Cyprodinil

NOTIFICATION OF AN ACTIVE SUBSTANCE UNDER COMMISSION REGULATION (EU) 844/2012

DOCUMENT M-CA, Section 8 Supplement

ECOTOXICOLOGICAL STUDIES ON THE ACTIVE SUBSTANCE

Version history

Date	Data points containing amendments or additions[1]	Document identifier or version number
31 May 2016	New data included in response to questions from RMS: Some algae statistics updated in order to attempt to derive E_rC_{50} values. Also updated some updated based on mean measured concentrations. Statistics were re-evaluated for Folsomia candida test with A8637C (All changes are highlighted in yellow)	CGA219417_11533 8 October 2015 updated 20/5/16
3 February 2017	New non-target arthropod data included at the recommendation of the RMS (Additions highlighted in green)	CGA219417_11533 8 October 2015 updated 20/5/16, 3/2/17
31 May 2017	Summary of biological screening for metabolites CGA321915 and CGA249287 has been included in Section CA 8.7. (All changes are highlighted in green)	CGA219417_11533 8 October 2015 updated 20/5/16, 3/2/17, 31/5/17

[1] Note how the amendments or additions are represented (italics/colour etc)

Table of Contents

CA 8 ECOTOXICOLOGICAL STUDIES 5

INTRODUCTION 5

CA 8.1 Effects on Birds and Other Terrestrial Vertebrates 6
CA 8.1.1 Effect on birds 6
CA 8.1.1.1 Acute oral toxicity to birds 7
CA 8.1.1.2 Short-term dietary toxicity to birds 16
CA 8.1.1.3 Sub-chronic and reproductive toxicity to birds 16
CA 8.1.2 Effects on terrestrial vertebrates other than birds 27
CA 8.1.2.1 Acute oral toxicity to mammals 27
CA 8.1.2.2 Long-term and reproductive toxicity to mammals 27
CA 8.1.3 Effects of active substance bioconcentration in prey of birds and mammals 28
CA 8.1.4 Effects on terrestrial vertebrate wildlife (birds, mammals, reptiles and amphibians) 38
CA 8.1.5 Endocrine disrupting properties 39
CA 8.2 Effects on Aquatic Organisms 40
CA 8.2.1 Acute toxicity to fish 49
CA 8.2.2 Long-term and chronic toxicity to fish 77
CA 8.2.2.1 Fish early life stage toxicity test 83
CA 8.2.2.2 Fish full life cycle test 93
CA 8.2.2.3 Bioconcentration in fish 93
CA 8.2.3 Endocrine disrupting properties 93
CA 8.2.4 Acute toxicity to aquatic invertebrates 94
CA 8.2.4.1 Acute toxicity to *Daphnia magna* 94
CA 8.2.4.2 Acute toxicity to an additional aquatic invertebrate species 110
CA 8.2.5 Long-term and chronic toxicity to aquatic invertebrates 167
CA 8.2.5.1 Reproductive and development toxicity to *Daphnia magna* 167
CA 8.2.5.2 Reproductive and development toxicity to an additional aquatic invertebrate species 180
CA 8.2.5.3 Development and emergence in *Chironomus riparius* 184
CA 8.2.5.4 Sediment dwelling organisms 194
CA 8.2.6 Effects on algal growth 194
CA 8.2.6.1 Effects on growth of green algae 194
CA 8.2.6.2 Effects on growth of an Additional Algal Species 226

CA 8.2.7 Effects on aquatic macrophytes....245
CA 8.2.8 Further testing on aquatic organisms....249
CA 8.3 Effects on Arthropods....259
CA 8.3.1 Effects on bees....259
CA 8.3.1.1 Acute toxicity to bees....259
CA 8.3.1.1.1 Acute oral toxicity....259
CA 8.3.1.1.2 Acute contact toxicity....266
CA 8.3.1.2 Chronic toxicity to bees....268
CA 8.3.1.3 Effects on honeybee development and other honeybee life stages....275
CA 8.3.1.4 Sub-lethal effects....281
CA 8.3.2 Effects on non-target arthropods other than bees....281
CA 8.3.2.1 Effects on *Aphidius rhopalosiphi*....284
CA 8.3.2.2 Effects on *Typhlodromus pyri*....292
CA 8.4 Effects on Non-Target Soil Meso- and Macrofauna....301
CA 8.4.1 Earthworms – sub-lethal effects....303
CA 8.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)....318
CA 8.4.2.1 Species level testing....318
CA 8.5 Effects on Nitrogen Transformation....346
CA 8.6 Effects on Terrestrial Non-Target Higher Plants....365
CA 8.6.1 Summary of screening data....365
CA 8.6.2 Testing on non-target plants....369
CA 8.7 Effects on Other Terrestrial Organisms (Flora and Fauna)....369
CA 8.8 Effects on Biological Methods for Sewage Treatment....370
CA 8.9 Monitoring Data....378

CA 8 ECOTOXICOLOGICAL STUDIES

Introduction

This document supports the application for renewal of the regulatory approval of cyprodinil under Commission Implementing Regulation (EU) 844/2012 of 18 September 2012. This document reviews the ecotoxicology, including additional data and risk assessments, for cyprodinil.

Cyprodinil was included in Annex I of Council Directive 91/414/EEC (***Commission Directive 2005/53/EC of 15 September 2005***). This active substance is an approved active substance under Regulation (EC) 1107/2009 (repealing Commission Directive 91/414/EEC) as specified in Commission Implementing Regulation (EU) No. 540/2011 of 25 May 2011.

In accordance with Commission Implementing Regulation (EU) 844/2012, this document summarises new information which are relevant for the renewal of the approval of cyprodinil under Regulation (EC) 1107/2009. Where appropriate this document refers to the Commission Implementing Regulation (EU) No. 540/2011 for cyprodinil and to the Review Report for cyprodinil (***SANCO/4343/2000 final (revised) 28 September 2006***), and in particular the endpoints provided in Appendices I and II thereof.

This document covers data and risk assessments which were not part of the original dossier and which are necessary to reflect changes:

- In requirements under Commission Regulation (EU) No Commission Regulation (EU) No 283/2013, and the associated Annex, which repeals Commission Regulation (EU) No 544/2011 which, under Regulation (EC) 1107/2009, replaced the requirements of Annex II to Directive 91/414/EEC

- In scientific and technical knowledge since the approval or last renewal of the approval

- To representative uses

Where the conclusions of the EU review had specific areas of concern on cyprodinil, new data and/or reviews and/or risk assessments have been provided. Where additional and/or new data on cyprodinil are provided, a justification has been included. Also a justification has been given if new data are required but none were provided.

Details of the literature search undertaken can be found in M-CA Section 9. If a relevant scientifically peer-reviewed open literature reference has been identified for cyprodinil or its major metabolites, it has been discussed within the relevant data point.

CA 8.1 Effects on Birds and Other Terrestrial Vertebrates

CA 8.1.1 Effect on birds

Table 8.1.1-1: Summary of endpoints for birds for cyprodinil

Organism	Test type	Endpoint		Endpoints used for the risk assessment	Reference (author, date, Syngenta File No.)
Mallard duck	Acute oral	EU	14 d LD_{50} >500 mg/kg bw	-	***Hakin & Rogers (1992) CGA219417/0062***
Bobwhite quail			14 d LD_{50} > 2000 mg/kg bw	14 d LD_{50} 3776 mg/kg bw [a]	***Hakin & Rogers (1992) CGA219417/0067***
Canary		New	5 d LD_{50} > 5620 mg/kg bw	-	***Hubbard (2015) CGA219417_50779***
Mallard duck	Short-term dietary	EU	14 d LC_{50} > 5200 mg/kg feed	-	***Hakin & Rogers (1992) CGA219417/0068***
Bobwhite quail			14 d LC_{50} > 5200 mg/kg feed	-	***Hakin & Rogers (1992) CGA219417/0066***
Mallard duck	Sub-chronic and reproductive	EU	22 week NOEC = 600 mg/kg diet; NOEL = 102 mg/kg bw	-	***Rogers (1995) CGA219417/0477***
		EC_{10}/EC_{20} [b]	Could not be estimated		***Taylor & Majdanik (2015) CGA219417_11590***
Bobwhite quail		EU	22 week NOEC = 600 mg/kg diet; NOEL = 64 mg/kg bw	64 mg/kg bw	***Rogers (1995) CGA219417/0478***
		EC_{10}/EC_{20} [b]	Could not be estimated		***Taylor & Allen (2015) CGA219417_11586***

[a] Value extrapolated according to EFSA 2009
[b] Estimated attempted in accordance with **Commission Regulation (EU) No 283/2013**

Note on acute oral studies.

Acute toxicity studies were performed with bobwhite quail and mallard duck. In all cases, no mortalities occurred and no toxic symptoms were seen. Regurgitation occurred in the mallard duck study at the two highest dose levels of 1000 and 2000 mg a.s./kg, and the endpoint in the LoEP was set at >500 mg a.s./kg.

In the EFSA guidance document the following is stated on this subject:

According to Annex II of Directive 91/414/EEC, the acute oral toxicity of an active substance to a quail species (Japanese quail, Coturnix japonica or bobwhite quail, Colinus virginianus) or to mallard duck (Anas platyrhynchos) must be determined. The highest dose used in tests need not normally exceed 2000 mg/kg body weight. Due to issues of regurgitation it is recommended not to use the mallard duck (EFSA, 2007). Where regurgitation or emesis occurs at doses used for risk assessment, additional information is essential to complete the risk assessment. The amount of regurgitated material should be assessed for determination of the ingested dose. In the absence of this information, the lowest overall no observed effect level (NOEL) must be used for risk assessment purposes. Where more than one study has been submitted, the study/studies where no regurgitation has occurred should be used. If, however, mortalities appear in the study in which regurgitation has occurred (at dose levels at or around the LD50 value for

the non-regurgitation study), then it is proposed to use the NOEL (for regurgitation or mortality, whichever is lower) from the study where regurgitation has occurred.

Since no other signs of toxicity other than vomiting were seen in the studies with the mallard, and no effects were seen in the study with the bobwhite quail, it is proposed to use the LD_{50} of >2000 mg a.s./kg.

Under Point 2.1.2 of the **Guidance Document**[1] a method has been proposed to extrapolate upwards the LD_{50} value. The extrapolation is carried out assuming a 50% binomial probability bound that mortality could have occurred but had simply been missed by chance in the test. The extrapolation factor are presented in Table 1 of the guidance document and are dependent upon the number of animals tested and whether no, or a single mortality, was observed in the study. The acute toxicity value from the study with the bobwhite quail has been extrapolated and is presented in the table below.

Table 8.1.1-2: Extrapolation of the acute oral toxicity values for cyprodinil

Test substance	Study	Test species	Experimental LD_{50} (mg/kg bw/d)	Number of animals tested	Number of mortalities	Extrapolation factor [a]	Corrected LD_{50} (mg/kg bw/d)
Cyprodinil	***Hakin & Rogers***	Bobwhite quail	>2000	10	0	1.888	3776

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

CA 8.1.1.1 Acute oral toxicity to birds

Since cyprodinil is not intended solely for use in enclosed spaces, an acute study on birds is a data requirement in accordance with **Commission Regulation (EU) No 283/2013**.

The studies previously submitted in the EU were summarised in point B.9.1.1 of the Draft Assessment Report (DAR)[2]. At the request of ANSES summaries have been included in order to aid dossier evaluation. ~~Therefore, summaries of these studies are not repeated here.~~ The endpoints are summarised in Table 8.1.1-1 above.

Report:	Summary submitted at the request of ANSES for ease of dossier evaluation: *KIIA 8.1.1.1. Hakin B. & Rodgers M. (1992). Acute Oral Toxicity (LD_{50}) to Mallard Duck. Report Number CBG526/901711. Huntingdon Research Centre Ltd., Huntingdon, England* *(Syngenta File No. CGA219417/0062).*

Guidelines

U.S. EPA FIFRA – Guideline No. 71-1.

GLP: Yes

[1] European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12):1438. doi:10.2903/j.efsa.2009.1438. Available online: www.efsa.europa.eu

[2] Draft Assessment Report (DAR) Initial risk assessment provided by the rapporteur Member State France for the existing active substance Cyprodinil of the second stage of the review programme referred to in Article 8(2) of Council Directive 91/414/EEC. June 2005

Executive Summary

In an acute toxicity test mallard ducks were fed a single oral dose of either 125, 250, 500, 1000 or 2000 mg CGA219417/kg bodyweight. A control group were fed corn oil, without the addition of the test substance. Observations, including mortality, clinical signs, bodyweight and food consumption were made for a 14-day period post-dosing. The birds were then euthanized and the high level dose group were subjected to a gross post-mortem examination.

