content and 54.08% of MWHC.

The study consisted of ten treatments: one control and nine product concentrations. Eight replicates (test units) were set up for the control, whereas four replicates were set up for each product concentration. All replicates consisted of ten *E. fetida* adults (80 control earthworms and 40 product treated earthworms). Earthworms were assigned randomly to the different treatments groups.

After application, earthworms were weighed individually; earthworms were washed with purified water prior to weighing and the excess water was removed by placing them briefly on blotting paper. Earthworms were then 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.

The exposure phase of the adults in the study lasted four weeks (28 days). Thereafter, surviving animals were removed from the test units and adult mortality and body weight were determined. Effects on reproduction were assessed after a further four weeks by counting the number of offspring present in the soil.

CA3301 / JOUST	Page 223 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

The nine product concentrations use in the study were 9.07, 16.3, 29.4, 52.9, 95.3, 171, 309, 556, and 1000 µL product/kg dry soil (spacing factor: 1.8), equivalent to 2.3, 4.1, 7.4, 13.2, 23.8, 42.8, 77.3, 139, and 250 mg a.s./kg dry soil (accounting for the nominal product a.s. content of 250 g/L). The control was tested in parallel and consisted of water only. For validation of the test system, treatment with boric acid was assessed separately, also in a 56-day test.

Since the product, CA3301, is emulsifiable in water, a stock solution was made by emulsifying 7.24 mL of CA3301 in 500 mL of ultrapure water (this stock was added directly to achieve the highest product concentration of 1000 µL product/kg dsw. The stock solution was stirred for 10 minutes before application. Aliquots of the stock solution were added to ultrapure water to achieve the remaining eight product concentrations, and were also continuously stirred for 10 minutes before application. The control treatment was prepared in the same way as the product treatments, but using only ultrapure water

The treated soil substrate needed for the replicates of each treatment was prepared as one batch. For each treatment, 2200 g of soil was pre-wetted with 300 mL ultrapure water one day before application. On application day, 30 mL of the application solutions described above were mixed into the pre-wetted soil along with 320 mL ultrapure water.

After 4 weeks (28 days), each adult earthworm was removed, counted, and observed for abnormal behaviour or symptoms of toxicity. The surviving earthworms were weighed (after being washed under ultrapure water and dried). The remaining soil (with adults removed) was then incubated for additional 4 weeks, under the same test conditions.

Changes in adult biomass was determined by comparing the initial weight of a worm to its final weight on day 28. Adult mortality was also assessed on day 28. Missing earthworms or that failed to respond to gentle stimulation were considered dead.

After the additional 4 weeks were over (after 56 days), test containers were place in a 47°C bath for 20 min, to heat-extract hatched juveniles to the soil surface. Juveniles were removed and counted. Additionally, the number of filled cocoons present in the soil were counted. The reproductive output of the worms in the product treatments was determined and compared to the control for both the number of juveniles and number of filled cocoons.

Statistically significant differences between the control and the product treatments were revealed with the statistical software ToxRat Professional, using a multiple Fisher's test (followed by Bonferroni-Holm corrections), for mortality (one-sided greater, $\alpha = 0.05$); an ANOVA for body weight at Day 0 ($\alpha = 0.05$, two-sided); a Dunnett's t-test, for body weight and body-weight change at Day 28 ($\alpha = 0.05$, one-sided smaller); and a William's t-test, for reproduction at Day 56 ($\alpha = 0.05$, one-sided smaller).

NOEC and LOEC values were determined based on the outcome of the statistics. EC_x calculations for reproduction were performed using non-linear regression [3-parametric normal cumulative distribution function, with the Downhill Simplex optimization method and 500 resamples, for calculation of the confidence intervals (CIs) by bootstrapping].

Results

Biological results

Biomass

A summary of the effect of CA3301 on adult biomass from the definitive test, after 28 days of exposure, is presented in the Table CP 10.4.1.1/01-01 below.

CA3301 / JOUST	Page 224 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Table CP 10.4.1.1/01-01. The effect of CA3301 (nominally 250 g a.s./L) on adult *E. fetida* biomass, after 28 days of exposure.

Nominal soil concentration		Rep.	Replicate weight (mg)		Mean values	
					Weight \pm SD (mg)	Change in weight \pm SD (%)
μ L product/kg	mg a.s./kg*		Day 0	Day 28	Day 28	
0 (control)	0	1	475	499	537 \pm 25.5	20 \pm 11
		2	491	536		
		3	433	523		
		4	474	536		
		5	433	573		
		6	446	510		
		7	449	556		
		8	408	560		
9.07	2.3	1	501	608	585 \pm 29.9	23 \pm 6
		2	460	553		
		3	460	612		
		4	472	565		
16.3	4.1	1	498	595	561 \pm 24.3	24 \pm 3
		2	449	543		
		3	420	561		
		4	439	544		
29.4	7.4	1	483	636	602 \pm 25.9	32 \pm 3
		2	462	608		
		3	421	577		
		4	456	588		
52.9	13.2	1	474	591	591 \pm 38.5	30 \pm 5
		2	456	582		
		3	471	642		
		4	422	549		
95.3	23.8	1	456	490	536 \pm 37.8	18 \pm 10
		2	470	523		
		3	469	577		
		4	429	554		
171	42.8	1	476	590	538 \pm 52.3	18 \pm 11
		2	457	478		
		3	445	573		
		4	446	512		
309	77.3	1	447	570	519 \pm 44.1	15 \pm 12
		2	447	538		
		3	446	495		
		4	474	471		
556	139	1	437	524	554 \pm 23.8	24 \pm 5
		2	433	548		
		3	450	579		
		4	476	566		
1000	250	1	532	639	556 \pm 56.9	20 \pm 12
		2	481	518		
		3	402	544		
		4	453	521		

No statistically significant differences, relative to the control, were revealed with one-sided Dunnett's t-tests ($\alpha = 0.05$).

*Accounting for the nominal product a.s. content of 250 g/L. SD, standard deviation; n.s., not significant; Rep., replicate.

At test initiation, the fresh weight of individual worms ranged from 300 to 600 mg, with an overall mean weight of 456 ± 25.4 mg/earthworm. Mean weight for individual replicates of the control and product treatment groups ranged from 449 to 473 mg/earthworm, with no significant differences in body weight.

CA3301 / JOUST	Page 225 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

The mean body weight at day 28 in the control was 537 ± 25.5 mg. The mean body weight at day 28 in the product treatment groups ranged from 519 to 602 mg/earthworm, with no significant differences in body weight.

The mean body weight in the control increased by 20%, whereas it increased between 15 and 32% in the product treatment groups, with no significant differences in body-weight change. The NOEC and LOEC values for adult body weight were determined to be 1000 and $>1000 \mu\text{L}$ product/kg sdw, equivalent to 250 and >250 g a.s./kg sdw (when accounting for nominal product a.s. content of 250 g/L), respectively. The EC_{10} , EC_{20} , and EC_{50} values were all estimated to be $>1000 \mu\text{L}$ product/kg sdw, equivalent to >250 g a.s./kg sdw.

Mortality

A summary of the effect of CA3301 on adult mortality from the definitive test, after 28 days of exposure, is presented in the Table CP 10.4.1.1/01-02 below.

CA3301 / JOUST	Page 226 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Table CP 10.4.1.1/01-02. The effect of CA3301 (nominally 250 g a.s./L) on adult *E. fetida* mortality, after 28 days of exposure.

Nominal dry soil concentration		Rep	Number of earthworms		Mortality (%)
			Live	Dead	
$\mu\text{L product/kg}$	mg a.s./kg^*	.	Day 0	Day 28	[P-value] [#]
0 (control)	0	1	10	10	1
		2	10	10	
		3	10	10	
		4	10	10	
		5	10	9	
		6	10	10	
		7	10	10	
		8	10	10	
9.07	2.3	1	10	10	0
		2	10	10	
		3	10	10	
		4	10	10	
16.3	4.1	1	10	10	0
		2	10	10	
		3	10	10	
		4	10	10	
29.4	7.4	1	10	10	0
		2	10	10	
		3	10	10	
		4	10	10	
52.9	13.2	1	10	10	1
		2	10	10	
		3	10	9	
		4	10	10	
95.3	23.8	1	10	10	0
		2	10	10	
		3	10	10	
		4	10	10	
171	42.8	1	10	10	0
		2	10	10	
		3	10	10	
		4	10	10	
309	77.3	1	10	10	1
		2	10	10	
		3	10	10	
		4	10	9	
556	139	1	10	10	0
		2	10	10	
		3	10	10	
		4	10	10	
1000	250	1	10	10	1
		2	10	9	
		3	10	10	
		4	10	10	

*Accounting for the nominal product a.s. content of 250 g/L. [#] Statistical significances revealed with a one-sided, multiple sequentially rejective Fisher's test, with Bonferroni-Holm corrections ($\alpha = 0.05$); n.s., not significant; Rep., replicate.

Adult mortality at day 28 was 1.3% in the control and between 0-2.5% in the product treatment groups, with no significant differences in mortality, relative to the control. The NOEC and LOEC values for adult mortality was determined to 1000 and >1000 $\mu\text{L product/kg sdw}$, equivalent to 250 and >250 g a.s./kg sdw (when accounting for nominal product a.s. content of 250 g/L), respectively. The LC_{10} , LC_{20} , and LC_{50}

CA3301 / JOUST	Page 227 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

values were all estimated to be >1000 µL product/kg sdw, equivalent to >250 g a.s./kg sdw.

Reproduction

Summaries of the effect of CA3301 on the number of cocoons and hatched juveniles during the definitive test, after 28 days of exposure, are presented in the Tables A2.4.1/01-03 A2.4.1/01-04 below.

CA3301 / JOUST	Page 228 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Table CP 10.4.1.1/01-03. The effect of CA3301 (nominally 250 g a.s./L) on cocoon production, after 56 days of exposure.

Nominal soil concentration		Rep.	Cocoons per vessel				Reduction in cocoon production (% control)
$\mu\text{L product/kg}$	mg a.s./kg*		No.	Mean \pm SD	CV (%)	% control	
0 (control)	0	1	3	0.9 ± 1.6	188.0	0.0	0.0
		2	0				
		3	4				
		4	0				
		5	0				
		6	0				
		7	0				
		8	0				
9.07	2.3	1	0	0.0 ± 0.0	0.0	0.0	100.0
		2	0				
		3	0				
		4	0				
16.3	4.1	1	0	0.3 ± 0.5	200.0	29.0	71.0
		2	0				
		3	1				
		4	0				
29.4	7.4	1	0	0.0 ± 0.0	0.0	0.0	100.0
		2	0				
		3	0				
		4	0				
52.9	13.2	1	0	0.0 ± 0.0	0.0	0.0	100.0
		2	0				
		3	0				
		4	0				
95.3	23.8	1	0	0.0 ± 0.0	0.0	0.0	100.0
		2	0				
		3	0				
		4	0				
171	42.8	1	0	0.0 ± 0.0	0.0	0.0	100.0
		2	0				
		3	0				
		4	0				
309	77.3	1	0	0.0 ± 0.0	0.0	0.0	100.0
		2	0				
		3	0				
		4	0				
556	139	1	0	0.0 ± 0.0	0.0	0.0	100.0
		2	0				
		3	0				
		4	0				
1000	250	1	0	0.3 ± 0.5	200.0	29.0	71.0
		2	0				
		3	1				
		4	0				

*Accounting for the nominal product a.s. content of 250 g/L. Rep., replicate; No., number; SD, standard deviation; CV, coefficient of variation.

CA3301 / JOUST	Page 229 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Table CP 10.4.1.1/01-04. The effect of CA3301 (nominally 250 g a.s./L) on juvenile production, after 56 days of exposure.

Nominal dry soil concentration*		Rep.	Surviving adults	Number of juveniles				Juveniles per surviving adult		
$\mu\text{L prod/kg}$	mg a.s./kg			#	Mean \pm SD	CV (%)	% control [#]	Mean \pm SD	CV (%)	% control
0 (control)	0	1	10	81	58.5 ± 16.7	28.5	0	5.9 ± 1.6	27.0	73.0
		2	10	62			n.s.			
		3	10	49						
		4	10	68						
		5	9	32						
		6	10	75						
		7	10	59						
		8	10	42						
9.07	2.3	1	10	76	76.8 ± 11.4	14.9	31	7.7 ± 1.1	14.9	30.0
		2	10	87			n.s.			
		3	10	61						
		4	10	83						
16.3	4.1	1	10	54	59.5 ± 4.7	7.8	2	6 ± 0.5	7.8	1.0
		2	10	58			n.s.			
		3	10	61						
		4	10	65						
29.4	7.4	1	10	59	55.5 ± 10.8	19.4	-5	5.6 ± 1.1	19.4	-6.0
		2	10	65			n.s.			
		3	10	40						
		4	10	58						
52.9	13.2	1	10	62	54.3 ± 6.1	11.3	-7	5.6 ± 0.5	8.5	-6.0
		2	10	56			n.s.			
		3	9	48						
		4	10	51						
95.3	23.8	1	10	54	51.3 ± 22.2	43.3	-12	5.1 ± 2.2	43.3	-13.0
		2	10	44			n.s.			
		3	10	80						
		4	10	27						
171	42.8	1	10	54	58.8 ± 8.8	15.0	0	5.9 ± 0.9	15.0	0.0
		2	10	59			n.s.			
		3	10	51						
		4	10	71						
309	77.3	1	10	42	38.5 ± 4	10.5	-34	4 ± 0.6	14.4	-33.0
		2	10	35			n.s.			
		3	10	35						
		4	9	42						
556	139	1	10	33	51.5 ± 18.1	35.2	-12	5.2 ± 1.8	35.2	-13.0
		2	10	45			n.s.			
		3	10	76						
		4	10	52						
1000	250	1	10	23	24.3 ± 2.2	9.1	-59	2.5 ± 0.4	14.2	-58.0
		2	9	27			sig.			
		3	10	22						
		4	10	25						

*Accounting for the nominal product a.s. content of 250 g/L. [#]Statistically significant differences revealed with a one-sided Dunnett's t-test ($\alpha = 0.05$). n.s., not significant; sig., significant; Rep., replicate; SD, standard deviation; CV, coefficient of variation.