There were no mortalities following dosing. Vomiting was observed within one hour of dosing, in seven birds in the 2000 mg/kg group and eight birds in the 1000 mg/kg dose group. Bodyweight and food consumption were unaffected and no macroscopic abnormalities were detected in any of the post-mortem examinations. The acute oral LD_{50} of CGA219417 to the mallard duck is >2000 mg/kg.

Materials

Test Material	CGA219417
Lot/Batch #:	P912004
Purity:	99.5 %
Description:	Off-white powder
Stability of test compound:	Not reported
Reanalysis/Expiry date:	February 1994
Treatments	
Test concentrations:	125, 250, 500, 1000 and 2000 mg/kg bodyweight alongside an untreated control
Control:	Untreated corn oil
Analysis of test concentrations	Dosing formulations were analysed for CGA219417 concentration
Test organisms	
Species:	Mallard duck (*Anas platyrhynchos*) approximately 15 months old
Source:	J. Cole, The County Game Farms, Ashford, Kent, England
Acclimatisation period:	14 days
Treatment for disease:	Not reported
Weight:	850 to 1195 g at test initiation
Test design	
Replication:	Two replicate pens for each treatment each containing either 5 male or 5 female test organisms.
No. of birds/pen :	5
Duration of test:	14 days
Environmental test conditions	
Temperature:	16 to 18 °C
Humidity:	78 %
Photoperiod:	10 hour light : 14 hour dark

Study Design and Methods

Experimental dates: 31th October to 28th November 1990

All mallard ducks were approximately 15 months old, and appeared to be in good health at initiation of the test. Birds were allocated to groups based on bodyweight, with the aim of all treatment groups having similar mean bodyweights and bodyweight distribution. Groups were assigned to treatments using a random number allocation system. The birds were individually identified by numbered wing tags.

A total of 10 birds, 5 males and 5 females were exposed to each treatment and control. The birds were housed by sex in groups of 5 according to treatment group. For application the test substance was administered as a suspension in corn oil. Each bird was given a single dose by oral intubation at a rate of 5 mL/kg bodyweight. Control birds were dosed with untreated corn oil. Samples of the dosing solutions were analysed by HPLC to confirm the CGA219417 concentrations.

After dosing the birds were observed daily for mortality, health and clinical signs of toxicity. Individual bodyweights of each bird was measured on Day -14, -7, 0 (immediately prior to dosing), 7 and 14. Group mean food consumption was determined weekly. At the end of the 14-day observation period a post-mortem examination was performed on all ten birds from the highest dose group.

There were no mortalities in this study. Therefore, it was not possible to perform the calculation of an LD_{50} value. The LD_{50} value was determined to be greater than the highest dosage tested.

Results and Discussion

Chemical analysis of the dosing solutions showed that all solutions were within 10% of the target nominal concentrations.

There were no mortalities observed in any treatment group during the 14-day observation period. During the 1 hour period immediately following dosing, vomiting was observed in seven birds in the 2000 mg/kg treatment group and eight birds in the 1000 mg/kg treatment group. Bodyweight changes were variable in all treatment groups and there was no evidence of any treatment-related effect. Food consumption was variable, with no evidence of treatment-related effects. No abnormalities were detected in any bird undergoing post-mortem examination.

Table 8.1.1.1-1: Summary of effects of CGA219417 on and growth and food consumption of the mallard duck following acute oral exposure

Treatment (mg a.s./kg)	Replicate (A:Male, B:Female)	Mean Weight Change Day 0-7 (g)	Mean Weight Change Day 7-14 (g)	Group Mean Food Consumption Day 1-7 (g/bird/day)	Group Mean Food Consumption Day 8-14 (g/bird/day)
Control	A	+56	-1	86	94
	B	+75	-14	131	91
125	A	+58	+17	86	100
	B	+54	-8	86	71
250	A	+47	+17	74	74
	B	+48	+36	114	97
500	A	+45	-8	83	106
	B	+74	+39	94	89
1000	A	+63	+37	83	80

Treatment (mg a.s./kg)	Replicate (A:Male, B:Female)	Mean Weight Change Day 0-7 (g)	Mean Weight Change Day 7-14 (g)	Group Mean Food Consumption Day 1-7 (g/bird/day)	Group Mean Food Consumption Day 8-14 (g/bird/day)
	B	+50	+26	97	103
2000	A	+42	-3	74	80
	B	+64	+8	91	89

Validity Criteria

Not reported

Conclusions

The acute oral LD_{50} value of CGA219417 to the mallard duck was determined to be greater than 2000 mg/kg bodyweight. The non-emetic level must lie between 500 and 1000 mg/kg bodyweight.

(Hakin *et al.* 1992)

Report:	Summary submitted at the request of ANSES for ease of dossier evaluation: *KIIA 8.1.1. Hakin B. & Rodgers M. (1992). Acute Oral Toxicity (LD_{50}) to Bobwhite Quail. Report Number CBG526/901712. Huntingdon Research Centre Ltd., Huntingdon, England* *(Syngenta File No. CGA219417/0067).*

Guidelines

U.S. EPA FIFRA – Guideline No. 71-1.

GLP: Yes

Executive Summary

In an acute toxicity test bobwhite quails were given a single oral dose of either 125, 250, 500, 1000 or 2000 mg CGA219417/kg bodyweight. A control group were fed corn oil, without the addition of the test substance. Observations, including mortality, clinical signs, bodyweight and food consumption were made for a 14-day period post-dosing. The birds were then euthanized and the high level dose group were subjected to a gross post-mortem examination.

There were no mortalities following dosing and all birds remained in good health throughout the study. Bodyweight and food consumption were unaffected and no macroscopic abnormalities were detected in any of the post-mortem examinations. The acute oral LD_{50} of CGA219417 to the bobwhite quail is >2000 mg/kg bodyweight.

Materials

Test Material CGA219417

Lot/Batch #: P912004

Purity: 99.5 %

Description:	Off-white powder
Stability of test compound:	Not reported
Reanalysis/Expiry date:	February 1994

Treatments

Test concentrations:	125, 250, 500, 1000 and 2000 mg/kg bodyweight alongside an untreated control
Control:	Untreated corn oil
Analysis of test concentrations	Dosing formulations were analysed for CGA219417 concentration

Test organisms

Species:	Bobwhite quail (*Colinus virginianus*) over 16 weeks old
Source:	D.R. & R.E. Wise, Monkfield, Bourn, Cambridgeshire, England
Acclimatisation period:	14 days (pre-treatment period)
Treatment for disease:	Not reported
Weight:	182 to 206 g at test initiation

Test design

Replication:	Two replicate pens for each treatment each containing either 5 male or 5 female test organisms.
No. of birds/pen :	5
Duration of test:	14 days post-dosing

Environmental test conditions

Temperature:	15 to 18 °C
Relative humidity:	80 %
Photoperiod:	10 hour light : 14 hour dark

Study Design and Methods

Experimental dates: 1st to 29th November 1990

All bobwhite quails were in apparent good health at initiation of the test. Birds were allocated to groups based on bodyweight, with the aim of all treatment groups having similar mean bodyweights and bodyweight distribution. The birds were individually identified by numbered wing tags.

A total of 10 birds, 5 males and 5 females were exposed to each treatment and control. The birds were housed by sex in groups of 5 according to treatment group. For application the test substance was administered as suspension in corn oil at a rate of 10 mL/kg. Each bird was given a single dose by oral intubation. Control birds were dosed with untreated corn oil. Samples of the dosing solutions were analysed by HPLC to confirm the CGA219417 concentrations.

After dosing the birds were observed daily for mortality, health and clinical signs of toxicity. Individual bodyweights of each bird was measured on Day -14, -7, 0 (immediately prior to dosing), 7 and 14. Group mean food consumption was determined weekly. At the end of the 14-day observation period a post-mortem examination was performed on all ten birds from the highest dose group.

There were no mortalities in this study. Therefore, it was not possible to perform the calculation of an LD_{50} value. The LD_{50} value was determined to be greater than the highest dosage tested.

Results and Discussion

Chemical analysis of the dosing solutions showed that all solutions were within 6% of the target nominal concentrations.

There were no mortalities observed in any treatment group during the 14-day observation period and all birds remained in good health throughout the study. Bodyweight changes were variable and there was no evidence of any treatment-related effect. Food consumption was variable, with no evidence of treatment-related effects. No abnormalities were detected in any bird undergoing post-mortem examination.

Table 8.1.1.1-2: Summary of effects of CGA219417 on and growth and food consumption of the bobwhite quail following acute oral exposure

Treatment (mg a.s./kg)	Replicate (A:Male, B:Female)	Mean Weight Change Day 0-7 (g)	Mean Weight Change Day 7-14 (g)	Group Mean Food Consumption Day 1-7 (g/bird/day)	Group Mean Food Consumption Day 8-14 (g/bird/day)
Control	A	+5	+4	17	18
	B	+5	+3	17	17
125	A	+5	+3	18	18
	B	+4	+4	17	17
250	A	+4	+4	17	19
	B	+8	+2	17	18
500	A	+3	+4	17	19
	B	+5	+3	18	16
1000	A	+5	+4	16	17
	B	+4	+4	17	18
2000	A	+1	+6	16	18
	B	0	+7	16	19

Validity Criteria

Not reported

Conclusions

The acute oral LD_{50} value of CGA219417 to the bobwhite quail was determined to be greater than 2000 mg/kg bodyweight. In the absence of any mortalities, clinical abnormalities, post-mortem findings and effects on bodyweight and food consumption, the no-effect level must also be in excess of 2000 mg/kg bodyweight.

(Hakin *et al.* 1992)

A study conducted for USA compliance on the canary (*Serinus canaria*) is summarised below.

Report:	Submitted for purposes of renewal as conducted on cyprodinil for USA compliance: K-CA 8.1.1.1/01. Hubbard M. (2015). Cyprodinil – A Dietary LC_{50} Study with the Canary, Report Number 528-415. Wildlife International. 8598 Commerce Drive, Easton, MD 21601 USA (Syngenta File No. CGA219417_50779)

Guidelines

U.S. EPA 850 –OPPTS Number 850.2200: *Avian Dietary Toxicity Test* (1996)

GLP: Yes

Executive Summary

In an acute toxicity test canaries were fed a daily diet of 5620 mg cyprodinil/kg total diet alongside an untreated control, for five consecutive days. Following the five-day exposure period both groups were given untreated basal diet for three days.

The dietary LC_{50} value for canary exposed to cyprodinil was determined to be greater than 5620 mg a.s./kg (940 mg a.s./kg bw/day or a cumulative dose of 4699 mg a.s./kg bw). The no mortality level and the no observed adverse effect concentration were 5620 mg a.s./kg.

Materials

Test Material	CGA219417 technical Cyprodinil technical
Lot/Batch #:	P.012011
Purity:	99.2 % w/w
Description:	Light beige crumbs
Stability of test compound:	Stable under standard conditions.
Reanalysis/Expiry date:	End of January 2018
Treatments	
Test rates:	A single concentration of 5620 mg cyprodinil/kg alongside an untreated control
Analysis of test concentrations	Verification of dose tested at test start and test termination via HPLC analysis
Test organisms	
Species:	Canaries (*Serinus canaria*) 4 to 34 month old adults
Source:	Maryland Exotic Birds of Pasadena, MD 21122
Acclimatisation period:	5 weeks
Treatment for disease:	The birds received no form of antibiotic medication during acclimation or the test
Weight:	18.4 to 29.5g at test initiation
Test design	
Replication:	10 pens of individually housed canaries per treatment group (5 male, 5 female)
No. of birds/pen :	1
Duration of test:	Study phases: Acclimation – 5 weeks Pre-exposure: 3 days Exposure – 5 days

Post-exposure observation – 3 days

Environmental test conditions

Temperature:	22.3 to 23.0 °C
Humidity:	44 to 77 %
Photoperiod:	8 hr light : 15.5 hr dark, with a 15 minute dawn and dusk period, average illumination of approximately 323 lux

Study Design and Methods

Experimental dates: 6th to 22nd October 2014

All canaries (*Serinus canaria*) were adults, ranging from 4 to 34 months old, and appeared to be in good health at initiation of the test. Birds were randomly assigned to a single treatment group or a control group. The treatment group contained ten birds (5 male, 5 female) as did the control group. Birds were individually housed in pens. Each pen had a floor space of approximately 29 x 26 cm, with a ceiling height of 31 cm.

Test diets were prepared by mixing the test substance with corn oil and mixing the solution directly into the feed using standard laboratory mixers. An amount of diet sufficient to last the five-day exposure period was prepared on the day of test initiation for each treatment and control group. Diets were presented daily to the birds during the exposure period.

Dietary test concentrations were corrected for purity of the test substance. Nominal dietary test concentrations used in this study were 0 and 5620 mg a.s./kg.

From test initiation until termination, all birds were observed at least twice daily. A record was maintained of all signs of toxicity and abnormal behaviour.

Individual body weights were measured at test initiation (Day 0), at the end of the exposure, on day 1, on day 5 and on test termination on day 8. Feed consumption values were determined daily during the pre-exposure period (Days -3 to 0), during the exposure period (day 0 to 5) and during the post-exposure observation period (Days 6-8) by pen for each treatment group and the control group. Feed consumption was determined by measuring the change in the weight of the feed presented to the birds over a given period of time. The accuracy of feed consumption values may have been affected by the unavoidable wastage of feed by the birds.

Gross necropsies were performed on three birds from the treatment group and three birds from the control group.

There were no mortalities in this study. Therefore, it was not possible to perform the calculation of an LC_{50} value. The LC_{50} value was determined to be greater than the highest dosage tested. No statistical analyses were applied to separate mean resources among treatment groups for the endpoints of food consumption and body weight.

Results and Discussion

Analysis of the control samples did not show any indication of the presence of the test substance or of the presence of a co-eluting substance at the characteristic retention time of the test substance. Diet samples were collected from the 5620 mg a.s./kg test concentration, and were analysed to evaluate the homogeneity of the test substance in the diet. The mean concentration and standard deviation was 4980 ± 93.1 mg a.s./kg. The coefficient of variation was 1.87%. Analysis of diet samples collected from feeders after being held at ambient temperature for five days averaged 90% of the Day 0 values.

Table 8.1.1.1-13: Summary of effects of CGA219417 on mortality and growth of the canary (*Serinus canaria)* following acute oral exposure

Treatment (mg a.s./kg)	Cumulative mortality	Mean weight change[1] day 0-5 (SD) (in g)	Mean weight change [1] day 5-8 (SD) (in g)	Total weight change (SD) (in g)
0	0/10	0.2	0.2	0.4
5620	0/10	0.3	0.1	0.4

There were no mortalities in the control group or in the 5620 ppm a.s. test concentration. No incidences of regurgitation were noted for the control birds or for any of the treatment birds. All birds in the control group were normal in appearance and behaviour throughout the test. At the 5620 mg a.s./kg test concentration, three birds were noted with a ruffled appearance or slight ruffled appearance during at least one observation from Day 2 to Day 4 of the test. All birds in the 5620 ppm a.s. test concentration were normal in appearance and behaviour from Day 5 to test termination.

When compared to the control group, there were no apparent treatment-related effects upon mean body weight,mean body weight changes or food consumption among the birds in the 5620 mg a.s./kg test concentration.

Gross necropsies were performed on three birds from the control group and three birds from the treatment group at test termination. No findings were noted for any of the birds that were necropsied.

Validity Criteria

The validity criteria for the test were met;

- Birds were randomly assigned to control and treatment pens.
- The mortality in the control group did not exceed 10%.
- Concentrations of the test substance in the diet were satisfactorily maintained (at least 80% of nominal) throughout the exposure period.
- The test substance was administered in diet for five consecutive days (5 ~ 24 hr. periods).
- A minimum of ten birds were used for each control and treatment group.

Conclusions

The dietary LC_{50} value for canary exposed to cyprodinil was determined to be greater than 5620 mg a.s./kg (940 mg a.s./kg bw/day or a cumulative dose of 4699 mg a.s./kg bw). The no mortality level and the no-observed-adverse-effect concentration were 5620 mg a.s./kg.

(Hubbard M, 2015)

For acute toxicity studies LD_{10} and LD_{20} values have not been estimated since they are not required by the recommended test guidelines set out in Commission Communication 2013/C 95/01 (OECD Test Guideline No 223: Avian acute oral toxicity study or US EPA OCSPP 850.2100: Avian oral toxicity test), or by the bird acute risk assessments according to the current EFSA Guidance Document (2009).

Metabolism of cyprodinil

The metabolism of cyprodinil has been investigated in three crop groups; fruit crops (apple, peach and tomato), root crops (potato) and cereals (wheat), following foliar applications (see MCA Section 6.2.1). It has been concluded that the metabolism pathway is similar in all crops with the parent compound remaining the dominant residue except in potato tubers where the metabolic pattern results from the translocation of degradation products through the plant from the soil metabolism of cyprodinil.

Where there is a direct contact of cyprodinil with the edible part of the crop, metabolism proceeds mainly via hydroxylation of the phenyl and pyrimidine rings forming metabolites which then undergo sugar conjugation. Lower levels of other hydroxylated metabolites were also detected. These metabolites were found in the rat metabolism study and are considered covered by the toxicological profile of parent cyprodinil.

Where the edible part of the crop is not exposed to the fungicide spray, metabolism occurs by the cleavage of the pyrimidine ring with other hydroxylated metabolites identified in both their free and conjugated forms. These potato- specific metabolites were not found in the rat metabolism study, but due to the low absolute levels at which they were found in the potato metabolism study, they are not of toxicological relevance.

The metabolism of cyprodinil was investigated in four confined rotational crops studies elucidating the nature of residues following different plant-back intervals. In these studies cyprodinil radiolabelled in the phenyl or pyrimidinyl rings was applied to bare soil or crops. When radiolabelled cyprodinil was applied on a primary crop at an application rate of 1.25 kg a.s./ha, no significant cyprodinil residues were found in any of the edible parts of the succeeding crops tested. When cyprodinil was applied to bare soil, the studies identified four major cyprodinil metabolites in the succeeding crops sown at any of the replant intervals. It is concluded that the metabolism of cyprodinil in rotational crops is sufficiently elucidated. Studies on the magnitude of residues in rotational crops confirmed the presence of two plant metabolites which were found at measurable levels at the earliest replanting interval of 30 DAT, whilst parent cyprodinil occurred rarely. However, these metabolites were found to be of no toxicological concern.

The nature of cyprodinil residues in commodities of animal origin was investigated in three metabolism studies, one study in lactating goats and two studies in laying hens, using radiolabelled cyprodinil. The metabolism studies in both ruminants and poultry show that cyprodinil is extensively metabolised and that metabolism proceeds predominantly via hydroxylation of the phenyl and pyrimidine rings and conjugation with sulphate or glucuronic acid. The majority of the radioactivity was eliminated in the urine and faeces. The four metabolites identified in the studies were all found in the rat metabolism study.

CA 8.1.1.2 Short-term dietary toxicity to birds

Short-term dietary studies were previously submitted and summarised in point B.9.1.2 of the DAR therefore additional study summaries are not repeated here. However, these studies are no longer data requirements under **Commission Regulation (EU) No 283/2013** as the mode of action or results from mammalian studies do not indicate a potential for the dietary LD_{50} measured by the short term dietary (5-day) study to be lower than the LD_{50} based on an acute oral study.

CA 8.1.1.3 Sub-chronic and reproductive toxicity to birds

Since cyprodinil is not intended solely for use in enclosed spaces, a chronic study on birds is a data requirement in accordance with **Commission Regulation (EU) No 283/2013**.

The studies previously submitted in the EU were summarised in point B.9.1.3 of the DAR. At the request of ANSES summaries have been included in order to aid dossier evaluation. ~~therefore additional study summaries are not repeated here.~~ The endpoints are summarised in Table 8.1.1-1 above.

Report:	Summary submitted at the request of ANSES for ease of dossier evaluation: *KIIA 8.1.3. Rodgers M.H. (1995). CGA 219417 Effects on Reproduction in Bobwhite Quail After Dietary Administration. Report Number CBG672/950770. Huntingdon Research Centre Ltd., Huntingdon, Cambridgeshire, England. (Syngenta File No. CGA219417/0478).*

Guidelines

U.S. Environmental Protection Agency. 1982. Pesticide Assessment Guidelines, Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms, Series 71 – Avian and Mammalian Testing, 71-4 Avian Reproduction test. (version: October 1982)

GLP: Yes

Executive Summary

The effects of CGA219417 on reproduction in the bobwhite quail (*Colinus virginianus*) were determined in a 22-week toxicity test. Mortality, body weight, food consumption, reproductive parameters, and any other clinical signs of toxicity, were assessed at nominal dietary concentrations of 100, 300 and 600 ppm CGA219417, alongside an untreated control group.

No significant treatment-related mortalities or effects were observed in any of the parameters tested, in adult or F1 generation birds. Therefore, the no-observed effect concentration was 600 ppm CGA219417, the highest concentration tested.

Materials

Test Material	CGA219417
Lot/Batch #:	P.012012
Purity:	99.2 %
Description:	Light beige powder
Stability of test compound:	Stable under test conditions
Reanalysis/Expiry date:	March 1995
Treatments	
Test doses:	100, 300 and 600 ppm CGA219417, alongside an untreated control
Food:	Basal diet (Protein: 25.08 %, Fibre: 2.92 %, Ash: 10.65 %).
Water:	Domestic quality potable water supplied by Anglian Water
Analysis of test concentrations:	Samples of spiked diet collected at time of mixing from each dietary level for weeks 1, 6, 12, 15, 18 and 22.
Test organisms	
Species:	Bobwhite quail (*Colinus virginianus*), approximately 7 months old
Source:	Mr D. R. Wise, Monkfield, Bourn, Cambridgeshire, England
Acclimatisation period:	Not reported. 14-day pre-treatment period
Treatment for disease:	On arrival the birds received a routine course of antibiotic
Weight:	173 to 232 g at test initiation
Test design	
Replication:	20 replicates per treatment and control
No. of birds/pen :	2, one male and one female
Duration of test:	22 weeks (10 weeks prior to the start of egg production and 12 weeks of egg production)

Parameters monitored:	Adult feed consumption, adult bodyweight, number of eggs laid per female, proportion of eggs damaged, egg shell thickness, number of viable embryos/number of eggs set, number of live 3-week embryos/number of viable eggs, number of normal hatchlings/number of live 3-week embryos, number of normal hatchlings/number of viable embryos, number of 14-day survivors/number of normal hatchlings, number of 14-day survivors/number of eggs laid, number of 14-day survivors/adult female, hatchling weights and 14-day survivor weights.

Environmental test conditions

Temperature:	Adult birds, mean daily minimum and maximum temperatures: 20 and 22 ºC Egg storage prior to incubation: 16 ºC Egg storage during incubation: 37.7 ºC Egg storage during hatching: 37.5 ºC Chicks, mean daily minimum and maximum temperatures: 26 and 29 ºC
Humidity:	Adult birds, mean daily relative humidity: 76 % Egg storage during incubation relative humidity: 55 % Chicks, mean daily relative humidity: 53 %
Photoperiod:	Adult birds: 7 hours light per day from test initiation to Week 5, increasing to 16 hours light per day until the end of egg production. Mean light intensity ranged from 5 – 50 lux. Chicks: 14 hour light photoperiod

Study Design and Methods

Experimental dates: 20th June 1994 to 12th January 1995

Adult bobwhite quails, approximately 7 months old, in good health and approaching first breeding season, were randomly assigned to one control group and three treatment groups. A pre-mix of suitable strength was prepared weekly by mixing the required quantity of test substance with untreated basal diet and the test substance doses were prepared by direct dilution of the pre-mix at treatment levels of 100, 300 and 600 ppm CGA219417. The mix for the control groups consisted of basal ration only. Samples were collected from spiked food from each dietary level for weeks 1, 6, 12, 15, 18 and 22 and analysed for CGA219417 content.