The average number of full cocoons on Day 56 in the control was 0.9 and ranged from 0.0 to 0.3 in the product treatment groups, and showed no concentration-dependent effect. The average number of juveniles

CA3301 / JOUST	Page 230 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

per vessel was 58.5 in the control and 38.5-76.8 in the product treatment groups up to and including 556 µL product/kg sdw; these differences were not statistically significant. However, there was significant reduction in juveniles in the highest product treatment group (1000 µL product/kg sdw), with a mean of 24.3 juveniles.

NOEC and LOEC values for reproduction were determined to be 556 and 1000 µL product/kg sdw (equivalent to 139 and 250 mg a.s./kg sdw, when accounting for nominal product a.s. content of 250 g/L), respectively. The EC₁₀, EC₂₀, and EC₅₀ values for reproduction were calculated to be 94.6, 216, and 1041 µL product/kg sdw (equivalent to 23.7, 54.0, and 260.3 mg a.s./kg sdw), respectively.

Validity

All validity criteria were met in accordance with the OECD 222 (2016) test guideline:

- Each control replicate to produce ≥ 30 juveniles by the end of the test (actual value: 32 to 81 juveniles per replicate in the control).
- The coefficient of variation (reproduction) in the control to be $\leq 30\%$ (actual value: 28.5%).
- Adult mortality in the control to be $\leq 10\%$ (actual value: 1.3%).

Conclusion

The 56-day chronic toxicity of CA3301 to *Eisenia fetida* was studied under in accordance with OECD 222 (2016) test guideline.

The 28-day NOEC and LOEC values (adult biomass and adult mortality) were determined to be 1000 and >1000 µL product/kg sdw, equivalent to 250 and >250 g a.s./kg sdw (when accounting for the nominal product a.s. content of 250 g/L), respectively. The 28-day EC₁₀, EC₂₀, and EC₅₀ values (adult biomass) were all estimated to be >1000 µL product/kg sdw, equivalent to >250 g a.s./kg sdw.

The NOEC and LOEC values (reproduction) were determined to be 556 and 1000 µL product/kg sdw (equivalent to 139 and 250 mg a.s./kg sdw, when accounting for the nominal product a.s. content of 250 g/L), respectively. The EC₁₀, EC₂₀, and EC₅₀ values (reproduction) were calculated to be 94.6, 216, and 1041 µL product/kg sdw (equivalent to 23.7, 54, and 260.3 mg a.s./kg sdw), respectively.

This study is considered acceptable.

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

CA3301 / JOUST	Page 231 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Study 1

Comments of zRMS:	The study was conducted to the OECD guideline 232 and according to the principles of GLP. All validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.
-------------------	--

Reference:	KCP 10.4.2.1/01
Report	CA3301 - A laboratory study to determine the acute and sublethal effects on the collembolan <i>Folsomia candida</i> (Arthropoda: Isotomidae) Frainout, C., 2021, report no. 029SRFR20C10
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 CA3301 (analysed a.s. content of 25.57% w/w prothioconazole) at 0 (control), 5.757, 10.36, 18.64, 33.55, 60.38, 108.6, 195.5, and 352 mg product/kg soil dry weight (dw), equivalent to 0, 1.472, 2.648, 4.766, 8.578, 15.44, 27.78, 50, and 90 mg a.s./kg soil dw, in artificial soil with 5% peat, according to OECD test guideline 232 (2016). The toxic reference chemical used was boric acid, which was tested in the same study at 100 mg boric acid/kg soil dw.

The control group comprised eight replicates and the product 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).

Treatment with CA3301 significantly increased mortality with the three-highest product concentrations. Thus, the 28-day NOEC and LOEC values, based on mortality, were determined to be 60.38 and 108.6 mg product/kg soil dw, equivalent to 15.44 and 27.78 mg a.s./kg soil dw, respectively. The 28-day LC₁₀, LC₂₀, and LC₅₀ values, based on mortality, were calculated to be 92.57, 106.1, and 134.1 mg product/kg soil dw, equivalent to 23.68, 27.15, and 34.34 mg a.s./kg soil dw, respectively.

Treatment with CA3301 significantly reduced reproduction with the three-highest product concentrations. Thus, the 28-day NOEC and LOEC values, based on reproduction, were determined to be 60.38 and 108.6 mg product/kg soil dw, equivalent to 15.44 and 27.78 mg a.s./kg soil dw, respectively. The 28-day reproductive EC₁₀, EC₂₀, and EC₅₀ values were calculated to be 87.32, 100.5, and 128.1 mg product/kg soil dw, equivalent to 22.29, 25.63, and 32.67 mg a.s./kg soil dw, respectively.

For validation of the test system, treatment with boric acid, as the reference item, resulted in >50% reduction in reproduction.

Overall, the study satisfies the OECD 232 (2016) test-guideline requirements for chronic toxicity with *Folsomia candida* and is considered acceptable.

CA3301 / JOUST	Page 232 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Materials and methods

Test materials

Test item

Name:	CA3301
Active substance:	Prothioconazole
Formulation type:	Emulsifiable concentrate (EC)
Source and lot/batch no.:	A19045
Product density:	0.9948 kg/L (nominal)
Active substance content:	250 g/L (nominal), 257.04 g/L or 25.57% w/w (analyzed)
Appearance:	Clear, straw-coloured liquid
Expiry date of lot/batch:	17 June 2022
Storage conditions:	12.5-25.5°C, protected from light

Reference item

Name:	Boric acid, was assessed separately, also in a 56-day test
Formulation type:	Technical
Density:	1 g/mL
Batch no.:	1860103
Purity:	>95% w/w or 950 g/kg
Expiry date:	18 June 2023

Test organism

Species:	<i>Folsomia candida</i>
Age at study initiation:	10-to-12-days old
Sex:	Females
Source:	Bias Labs, Kirkcaldy, Fife, UK
Feeding during test:	2 mg of granulated dry yeast was added to each test unit, at beginning of test and 14 days after application

Test medium:

Soil type:	Artificial soil 5% sphagnum peat 20% kg kaolin clay (kaolinite content > 30%), 50% industrial quartz sand (air dried, particle size 0.05 mm to 0.2 mm), 24.8% industrial quartz sand (air dried, particle size ≥ 0.2 mm) 0.2% CaCO ₃ , to obtain optimal pH of 6.0 ± 0.5.
pH:	6.35-7.29
Water-holding capacity:	35.03%

Test conditions

Test temperature:	17.7 – 22.4°C (mean value of 20.2°C)
Photoperiod:	16 h light:8 h dark
Light intensity:	574 lux

The test units consisted of a transparent glass container (allowing light transmission) of 100-mL capacity and 5-cm diameter (actual soil depth within a test unit was 2-4 cm). The test units had screw lids designed to reduce water evaporation and sufficient headspace between the substrate and the lid to allow gas exchange between the soil and the air. Each test unit represented a replicate, and there were 10 female collembola per replicate; there were four replicates for the product and toxic-reference treatments, and eight replicates for the control group.

There were 10 treatment groups – a distilled water control, eight product treatments, and a toxic reference. Collembola were exposed to concentrations of 0, 5.757, 10.36, 18.64, 33.55, 60.38, 108.6, 195.5, and 352

CA3301 / JOUST	Page 233 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

mg product/kg soil dw, equivalent to 0, 1.472, 2.648, 4.766, 8.578, 15.44, 27.78, 50, and 90 mg a.s./kg soil dw, (when accounting for the product a.s. content of 25.57% w/w), as well as 100 mg boric acid technical/kg soil dw. Stock solutions (191.0 mg a.s. dissolved in 500 mL distilled water, equivalent to 747 mg product) and dilutions of the product were made on the day of the application, which were then mixed evenly into the pre-moistened soil. Test soils were mixed manually by an operator, for between 120 and 240 s, with a steel spatula. Each test unit received 34.08 to 36.43 g soil

28 days after treatment application, the number of collembola were counted by transferring the contents of a test unit into a container and filled with water that contained blue ink. The collembola floated to the surface of the water, allowing for the counting of adults and juveniles to be quantified. Adult collembola not found were considered dead. Mortality data were corrected for control mortality, using the Abbott's formula.

The mortality and reproduction (fecundity) data were analysed with the statistical software R (version 3.3.1). All the statistical analyses were performed at significance level of $\alpha = 0.05$. A Spearman's correlation test confirmed the monotonicity of the mortality data, which allowed for a step-down Cochran-Armitage trend test to reveal any statistically significant differences in mortality between the control and product treatment groups. A second Spearman's correlation test then revealed that the mortality data of the non-significant groups were not monotonic; thus, these data were compared using a single-step Fisher's exact pairwise test, with Bonferroni-Holm corrections. LC_x values were calculated with a log-logistic binomial model.

Reproductive (fecundity) data was tested for outliers, using Tukey's rule, which were not analyzed. These data were normally distributed (Shapiro-Wilk's test) and had equal variances (Bartlett's test). Therefore, significant differences in reproduction were revealed with an ANOVA, followed by a Dunnett's test. EC_x values were calculated with a log-logistic gamma-Poisson model.

Results

Biological results

Mortality

A summary of the effect of CA3301 on adult mortality from the definitive test, after 28 days of exposure, is presented in the Table CP 10.4.2.1/01-01 below.

Table CP 10.4.2.1/01-01. The effect of CA3301 on adult *F. candida* mortality, after 28 days of exposure.

Soil concentration		Rep.	Number of collembola		Mortality	
mg product/kg sdw	mg a.s./kg sdw		Initial	Surviving	Mean (%)	Corrected (%) ^s
0 (control)	0		10	10	6.25	0.0
		2	10	10		
		3	10	8		
		4	10	7		
		5	10	10		
		6	10	10		
		7	10	10		
		8	10	10		
5.757	1.472	1	10	9	7.5	+1.333
		2	10	10		
		3	10	8		
		4	10	10		
10.356	2.648	1	10	8	20.0	+14.67

CA3301 / JOUST	Page 234 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Soil concentration		Rep.	Number of collembola		Mortality	
mg product/kg sdw	mg a.s./kg sdw		Initial	Surviving	Mean (%)	Corrected (%) ^{\$}
		2	10	8		
		3	10	8		
		4	10	8		
18.639	4.766	1	10	10	0.0	-6.667
		2	10	10		
		3	10	10		
		4	10	10		
33.55	8.578	1	10	9	12.5	+6.667
		2	10	9		
		3	10	9		
		4	10	8		
60.38	15.44	1	10	10	2.5	-4.0
		2	10	10		
		3	10	9		
		4	10	10		
108.64	27.78	1	10	4	30.0	+25.33*
		2	10	9		
		3	10	7		
		4	10	8		
195.54	50	1	10	0	90.0	+89.33*
		2	10	1		
		3	10	1		
		4	10	2		
352.0	90	1	10	0	100.0	+100.0*
		2	10	0		
		3	10	0		
		4	10	0		
100 mg dimethoate technical/kg sdw		1	10	6	17.5	+12.0
		2	10	9		
		3	10	8		
		4	10	10		

Significant differences in mortality were revealed with either a step-down Cochran-Armitage trend test or a single-step Fisher's exact pairwise test, with Bonferroni-Holm corrections, when $p < 0.05$, and are denoted with asterisks (*). ^{\$}Mortality was corrected using the Abbott formula; positive and negative values indicate an increase and decrease in mortality, respectively, relative to the control group. Rep., replicate.

There were significant differences in mortality between the control group and the three-highest product concentrations. The 28-day NOEC and LOEC values, based on mortality, were determined to be 60.38 and 108.6 mg product/kg soil dw, equivalent to 15.44 and 27.78 mg a.s./kg soil dw, respectively. The 28-day LC₁₀, LC₂₀, and LC₅₀ values, based on mortality, were calculated to be 92.57, 106.1, and 134.1 mg product/kg soil dw, equivalent to 23.68, 27.15, and 34.34 mg a.s./kg soil dw, respectively.

Reproduction

A summary of the effect of CA3301 on reproduction from the definitive test, after 28 days of exposure, is presented in the Table CP 10.4.2.1/01-02 below.