The birds were housed in pens measuring 0.31 x 0.39 x 0.24 m. The pens were constructed of polythene coated steel wire and had sloping floors with 0.1 m egg-catchers. Each pen housed one study replicate of a single male and a single female quail. During the test all birds were given food and water *ad libitum*. The treated food was supplied in a food hopper and the test diets were fed to the birds for 10 weeks during the pre-egg laying period and for 12-week egg production period. Food hopper contents were weighed at the beginning and end of each time period and food consumption calculated.

Adult birds were observed daily for mortality and clinical signs. Individual adult bodyweights were recorded in weeks -2, 0 (immediately prior to introduction of test diets), 2, 4, 6 and at termination (week 22). Feed consumption per replicate was recorded weekly. Offspring were observed daily from hatching until 14 days old for mortality and clinical signs. Individual chick bodyweight was recorded within 24 hours of hatching and at the end of the observation period. At the conclusion of the experiment, all adult birds were examined following sacrifice by cervical dislocation. All sporadic mortalities were subject to macroscopic internal and external examination.

Eggs were collected daily from the start of week 11 and stored on plastic egg trays in a refrigerator at 16 ºC. Following each 7-day period, eggs were removed from the refrigerator, and allowed to reach room

temperature for at least 12 hours prior to incubation and candling. The total number of eggs collected and the number of uncracked and unblemished eggs were recorded. Non-defective eggs were placed in an incubator maintained at 37.7 ºC and 55 % relative humidity and turned once hourly until incubation day 21, when they were placed in a still air Bristol hatcher set to maintain 37.5 ºC.

Eggs laid on the first days of weeks 11, 13, 15, 17, 19 and 21 were examined for shell thickness. Embryo viability and survival, unhatched eggs, hatchlings, and weight and viability of chicks, were recorded for each hatch. Chicks were not examined *post mortem* upon study completion.

Williams' test ($P < 0.05$ and 0.01) was used to analyse statistical significance compared to the control for adult bodyweight, food consumption, egg production, proportion of eggs damaged, egg shell thickness, egg viability, and chick survival and bodyweight. Sample units were individual pens within experimental groups for each analysis.

Results and Discussion

The mean concentrations of CGA219417 in test diet formulations analysed during the study were within 10% of the nominal concentrations, with the exception of one result (Week 12, group 3) which was 66% above nominal. CGA219417 was confirmed to be stable at ambient temperature for 18 days in the diet formulations. A summary of the analytical results is presented below.

Table 8.1.1.3-1: Results of chemical analysis of the diet formulations for CGA219417

Week of Study	Group	Nominal Concentration (ppm)	Mean Measured Concentration (ppm)	Relative Mean Error
1	Control	0	Not detected	-
	2	100	96.1	-3.9
	3	300	299	-0.3
	4	600	641	+6.8
6	Control	0	Not detected	-
	2	100	96.0	-4.0
	3	300	305	+1.7
	4	600	644	+7.3
12	Control	0	Not detected	-
	2	100	97.4	-2.6
	3	300	499	+66.3
	4	600	543	-9.5
15	Control	0	Not detected	-
	2	100	99.1	-0.9
	3	300	298	-0.7
	4	600	585	-2.5
18	Control	0	Not detected	-
	2	100	95.5	-4.5
	3	300	298	-0.7
	4	600	615	+2.5

Week of Study	Group	Nominal Concentration (ppm)	Mean Measured Concentration (ppm)	Relative Mean Error
22	Control	0	Not detected	-
	2	100	99.3	-0.7
	3	300	301	+0.3
	4	600	589	-1.8

Fecundity is summarised in the table below.

Table 8.1.1.3-2: Summary of effects of CGA219417 on fecundity of bobwhite quail (*Colinus virginianus*) following dietary exposure

Parameter	Control	100 ppm CGA219417	300 ppm CGA219417	600 ppm CGA219417
Eggs laid per female	60.2	56.1	46.1	58.1
Eggs damaged	49	32	34	43
Eggs damaged of eggs laid (%)	4.2	3.6	4.6	3.9
Mean egg shell thickness (mm)	0.21	0.21	0.21	0.21
Eggs set	1019	770	635	971
Viable embryos	980	699	608	932
Viable embryos of eggs set (%)	96	91	96	96
Live 3-week embryos	940	659	590	903
Live 3-week embryos of viable embryos (%)	96	94	97	97
Normal hatchlings	864	606	551	829
Normal hatchlings of viable embryos (%)	88	87	91	89
Normal hatchlings of live 3-week embryos (%)	92	92	93	92
14-day old survivors	821	566	517	777
14-day old survivors of eggs laid (%)	70	64	71	70
14-day old survivors of normal hatchlings (%)	95	93	94	94
14-day old survivors per female	42.4	35.7	32.2	40.6
Chick bodyweights at hatching (g)	7.0	6.9	6.8	6.9
Chick bodyweights at 14 days (g)	29	29	28	30

There were no treatment-related signs of toxicity during the experimental period and in the *post mortem* examination. The majority of sporadic mortalities examined were found to have reduced muscle and subcutaneous fat but there was insufficient evidence to suggest that results were treatment-related.

There was no evidence to suggest a treatment-related effect on the incidence of mortality as a greater of deaths occurred in both the 100 ppm and 300 ppm than in the highest treatment group. Overall the number of deaths observed in the 100 and 300 ppm treatments were considered to be within the normal limits.

No statistically significant effects from treatment with CGA219417 were observed for bodyweight, food consumption and egg production.

There were no statistically significant treatment-related effects for egg quality, egg viability, egg shell thickness, chick survival and bodyweight compared to the control.

Conclusions

No treatment-related mortalities or effects were observed in any of the parameters tested, in adult or F1 generation birds. Therefore, the no-observed effect concentration was 600 ppm CGA219417, the highest concentration tested.

(Rodgers, 1995)

In accordance with **Commission Regulation (EU) No 283/2013**, attempts have been made to estimate EC_{10} and EC_{20} values. ~~for the two avian reproduction studies conducted by Rogers in 1995 (CGA219417/0477 and CGA219417/0478).~~ A summari~~es~~y of the attempted re-evaluation~~s~~ ~~are~~ is provided below.

Report:	Submitted for purposes of renewal due to change in data requirements guidance: K-CA 8.1.1.3/01 Taylor S. & Allen M. (2015) Cyprodinil (CGA219417) – Effects on reproduction of the bobwhite quail after dietary administration. Statistical Re-analysis. Report Number CEA.1413. Cambridge Environmental Assessments, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK. (Syngenta File No: CGA219417_11586)

Summary

Report number CBG672/950770 (Rodgers, 1995) for the reproductive toxicity test of cyprodinil, at nominal dose rates of 100, 300 and 600 ppm with the bobwhite quail (*Colinus virginianus*), did not provide estimates of the EC_{10} and EC_{20} for the parameters of adult bodyweight, adult food consumption, number of eggs laid, proportion of eggs damaged, egg shell thickness, viable embryos as a proportion of eggs set, live 3-week embryos as a proportion of those viable, normal hatchlings as a proportion of live 3-week embryos, 14-day survivors as a proportion of normal hatchlings and as a proportion of eggs laid, number of 14 day survivors and chick body weight at hatch and at 14 days. Consequently the data have been re-analysed in order to estimate these values.

Statistical analysis of the parameters revealed that no reliable EC_{10} and EC_{20} values could be calculated for any of the measured parameters.

Statistical Analysis

The effective concentrations for viable embryos, live 3-week embryos, normal hatchlings and 14 day survivors as a proportion of both normal hatchlings and of eggs laid were assessed. Probit analysis with linear maximum likelihood regression was used to determine the concentration response function. Chi^2 was used as a goodness of fit measure.

The effective concentrations for male and female adult bodyweights, adult food consumption, eggs damaged, total number of eggs laid, chick bodyweights and eggshell thickness were assessed. Probit analysis with linear maximum likelihood regression was used to determine the concentration response function. Chi^2 was used as a goodness of fit measure, and the proportion of variance explained by the dose/response function was determined.

Where no meaningful concentration/response was found ($p(F) > 0.05$) the calculated EC_x's were not valid due to the absence of a concentration/response. All computations were carried out in ToxRat Professional version 2.10 (ToxRat Solutions GmbH, 2001-2010).

Results

In the parameters of numbers of eggs laid and number of eggs set there were significant dose responses ($p(F) \leq 0.05$), however, due to the low goodness of fit ($\chi^2 < 0.1$) or lack of meaningful dose response (slope = 0.000), EC_x could not be reliably calculated.

Conclusion

Statistical analysis of the parameters revealed that no reliable EC_{10} and EC_{20} values could be calculated for any of the measured parameters.

(Taylor S, Allen M, 2015)

Report:	Summary submitted at the request of ANSES for ease of dossier evaluation: *KIIA 8.1.3. Rodgers M.H. (1995). CGA 219417 Effects on Reproduction in Mallard Duck After Dietary Administration. Report Number CBG671/950769. Huntingdon Research Centre Ltd., Huntingdon, Cambridgeshire, England. (Syngenta File No. CGA219417/0477).*

Guidelines

U.S. Environmental Protection Agency. 1982. Pesticide Assessment Guidelines, Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms, Series 71 – Avian and Mammalian Testing, 71-4 Avian Reproduction test. (version: October 1982)

GLP: Yes

Executive Summary

The effects of CGA219417 on reproduction in the mallard duck (*Anas platyrhynchos*) were determined in a 22-week toxicity test. Mortality, body weight, food consumption, reproductive parameters, and any other clinical signs of toxicity, were assessed at nominal dietary concentrations of 100, 300 and 600 ppm CGA219417, alongside an untreated control group.

No significant treatment-related mortalities or effects were observed in any of the parameters tested, in adult or F1 generation birds. Therefore, the no-observed effect concentration was 600 ppm CGA219417, the highest concentration tested.

Materials

Test Material	CGA219417
Lot/Batch #:	P.012012
Purity:	99.2 %
Description:	Light beige powder
Stability of test compound:	Stable under test conditions
Reanalysis/Expiry date:	March 1995
Treatments	
Test doses:	100, 300 and 600 ppm CGA219417, alongside an untreated control
Food:	Adults: Basal diet (Protein: 25.08 %, Fibre: 2.92 %, Ash: 10.65 %)

	Ducklings: HRC duckling diet
Water:	Domestic quality potable water supplied by Anglian Water
Analysis of test concentrations:	Samples of spiked diet collected, at time of mixing, from each dietary level for weeks 1, 6, 12, 15, 18 and 22.
Test organisms	
Species:	Mallard ducks (*Anas platyrhynchos*), approximately 7.5 months old
Source:	J. Coles, The County Game Farms, Ashford, Kent, England
Acclimatisation period:	Not reported. 14-day pre-treatment period
Treatment for disease:	Not reported
Weight:	840 to 1200 g at test initiation
Test design	
Replication:	6 replicates per treatment and control
No. of birds/pen :	7, two male and five female
Duration of test:	22 weeks (10 weeks prior to the start of egg production and 12 weeks of egg production)
Parameters monitored:	Adult feed consumption, adult bodyweight, number eggs laid per female, proportion of eggs damaged, egg shell thickness, number of viable embryos/number of eggs set, number of live 3-week embryos/number of viable eggs, number of normal hatchlings/number of live 3-week embryos, number of normal hatchlings/number of viable embryos, number of 14-day survivors/number normal hatchlings, number of 14-day survivors/number of eggs laid, number of 14-day survivors/adult female, hatchling weights and 14-day survivor weights.
Environmental test conditions	
Temperature:	Adult birds, mean daily minimum and maximum temperatures: Room 7E 18 and 21 ºC. Room 7F 18 and 22 ºC. Egg storage prior to incubation: 16 ºC Egg storage during incubation: 37.7 ºC Egg storage during hatching: 37.5 ºC Ducklings, mean daily minimum and maximum temperatures: 23 and 26 ºC
Humidity:	Adult birds, mean daily relative humidity: 87 % room 7E and 89 % room 7F Egg storage during incubation relative humidity: 55 % Ducklings, mean daily relative humidity: 67 %
Photoperiod:	Adult birds: 7 hours light per day from test initiation to Week 8, increasing to 16 hours light per day until test termination. Mean light intensity ranged from 110 – 200 lux. Ducklings: 14 hour light photoperiod

Study Design and Methods

Experimental dates: 22nd June 1994 to 16th January 1995

Adult mallard ducks, approximately 7.5 months old, in good health and approaching first breeding season, were employed in the study. The birds were housed in pens measuring 0.73 x 1.48 m with each pen containing two males and five females and constituting a single replicate. The pens were constructed of galvanised steel wire and had solid sides and wire mesh doors and floors. During the egg production

phase the floors were covered with plastic pillow matting to minimise the chance of egg damage. The pens were assigned to treatments based on a randomised block design.