CA3301 / JOUST	Page 235 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Table CP 10.4.2.1/01-02. The effect of CA3301 on *F. candida* reproduction (fecundity), after 28 days of exposure.

Soil concentration		Rep.	Number of juveniles		Reproduction	
mg product/kg sdw	mg a.s./kg sdw		Per rep.	Mean	CV (%)	Change (% control)
0 (control)	0		523	387.1	18.1	0
		2	406			
		3	334			
		4	310			
		5	330			
		6	361			
		7	440			
		8	393			
5.757	1.472	1	507	379.5	22.73	-1.97
		2	357			
		3	332			
		4	322			
10.356	2.648	1	285	341	20.25	-11.91
		2	345			
		3	437			
		4	297			
18.639	4.766	1	292	355.8	13.16	-8.1
		2	350			
		3	396			
		4	385			
33.55	8.578	1	339	452.8	19.62	16.95
		2	556			
		3	455			
		4	461			
60.38	15.44	1	581	560.3 [#]	11.00 [#]	+44.74 [#]
		2	491			
		3	321[3]			
		4	609			
108.64	27.78	1	191	218.8*	28.99	-43.49
		2	244			
		3	293			
		4	147			
195.54	50	1	0	33.5*	72.49	-91.35
		2	55			
		3	32			
		4	47			
352.0	90	1	0	0.0*	0.0	-100
		2	0			
		3	0			
		4	0			
100 mg dimethoate technical/kg sdw		1	129	189.3	23.98	-51.11
		2	219			
		3	229			
		4	180			

Significant differences in mortality were revealed with an ANOVA, followed by a Dunnett's test, when $p < 0.05$, and are denoted

CA3301 / JOUST	Page 236 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

with asterisks (*). #One replicate was considered an outlier, based on Tukey's rule, and was not analyzed. Positive and negative changes in reproduction values indicate an increase and decrease in the number of juveniles per replicate, respectively, relative to the control group. Rep., replicate; CV, coefficient of variation.

Treatment with CA3301 caused significant reductions in reproduction, when collembola were exposed to the three-highest concentrations. Thus, the 28-day NOEC and LOEC (reproduction) values were determined to be 60.38 and 108.6 mg product/kg soil dw, equivalent to 15.44 and 27.78 mg a.s./kg soil dw, respectively. The 28-day reproductive EC₁₀, EC₂₀, and EC₅₀ values (with 95% confidence intervals) were calculated to be 87.32 (66.74 – 159.8), 100.5 (80.80 – 166.2), and 128.1 (109.6 – 177.8) mg product/kg soil dw, respectively. These are equivalent to 28-day reproductive EC₁₀, EC₂₀, and EC₅₀ values (with 95% confidence intervals) of 22.29 (16.97 – 40.37), 25.63 (20.50 – 42.10), and 32.67 (27.87 – 45.16) mg a.s./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 to be <20% (actual value: 6.25%)
- The mean number of juveniles per replicate in control to be >100 (actual value: 387.1)
- The coefficient of variation calculated for the number of juveniles in the control to be <30% (actual value: 18.1%)
- The reduction of fecundity in the toxic reference, relative to the control to be >50% (actual value: -51.11%)

Conclusion

The 28-day chronic toxicity of CA3301 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 for mortality were determined to be 60.38 and 108.6 mg product/kg soil dw, equivalent to 15.44 and 27.78 mg a.s./kg soil dw, respectively. The 28-day LC₁₀, LC₂₀, and LC₅₀ values were calculated to be 92.57, 106.1, and 134.1 mg product/kg soil dw 23.68, equivalent to 27.15, and 34.34 mg a.s./kg soil dw, respectively.

The 28-day NOEC and LOEC (reproduction) values were determined to be 60.38 and 108.6 mg product/kg sdw, equivalent to 15.44 and 27.78 mg a.s./kg sdw, respectively. The 28-day reproductive EC₁₀, EC₂₀, and EC₅₀ values were calculated to be 87.32, 100.5, and 128.1 mg product/kg soil dw, equivalent to 22.29, 25.63, and 32.67 mg a.s./kg soil dw, respectively.

This study is considered acceptable.

CA3301 / JOUST	Page 237 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Study 2

Comments of zRMS:	The study was conducted to the OECD guideline 226 and according to the principles of GLP. All validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.
-------------------	--

Reference:	KCP 10.4.2.1/02
Report	CA3301 - A laboratory study to determine the acute and sublethal effects on the predatory mite <i>Hypoaspis aculeifer</i> (Acari: Laelapidae) Frainout, C., 2021, report no. 029SRFR20C11
Guideline(s):	Yes. 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

A 14-day reproductive study of a predatory soil mite, *Hypoaspis aculeifer*, with the product, CA3301 (analyzed a.s. content of 25.57% w/w) was conducted in artificial soil, with eleven treatment groups of 0 (water control), 1.779, 3.199, 5.757, 10.36, 18.64, 33.55, 60.38, 108.6, 195.5, and 352 mg product/kg soil dry weight (dw), equivalent to 0, 0.455, 0.818, 1.472, 2.648, 4.766, 8.578, 15.44, 27.78, 50, and 90 mg a.s./kg soil dw and a toxic reference, boric acid (purity >95% w/w).

The control and toxic reference groups comprised eight replicates and the product treatment groups comprised four replicates each, with ten adult mites per replicate. After 14 days' exposure, adult and juvenile mites were removed from their test units, to assess adult mortality and reproduction (fecundity).

Treatment with CA3301 significantly increased mortality with the two highest product concentrations. Thus, the 14-day NOEC and LOEC values for mortality were determined to be 108.6 mg product/kg soil dw (equivalent to 27.78 mg a.s./kg soil dw) and 195.5 mg product/kg soil dw (equivalent to 50.00 mg a.s./kg soil dw), respectively. The 14-day LC₅₀ value was estimated to be >352.0 mg product/kg soil dw (equivalent to >90.0 mg a.s./kg soil dw).

Treatment with CA3301 significantly reduced reproduction with the three highest product concentrations. Thus, the 14-day NOEC and LOEC values, based on reproduction, were determined to be 60.38 and 108.6 mg product/kg soil dw (equivalent to 15.44 and 27.78 mg a.s./kg soil dw), respectively. The 14-day EC₁₀, EC₂₀, and EC₅₀ values (reproduction) were calculated to be 90.18, 124.8, and 218.2 mg product/kg soil dw (equivalent to 23.03, 31.87, and 55.83 mg a.s./kg soil dw), respectively.

For validation of the test system, treatment with boric acid, as the reference item, resulted in >50% in reproduction.

Overall, the study satisfies the OECD 226 (2016) test-guideline requirements for chronic toxicity with *Hypoaspis aculeifer* and is considered acceptable.

Materials and methods

Test materials

CA3301 / JOUST	Page 238 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Test item

Name:	CA3301
Active substance:	Prothioconazole
Formulation type:	Emulsifiable concentrate (EC)
Source and lot/batch no.:	A19045
Product density:	0.9948 kg/L (nominal)
Active substance content:	250 g/L (nominal), 257.04 g/L or 25.57% w/w (analyzed)
Appearance:	Clear, straw-coloured liquid
Expiry date of lot/batch:	17 June 2022
Storage conditions:	9.2-25.5°C, protected from light

Reference item

Name:	Boric acid
Formulation type:	Technical
Batch no.:	1860103
Purity:	>95% w/w or 950 g/kg
Expiry date:	18 June 2023

Test organism

Species:	<i>Hypoaspis (Geolaelaps) aculeifer</i> Canestrini (Acari: Laelapidae)
Age at study initiation:	32 and 35 days after the start of the egg laying in the synchronization
Sex:	Adult females
Source:	Synchronized cohort from Bias Labs, Kirkcaldy, Fife, UK
Feeding during test:	Cheese mites (<i>Tyrophagus putrescentiae</i>) <i>ad libitum</i> , 3x weekly, alongside distilled water.

Test medium:

Soil type:	Artificial soil 5% sphagnum peat 20% kg kaolin clay (kaolinite content > 30%), 50% industrial quartz sand, CaCO ₃ , to obtain optimal pH of 6.0 ± 0.5.
pH:	5.96-6.69 (test initiation), 6.32-6.84 (test termination), mean of 6.31
Water-holding capacity:	mean of 50%

Test conditions

Test temperature:	17.5 – 22.9°C (mean value of 19.7°C)
Photoperiod:	16 h light:8 h dark
Light intensity:	468 lux

20 ± 1 g of dry artificial soil (equivalent to 22.6 ± 1.13 g wet soil) were placed into each plastic test unit (100-mL capacity and 4-5-cm diameter) that were covered translucent screw lids, which reduced evaporation, but allowed gas exchange.

Stock solutions and dilutions of the product were made on the day of the application, which were then mixed evenly into the pre-moistened soil. Test soils were mixed manually by an operator, for about 120 s, with a steel spatula. The study consisted of twelve treatments: one control, nine product concentrations, and one toxic reference-item group. Eight replicates (test units) were set up for the control, and reference-item concentrations, and four replicates for each product concentration. All replicates consisted of ten adult females (80 control and reference-item mites and 40 mites for each product group). Mites were assigned randomly to the different treatment groups.

Each test unit was weighed, to monitor the water content throughout the test. Test units were positioned

CA3301 / JOUST	Page 239 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

randomly in a temperature-controlled room and these positions were re-randomized weekly. The exposure phase of the adults in the study lasted four weeks (14 days). Thereafter, mites were removed from the test units using light and heat extraction, then adult mortality and reproduction (the number of juveniles found) were determined.

The eleven product treatment groups were 0 (water control), 1.779, 3.199, 5.757, 10.36, 18.64, 33.55, 60.38, 108.6, 195.5, and 352 mg product/kg soil dw, equivalent to 0, 0.455, 0.818, 1.472, 2.648, 4.766, 8.578, 15.44, 27.78, 50, and 90 mg a.s./kg soil dw (when accounting for the product a.s. content of 25.57% w/w), respectively. The toxic-reference group used 250 mg boric acid/kg soil dw.

14 days after treatment application, the surviving mites were extracted from the soil via light & heat extraction. Before heat extraction, any visible mites in the test units were removed. After this, the test units were turned upside-down and heat extracted. The numbers of extracted juveniles and adults were counted and recorded under microscope; any adult mites not found was recorded as dead.

The 14-day mortality and reproduction (fecundity) data were analysed with the statistical software R (version 3.3.1). All the statistical analyses were performed at significance level of $\alpha = 0.05$.

A Spearman's correlation test confirmed the monotonicity of the mortality data, which allowed for a step-down Cochran-Armitage test to reveal any statistically significant differences in mortality between the control and product treatment groups. A second Spearman's correlation test then revealed that the mortality data of the non-significant groups were not monotonic; thus, these data were compared using a single-step Fisher's exact pairwise test, with Bonferroni-Holm corrections. Since no product concentrations showed effects on mortality exceeding 50%, no statistical dose-response model was relevant to calculate the LC_x values.

The reproductive (fecundity) data were normally distributed (Shapiro-Wilk's test) and had equal variances (Barlett's test). Thus, reproductive data were compared with an ANOVA, with pairwise Dunnett's corrections. EC_x were calculated using the Bayesian inference model MORSE (log-logistic Gamma-Poisson model).

Results

Biological results

Mortality

The observations of female adult mortality were carried out from the day of application (day 0) to until 14 days after the treatment incorporation, detailed in the table below.

Table CP 10.4.2.1/02-01. The effects of CA3301 on adult *H. aculeifer* mean mortality, after 14 days of exposure.

Soil concentration		Rep.	Number of mites		Mortality	
mg product/kg sdw	mg a.s./kg sdw		Initial	Surviving	Mean (%)	Corrected (%) ^s
0 (control)	0	1	10	10	8.75	0
		2	10	8		
		3	10	10		
		4	10	7		
		5	10	10		
		6	10	8		
		7	10	10		
		8	10	10		
1.779	0.455	1	10	10	2.5	-6.849

CA3301 / JOUST	Page 240 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

		2	10	10		
		3	10	10		
		4	10	9		
3.199	0.818	1	10	8	7.5	-1.37
		2	10	10		
		3	10	10		
		4	10	9		
5.757	1.472	1	10	8	20	+12.33
		2	10	8		
		3	10	8		
		4	10	8		
10.36	2.648	1	10	10	7.5	-1.37
		2	10	8		
		3	10	10		
		4	10	9		
18.64	4.766	1	10	10	0	-9.589
		2	10	10		
		3	10	10		
		4	10	10		
33.55	8.578	1	10	10	10	+1.37
		2	10	9		
		3	10	7		
		4	10	10		
60.38	15.44	1	10	9	20	+12.33
		2	10	9		
		3	10	10		
		4	10	4		
108.6	27.78	1	10	8	10	+1.37
		2	10	9		
		3	10	9		
		4	10	10		
195.5	50	1	10	6	27.5*	+20.55*
		2	10	7		
		3	10	7		
		4	10	9		
352	90	1	10	5	47.5*	+42.47*
		2	10	6		
		3	10	5		
		4	10	5		
250.0 mg boric acid/kg sdw		1	10	8	13.75	+5.48
		2	10	9		
		3	10	6		
		4	10	9		
		5	10	10		
		6	10	10		
		7	10	7		
		8	10	10		

CA3301 / JOUST	Page 241 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Significant differences in mortality between the product and control treatment groups were revealed with a pairwise Fisher's exact test, with Holm-Bonferroni corrections, and are denoted with asterisks (*). ^sData were corrected for control mortality using the Abbott formula. Negative/positive values mean lower/higher mortality, compared to the control.