A pre-mix of suitable strength was prepared weekly by mixing the required quantity of test substance with untreated basal diet and the test substance doses were prepared by direct dilution of the pre-mix at treatment levels of 100, 300 and 600 ppm CGA219417. The mix for the control groups consisted of basal ration only. Samples were collected from spiked food from each dietary level for weeks 1, 6, 12, 15, 18 and 22 and analysed for CGA219417 content.

During the test all birds were given food and water *ad libitum*. The treated food was supplied in a food hopper and the test diets were fed to the birds for 10 weeks during the pre-egg laying period and for 12-week egg production period. Food hopper contents were weighed at the beginning and end of each time period and food consumption calculated.

Adult birds were observed daily for mortality and clinical signs. Individual adult bodyweights were recorded in weeks -2, 0 (immediately prior to introduction of test diets), 2, 4, 6, 8 and at termination (Week 22). Feed consumption per replicate was recorded weekly. Offspring were observed daily from hatching until 14 days old (end of the observation period) for mortality and clinical signs. Individual duckling bodyweight was recorded within 24 hours of hatching and at the end of the observation period. At the conclusion of the experiment, all adult birds were examined following sacrifice by cervical dislocation. All sporadic mortalities were subject to macroscopic internal and external examination.

Eggs were collected daily from the start of Week 11 and stored on plastic egg trays in a refrigerator at 16 ºC. Following each 7-day period, eggs were removed from the refrigerator, and allowed to reach room temperature for at least 12 hours prior to incubation and candling. The total number of eggs collected and the number of uncracked and unblemished eggs were recorded. Non-defective eggs were placed in an incubator maintained at 37.7ºC and 55% relative humidity and turned once hourly until incubation day 23, when they were placed in a Bristol hatcher set to maintain 37.5ºC.

Eggs laid on the first days of weeks 11, 13, 15, 17, 19 and 21 were examined for shell thickness. Embryo viability and survival, unhatched eggs, hatchlings, and weight and viability of ducklings, were recorded for each hatch. Ducklings were not examined *post mortem* upon study completion.

Williams' test ($P < 0.05$ and 0.01) was used to analyse statistical significance compared to the control for adult bodyweight, food consumption, egg production, proportion of eggs damaged, egg shell thickness, egg viability, and duckling survival and bodyweight. Sample units were individual pens within experimental groups for each analysis.

Results and Discussion

The mean concentrations of CGA219417 in test diet formulations analysed during the study were within 10% of the nominal concentrations, with the exception of one result (Week 12, Group 3) which was 66% above nominal. CGA219417 was confirmed to be stable at ambient temperature for 18 days in the diet formulations. A summary of the analytical results is presented below.

Table 8.1.1.3-3: Results of chemical analysis of the diet formulations for CGA219417

Week of Study	Group	Nominal Concentration (ppm)	Mean Measured Concentration (ppm)	Relative Mean Error
1	Control	0	Not detected	-
	2	100	96.1	-3.9
	3	300	299	-0.3
	4	600	641	+6.8
6	Control	0	Not detected	-
	2	100	96.0	-4.0
	3	300	305	+1.7
	4	600	644	+7.3
12	Control	0	Not detected	-
	2	100	97.4	-2.6
	3	300	499	+66.3
	4	600	543	-9.5
15	Control	0	Not detected	-
	2	100	99.1	-0.9
	3	300	298	-0.7
	4	600	585	-2.5
18	Control	0	Not detected	-
	2	100	95.5	-4.5
	3	300	298	-0.7
	4	600	615	+2.5
22	Control	0	Not detected	-
	2	100	99.3	-0.7
	3	300	301	+0.3
	4	600	589	-1.8

Fecundity is summarised in the table below.

Table 8.1.1.3-4: Summary of effects of CGA219417 on fecundity of mallard duck (*Anas platrhynchos*) following dietary exposure

Parameter	Control	100 ppm CGA219417	300 ppm CGA219417	600 ppm CGA219417
Eggs laid per female	25.5	27.3	30.8	27.5
Eggs damaged	16	18	24	15
Eggs damaged of eggs laid (%)	2.1	2.2	2.6	1.9
Mean egg shell thickness (mm)	0.34	0.35	0.34	0.34
Eggs set	696	745	835	708
Viable embryos	622	711	705	593
Viable embryos of eggs set (%)	89	95	84	84
Live 3-week embryos	581	652	639	544
Live 3-week embryos of viable embryos (%)	93	92	91	92
Normal hatchlings	352	403	437	308
Normal hatchlings of viable embryos (%)	57	57	62	52

Parameter	Control	100 ppm CGA219417	300 ppm CGA219417	600 ppm CGA219417
Normal hatchlings of live 3-week embryos (%)	61	62	68	57
14-day old survivors	337	380	413	294
14-day old survivors of eggs laid (%)	44	47	45	38
14-day old survivors of normal hatchlings (%)	96	94	95	95
14-day old survivors per female	11.2	12.7	13.9	10.4
Duckling bodyweights at hatching (g)	36	35	35	36
Duckling bodyweights at 14 days (g)	277	267	271	273

There were no treatment-related signs of toxicity during the experimental period and in the *post mortem* examination. There was no evidence to suggest a treatment-related effect on the incidence of mortality.

No statistically significant effects from treatment with CGA219417 were observed for bodyweight, food consumption and egg production.

There were no statistically significant treatment-related effects for egg quality, egg viability, egg shell thickness, duckling survival and bodyweight compared to the control.

Conclusions

No treatment-related mortalities or effects were observed in any of the parameters tested, in adult or F1 generation birds. Therefore, the no-observed effect concentration was 600 ppm CGA219417, the highest concentration tested.

(Rodgers, 1995)

In accordance with **Commission Regulation (EU) No 283/2013**, attempts have been made to estimate EC_{10} and EC_{20} values. A summary of the attempted re-evaluation is provided below.

Report:	Submitted for purposes of renewal due to change in data requirements guidance: K-CA 8.1.1.3/02 Taylor S. & Majdanik V. (2015) Cyprodinil (CGA219417) – Effects on reproduction in Mallard duck after dietary administration. Statistical Re-analysis. Report Number CEA.1419. Cambridge Environmental Assessments, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK. (Syngenta File No: CGA219417_11590)

Summary

Report number CBG671/950769 (Rodgers *et al.*, 1995) for the reproductive toxicity test of cyprodinil, at nominal dose rates of 100, 300 and 600 ppm with the mallard duck (*Anas platyrhynchos*), did not provide estimates of the EC_{10} and EC_{20} for the parameters of adult bodyweight, adult food consumption, number of eggs laid, proportion of eggs damaged, egg shell thickness, viable embryos as a proportion of eggs set, live 3-week embryos as a proportion of those viable, normal hatchlings as a proportion of viable embryos, 14-day survivors as a proportion of normal hatchlings and as a proportion of eggs laid, number of 14 day survivors and duckling body weight at hatch and at 14 days. Consequently the data have been re-analysed in order to estimate these values.

Statistical analysis of the parameters revealed that no reliable EC_{10} and EC_{20} values could be calculated for any of the measured parameters.

Statistical Analysis and Results

Statistical analysis in the original report demonstrated that no reliable statistically significant treatment related effects could be determined. Data were meaningless for reliable EC_{10} and EC_{20} derivation and therefore no further statistical analysis was conducted.

Conclusion

Statistical analysis of the parameters revealed that no reliable EC_{10} and EC_{20} values could be calculated for any of the measured parameters.

(Taylor S, Majdanik V, 2015)

Relevant Literature on Birds

No relevant scientifically peer-reviewed open literature could be found on cyprodinil or its major metabolites. Details of the literature search undertaken can be found in M-CA Section 9 of the current submission.

CA 8.1.2 Effects on terrestrial vertebrates other than birds

Table 8.1.2-1: Summary of endpoints for vertebrates other than birds for cyprodinil

Organism	Test item	Test type	Endpoint		Endpoints used for the risk assessment	Reference (author, date, Syngenta File No.)
Rat	A8779A (UNIX 75 WG)	Acute oral	EU	LD_{50} = >2000 mg/kg bw (>1500 mg a.s./kg bw)	-	***Hartmann (1992) CGA219417/0083***
Rat	Cyprodinil			LD_{50} = >2000 mg/kg bw	LD_{50} = >2000 mg/kg bw	***Hartmann (1992) CGA219417/0020***
Mouse				LD_{50} = >5000 mg/kg bw	-	***Winkler (1995) CGA219417/0593***
Rat		2-generation		NOAEL = 72.7 mg/kg bw/day [a]	NOAEL = 72.7 mg/kg bw/day	***Khali (1993) CGA219417/0162***

[a] The lowest overall mean value was calculated from all of the mean weekly consumption values for the individual sexes (72.7 mg/kg bw/day for males and 96.6 mg/kg bw/day for females)

CA 8.1.2.1 Acute oral toxicity to mammals

Since cyprodinil is not intended solely for use in enclosed spaces, an acute study on mammals is a data requirement in accordance with **Commission Regulation (EU) No 283/2013**. Please refer to the original EU review. No new data are provided. Studies have been carried out with cyprodinil for the toxicology assessment; please refer to M-CA Section 5 (Toxicology) for studies with the active substance and major metabolites.

The endpoints relevant for the ecotoxicological risk assessment are summarised in Table 8.1.2-1 above.

For acute toxicity studie,s LD_{10} and LD_{20} values have not been estimated since they are not required by the recommended test guidelines set out in Commission Communication 2013/C 95/01, or for the wild mammal acute risk assessments according to the current EFSA Guidance Document (2009).

CA 8.1.2.2 Long-term and reproductive toxicity to mammals

Since cyprodinil is not intended solely for use in enclosed spaces, a chronic study on mammals is a data requirement in accordance with **Commission Regulation (EU) No 283/2013**. Studies have been carried

out for the toxicology assessment; please refer to point M-CA Section 5 (Toxicology) for studies with the active substance and major metabolites.

The endpoints relevant for the ecotoxicological risk assessment are summarised in Table 8.1.2-1 above.

Relevant Literature on Terrestrial Vertebrates other than Birds

No relevant scientifically peer-reviewed open literature could be found on cyprodinil or its major metabolites from an ecotoxicological perspective on wild mammals. Please refer to point CA 5 (Toxicology Section) for a review of literature on mammals from a human toxicological perspective. Details of the literature search undertaken can be found in M-CA Section 9 of the current submission.

CA 8.1.3 Effects of active substance bioconcentration in prey of birds and mammals

Studies on potential bioconcentration of active substances with a log P_{ow} >3 may be required if indicated by an assessment of the risk posed by bioconcentration of the substance in the prey of birds and mammals, presented in document M-CP Section 10. The highest log P_{OW} values for metabolites CGA249287 and CGA321915 are 1.5 and -0.10, respectively and further consideration is therefore not necessary. For CGA275335, the highest log P_{OW} value is 3.3, but given that the metabolite will be found at lower concentrations than the parent compound, the risk assessment for the parent compound is considered to address this metabolite also.

For cyprodinil, the log P_{ow} is 4.0; therefore bioconcentration studies have been conducted. The studies previously submitted in the EU were summarised in Point B 9.2.3 of the DAR. At the request of ANSES summaries have been included in order to aid dossier evaluation. ~~therefore additional study summaries are not repeated here.~~ The endpoints are summarised in Table 8.1.3-1 below.