There were significant differences in mortality between the control group and the two highest product concentrations (195.5 and 352.0 mg product/kg soil dw). The 14-day NOEC and LOEC values for mortality were determined to be 108.6 mg product/kg soil dw (equivalent to 27.78 mg a.s./kg soil dw) and 195.5 mg product/kg soil dw (equivalent to 50.00 mg a.s./kg soil dw), respectively. Since the effects on mortality did not exceed 50% in any product treatment group, no statistical model was relevant to calculate the LC_x values. Therefore, the 14-day LC₅₀ value was estimated to be >352.0 mg product/kg soil dw (equivalent to >90.0 mg a.s./kg soil dw). Mean adult mortality was 8.75% in the distilled water control and 13.75% in the toxic reference item treatment after 14 days.

Reproduction

Observations on reproductive output of *Hypoaspis aculeifer* were carried out 14 days after the treatment incorporation, in the control, toxic reference, and product treatment groups, detailed in the table below.

Table CP 10.4.2.1/02-02. The effects of CA3301 on *H. aculeifer* reproduction (fecundity), after 14 days of exposure.

Soil concentration		Rep.	Number of juveniles		Reproduction	
mg product/kg sdw	mg a.s./kg sdw		Per rep.	Mean	CV (%)	Change (% control) ^s
0 (control)	0	1	89	89.38	18.83	0
		2	87			
		3	88			
		4	74			
		5	90			
		6	63			
		7	109			
		8	115			
1.779	0.455	1	93	82	14.87	-8.252
		2	65			
		3	88			
		4	82			
3.199	0.818	1	74	80	27.06	-10.49
		2	107			
		3	84			
		4	55			
5.757	1.472	1	73	87.5	16.34	-2.098
		2	78			
		3	96			
		4	103			
10.36	2.648	1	67	68.25	9.673	-23.64
		2	68			
		3	77			
		4	61			
18.64	4.766	1	83	81.5	11.62	-8.811
		2	94			
		3	72			
		4	77			
33.55	8.578	1	69	92.5	23.53	+3.497

CA3301 / JOUST	Page 242 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Soil concentration		Rep.	Number of juveniles		Reproduction	
mg product/kg sdw	mg a.s./kg sdw		Per rep.	Mean	CV (%)	Change (% control) ^s
		2	100			
		3	82			
		4	119			
60.38	15.44	1	80	86.0	7.415	-3.776
		2	91			
		3	81			
		4	92			
108.6	27.78	1	57	65.0*	12.5	-27.27
		2	75			
		3	60			
		4	68			
195.5	50	1	49	44.5*	14.51	-50.21
		2	50			
		3	36			
		4	43			
352	90	1	29	15.75*	59.87	-82.38
		2	15			
		3	7			
		4	12			
250.0 mg boric acid/kg sdw		1	40	27.38*	42.28	-69.37
		2	11			
		3	25			
		4	24			
		5	12			
		6	41			
		7	31			
		8	35			

Significant differences in reproduction, relative to the control group, were revealed with an ANOVA, with Dunnett's test corrections, and are denoted with asterisks (*). ^sNegative/positive values mean lower/higher fecundity compared to the distilled water control. Rep., replicate; CV, coefficient of variation.

There were significant differences in reproduction between the control group and three highest product treatment groups. The 14-day NOEC and LOEC values, based on reproduction, were determined to be 60.38 and 108.6 mg product/kg soil dw (equivalent to 15.44 and 27.78 mg a.s./kg soil dw), respectively.

The 14-day EC₁₀, EC₂₀, and EC₅₀ values (with 95% CIs) were calculated to be 90.18 (52.68 – 135.6), 124.8 (84.57 – 169.8), and 218.2 (181.1 – 259.8) mg product/kg soil dw, respectively. These values were equivalent to 14-day EC₁₀, EC₂₀, and EC₅₀ values (with 95% CIs) of 23.03 (13.44 – 34.56), 31.87 (21.68 – 43.29), and 55.83 (46.40 – 66.57) mg a.s./kg soil dw,, respectively.

The mean number of juveniles per replicate was 89.38 in the control and 27.38 in the toxic reference item after 14 days.

Validity

All validity criteria were met in accordance with OECD 226 (2016) test guideline:

- The mean adult mortality in the control to be <20% (actual value: 8.75%)
- The mean number of juveniles per replicate in control to be >50 (actual value: 89.38)
- The coefficient of variation calculated for the number of juveniles in the control to be <30% (actual value: 18.83%)

CA3301 / JOUST	Page 243 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

- The reduction of fecundity in the toxic reference, relative to the control to be >50% (actual value: -69.37%)

Conclusion

The 14-day chronic toxicity of CA3301 to *Hypoaspis aculeifer* was studied under in accordance with the OECD 226 (2016) test guideline.

The 14-day NOEC and LOEC values for mortality were determined to be 108.6 mg product/kg soil dw (equivalent to 27.78 mg a.s./kg soil dw) and 195.5 mg product/kg soil dw (equivalent to 50.00 mg a.s./kg soil dw), respectively. The 14-day LC₅₀ value was estimated to be >352.0 mg product/kg soil dw (equivalent to >90.0 mg a.s./kg soil dw).

The 14-day NOEC and LOEC values, based on reproduction, were determined to be 60.38 and 108.6 mg product/kg soil dw (equivalent to 15.44 and 27.78 mg a.s./kg soil dw), respectively. The 14-day EC₁₀, EC₂₀, and EC₅₀ values (reproduction) were calculated to be 90.18, 124.8, and 218.2 mg product/kg soil dw (equivalent to 23.03, 31.87, and 55.83 mg a.s./kg soil dw), respectively.

This study is considered acceptable.

A 2.4.2.2	KCP 10.4.2.2	Higher-tier testing
A 2.5	KCP 10.5	Effects on soil nitrogen transformation
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 to the OECD guideline 227 and according to the principles of GLP. All validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.
-------------------	--

Reference:	KCP 10.6.2/01
Report	CA3301 - A study to determine the effects on the Vegetative Vigour of terrestrial plants Frainout, C., 2021, report no. 029SRFR20C13
Guideline(s):	Yes. OECD 227 (2006)
Deviations:	No
GLP:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability:	Yes
Duplication (if vertebrate study)	No

CA3301 / JOUST	Page 244 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Executive summary

This study was performed as a limit test to evaluate the effects of CA3301 (analyzed product a.s. content of 257.04 g prothioconazole/L) on vegetative vigour, using ten selected non-target plant species, following application of 0 (untreated water control) and 1.6 L product/ha (equivalent to 406.99 g a.s./ha). 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 CA3301.

CA3301 did not significantly reduce survival in any of the plant species tested. Thus, NOER and LOER (survival) values for all species were determined to be 1.6 L and >1.6 L product/ha, equivalent to 406.99 and >406.99 g a.s./ha, respectively (based on nominal concentrations).

CA3301 significantly decreased shoot height in soybean, cucumbers, and tomatoes. Thus, for soybean, cucumber and tomato, the NOER and LOER (shoot height) values were determined to be <1.6 and 1.6 L product/ha, equivalent to <406.99 and 406.99 g a.s./ha, respectively (based on nominal concentrations). The ER_{50} (shoot height) was estimated to be >1.6 L product/ha, equivalent to >406.99 g a.s./ha.

CA3301 significantly decreased dry shoot weight in cucumber and tomato. Thus, for cucumber and tomato, the NOER and LOER (shoot height) values were <1.6 and 1.6 L product/ha, equivalent to <406.99 and 406.99 g a.s./ha, respectively (based on nominal concentrations). The ER_{50} (shoot dry weight) was estimated to be >1.6 L product/ha, equivalent to >406.99 g a.s./ha.

Given these results, the most sensitive species after 21 days of exposure were soybeans, cucumber, and tomatoes. Overall, the study satisfies the OECD 227 (2006) test-guideline requirements for a vegetative-vigour study, and is considered acceptable.

Materials and methods

Test materials

Test item

Name:	CA3301
Density:	0.9948 g/mL
Active substance:	Prothioconazole
Formulation type:	Emulsifiable concentrate (EC)
Source and lot/batch no.:	A19045
Active-substance content:	250 g/L (nominal), 257.04 g/L or 25.57% w/w (analyzed)
Appearance:	Clear, straw-coloured liquid
Expiry date of lot/batch:	17 June 2022
Storage:	Between 15.9°C and 26.4°C, protected from light

Reference item

Name:	Roundup Innov
Density:	1.3426 g/mL
Active substance:	Glyphosate K-salt
Formulation type:	Soluble concentrate
Source and lot/batch no.:	AZM012312C
Active-substance content:	44% w/w
Storage:	Between 19.2°C and 24.6°C

Test organisms

Clade	Plant	Species	Family	Variety	Seed source	Seed lot ID.
Monocot	Ryegrass	<i>Lolium perenne</i> L.	Poaceae	Indra	Gamm vert	1943F0674R2B0161C
	Sorghum	<i>Sorghum sudanense</i> L.	Poaceae	Cert. Piper	USA	Z020SP1004
	Onion	<i>Allium cepa</i> L.	Liliaceae	Paille des vertus	Truffaut	I04085
	Corn	<i>Zea mays</i> L.	Poaceae	Monloui	Caussade semence	F0252EA01940
Dicot	Carrot	<i>Daucus carota</i> L.	Apiaceae	Bolero F1	Vilmorin	N60675
	Radish	<i>Rafanus sativus</i> L.	Brassicaceae	Flamboyant 2	Ferme de Sainte Marthe	20-03024
	Soybean	<i>Glycine max</i> L.	Fabaceae	n.d.v	SynTech Research 2017	VBA0191/704
	Tomato	<i>Solanum lycopersicum</i> L.	Solanaceae	Montfavet 63-5F1	Vilmorin	L41905
	Cucumber	<i>Cucumis sativus</i> L.	Cucurbitaceae	Serit F1	Hazera	L60606/M
	Lettuce	<i>Lactuca sativa</i> L.	Asteraceae	Laitue beure plein champs	Vilmorin	N39563

Test medium:

Soil type:

Artificial soil

Sandy soil: (coarse sand 87%, fine sand 8.6%, fine silt 1.6%, coarse silt 1.6%, clay 1.1%) containing 1.8% of organic matter, 1.01% of organic carbon

92.85% of quartz sand (air dried, particle size < 2 mm)

7.15% of universal horticultural compost (air dried and with no visible plant remains)

pH:

6.8

Conductivity:

0.08 mS/cm

Test conditions

Test temperature:

Treatment application: 27.1 – 31.8°C,

Experimental phase: 18.9 – 31°C (daytime), 15.1 – 22°C (nighttime)

Relative humidity:

Treatment application: 31 – 46%,

Experimental phase: 52.1 – 86.8%

Photoperiod:

16 h light:8 h dark

Light intensity:

16200 – 32800 lux, 350 ± 50 µE/m²/s

Ten higher terrestrial plant species were used for this study (four monocotyledons and six dicotyledons). Within a given species, all test plants seeds, including the control, were from the same source. Plants were grown from seeds at the test site of SynTech Research France SAS, (Nimes, France). Within 48 h prior to the application of the treatments, plants of similar size and condition were randomly selected and assigned to the different treatment groups. The plants were between 2-to-4 true-leaf stage at spray application.

CA3301 / JOUST	Page 246 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Plants were grown in test units consisting of plastic pots (14-cm diameter), containing about 1.3 L of soil. Each pot was filled to a depth of 90 mm of soil, with 1 to 5 plants per pot, depending on the size of the plant species. Briefly, for ryegrass, sorghum, and onion, there were two pots per replicate, with five plants per pot; for corn, carrot, radish, and soybean, there were five pots per replicate, with 2 plants per pot; and for cucumber, tomato, and lettuce, there were ten pots per replicate, with one plant per pot. Germination of plants was between 91 and 100%.

The study was conducted as a limit test with three treatment groups: a distilled-water control, one product treatment group, and one toxic-reference group. The treatment rates were 0 (control) and 1.6 L product/ha (equivalent to 406.99 g a.s./ha), and 8 L Roundup/ha (4.73 mg glyphosate K-salt/ha, when accounting for the nominal product density of 1.3426 g/mL and a.s. content of 44% w/w). There were 4 replicates per plant species per treatment group and each replicate consisted of 10 seedlings (40 seedlings per species). For the treatment spray solutions, 7.9585 g of product was dissolved in 2000 mL distilled water (equivalent to 1028 mg a.s./L) and 53.7038 g of Roundup was dissolved in 2000 mL of distilled water. Only distilled water was used for the control treatment. The spray solutions were applied at a rate of 400 L/ha.