Table 8.1.3-1: Summary of endpoints for bioconcentration of cyprodinil in fish

Organism	Endpoint		Endpoint used for the risk assessment	Reference (author, date, Syngenta File No.)
Bluegill sunfish (*Lepomis macrochirus*)	EU	BCF (whole fish) = 399/387 [a]	393 [b]	***Van Dijk (1994) CGA219417/0267***
	-	BCF (whole fish) = 397 [c] /407	-	***Wyness & Hill (1996) CGA219417/0698***

[a] represents values derived for two different exposure groups
[b] mean value and EU endpoint
[c] derived from exposure to phenyl-labelled cyprodinil
[d] derived from exposure to pyrimidyl-labelled cyprodinil

Report:	Summary submitted at the request of ANSES for ease of dossier evaluation: *KIIA 8.2.3 van Dijk A. (1994). Accumulation and Elimination of [U-^{14}C-Phenyl]-CGA219417 by Bluegill Sunfish in a Dynamic Flow-through System, Report Number 322277, RCC UMWELTCHEMIE AG, Itingen, Switzerland. (Syngenta File No. CGA219417/0267)*

Guidelines

EPA 540/9-82-021, Subdivision N, Series 165-4, October 1982

EPA 540/09-88-051, Subdivision N, PB88-191739, Series 165-4, Add. 8 on Data Reporting

OECD Guidelines for Testing of Chemicals, Test Guideline 305E, Bioaccumulation: Flow-through Fish Test, adopted May 1981

GLP: Yes

Executive Summary

The study was undertaken to determine the bioconcentration factor of [^{14}C]-CGA219417 in bluegill sunfish (*Lepomis macrochirus*). The study was conducted with a flow-through system where duplicate tanks of fish were exposed to a single nominal test concentration of 0.1 mg/L (mean measured of 0.107 and 0.104 mg/L) for a 28 day uptake phase, followed by a 14 day depuration phase.

Steady-state concentrations in whole fish were reached within 7-14 days during the uptake phase. Rapid depuration was observed during the depuration phase with half-life values of 0.3-0.8 days and >98 % elimination by Day 14. The mean steady-state BCF (BCF_{SS}) value based on whole fish was 393; the kinetic BCF (BCF_k) was not determined.

Analysis of extracts from fish samples at Day 14 and 21 of the uptake phase indicated that 17-29% of the radioactivity was present as parent. Six metabolites were detected, of which, only one was >5 %, 4-(4-cyclopropyl-6-methyl-pyrimidin-2-ylamino)-phenyl hydrogensulfate, which accounted for 53-67% of the radioactivity in the extracts.

Materials

Test Material:	(a) CGA219417 (b) [U-^{14}C-Phenyl]-CGA219417
Description:	(a) Not reported (b) Not reported
Lot/Batch #:	(a) M-334.2A (b) P.912006
Purity/radiochemical purity:	(a) 99.6 % (b) 98.4-99.2%
Stability of test compound:	Stable under test conditions
Test concentrations:	Dilution water solvent control and nominal concentration of 0.1 mg CGA219417/L (corresponding mean measured concentration of 0.107 and 0.104 mg/L).
Test medium:	Tap water
Vehicle and/or positive control:	Acetone / no positive control
Analysis of test concentrations:	Yes – LSC and TLC
Test animals	
Species:	Bluegill sunfish (*Lepomis macrochirus*)
Source:	Osage Catfisheries, MO 65 065, USA
Acclimatisation period:	One week
Treatment for disease:	None reported
Weight and length of dilution water control fish at start of exposure period:	Weight: mean range 0.86 – 1.14 g Length: not reported
Feeding:	Commercial fish food (Kliba Forellenfutter) 2 % of body weight daily

Environmental conditions

Test temperature:	17.4 to 20.2°C)
pH range:	7.9 -8.3
Dissolved oxygen:	8 – 10 mg/L
Total hardness of dilution water:	Sample of tap water 41.2 mg/L $CaCO_3$
Conductivity of dilution water	Sample of tap water 734 µS/cm
Lighting:	12 -16 hours light
Length of test:	Uptake phase: 28 days Depuration phase: 14 days

Study Design and Methods

Experimental dates: 22nd October 1992 to 1st November 1993

Apparatus

A continuous flow-through system was used to expose fish to the nominal concentration of 0.1 mg/L for a 28-day exposure period, followed by a 14-day depuration period. In addition, a dilution water control was used. A glass aquarium (one per test concentration duplicate vessel and control), holding a volume of 100 L, was set up with a continuous flow-through of 300 L per day.

[U-^{14}C-Phenyl]-CGA219417 was radiodiluted with non-labelled CGA219417 in acetone, yielding material with a specific activity of 1.99 µCi/mg. An aliquot (680 µL) of this acetone stock solution was mixed with Tween 80 (1.8 g) and 9 L of tap water. For each test item treatment tank, the dose solution was delivered via a Hamilton dispenser unit at a rate of 4.5 L per 24 h into a mixing vessel which was also receiving tap water at a rate of 300 L per 24 h. The contents of the mixing vessel flowed directly into the exposure vessels. The control vessel was prepared in an identical manner, excluding the test item.

Test procedure

The uptake phase (day 0 to day 28) was initiated by transferring fish to each of the control and treatment vessels. The population of fish in each tank were fed commercial fish food at a rate of approximately 2 % of the total fish weight per day. During the depuration phase, the fish were transferred to equivalent tanks without the test item. The depuration phase lasted 14 days.

Analysis of fish tissues

Fish from each tank were taken at 0, 3, 7, 14, 21 and 28 days during the uptake phase and 0, 1, 3, 7, 10 and 14 days during the depuration phase. Four fish from each exposure tank were analysed for total radioactivity at each sampling point. One whole fish was directly analysed and three fish were separated into edible (fillet and skin) and non-edible parts (head, fins, viscera and skeleton). The whole fish and the individual fish parts were each pooled, weighed and solubilized. Total radioactivity in duplicate solubilized subsamples was measured by LSC. Additionally, fish edibles and non-edibles at days 14 and 21 were analysed for parent compound and metabolites.

For additional analyses, pooled samples were homogenized and extracted three times with acetonitrile and additionally with either methanol (non-edibles, Day 14) or acetonitrile/methanol (50:50%, v/v; edibles, Day 21). The homogenates of day 14, non-edibles and day 21, edibles were additionally extracted by means of a methanol Soxhlet. After extraction remaining fish parts were air-dried, homogenized and remaining radioactivity determined by combustion.

Organic extracts were combined and either directly analysed or concentrated for TLC analyses. For HPLC analyses, 2 mL of the selected extract of fish non-edibles, Day 14 were evaporated to dryness and re-suspended in methanol.

Analysis of test water

Water sampling and analysis were performed on days -1, 0, 3, 7, 14, 21 and 28 days during the uptake phase and 0, 1, 3, 7, 10 and 14 days during the depuration phase. Duplicate aliquots of 4 mL were taken for LSC. The additional 200 mL water aliquots were extracted twice into dichloromethane and ethylacetate (only time point Day 21). Extracts were pooled and concentrated before analysis for parent. At one time point the remaining aqueous phase was lyophilized, the residue re-suspended in methanol and centrifuged. After removal of the supernatant, the pellet was washed with methanol/water (80:20%, v/v), which was added to the previous methanol extractant. Samples were analysed by TLC.

Physical and chemical parameters

Dissolved oxygen, pH and temperature were measured daily throughout the study.

Calculation of Bioconcentration Factors (BCF)

The BCF, uptake and depuration rate constants were calculated by the following equations:

The steady state bioconcentration factor (BCF) at various sample points:

$$BCF = \frac{C_{f(t)}}{C_{w(t)}}$$

Where:

$C_{f(t)}$: Concentration of radioactivity (parent equivalents) in the fish during exposure at time interval t

$C_{w(t)}$: Concentration of CGA219417 in the exposure water at time interval t

The plateau value for bioconcentration (BCF_{ss}) was calculated by using non-linear parameter estimation:

$$BCF_t = BCF_{ss} \times \left(1 - e^{\left(-k_{bcf} \times t\right)}\right)$$

Where:

BCFt: The bioconcentration factor at exposure time t

BCF_{ss}: The final plateau bioconcentration factor

k_{bcf}: The rate constant for bioaccumulation

t: The exposure time (days)

Results and Discussion

[^{14}C]-CGA219417 in water and fish

The concentration of [^{14}C]-CGA219417 from test tanks was determined by LSC during the uptake phase. The measured range was between 0.099 to 0.113 mg/L. The mean concentrations of the uptake phase were 0.107 ± 0.006 and 0.104 ± 0.004 mg/L for the individual duplicate tanks. During the depuration

phase concentrations were below the LOQ (0.002 mg/L). Extraction efficiency into the organic phase for the bulk 200 mL samples was generally >90 %, with between 0.8 – 13.9 % remaining in the aqueous phase. Radioactivity increased in the aqueous phase during the first 7 days, which was presumed to be due to increasing presence of excreted soluble metabolites. Analysis of the organic phase indicated that [^{14}C]-CGA219417 accounted for >90 % of the radioactivity. In the aqueous phase metabolites were mainly detected, with 4-(4-cyclopropyl-6-methyl-pyrimidin-2-ylamino)-phenyl hydrogensulfate being predominant, present at 8.0 – 11.8 % of the radioactivity.

[^{14}C]-CGA219417 residues were accumulated in bluegill sunfish. In both duplicate tanks, plateau levels of total radioactivity in edible tissues, non-edible tissues and whole fish were reached after 7-14 days. Plateau levels as parent equivalents were 5.909 - 8.794, 61.523 - 63.413 and 39.622 - 38.066 mg/kg for edible tissues, non-edible tissues and whole fish, respectively.

Extractable radioactivity from edible tissue at steady-state (days 14 and 21) was 93.6 % - 99.4 %; non-extractable residue accounted for 0.6 - 6.4 %. At Day 14, parent accounted for 19.5 % of radioactivity in extracts, with at least four metabolites. A major metabolite accounting for 54.8 % was characterized as 4-(4-cyclopropyl-6-methyl-pyrimidin-2-ylamine)-phenyl hydrogensulfate, all other metabolite were minor, present at 5.5 - 8.5%. At Day 21 parent accounted for 29.3 % and 4-(4-cyclopropyl-6-methyl-pyrimidin-2-ylamine)-phenyl hydrogensulfate 53.2 %, all other metabolites were <6.7 %.

Extractable radioactivity from non-edible tissue at steady-state (days 14 and 21) was 94.2 % - 99.1 %; non-extractable residue accounted for 0.9 – 5.8 %. A similar pattern of metabolites was noted in the non-edible samples from the same time points. At Day 14 parent accounted for 17.3 % and 4-(4-cyclopropyl-6-methyl-pyrimidin-2-ylamine)-phenyl hydrogensulfate 67.4 %, all other metabolites were <5.3 %. At Day 21 parent accounted for 17.1 % and 4-(4-cyclopropyl-6-methyl-pyrimidin-2-ylamine)-phenyl hydrogensulfate 60.8 %, all other metabolites were <8.0 %.

Depuration of radioactivity from bluegill sunfish during the 14 day depuration period was rapid. [^{14}C]-CGA219417 residues had a half-life ($t_{0.5}$) values of 0.36 – 0.44, 0.32 – 0.64 and 0.26 – 0.75 days for edible tissues, non-edible tissues and whole fish, respectively. Time to depurate 95% ($t_{0.95}$) of [^{14}C]-CGA219417 residues was 1.54 - 1.92, 1.40 - 2.78 and 1.11 - 3.24 days for edible tissues, non-edible tissues and whole fish, respectively.

Bioconcentration factors are summarized below:

Table 8.1.3-1: Bioconcentration factors (BCF)

Sample	Time point (days)	Replicate tank 1			Replicate tank 2			Mean BCF	BCF_{ss}	k_d	Lipid content (%)
		C_f	C_w	BCF	C_f	C_w	BCF				
Edible	0	2.445	0.106	23.07	2.436	0.103	23.65	23.36	72	1.562 - 1.942	NR
	3	4.746	0.099	47.94	8.19	0.096	85.31	66.63			
	7	4.177	0.089	46.93	3.83	0.088	43.52	45.23			
	21	6.124	0.099	61.86	8.654	0.095	91.1	76.48			
	28	7.964	0.094	84.72	11.325	0.098	115.56	100.14			

Sample	Time point (days)	Replicate tank 1			Replicate tank 2			Mean BCF	BCF_{ss}	k_d	Lipid content (%)
		C_f	C_w	BCF	C_f	C_w	BCF				
Non-edible	0	9.247	0.106	87.24	7.107	0.103	69	78.12	677	1.078 - 2.137	NR
	3	76.481	0.099	772.54	75.003	0.096	781.28	776.91			
	7	67.007	0.089	752.89	63.45	0.088	721.02	736.96			
	21	59.989	0.099	605.95	65.332	0.095	687.71	646.83			
	28	54.838	0.094	583.38	49.966	0.098	509.86	546.62			
Whole fish	0	4.958	0.106	46.77	4.541	0.103	44.09	45.43	393	2.700 - 0.925	NR
	3	29.09	0.099	293.84	45.804	0.096	477.13	385.48			
	7	45.494	0.089	511.17	37.115	0.088	421.76	466.47			
	21	44.32	0.099	447.68	38.045	0.095	400.47	424.08			
	28	32.333	0.094	343.97	24.538	0.098	250.39	297.18			

k_d: Depuration rate constant
NR: Not reported

Conclusions

During the uptake phase, the concentration of [^{14}C]-CGA219417 residues in fish increased rapidly, reaching a steady-state within 7 – 14 days; the BCF_{ss} for total radioactive residues (non-normalised for lipid content or growth) in whole fish was 393. During the depuration phase, [^{14}C]-CGA219417 was rapidly depurated from fish. More than 50 % of the concentration at steady-state was depurated within 1 day. Also, more than 95 % of the average concentration a steady-state was depurated after 3.25 days.

In conclusion, CGA219417 had limited bioconcentration potential in bluegill sunfish (*Lepomis macrochirus*) and the bioconcentrated residues were rapidly eliminated when the fish were placed in clean water. Therefore, bioconcentration potential of CGA219417 is considered to be low.

(van Dijk. 1994)

Report: Summary submitted at the request of ANSES for ease of dossier evaluation:

KIIA 8.2.3 Wyness L.E. & Hill A.D. (1996). CGA219417: Semi-Static Fish Bioconcentration Test with Lepomis macrochirus. Report Number HE 380/230, Corning Hazleton (Europe), Harrogate, UK. (Syngenta File No. CGA219417/0698)

Guidelines

EPA 540/9-82-021, Subdivision N, Series 165-4, October 1982

GLP: Yes

Executive Summary

The study was undertaken to determine the accumulation of [U-^{14}C-Phenyl]-CGA219417 and [2-^{14}C-Pyrimidinyl]-CGA219417 in bluegill sunfish (*Lepomis macrochirus*). The study was conducted with a semi-static renewal regimen where where fish were exposed to a nominal test concentration of 0.25 mg/L (mean measured of 0.242 and 0.244 mg/L for phenyl and pyrimidinyl labels respectively). Individual exposure tanks were prepared for each label in which fish were exposed for a 96 hour phase.

Total radioactivity was measured in test water and in edible and non-edible fish samples. Static bioconcentration factors after 96 hours were calculated from the ratio of residue concentration in fish at 96 h and the mean measured residue concentration in water. The static BCF values after 96 hours exposure were 68.6 and 63.1, 798 and 868, 379 and 407 for phenyl and pyrimidinyl labels and for edible, non-edible and whole fish tissues, respectively.

Analysis of extracts from fish samples at the end of the exposure period indicated that [^{14}C]-CGA219417 was extensively metabolised in the fish to five significant components, four of which were identified as 4-(4-cyclopropyl-6-methyl-pyrimidin-2-ylamino)-phenol and 3 conjugates thereof (a glucuronic acid conjugate and two hydrogensulphate conjugates). After 96 hours exposure only 13 – 46 % AR was present in fish as CGA219417.

The metabolite profiles and bioconcentration factors obtained in this study were qualitatively consistent with those obtained in the flow-through study on this molecule conducted in 1992 and 1993 (RCC Project 322277).

Materials

Test Material:	(a) CGA219417 (b) [U-^{14}C-Phenyl]-CGA219417 (c) [2-^{14}C-Pyrimidinyl]-CGA219417
Description:	(a) Colourless crystals (b) Not reported (c) Not reported
Lot/Batch #:	(a) AMS 452/102 (b) M-334.2A (HD: 746/94-380) (c) M-336.4A (HD: 745/94-380)
Purity/radiochemical purity:	(a) 99 % (b) 98 % (c) 97 %
Stability of test compound:	Stable under test conditions (hydrolytically stable DT_{50} >200 days)
Test concentrations:	Dilution water solvent control and nominal concentration of 0.25 mg CGA219417/L (mean measured concentration of 0.242 – 0.244 mg/L).
Test medium:	Treated mains water (particulate and carbon filtered)
Vehicle and/or positive control:	Vehicle control (acetone / Tween 80) / no positive control
Analysis of test concentrations:	Yes – LSC and TLC / HLPC
Exposure regime:	Semi-static, test media renewal at 24 hour intervals
Test animals	
Species:	Bluegill sunfish (*Lepomis macrochirus*)
Source:	Fish Network Ltd, Blackborough, UK

Acclimatisation period:	>14 days
Treatment for disease:	None reported
Weight and length of dilution water control fish at end of exposure period:	Weight: mean range 0.73 – 1.18 g Length: not reported
Feeding:	Not feed during the exposure period
Environmental conditions	
Test temperature:	18.2 to 22.7°C
pH range:	7.0 - 7.8
Dissolved oxygen:	69 – 118 % air saturation
Total hardness of dilution water:	40.5 – 47.0 mg/L $CaCO_3$
Conductivity of dilution water	137 - 177 µS
Lighting:	16 hours light
Length of test:	Uptake phase: 96 hours

Study Design and Methods

Experimntal dates: 20th March 1995 to 30th January 1996

Apparatus

A semi-static system with a 24 hour renewal period, was used to expose fish to the nominal concentration of 0.25 mg/L for a 96 hour exposure period. A 80 L volume glass aquarium was prepared for each of the phenyl and pyrimidinyl labels individually, each tank held 60 L of test medium. A vehicle control (dilution water and vehicle only) tank of 25 L, holding 20 L of control medium was also prepared. Forty fish were added to each tank containing CGA219417 and 11 fish were added to the control tank.

[U-^{14}C-Phenyl]-CGA219417 and [2-^{14}C-Pyrimidinyl]-CGA219417 were radiodiluted with non-labelled CGA219417 in acetone, yielding material with a specific activity of 32.30 and 32.99 µCi/mg, respectively. The equivalent concentration of the radiodiluted stock solutions was 2.55 and 2.52 mg/mL for the phenyl and pyrimidinyl labels respectively. An aliquot (6 mL) of each acetone stock was added to a volumetric flask, this acetone stock solution was mixed with Tween 80 and dilution water. The contents of the flask was shaken and rinsed out into the pre-filled exposure vessels. The control vessel was prepared in an identical manner, excluding the test item. This procedure was repeated every 24 hours to prepare fresh test and control medium. The final nominal exposure concentrations of acetone and Tween 80 were 100 and 10 µg/L respectively.

Test procedure

The test was was initiated by transferring fish to each of the control and treatment vessels. Fish were transferred to freshly prepared test or control medium every 24 hours. The fish were not fed during the test.

Analysis of fish tissues

At the end of the exposure period three fish from each test vessel were removed, weighed and dissected into edible and non-edible portions. Total radioactivity was measured by LSC, after solubilisation of the tissues.

The remaining fish were dissected into edible and non-edible portions for each label and control separately. The portions were homogenised and extracted with acetonitrile three times followed by a final methanol wash. The extracts were then pooled and the profiled by HPLC and TLC and the components identified by LCMS.

Analysis of test water

Duplicate 1 mL water samples from were taken from a mid-tank position at the start and end of each 24 hour renewal period and analysed for total radioactivity by LSC.

Additional duplicate 1 L samples were taken from the control and CGA219417 tanks at the start and end of the exposure period. Each sample was extracted twice with dichloromethane, the organic phases were then concentrated to dryness and reconstituted in methanol. Aliquots of these concentrated methanol samples were analysed by HPLC and TLC, co-chromatographed with authentic reference standards.

Physical and chemical parameters

Dissolved oxygen, pH, total hardness, alkalinity, conductivity and temperature were measured in freshly prepared and 24 hour old solutions throughout the study. Residual chlorine was determined in each fresh preparation of the control test medium.

Calculation of Bioconcentration Factors (BCF)

The BCF, was calculated by the following equation:

$$BCF = \frac{C_{f(96\ h)}}{C_w}$$

Where:

$C_{f(96\ h)}$: Concentration of radioactivity (parent equivalents) in fish at 96 hours

C_w: Mean measured concentration of radioactivity (parent equivalents) in water over the exposure period

Results and Discussion

[^{14}C]-CGA219417 in water and fish

The mean measured concentrations of [^{14}C]-CGA219417 in the test medium during the 96 h exposure period were 0.242 and 0.244 mg/L, for the [U-14C-Phenyl] and [2-14C-Pyrimidinyl]-CGA219417 label treatments respectively. The concentrations of radioactivity in the control test medium were below the background determinations.

Extraction efficiency into the organic phase for the bulk 1 L samples was >96% of the radioactivity measured in the exposure water at Day 0. After four days this decreased to 81.31% and 77.56% phenyl and pyrimidinyl labels respectively. HPLC and TLC analyses of the concentrated organic phases indicated that >98% was parent CGA219417.

[^{14}C]-CGA219417 residues were accumulated in bluegill sunfish. After 96 h exposure to phenyl and pyrimidinyl labelled CGA219417, the concentrations of [^{14}C]-residues in edible fish tissues were 16.6 and 15.4 µg/g respectively. For non-edible tissues the concentrations were 193 and 212 µg/g and for total fish tissues 91.6 and 99.4 µg/g for the phenyl and pyrimidinyl labels respectively.

Extractable radioactivity from edible tissue was 96.0% and 96.7% for the phenyl and pyrimidinyl labels respectively. HPLC and TLC analyses of the concentrated organic phases indicated that >98% was parent CGA219417. Concentrations of CGA219417 in edible tissues were 9.08 and 10.0 µg/g or 35 and 48% of AR for the phenyl and pyrimidinyl labels respectively. Five other significant components to the profile were identified, with concentrations ranging from 0.49 to 5.46 µg/g (2 – 21% AR). No significant differences were observed between the profiles of the samples treated with test compound radiolabelled in the two positions.

Extractable radioactivity from non-edible tissue was 95.2% and 103.2% for the phenyl and pyrimidinyl labels respectively. HPLC and TLC analyses of the concentrated organic phases indicated that >98% was parent CGA219417. Concentrations of CGA219417 in non-edible tissues were 24.3 and 27.9 µg/g or 13 and 14 % of total applied radioactivity for the phenyl and pyrimidinyl labels respectively. Five other significant components to the profile were identified, with concentrations ranging from 15.1 to 43.2 µg/g (8 – 21% AR).

Four of the significant metabolites were identified as 4-(4-cyclopropyl-6-methyl-pyrimidin-2-ylamino)-phenol (CGA304075; 2 – 9% AR), a glucuronic acid conjugate of CGA304075 (6 – 10 % AR) and two CGA304075 hydrogensulphate conjugates at the phenyl ring (13 - 22% AR) and pyrimidinyl ring (7 – 15 % AR). The fifth metabolite (5 – 9% AR) remained unidentified.

Static bioconcentration factors after 96 hours exposure are summarized below:

Table 8.1.3-2: Static bioconcentration factors (BCF) after 96 h

Sample	[U-^{14}C-Phenyl]-CGA219417	[2-^{14}C-Pyrimidinyl]-CGA219417
Edible	68.6	63.1
Non-edible	798	869
Whole fish	379	407

Conclusions

[^{14}C]-CGA219417 residues were accumulated in fish over the 96 hour exposure period; the static BCF for total radioactive residues (non-normalised for lipid content or growth) in whole fish was 379 and 407 for the phenyl and pyrimidinyl labels respectively. [^{14}C]-CGA219417 was extensively metabolised in the fish to five significant components, four of which were identified as 4-(4-cyclopropyl-6-methyl-pyrimidin-2-ylamino)-phenol and 3 conjugates thereof (a glucuronic acid conjugate and two hydrogensulphate conjugates). After 96 hours exposure only 13 – 46% AR was present in fish as CGA219417.

(Wyness & Hill 1996)

CA 8.1.4 Effects on terrestrial vertebrate wildlife (birds, mammals, reptiles and amphibians)

An acute toxicity study has been conducted with *Xenopus laevis*. A summary is presented below.

Report:	Submitted for purposes of renewal due to Chinese data requirements: K-CA 8.1.4/01 Zhao Y.Y. (2009) Cyprodinil (CGA219417) - Acute toxicity test in frog (*Xenopus laevis*). Report Number H093453390, Supervision and Test Center for Pesticide Safety Evaluation and Quality Control, 600 Shenliao Road, Tiexi District, Shengyang 110141, Liaoning Province, P.R. China. (Syngenta File No. CGA219417_11635)

Guidelines

Testing Guidelines for Environmental Safety Evaluation of Chemical Pesticides (2004, Draft) issued by State Environmental Protection Administration (SEPA).