Survival, mortality, and phytotoxicity were assessed 1x weekly, from day 0 (test initiation) to day 21 (test termination). Phytotoxicity was assessed visually, by observing morphology, specifically chlorosis, necrosis, pigmentation, leaf curling, wilting, deformities, and stunting. At the end of assessment for a given species, shoot height of surviving plants was first recorded, then the surviving plants were dried (at 60°C) and their shoots weighed. Dry weights of each replicate were measured (not individual plants).

Data were analysed with the statistical software R (version 3.3.1), at a significance level of $\alpha = 0.05$, to determine any significant differences in survival, shoot height, dry weight, and phytotoxicity. Survival data were analysed with a unilateral Fisher's exact test, whereas data for shoot height, shoot dry weight, and phytotoxicity was tested for normality (Shapiro-Wilk's test) and homoscedasticity (Bartlett's test). When normality and homoscedasticity were confirmed, data were analysed with two-sided Student's t-tests. For non-normal and/or non-homoscedastic data, data were log10-transformed (shoot height and shoot dry weight) or arcsine-square-root-transformed (phytotoxicity) when needed. When normality and/or homoscedasticity could not be achieved, even after transformation, a Mann-Whitney-Wilcoxon's test was performed. Based on the results of these tests, the NOER and LOER values were determined.

Analytical verification of the stock product spray solution was conducted during the analytical phase of the study (analytical test site code: 20070-06R). Four representative samples of the spray solutions (50 mL each) were collected (product was diluted in distilled water) during application of the study: two samples (one for analysis and one as a retained sample) were collected just prior to the application and two samples just after the application.

Results

Analytical results

Table CP 10.6.2/01-01. Summary of the analytical results in the spray solutions.

Sampling Time	Prothioconazole concentration (mg a.s./L) [#]		Recovery (%)		
	Target*	Measured	Nominal	Mean	Deviation
Before Application	1028	885.9	86.2	89.4	-13.8
After Application	1028	952.4	92.6		-7.4

LOQ: 10 mg/kg; a.s. = active substance. *Calculated from the analyzed product a.s. content of 257.04 g/L. [#]Calculated considering the density of water at 20°C (0.9982 g/mL).

The analytical recoveries were all within $\pm 20\%$ of nominal concentrations, therefore, results and endpoints are based on nominal concentrations.

CA3301 / JOUST	Page 247 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Biological results

Survival and mortality

The effect of CA3301 on survival and mortality, after 21 days of exposure, is presented in the tables below.

Table CP 10.6.2/01-02. The effect of CA3301 on the mortality of 10 plant species, after 21 days of exposure.

Application rate		Rep.	Mortality					
L product/ha	g a.s./ha		No.	Mean (%)	CV (%)	No.	Mean (%)	CV (%)
			Ryegrass			Radish		
0 (control)	0	1	0	0.0 ± 0.0	0.0	0	0.0 ± 0.0	0.0
		2	0			0		
		3	0			0		
		4	0			0		
1.6	406.99	1	0	0.0 ± 0.0	0.0	0	0.0 ± 0.0	0.0
		2	0			0		
		3	0			0		
		4	0			0		
8 L Roundup/ha		1	10	100.0 ± 0.0	-	10	100.0 ± 0.0	-
		2	10			10		
		3	10			10		
		4	10			10		
			Sorghum			Soybean		
0 (control)	0	1	0	0.0 ± 0.0	0.0	0	0.0 ± 0.0	0.0
		2	0			0		
		3	0			0		
		4	0			0		
1.6	406.99	1	0	0.0 ± 0.0	0.0	0	0.0 ± 0.0	0.0
		2	0			0		
		3	0			0		
		4	0			0		
8 L Roundup/ha		1	10	100.0 ± 0.0	-	6	62.5 ± 17.08	45.54
		2	10			4		
		3	10			8		
		4	10			7		
			Onion			Cucumber		
0 (control)	0	1	0	0.0 ± 0.0	0.0	0	0.0 ± 0.0	0.0
		2	0			0		
		3	0			0		
		4	0			0		
1.6	406.99	1	0	0.0 ± 0.0	0.0	0	0.0 ± 0.0	0.0
		2	0			0		
		3	0			0		
		4	0			0		
8 L Roundup/ha		1	10	100.0 ± 0.0	-	2	47.5 ± 26.3	50.09
		2	10			3		
		3	10			7		
		4	10			7		
			Corn			Tomato		
0 (control)	0	1	0	0.0 ± 0.0	0.0	0	0.0 ± 0.0	0.0
		2	0			0		
		3	0			0		
		4	0			0		

CA3301 / JOUST	Page 248 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

1.6	406.99	1	0	0.0 ± 0.0	0.0	0	0.0 ± 0.0	0.0
		2	0			0		
		3	0			0		
		4	0			0		
8 L Roundup/ha		1	10	100.0 ± 0.0	-	10	100.0 ± 0.0	-
		2	10			10		
		3	10			10		
		4	10			10		
		Carrot				Lettuce		
0 (control)	0	1	0	0.0 ± 0.0	0.0	0	0.0 ± 0.0	0.0
		2	0			0		
		3	0			0		
		4	0			0		
1.6	406.99	1	0	0.0 ± 0.0	0.0	0	0.0 ± 0.0	0.0
		2	0			0		
		3	0			0		
		4	0			0		
8 L Roundup/ha		1	9	80.0 ± 11.55	57.74	10	100.0 ± 0.0	-
		2	7			10		
		3	9			10		
		4	7			10		

Rep., replicate; no., number; CV, coefficient of variation.

Table CP 10.6.2/01-03. The effect of CA3301 on the survival of 10 plant species, after 21 days of exposure.

Plant	21-day mean survival and mortality				
	Control survival (%)	Product treatment		Toxic-reference treatment	
		Survival (%)	Mortality (%) [*]	Survival (%)	Mortality (%) [*]
Ryegrass	100.0	100.0	0.0	0.00	100.0
Sorghum	100.0	100.0	0.0	0.00	100.0
Onion	100.0	100.0	0.0	0.00	100.0
Corn	100.0	100.0	0.0	0.00	100.0
Carrot	100.0	100.0	0.0	20.0	80.00
Radish	100.0	100.0	0.0	0.00	100.0
Soybean	100.0	100.0	0.0	37.50	62.50
Cucumber	100.0	100.0	0.0	52.50	47.50
Tomato	100.0	100.0	0.0	0.00	100.0
Lettuce	100.0	100.0	0.0	0.00	100.0

^{*}Given that there was no effect on survival, the data were not considered significant. [#]Mortality data were normalized to the control mortality.

The mean survival of plants in the control and product treatment groups was 100%, whereas it ranged between 0 and 52.5% in the toxic-reference group. There were no significant differences in survival or mortality between the control and product treatment groups. Thus, NOER and LOER (survival) values for all species was 1.6 L and >1.6 L product/ha, equivalent to 406.99 and >406.99 g a.s./ha, respectively (based on nominal concentrations).

Shoot height and shoot dry weight

A summary of the effect of CA3301 on shoot height and dry shoot weight, after 21 days of exposure, is presented in tables below.

CA3301 / JOUST	Page 249 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Table CP 10.6.2/01-04. The effect of CA3301 on shoot height of 10 plant species, after 21 days of exposure.

Application rate	Rep.	Shoot height					
L product/ha [g a.s./ha]		Mean (%)	CV (%)	Inhibition (% control)	Mean (%)	CV (%)	Inhibition (% control)
		Ryegrass			Radish		
0 (control)	1	45.06 ± 6.61	14.67	0.00	0 ± 16.19	2.75	16.97
	2						
	3						
	4						
1.6 [406.99]	1	45.53 ± 5.76	12.65	-1.03	-1.03 ± 16.95	2.17	12.83
	2						
	3						
	4						
Rate		Sorghum			Soybean		
0 (control)	1	51.5 ± 7.74	15.02	0.00	0 ± 45.31	5.68	12.55
	2						
	3						
	4						
1.6 [406.99]	1	53.21 ± 9.59	18.03	-3.3	-3.3 ± 41.89	4.95	11.82
	2						
	3						
	4						
Rate		Onion			Cucumber		
0 (control)	1	31.4 ± 5.47	17.41	0.00	0 ± 19.28	1.9	9.88
	2						
	3						
	4						
1.6 [406.99]	1	32.49 ± 2.93	9.01	-3.46	-3.46 ± 17.44	2.3	13.21
	2						
	3						
	4						
Rate		Corn			Tomato		
0 (control)	1	69.86 ± 7.34	10.51	0.00	0 ± 38.36	4.63	12.06
	2						
	3						
	4						
1.6 [406.99]	1	70.81 ± 6.61	9.34	-1.36	-1.36 ± 34.89	6.13	17.58
	2						
	3						
	4						
Rate		Carrot			Lettuce		
0 (control)	1	38.59 ± 5.02	13	0.00	0 ± 11.15	1.47	13.21

CA3301 / JOUST	Page 250 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

	2					
	3					
	4					
1.6 [406.99]	1	38.41 ± 5.33	13.87	0.45	0.45 ± 10.61	1.22 11.52
	2					
	3					
	4					

Data for shoot height were normally distributed and had equal variances, thus, statistically significant differences in shoot height were revealed with Student's t-tests, which are denoted by asterisks (*). #Negative and positive signs indicated stimulatory and inhibitory effect on shoot height, respectively, relative to the control. DAE, days after exposure; Rep., replicate; SD, standard deviation; CV, coefficient of variation.

There were significant decreases in shoot height for soybean, cucumbers, and tomatoes. Thus, for soybean, cucumber and tomato, the NOER and LOER (shoot height) values were <1.6 and 1.6 L product/ha, equivalent to <406.99 and 406.99 g a.s./ha, respectively (based on nominal concentrations). Given that CA3301 did not have a >50% effect on shoot height for any tested species, the ER₅₀ (shoot height) was estimated to be >1.6 L product/ha, equivalent to >406.99 g a.s./ha.

Table CP 10.6.2/01-05. The effect of CA3301 on dry shoot weight of 10 plant species, after 21 days of exposure.

Application rate	Rep.	Shoot weight (g)							
L product/ha [g a.s./ha]		Mean (g)	Mean ± SD (g)	CV (%)	Inhibition (% control)#	Mean (g)	Mean ± SD (g)	CV (%)	Inhibition (% control)#
		Ryegrass (21 DAE)				Radish (21 DAE)			
0 (control)	1	0.48	0.43 ± 0.04	10.2	0.0	0.61	0.52 ± 0.06	12.1	0.0
	2	0.45				0.48			
	3	0.37				0.49			
	4	0.41				0.50			
1.6 [406.99]	1	0.43	0.45 ± 0.04	7.8	-6.3	0.51	0.54 ± 0.05	9.9	-3.8
	2	0.45				0.54			
	3	0.51				0.62			
	4	0.43				0.50			
		Sorghum (21 DAE)				Soybean (21 DAE)			
0 (control)	1	0.23	0.26 ± 0.02	8.4	0.0	1.44	1.39 ± 0.05	3.7	0.0
	2	0.29				1.33			
	3	0.26				1.37			
	4	0.26				1.43			
1.6 [406.99]	1	0.25	0.29 ± 0.03	10.1	-12.0	1.32	1.34 ± 0.04	3.3	+3.6
	2	0.30				1.40			
	3	0.28				1.35			
	4	0.32				1.29			
		Onion (21 DAE)				Cucumber (21 DAE)			
0 (control)	1	0.08	0.12 ± 0.04	33.9	0.0	2.18	2.28 ± 0.08	3.7	0.0
	2	0.12				2.24			
	3	0.18				2.36			
	4	0.10				2.35			
1.6 [406.99]	1	0.10	0.13 ± 0.03	23.1	-5.4	1.62	1.78 ± 0.17*	9.4	+21.9

	2	0.10			1.66
	3	0.16			1.92
	4	0.14			1.93
		Corn (21 DAE)			
0 (control)	1	1.14	1.33 ± 0.19	14.6	0.0
	2	1.32			
	3	1.60			
	4	1.27			
		Tomato (21 DAE)			
1.6 [406.99]	1	1.21	1.41 ± 0.15	10.4	-6.1
	2	1.39			
	3	1.53			
	4	1.51			
		Carrot (21 DAE)			
0 (control)	1	0.77	0.74 ± 0.04	5.0	0.0
	2	0.77			
	3	0.71			
	4	0.70			
		Lettuce (21 DAE)			
1.6 [406.99]	1	0.63	0.79 ± 0.13	16.5	-6.7
	2	0.80			
	3	0.95			
	4	0.76			

Data for dry weight height were normally distributed and had equal variances (except radishes), thus, statistically significant differences in shoot height were revealed with Student's t-tests, which are denoted by asterisks (*). Radish data were analysed with a Mann-Whitney-Wilcoxon test and were not significantly different than the control. (*). #Negative and positive signs indicated stimulatory and inhibitory effect on shoot height, respectively, relative to the control. DAE, days after exposure; Rep., replicate; SD, standard deviation; CV, coefficient of variation.

There were significant decreases in dry shoot weight for cucumbers and tomatoes. Thus, for cucumber and tomato, the NOER and LOER (shoot height) values were <1.6 and 1.6 L product/ha, equivalent to <406.99 and 406.99 g a.s./ha, respectively (based on nominal concentrations). Given that CA3301 did not have a >50% effect on dry shoot weight for any tested species, the ER₅₀ (shoot height) was estimated to be >1.6 L product/ha, equivalent to >406.99 g a.s./ha.

Phytotoxicity

A summary of the phytotoxic effects of CA3301 and the toxic reference, after 21 days of exposure, is presented in tables below.

Table CP 10.6.2/01-06. The phytotoxic effects of CA3301 on 10 plant species, after 21 days of exposure.

Plant	Mean value (number) after 21 days of exposure													
	Chlorosis		Necrosis		Pigmentation		Wilting		Curling		Stunting		Deformities	
	Control	Product	Control	Product	Control	Product	Control	Product	Control	Product	Control	Product	Control	Product
Ryegrass	0.0	0.0	0.0	5.0*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.0*
Sorghum	0.0	0.0	0.0	10.0*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.75*
Onion	0.0	0.0	0.0	15.0*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Corn	0.0	0.0	0.0	20.0*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Carrot	0.0	0.0	0.0	10.0*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Radish	0.0	0.0	0.0	40.0*	0.0	0.0	0.0	0.0	0.0	20.0*	0.0	0.0	0.0	0.0
Soybean	0.0	10*	0.0	13.75*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10.0*	0.0	0.0
Cucumber	0.0	10*	0.0	40.0*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10.0*	0.0	0.0
Tomato	0.0	10*	0.0	20.0*	0.0	0.0	0.0	0.0	0.0	10.0*	0.0	5.0*	0.0	0.0
Lettuce	0.0	0.0	0.0	15.0*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10.0*	0.0	0.0

Statistically significant differences, relative to the control, are denoted with asterisks (*). The following tests were used to reveal statistically significant differences. Chlorosis: data were analysed with Student's t-tests, except for soybean, cucumber, and tomato, which were analysed with Mann-Whitney-Wilcoxon tests. Necrosis: data were analysed with Mann-Whitney-Wilcoxon tests. Pigmentation: data were analysed with Student's t-tests. Wilting: data were analysed with Student's t-tests. Curling: data were analysed with Student's t-tests, except for radish and tomato, which were analysed with Mann-Whitney-Wilcoxon tests. Stunting: data were analysed with Student's t-tests, except for soybean, cucumber, tomato, and lettuce, which were analysed with Mann-Whitney-Wilcoxon tests. Deformities: data were analysed with Mann-Whitney-Wilcoxon tests, except for ryegrass, which were analysed with a Student's t-test.

Table CP 10.6.2/01-07. The phytotoxic effects of the toxic reference, Roundup Innov, on 10 plant species, after 21 days of exposure.

Plant	Mean value (number) after 21 days of exposure													
	Chlorosis		Necrosis		Pigmentation		Wilting		Curling		Stunting		Deformities	
	Control	Roundup	Control	Roundup	Control	Roundup	Control	Roundup	Control	Roundup	Control	Roundup	Control	Roundup
Ryegrass	0.0	0.0	0.0	90.0	0.0	0.0	0.0	90.0	0.0	0.0	0.0	80.0	0.0	0.0
Sorghum	0.0	n.d.	0.0	n.d.	0.0	n.d.	0.0	n.d.	0.0	n.d.	0.0	n.d.	0.0	n.d.
Onion	0.0	n.d.	0.0	n.d.	0.0	n.d.	0.0	n.d.	0.0	n.d.	0.0	n.d.	0.0	n.d.
Corn	0.0	n.d.	0.0	n.d.	0.0	n.d.	0.0	n.d.	0.0	n.d.	0.0	n.d.	0.0	n.d.
Carrot	0.0	15.0	0.0	95.0	0.0	0.0	0.0	30.0	0.0	0.0	0.0	80.0	0.0	0.0
Radish	0.0	60.0	0.0	90.0	0.0	0.0	0.0	90.0	0.0	0.0	0.0	90.0	0.0	0.0
Soybean	0.0	30.0	0.0	70.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	80.0	0.0	80.0
Cucumber	0.0	70.0	0.0	90.0	0.0	0.0	0.0	60.0	0.0	0.0	0.0	80.0	0.0	0.0
Tomato	0.0	n.d.	0.0	n.d.	0.0	n.d.	0.0	n.d.	0.0	n.d.	0.0	n.d.	0.0	n.d.
Lettuce	0.0	n.d.	0.0	n.d.	0.0	n.d.	0.0	n.d.	0.0	n.d.	0.0	n.d.	0.0	n.d.

There was 100% mortality, due to the use of Roundup Innov, thus, assessments of phytotoxicity could not be determined (n.d.).

Treatment with CA3301 resulted caused significant phytotoxicity in several of the plants tested. chlorosis for soybean, cucumber, and tomato; necrosis for all species; leaf curling for radish and tomato; stunting for soybean, cucumber, tomato, and lettuce; deformation for ryegrass. CA3301 did not cause any changes in pigmentation or wilting.

Validity

All validity criteria were met in accordance with the OECD 227 (2006) test guideline:

- There should be no visible phytotoxic effects in the control groups (actual result: no control plant species exhibited signs of phytotoxicity).

CA3301 / JOUST	Page 253 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

- At the end of the test, mean survival of seedlings to be $\geq 90\%$ in the distilled water control (actual values were 100% for all the plant species)
- For a given species, environmental conditions were identical (actual result: control and product treatment groups were all exposed to identical conditions)
- For a given species, all seedlings used in the test to be from the same cultivation group and source (actual result: seedlings from the control and product treatment groups were all from same cultivation group and source)

Conclusion

The 21-day test of CA3301 to ten species of monocot and dicot species was conducted as a limit test and in accordance with OECD 227 (2006) test guideline.

The 21-day NOER and LOER (survival) values for all species was 406.99 and >406.99 g a.s./ha, equivalent to 1.6 L and >1.6 L product/ha, respectively (based on nominal concentrations). The ER₅₀ (shoot height) was estimated to be >1.6 L product/ha, equivalent to >406.99 g a.s./ha.

For soybean, cucumber and tomato, the NOER and LOER (shoot height) values were <406.99 and 406.99 g a.s./ha, equivalent to <1.6 and 1.6 L product/ha, respectively (based on nominal concentrations). The ER₅₀ (shoot height) was estimated to be >1.6 L product/ha, equivalent to >406.99 g a.s./ha.

For cucumber and tomato, the NOER and LOER (shoot dry weight) values were <406.99 and 406.99 g a.s./ha, equivalent to <1.6 and 1.6 L product/ha, respectively (based on nominal concentrations). The ER₅₀ (shoot dry weight) was estimated to be >1.6 L product/ha, equivalent to >406.99 g a.s./ha.

Given these results, the most sensitive species after 21 days of exposure were soybeans, cucumber, and tomatoes.

This study is considered acceptable.

Study 2

Comments of zRMS:	The study was conducted to the OECD guideline 208 and according to the principles of GLP. All validity criterions were met. The study is considered to be reliable and suitable for the risk assessment.
-------------------	--

Reference:	KCP 10.6.2/02
Report	CA3301 - A study to determine the effects on the Seedling Emergence and Growth of terrestrial plants Fraimout, C., 2021, report no. 029SRFR20C12
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

This study was performed as a limit test to evaluate the effects of CA3301 (analyzed product a.s. content

CA3301 / JOUST	Page 254 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

of 257.04 g prothioconazole/L) on seedling emergence and growth, using ten selected non-target plant species, following application of 0 (untreated water control) and 1.6 L product/ha (equivalent to 406.99 g a.s./ha). 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 CA3301.

Treatment with CA3301 did not cause significant in emergence. Thus, NOER, LOER, and ER₅₀ (emergence) values for all species were estimated to be 1.6, >1.6, and >1.6 L product/ha, equivalent to 406.99, >406.99, and >406.99 g a.s./ha, respectively (based on nominal concentrations). Likewise, treatment with CA3301 did not cause significant in survival. Thus, NOER, LOER, and ER₅₀ (survival) values for all species were estimated to be 1.6, >1.6, and >1.6 L product/ha, equivalent to 406.99, >406.99, and >406.99 g a.s./ha, respectively (based on nominal concentrations).

Treatment with CA3301 caused a significant increase in shoot height for ryegrass, but a significant decrease in shoot height for carrot. Thus, for ryegrass and carrot, the NOER and LOER (shoot height) values were <1.6 and 1.6 L product/ha, equivalent to <406.99 and 406.99 g a.s./ha, respectively (based on nominal concentrations). For the other species, the NOER and LOER (shoot height) values were 1.6 and >1.6 L product/ha, equivalent to 406.99 and >406.99 g a.s./ha, respectively (based on nominal concentrations). Since the effects on shoot height were not >50%, the ER₅₀ value is estimated to be >1.6 L product/ha, equivalent to >406.99 g a.s./ha.

Treatment with CA3301 did not cause significant in dry shoot weight. Thus, NOER, LOER, and ER₅₀ (dry shoot weight) values for all species were estimated to be 1.6, >1.6, and >1.6 L product/ha, equivalent to 406.99, >406.99, and >406.99 g a.s./ha, respectively (based on nominal concentrations).

Given these results, the most sensitive species after 14 or 21 days of exposure were ryegrass and carrot. Overall, the study satisfies the OECD 208 (2006) test-guideline requirements for a seedling-emergence and growth study, and is considered acceptable.

Materials and methods

Test materials

Test item

Name:	CA3301
Density:	0.9948 g/mL
Active substance:	Prothioconazole
Formulation type:	Emulsifiable concentrate (EC)
Source and lot/batch no.:	A19045
Active-substance content:	250 g/L (nominal), 257.04 g/L or 25.57% w/w (analyzed)
Appearance:	Clear, straw-coloured liquid
Expiry date of lot/batch:	17 June 2022
Storage:	Between 15.3°C and 26.4°C, protected from light

Reference items

Name:	Mission
Density:	N/A (solid)
Active substance:	Flazasulfuron
Formulation type:	WG
Source and lot/batch no.:	20005
Active-substance content:	25% w/w
Expiry:	26 Nov 2021

Name:	Rami
Density:	N/A (solid)

CA3301 / JOUST	Page 255 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Active substance: Flumioxazin
 Formulation type: WP
 Source and lot/batch no.: 269379G911
 Active-substance content: 50% w/w
 Expiry: 19 July 2021
 Storage: Between 15.3°C and 24.6°C (both items)

Test organisms

Clade	Plant	Species	Family	Variety	Seed source	Seed lot ID.
Monocot	Corn	<i>Zea mays</i> L.	Poaceae	Monloui	Caussade semente	F0252EA01940
	Ryegrass	<i>Lolium perenne</i> L.	Poaceae	Indra	Gamm vert	1943F0674R2B0161C
	Sorghum	<i>Sorghum sudanense</i> L.	Poaceae	Cert. Piper	Syntech Research, USA	Z020SP1004
	Onion	<i>Allium cepa</i> L.	Liliaceae	Paille des vertus	Truffaut	I04085
Dicot	Carrot	<i>Daucus carota</i> L.	Apiaceae	Bolero F1	Vilmorin	N60675
	Cucumber	<i>Cucumis sativus</i> L.	Cucurbitaceae	Serit F1	Hazera	L60606/M
	Lettuce	<i>Lactuca sativa</i> L.	Asteraceae	Laitue beure d'abri	Vilmorin	L44817
	Radish	<i>Rafanus sativus</i> L.	Brassicaceae	Flamboyant 2	Ferme de Sainte Marthe	20-03024
	Soybean	<i>Glycine max</i> L.	Fabaceae	NAv	SynTech Research, France	VBA0191/704
	Tomato	<i>Solanum lycopersicum</i> L.	Solanaceae	Montfavet 63-5F1	Vilmorin	L41905

Test medium:

Soil type: Artificial soil
 Sandy soil: (coarse sand 87%, fine sand 8.6%, fine silt 1.6%, coarse silt 1.6%, clay 1.1%) containing 1.8% of organic matter, 1.01% of organic carbon
 92.85% of quartz sand (air dried, particles size < 2 mm)
 7.15% of universal horticultural compost (air dried and with no visible plant remains)
 pH: 6.8
 Conductivity: 0.08 mS/cm

Test conditions

Test temperature: Treatment application: 17.1– 31°C (first trial), 18.6 – 19.5°C (second trial, Experimental phase: 17.1– 31°C (daytime), 13.5 – 22°C (nighttime)
 Relative humidity: Treatment application: 31 – 60%, Experimental phase: 48.7 – 92.1%
 Photoperiod: 16 h light:8 h dark
 Light intensity: 16200 – 32800 lux, 350 ± 50 µE/m²/s

Ten higher terrestrial plant species were used for this study (four monocotyledons and six dicotyledons). Within a given species, all test plants seeds, including the control, were from the same source. Plants were grown from seeds at the test site of SynTech Research France SAS, (Nimes, France).

Plants were grown in test units consisting of plastic pots (14-cm diameter), containing about 1.3 L of soil. Each pot was filled to a depth of 90 mm of soil, with 1 to 5 plants per pot, depending on the size of the plant

CA3301 / JOUST	Page 256 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

species. Briefly, for ryegrass, sorghum, and onion, there were two pots per replicate, with five plants per pot; for corn, carrot, cucumber, radish, soybean, and tomato, there were five pots per replicate, with 2 plants per pot; and for lettuce, there were ten pots per replicate, with one plant per pot. Germination of plants was between 91 and 100%.

The study was conducted as a limit test with three treatment groups: a distilled-water control, one product treatment group, and one toxic-reference group (a mixture of two pesticides). The treatment rates were 0 (control) and 1.6 L product/ha (equivalent to 406.99 g a.s./ha), and 1.4 kg toxic reference/ha (0.2 kg Mission + 1.2 kg Rami/ha, equivalent to 0.05 kg flazasulfuron + 0.6 kg flumioxazine/ha). There were 4 replicates per plant species per treatment group and each replicate consisted of 10 seedlings (40 seedlings per species). For the treatment spray solutions, 7.9585 g of product was dissolved in 2000 mL distilled water and nominally 1.0 g Mission + 6 g Rami was dissolved in 2000 mL of distilled water. Only distilled water was used for the control treatment. The spray solutions were applied at a rate of 400 L/ha.

Seedling emergence in the control group was checked daily, until 50% of the sowed seeds emerged. From this date, emergence, survival, mortality, and phytotoxicity were assessed weekly in all treatments. For carrot plants, the control group exhibited symptoms of phytotoxicity between 7 and 14 days post-emergence; consequently, the test for carrots was extended to 21 days post-emergence. Phytotoxicity was assessed visually, by observing morphology, specifically chlorosis, necrosis, pigmentation, leaf curling, wilting, deformities, and stunting. At the end of assessment for a given species, shoot height of surviving plants was first recorded, then the surviving plants were dried (at 60°C) and their shoots weighed. Dry weights of each replicate were measured (not individual plants).

Data were analysed with the statistical software R (version 3.3.1), at a significance level of $\alpha = 0.05$, to determine any significant differences in survival, shoot height, dry weight, and phytotoxicity. Survival and emergence data were analysed with a unilateral Fisher's exact test, whereas data for shoot height, shoot dry weight, and phytotoxicity were tested for normality (Shapiro-Wilk's test) and homoscedasticity (Bartlett's test). When normality and homoscedasticity were confirmed, data were analysed with two-sided Student's t-tests. For non-normal and/or non-homoscedastic data, data were log10-transformed (shoot height and shoot dry weight) or arcsine-square-root-transformed (phytotoxicity) when needed. When normality and/or homoscedasticity could not be achieved, even after transformation, a Mann-Whitney-Wilcoxon's test was performed. Based on the results of these tests, the NOEC and LOEC values were determined.

Analytical verification of the stock product spray solution was conducted during the analytical phase of the study. Eight representative samples of the spray solutions (50 mL each) were collected (product was diluted in distilled water) during application of the study: two samples (one for analysis and one as a retained sample) were collected just prior to the application and two samples just after the application.

Results

Analytical results

Table CP 10.6.2/02-01. Summary of the analytical results in the spray solutions.

Trial	Sampling Time	Prothioconazole concentration (mg a.s./L)		Recovery (%)	
		Nominal (target)*	Measured	Nominal	Deviation
1	Before Application	1028	886.2	86.2	-13.8
	After Application	1028	955.6	93.0	-7.0
2	Before Application	1028	943.3	91.8	-8.2
	After Application	1028	787.0	76.6	-23.4

The analytical recoveries were all within $\pm 20\%$ of nominal concentrations, therefore, results and endpoints are based on nominal concentrations.

CA3301 / JOUST	Page 257 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Biological results

Emergence, survival, and mortality

The effect of CA3301 on emergence, after 14 or 21 days of exposure, is presented in the table below.

Table CP 10.6.2/02-02. The effect of CA3301 on the emergence of 10 plant species, after 14 or 21 days of exposure (N = 10 seedlings per replicate).

Application rate L product/ha [g a.s./ha]	Rep.	Emergence							
		No.	Mean ± SD (%)	CV (%)	Effect (%)	No.	Mean ± SD (%)	CV (%)	Effec (%)
		Ryegrass (14 DAE)				Radish (14 DAE)			
0 (control)	1	10	90 ± 8.16	9.07	0.0	10	90 ± 8.16	9.07	0.0
	2	8				9			
	3	9				9			
	4	9				8			
1.6 [406.99]	1	9	92.5 ± 5	5.41	2.78	8	92.5 ± 9.57	10.35	2.78
	2	9				10			
	3	9				9			
	4	10				10			
1.4 kg toxic reference/ha	1	5	40 ± 8.16	20.41	-55.56	4	30 ± 14.14	47.14	-66.67
	2	3				1			
	3	4				4			
	4	4				3			
		Sorghum (14 DAE)				Soybean (14 DAE)			
0 (control)	1	10	97.5 ± 5	5.13	0.0	8	75 ± 12.91	17.21	0.0
	2	10				9			
	3	9				7			
	4	10				6			
1.6 [406.99]	1	9	85 ± 5.77	6.79	-12.82	7	75 ± 5.77	7.7	0.0
	2	9				8			
	3	8				7			
	4	8				8			
1.4 kg toxic reference/ha	1	3	27.5 ± 5	18.18	-71.79	3	20 ± 8.16	40.82	-73.33
	2	2				2			
	3	3				2			
	4	3				1			
		Onion (14 DAE)				Cucumber (14 DAE)			
0 (control)	1	8	90 ± 11.55	12.83	0.0	9	85 ± 5.77	6.79	0.0
	2	10				8			
	3	10				8			
	4	8				9			
1.6 [406.99]	1	7	82.5 ± 15	18.18	-8.33	9	97.5 ± 5	5.13	14.71
	2	7				10			
	3	10				10			
	4	9				10			
1.4 kg toxic reference/ha	1	5	45 ± 5.77	12.83	-50	4	47.5 ± 9.57	20.16	-44.12
	2	4				5			
	3	5				6			
	4	4				4			
		Corn (14 DAE)				Tomato (14 DAE)			
0 (control)	1	10	90 ± 8.16	9.07	0.0	10	95 ± 5.77	6.08	0.0
	2	8				9			

CA3301 / JOUST	Page 258 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

	3	9				9			
	4	9				10			
1.6 [406.99]	1	10	90 ± 8.16	9.07	0.0	10	100 ± 0	0.0	5.26
	2	9				10			
	3	9				10			
	4	8				10			
1.4 kg toxic reference/ha	1	3	27.5 ± 5	18.18	-69.44	0	5 ± 5.77	115.47	-94.74
	2	2				1			
	3	3				0			
	4	3				1			
		Carrot (21 DAE)				Lettuce (14 DAE)			
0 (control)	1	9	85 ± 12.91	15.19	0.0	10	100 ± 0	0.0	0.0
	2	10				10			
	3	7				10			
	4	8				10			
1.6 [406.99]	1	10	100 ± 0	0.0	17.65	9	97.5 ± 5	5.13	-2.5
	2	10				10			
	3	10				10			
	4	10				10			
1.4 kg toxic reference/ha	1	0	0 ± 0	-	-100	3	22.5 ± 9.57	42.55	-77.5
	2	0				1			
	3	0				3			
	4	0				2			

*Corrected percentages from the control, positive and negative values indicate higher and lower emergence, relative to the control. DAE, days after exposure; Rep. replicate; no., number.

The mean emergence of plants in the control and product treatment groups ranged between 75 and 100%, whereas it ranged between 0 and 47.5% in the toxic-reference group. Treatment with CA3301 caused neither significant nor >50% changes in emergence. Thus, NOER, LOER, and ER₅₀ (emergence) values for all species were estimated to be 406.99, >406.99, and >406.99 g a.s./ha, equivalent to 1.6, >1.6, and >1.6 L product/ha, respectively (based on nominal concentrations).

A summary of the effect of CA3301 on survival and mortality, after 14 or 21 days of exposure, is presented in the Table CP 10.6.2/02-03 below.

Table CP 10.6.2/02-03. The effect of CA3301 on the survival of 10 plant species, after 14 or 21 days of exposure.

Application rate		Rep.	Survival							
L product/ha [g a.s./ha]			No. dead	No. alive	Mean ± SD (%)	CV (%)	No. dead	No. alive	Mean ± SD (%)	CV (%)
			Ryegrass (14 DAE)				Radish (14 DAE)			
0 (control)	1	0	10	100 ± 0	0.0	0	9	100 ± 0	0.0	
	2	0	8			0	9			
	3	0	9			0	9			
	4	0	9			0	8			
1.6 [406.99]	1	0	9	100 ± 0	0.0	0	8	100 ± 0	0.0	
	2	0	9			0	10			
	3	0	9			0	9			
	4	0	10			0	10			
8 L Roundup/ha	1	5	0	8.33 ± 16.67	200	4	0	0 ± 0	-	
	2	2	1			1	0			
	3	4	0			4	0			

CA3301 / JOUST	Page 259 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

	4	4	0		3	0
		Sorghum (14 DAE)			Soybean (14 DAE)	
0 (control)	1	0	10	100 ± 0	0.0	0 8 100 ± 0 0.0
	2	0	10			0 9
	3	0	9			0 7
	4	0	10			0 6
1.6 [406.99]	1	0	9	100 ± 0	0.0	0 7 100 ± 0 0.0
	2	0	9			0 8
	3	0	8			0 7
	4	0	8			0 8
8 L Roundup/ha	1	3	0	41.67 ± 50	120	0 3 100 ± 0 0
	2	0	2			0 2
	3	1	2			0 2
	4	3	0			0 1
		Onion (14 DAE)			Cucumber (14 DAE)	
0 (control)	1	0	8	100 ± 0	0.0	0 9 100 ± 0 0.0
	2	0	10			0 8
	3	0	9			0 8
	4	0	8			0 9
1.6 [406.99]	1	0	7	100 ± 0	0.0	0 9 100 ± 0 0.0
	2	0	7			0 10
	3	0	10			0 10
	4	0	9			0 10
8 L Roundup/ha	1	5	0	11.25 ± 13.15	116.89	4 0 0 ± 0 -
	2	3	1			5 0
	3	4	1			6 0
	4	4	0			4 0
		Corn (14 DAE)			Tomato (14 DAE)	
0 (control)	1	0	10	100 ± 0	0.0	0 100 ± 0 -
	2	0	9			0
	3	0	9			0
	4	0	9			0
1.6 [406.99]	1	0	10	100 ± 0	0.0	0 100 ± 0 -
	2	0	9			0
	3	0	9			0
	4	0	8			0
8 L Roundup/ha	1	1	2	75 ± 31.91	42.55	0.0 ± 0.0 -
	2	0	2			
	3	2	1			
	4	0	3			
		Carrot (21 DAE)			Lettuce (14 DAE)	
0 (control)	1	0	9	100 ± 0	0.0	0 10 100 ± 0 0.0
	2	0	10			0 10
	3	0	7			0 10
	4	0	8			0 10
1.6 [406.99]	1	0	10	100 ± 0	0.0	0 9 100 ± 0 0.0
	2	0	10			0 10
	3	0	9			0 10
	4	0	10			0 10
8 L Roundup/ha	1	-	-	-	-	3 0 45.83 ± 41.67 90.91
	2	-	-			0 1
	3	-	-			2 1

4	-	-	1	1
---	---	---	---	---

DAE, days after exposure; Rep., replicate; no., number.

The mean survival of plants in the control and product treatment groups was 100% for all species, whereas it ranged between 0 and 100% in the toxic-reference group. Treatment with CA3301 caused neither significant nor >50% changes in survival. Thus, NOER, LOER, and ER₅₀ (survival) values for all species were estimated to be 406.99, >406.99, and >406.99 g a.s./ha, equivalent to 1.6, >1.6, and >1.6 L product/ha, respectively (based on nominal concentrations).

Shoot height and shoot dry weight

Summaries of the effect of CA3301 on shoot height and dry shoot weight, after 14 or 21 days of exposure, are presented in tables below.

Table CP 10.6.2/02-03. The effect of CA3301 on shoot height of 10 plant species, after 14 or 21 days of exposure.

Application rate	Rep.	Shoot height							
L product/ha [g a.s./ha]		Mean (cm)	Mean ± SD (cm)	CV (%)	Inhibition (% control) [#]	Mean (cm)	Mean ± SD (cm)	CV (%)	Inhibition (% control) [#]
		Ryegrass (14 DAE)				Radish (14 DAE)			
0 (control)	1	36.3				17.6			
	2	35.8				14.1			
	3	33.6	34.5 ± 5.2	15.0	0.0	14.3	15.5 ± 4	25.5	0.0
	4	32.2				16.3			
1.6 [406.99]	1	39.2				16.3			
	2	39.7	39.8 ± 5.8*	14.7	-15.5	15.1	16.5 ± 2.8	16.9	-6.2
	3	41.2				18.7			
	4	39.2				16.1			
		Sorghum (14 DAE)				Soybean (14 DAE)			
0 (control)	1	40.1				11.1			
	2	39.6				15.8			
	3	41.4	39.9 ± 8.2	20.6	0.0	18.4	15.1 ± 6.5	43.1	0.0
	4	38.6				15.7			
1.6 [406.99]	1	35				18.8			
	2	51.6	44 ± 13.7	31.1	-10.3	15.1	17.2 ± 5.4	31.4	-6.2
	3	48.6				18.6			
	4	40.9				16.8			
		Onion (14 DAE)				Cucumber (14 DAE)			
0 (control)	1	23.9				14			
	2	23				13.4			
	3	20.4	22.4 ± 4.9	22.0	0.0	14.8	14.1 ± 1.5	10.6	0.0
	4	22.5				14.2			
1.6 [406.99]	1	21				14.7			
	2	23.8	23.2 ± 4	17.2	-3.5	14.7	14.7 ± 1.6	10.6	-4.2
	3	24.6				15.3			
	4	23				14.2			
		Corn (14 DAE)				Tomato (14 DAE)			
0 (control)	1	60.8	59.4 ± 7	11.9	0.0	21.5	22.1 ± 3.7	16.9	0.0

CA3301 / JOUST	Page 261 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

	2	52.7				23.8			
	3	61.7				20.8			
	4	61.7				22.5			
1.6 [406.99]	1	59.1				22.1			
	2	60.9	60.4 ± 6.8	11.2	-1.7	24.5	23.6 ± 3.4	14.2	-6.7
	3	61.3				23.8			
	4	60.4				24.1			
		Carrot (21 DAE)				Lettuce (14 DAE)			
0 (control)	1	24.4				11.3			
	2	25	25 ± 3.7	14.8	0.0	10.5	9.5 ± 3.1	32.2	0.0
	3	25.6				8.3			
	4	25.4				7.8			
1.6 [406.99]	1	23.7				9.8			
	2	20.8	22.2 ± 4.7*	20.9	11.2	11.9	11.5 ± 2.4	21.0	-21.6
	3	20.4				12.1			
	4	24.2				12			

Statistically significant differences in shoot height are denoted by asterisks (*); data for ryegrass, onion, carrot, radish, soybean, cucumber, tomato, and lettuce were normally distributed and had equal variances, thus, were analyzed with Student's t-tests; data for sorghum and corn were not normally distributed or have equal variances, thus, were analyzed with Mann-Whitney-Wilcoxon tests. #Negative and positive signs indicated stimulatory and inhibitory effect on shoot height, respectively, relative to the control. DAE, days after exposure; Rep., replicate; SD, standard deviation; CV, coefficient of variation.

Treatment with CA3301 caused a significant increase in shoot height for ryegrass, and a significant decrease in shoot height for carrot. Thus, for ryegrass and carrot, the NOER and LOER (shoot height) values were <406.99 and 406.99 g a.s./ha, equivalent to <1.6 and 1.6 L product/ha, respectively (based on nominal concentrations). For the other species, the NOER and LOER (shoot height) values were 406.99 and >406.99 g a.s./ha, equivalent to 1.6 and >1.6 L product/ha, respectively (based on nominal concentrations). Since the effects on shoot height were not >50%, the EC₅₀ value is estimated to be >406.99 g a.s./ha, equivalent to >1.6 L product/ha.

Table CP 10.6.2/02-04. The effect of CA3301 on dry shoot weight of 10 plant species, after 14 or 21 days of exposure.

Application rate		Rep.	Dry shoot weight (g)							
L product/ha [g a.s./ha]			Mean (g)	Mean ± SD (g)	CV (%)	Inhibition (% control) [#]	Mean (g)	Mean ± SD (g)	CV (%)	Inhibition (% control) [#]
			Ryegrass (14 DAE)				Radish (14 DAE)			
0 (control)		1	0.13	0.11 ± 0.02	16.3	0.0	0.46	0.34 ± 0.08	23.8	0.0
		2	0.09				0.28			
		3	0.11				0.32			
		4	0.10				0.30			
1.6 [406.99]		1	0.11	0.12 ± 0.01	6.2	-11.9	0.33	0.33 ± 0.01	4.5	+3.8
		2	0.11				0.31			
		3	0.12				0.34			
		4	0.13				0.33			
		Sorghum (14 DAE)					Soybean (14 DAE)			
0 (control)		1	0.13	0.13 ± 0.02	15.8	0.0	0.20	0.23 ± 0.04	16.1	0.0
		2	0.12				0.23			
		3	0.16				0.28			
		4	0.12				0.21			
1.6 [406.99]		1	0.13	0.14 ± 0.01	5.4	-2.9	0.31	0.25 ± 0.08	32.9	-9.9
		2	0.13				0.13			
		3	0.15				0.31			
		4	0.14				0.26			
		Onion (14 DAE)					Cucumber (14 DAE)			
0 (control)		1	0.03	0.03 ± 0	9.0	0.0	0.71	0.66 ± 0.13	19.9	0.0
		2	0.03				0.47			
		3	0.03				0.75			
		4	0.03				0.73			
1.6 [406.99]		1	0.02	0.028 ± 0	11.1	7.8	0.82	0.77 ± 0.06	7.5	-15.4
		2	0.03				0.75			
		3	0.03				0.80			
		4	0.03				0.69			
		Corn (14 DAE)					Tomato (14 DAE)			
0 (control)		1	0.70	0.76 ± 0.2	26.9	0.0	0.42	0.45 ± 0.06	12.9	0.0
		2	0.55				0.54			
		3	0.75				0.43			
		4	1.04				0.41			
1.6 [406.99]		1	0.71	0.72 ± 0.08	10.7	5.5	0.45	0.51 ± 0.04	8.7	-6.7
		2	0.65				0.49			
		3	0.83				0.53			
		4	0.68				0.55			
		Carrot (21 DAE)					Lettuce (14 DAE)			
0 (control)		1	0.25	0.25 ± 0.03	12.2	0.0	1.16	0.74 ± 0.35	47.6	0.0
		2	0.24				0.91			
		3	0.30				0.52			
		4	0.22				0.39			
1.6 [406.99]		1	0.27	0.21 ± 0.05	24.6	16.8	0.75	0.9 ± 0.1	11.7	-21.0
		2	0.16				0.90			
		3	0.17				0.97			
		4	0.24				0.98			

No statistically significant differences in shoot weight were found with statistical analyses; data for ryegrass, sorghum, carrot, corn, soybean, cucumber, tomato, and lettuce were normally distributed and had equal variances, thus, were analyzed with Student's t-tests; data for onion and radish were not normally distributed or have equal variances, thus, were analyzed with Mann-Whitney-Wilcoxon tests. [#]Negative and positive signs indicated stimulatory and inhibitory effect on shoot weight, respectively, relative to the control. DAE, days after exposure; Rep., replicate; SD, standard deviation; CV, coefficient of variation.

CA3301 / JOUST	Page 263 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

Treatment with CA3301 caused neither significant nor >50% changes in dry shoot weight. Thus, NOER, LOER, and ER₅₀ (dry shoot weight) values for all species were estimated to be 406.99, >406.99, and >406.99 g a.s./ha, equivalent to 1.6, >1.6, and >1.6 L product/ha, respectively (based on nominal concentrations).

Phytotoxicity

A summary of the phytotoxic effects of CA3301 and the toxic reference, after 21 days of exposure, is presented in Tables CP 10.6.2/02-05 and 02-06 below.

Table CP 10.6.2/02-05. The phytotoxic effects of CA3301 on 10 plant species, after 14 or 21 days of exposure.

Plant	Mean value (number) after 14 or 21 days													
	Chlorosis		Necrosis		Pigmentation		Wilting		Curling		Stunting		Deformities	
	Control	Product	Control	Product	Control	Product	Control	Product	Control	Product	Control	Product	Control	Product
Ryegrass	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.5
Sorghum	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	15.0	0.0	2.5
Onion	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Corn	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Carrot	0.0	5.0*	0.0	5.0*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7.5	0.0	0.0
Radish	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7.5
Soybean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cucumber	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Tomato	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.5	0.0	0.0
Lettuce	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Statistically significant differences, relative to the control, are denoted with asterisks (*). The following tests were used to reveal statistically significant differences. Chlorosis and necrosis: data were analysed with Student's t-tests, except for carrot data, which were analysed with Mann-Whitney-Wilcoxon tests. Pigmentation: data were analysed with Student's t-tests. Wilting and curling: data were analysed with Student's t-tests. Stunting: data were analysed with Student's t-tests, except for sorghum, carrot, and tomato, which were analysed with Mann-Whitney-Wilcoxon tests. Deformities: data were analysed with Student's t-tests, except for ryegrass, sorghum, and radish, which were analysed with Mann-Whitney-Wilcoxon tests.

Table CP 10.6.2/02-06. The phytotoxic effects of the toxic reference (Mission + Rami mixture), on 10 plant species, after 21 days of exposure.

Plant	Mean value (number) after 14 or 21 days													
	Chlorosis		Necrosis		Pigmentation		Wilting		Curling		Stunting		Deformities	
	Control	Product	Control	Product	Control	Product	Control	Product	Control	Product	Control	Product	Control	Product
Ryegrass	0.0	0.0	0.0	60.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	80.0	0.0	0.0
Sorghum	0.0	0.0	0.0	70.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	80.0	0.0	0.0
Onion	0.0	0.0	0.0	80.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	80.0	0.0	0.0
Corn	0.0	0.0	0.0	50.0	0.0	0.0	0.0	0.0	0.0	60.0	0.0	80.0	0.0	30.0
Carrot	0.0	n.d.	0.0	n.d.	0.0	n.d.	0.0	n.d.	0.0	n.d.	0.0	n.d.	0.0	n.d.
Radish	0.0	n.d.	0.0	n.d.	0.0	n.d.	0.0	n.d.	0.0	n.d.	0.0	n.d.	0.0	n.d.
Soybean	0.0	0.0	0.0	10.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	50.0	0.0	0.0
Cucumber	0.0	n.d.	0.0	n.d.	0.0	n.d.	0.0	n.d.	0.0	n.d.	0.0	n.d.	0.0	n.d.
Tomato	0.0	n.d.	0.0	n.d.	0.0	n.d.	0.0	n.d.	0.0	n.d.	0.0	n.d.	0.0	n.d.
Lettuce	0.0	0.0	0.0	10.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	46.7	0.0	0.0

There was 100% mortality, due to the use of toxic reference (Mission + Rami), thus, assessments of phytotoxicity could not be determined (n.d.).

Treatment with CA3301 resulted caused significant increases in chlorosis and necrosis in carrot only.

Validity

CA3301 / JOUST	Page 264 /264
Part B – Section 9 - Core Assessment	Chemical PPP
zRMS version	Version August 2022

All validity criteria were met in accordance with the OECD 208 (2006) test guideline:

- There should be no visible phytotoxic effects in the control groups (actual result: no control plant species exhibited signs of phytotoxicity).
- At the end of the test, mean seedling emergence to be $\geq 70\%$ in the distilled water control (actual values were ranged between 75% and 100% for all species tested)
- At the end of the test, mean seedling survival to be $\geq 90\%$ in the distilled water control (actual values were 100% for all species tested)
- For a given species, environmental conditions were identical (actual result: control and product treatment groups were all exposed to identical conditions)
- For a given species, all seedlings used in the test were from the same cultivation group and source (actual result: seedlings from the control and product treatment groups were all from same cultivation group and source)

Conclusion

The toxicity test of CA3301 to ten species of monocot and dicot species was conducted as a limit test and in accordance with OECD 208 (2006) test guideline.

The NOER, LOER, and ER₅₀ (emergence) values for all species were estimated to be 1.6, >1.6, and >1.6 L product/ha, equivalent to 406.99, >406.99, and >406.99 g a.s./ha, respectively (based on nominal concentrations).

The NOER, LOER, and ER₅₀ (survival) values for all species were estimated to be 1.6, >1.6, and >1.6 L product/ha, equivalent to 406.99, >406.99, and >406.99 g a.s./ha, respectively (based on nominal concentrations).

For ryegrass and carrot, the NOER and LOER (shoot height) values were <1.6 and 1.6 L product/ha, equivalent to <406.99 and 406.99 g a.s./ha, respectively (based on nominal concentrations). For the other species, the NOER and LOER (shoot height) values were 1.6 and >1.6 L product/ha, equivalent to 406.99 and >406.99 g a.s./ha, respectively (based on nominal concentrations). Since the effects on shoot height were not >50%, the EC₅₀ value is estimated to be >1.6 L product/ha, equivalent to >406.99 g a.s./ha.

The NOER, LOER, and ER₅₀ (dry shoot weight) values for all species were estimated to be 1.6, >1.6, and >1.6 L product/ha, equivalent to 406.99, >406.99, and >406.99 g a.s./ha, respectively (based on nominal concentrations).

Given these results, the most sensitive species after 14 or 21 days of exposure were ryegrass and carrot.

This study is considered acceptable.

A 2.6.3	KCP 10.6.3	Extended laboratory studies on non-target plants
A 2.7	KCP 10.7	Effects on other terrestrial organisms (flora and fauna)
A 2.8	KCP 10.8	Monitoring data