GLP: Yes

Executive Summary

The acute toxicity of cyprodinil (CGA219417) to tadpoles of a species of frog (*Xenopus laevis*) was determined. Tadpoles were exposed to concentrations of 5.00, 7.1, 10.0, 14.1 and 20.0 mg/L, and a control (acetone water solution). The 48 hour LC_{50} was 12.3 mg /L (95% confidence intervals 11.3 – 13.6 mg/L).

Materials

Test Material	Cyprodinil (CGA219417) technical Batch No. 2009-2-2090
Test concentrations:	Nominal 5.00, 7.1, 10.0, 14.1 and 20.0mg/L and a control (acetone water solution)
Vehicle and/or positive control:	Acetone; none
Analysis of test concentrations:	No
Test animals	
Species:	Frog (*Xenopus laevis*) tadpole
Test design	
Exposure regime:	static
Aeration:	48h
Replication:	3
No of tadpoles per replicate:	10

Environmental conditions

Test temperature:	20 - 22°C
pH:	7.78 - 7.82
Dissolved oxygen:	7.43 - 7.62mg/L
Hardness of water:	250 ± 25 mg/L (by $CaCO_3$)
Lighting:	16 hours light and 8 hours dark
Length of test:	48 hours

Study Design and Methods

Tadpoles of the frog species (*Xenopus laevis*) were exposed for 48 h to cyprodinil (98%). Following a range finding test, the definitive study was conducted at test concentrations 5.00, 7.1, 10.0, 14.1 and 20.0 mg/L.

Test water was standard water dilution. Three replicates were prepared for each treatment and control group. Tadpoles were distributed into groups of 10 per group. Signs of toxic symptoms and mortality of tadpoles at 24h and 48h were observed and recorded.

Results and Discussion

The LC_{50} at 24 h was estimated to be 14.1 mg/L (95% confidence intervals were 13.1 to 15.3 mg/L).

The 48 hour LC_{50} was 12.3 mg/L (95% confidence intervals were 11.3 to 13.6 mg/L).

Conclusions

The acute toxicity of cyprodinil (98%) to tadpoles of a species of frog (*Xenopus laevis*) was determined. The 48 hour LC_{50} was 12.3 mg/L (95% confidence intervals 11.3 – 13.6 mg /L).

(Zhao Y, 2009)

Relevant Literature on Other Terrestrial Vertebrate Wildlife

No relevant scientifically peer-reviewed open literature could be found on cyprodinil or its major metabolites. Details of the literature search undertaken can be found in M-CA Section 9 of the current submission.

CA 8.1.5 Endocrine disrupting properties

There are no indications of endocrine-disrupting effects from the existing database for this active substance. No conclusions regarding ED effects were made in the EU review.

Report:	K-CA 8.1.5/01 Charlton A.J. (2015) Cyprodinil – Review for Potential for Endocrine Disruption in Mammalian Species. Task Number TK0223684, Syngenta, Jealott's Hill International Research Centre, Bracknell, Berkshire, RG42 6EY. (Syngenta File No. CGA219417_11641).

Cyprodinil has been extensively tested, with the relevant data from the regulatory and open scientific literature studies covering a wide range of study types, both *in vitro* and *in vivo*. These data fall into levels 1, 2, 4 and 5 of the OECD Conceptual Framework.

Following evaluation of each of the studies individually and a subsequent weight of evidence evaluation, it can be concluded that cyprodinil is not an endocrine disrupter, as defined by WHO/IPCS (2002):

"An endocrine disrupter is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations."

Relevant Literature on Endocrine Disrupting Properties

Scientifically peer-reviewed open literature considered relevant for determining effects on endocrine disruption were summarised in the above referenced document. Details of the literature search undertaken can be found in M-CA Section 9 of the current submission.

CA 8.2 Effects on Aquatic Organisms

Marine and estuarine species are not data requirements under **Commission Regulation (EU) No 283/2013**. However, data are available from testing required for the US EPA, and therefore these are included here and considered for relevance in the risk assessment in Document M-CP Section 10.

Table 8.2-1: Summary of endpoints for acute fish toxicity of cyprodinil

Organism	Endpoints (mg a.s./L)		Endpoint used for the risk assessment	Reference (author, date, Syngenta File No.)
Rainbow trout (*Oncorhynchus mykiss*)	EU	96 h LC_{50} = $2.41_{(mm)}$ (static test)	-	***Boeri et al. (1995) CGA219417/0486***
Bluegill sunfish (*Lepomis macrochirus*)		96 h LC_{50} = $2.17_{(mm)}$ (static test)	-	***Boeri et al. (1995) CG219417/0507***
		96 h LC_{50} = $3.2_{(mm)}$ (flow-through test)	-	***Ward et al. (1995) CGA219417/0651***
Sheepshead minnow *(Cyprinodon variegatus)*	Not previously submitted	96 h LC_{50} = $1.25_{(mm)}$ (flow-through test)	96 h LC_{50} = $1.25_{(mm)}$	***Ward et al. (1995) CGA219417/0652***
Carp *(Cyprinus carpio)*	New	96 h LC_{50} = $3.0_{(mm)}$ (semi-static test)	-	***Liedtke (2011) CGA219417/11450***

mm = Endpoint derived using mean measured concentration
LIT = peer-reviewed literature article

Table 8.2-2: Summary of endpoints for acute fish toxicity for metabolites of cyprodinil

Organism	Test item	Endpoints (mg a.s./L)		Endpoints used for the risk assessment	Reference (author, date, Syngenta File No.)
Rainbow trout	CGA249287	EU	96 h LC_{50} = $55_{(nom)}$ (static test)	96 h LC_{50} = $55_{(nom)}$	***Maetzler (1999) CGA249287/0007***
	CGA275535		96 h LC_{50} = $2.1_{(mm)}$ (static test)	96 h LC_{50} = $2.1_{(mm)}$	***Pfeifle (2001) CGA275535/0017***
	CGA263208 (phenyl guanidine)	Not previously submitted	96 h LC_{50} = $2.1_{(nom)}$ (static test)	96 h LC_{50} = $2.1_{(nom)}$	***Vial (1991) CA1059/0009***
	CA1139A (carbonate salt of phenyl guanidine)		96 h LC_{50} = >100 $_{(nom)}$ (static test)		***Grade (1992) CA1139/0008***

nom = Endpoint derived using nominal concentration
mm = Endpoint derived using mean measured concentration

Table 8.2-3: Summary of endpoints for chronic toxicity to fish for cyprodinil

Organism	Test type	Endpoints (mg a.s./L)		Endpoint used for the risk assessment	Reference (author, date, Syngenta File No.)
Rainbow trout	Prolonged toxicity (flow-through)	EU	21d NOEC $_{(length)}$ = $0.083_{(mm)}$	-	***Vial (1991) CGA219417/0009 EC_{10}/EC_{20} estimate Taylor & Arenas (2015) CGA219417_11597***
		EC_{10}/EC_{20} [a]	Could not be estimated	-	
Fathead minnow (*Pimephales promelas*)	Fish early life cycle (flow-through)	EU	36 d NOEC $_{(survival)}$ = $0.231_{(mm)}$	36 d NOEC $_{(survival)}$ = $0.231_{(mm)}$	***Ward et al. (1995) CGA 219417/0653 EC_{10}/EC_{20} estimate Taylor & Majdanik (2015) CGA219417_11595***
		EC_{10}/EC_{20} [a]	36 day $EC_{10\ (survival)}$ = 0.253 [b] 36 day $EC_{20\ (survival)}$ = 0.334 [b]		
Sheepshead minnow	Fish early life cycle (flow-through)	New study	34 day NOEC (growth) = $0.0406_{(mm)}$ EC_{10} (survival) = $0.130_{(mm)}$	34 day NOEC (growth) = $0.0406_{(mm)}$	***Minderhout et al. (2014) CGA219417_50676***

[a] Values estimated in accordance with **Commission Regulation (EU) No 283/2013**
[b] Represents the worst case endpoint
mm = Endpoint derived using mean measured concentration

Table 8.2-4: Summary of acute endpoints for aquatic invertebrates for cyprodinil

Organism	Test type	Endpoints (mg a.s./L)		Endpoints used for the risk assessment	Reference (author, date, Syngenta File No.)
Daphnia magna	Acute toxicity (flow-through)	EU	48 h EC_{50} = 0.033$_{(mm)}$	48 h EC_{50} = 0.033$_{(mm)}$	***Boeri et al (1995) CGA219417/0461***
Daphnia longispina	Acute toxicity (static)		48 h EC_{50} = 0.22$_{(mm)}$	48 h EC_{50} = 0.22$_{(mm)}$	***Peither (2000) CGA219417/0993***
Daphniopsis sp.			24 h EC_{50} = 0.21$_{(mm)}$	24 h EC_{50} = 0.21$_{(mm)}$	***Peither (2000) CGA219417/0990***
Simocephalus vetulus			48 h EC_{50} = 0.15$_{(mm)}$	48 h EC_{50} = 0.15$_{(mm)}$	***Peither (2000) CGA219417/0994***
Gammarus sp.			48 h EC_{50} = 1.8$_{(mm)}$	48 h EC_{50} = 1.8$_{(mm)}$	***Peither (2000) CGA219417/0998***
Thamnocephalus platyurus			24 h EC_{50} = 0.12$_{(mm)}$	24 h EC_{50} = 0.12$_{(mm)}$	***Peither (2000) CGA219417/0991***
Ostracoda			48 h EC_{50} = 1.1$_{(mm)}$	48 h EC_{50} = 1.1$_{(mm)}$	***Peither (2000) CGA 249417/0995***
Brachionus calyciflorus			24 h EC_{50} >9.5$_{(mm)}$	24 h EC_{50} >9.5$_{(mm)}$	***Peither (2000) CGA219417/0992***
Cloeon sp.			48 h EC_{50} = 3.5$_{(mm)}$	48 h EC_{50} = 3.5$_{(mm)}$	***Peither (2000) CGA219417/0996***
Chaoborus sp.			48 h EC_{50} = 4.0$_{(mm)}$	48 h EC_{50} = 4.0$_{(mm)}$	***Peither (2000) CGA219417/0999***
Bay shrimp (*Mysidopsis bahia*)	Acute toxicity (flow-through)	Not previously submitted	96 h LC_{50} = 0.00805$_{(mm)}$	96 h LC_{50} = 0.00805$_{(mm)}$ [a]	***Ward et al. (1995) CGA219417/0649***
Lymnea stagnalis	Acute toxicity (static)	EU	48 h EC_{50} = 2.9$_{(mm)}$	48 h EC_{50} = 2.9$_{(mm)}$	***Peither (2000) CGA219417/0997***
Crassostrea virginica	Acute shell deposition (flow-through)	Not previously submitted	48 h EC_{50} = 0.36$_{(mm)}$	-	***Ward et al. (1995) CGA219417/0650***
Asellus aquaticus	Acute adult toxicity (static)	New study	96 h EC_{50} = 1.96$_{(nom)}$	96 h EC_{50} = 1.96$_{(nom)}$	***Maynard (2011) CGA219417_11454***
	Acute nymph toxicity (static)		96 h EC_{50} = 2.64$_{(nom)}$	-	***Maynard (2011) CGA219417_11453***
Grandidierella japonica	Acute adult toxicity (static)	EU	10 day LC_{50} = 0.42 mg a.s./kg sediment (mm)	10 day LC_{50} = 0.42 mg a.s./kg sediment (mm)	***Kreuger & Sutherland (1998) CGA219417/0893***
Hyalella azteca	Acute adult toxicity (static)	New study	10 day LC_{50} = 0.73 mg a.s./kg sediment (mm)	-	***Sutherland & Krueger (1998) CGA219417/0892***
~~*Gammarus pulex*~~	~~Acute adult toxicity (static)~~	~~LIT~~	~~96 h LC_{50} = 0.69~~	~~96 h LC_{50} = 0.69~~	~~***Beketov & Liess (2008)***~~

mm = Endpoint derived using mean measured concentration
nom = Endpoint derived using nominal concentration
[a] endpoint used for the initial risk assessment
~~LIT = peer-reviewed literature article~~

Request from RMS: *Grandidierella japonica and Hyalella azteca being organisms living at the surface of the sediment rather than into the sediment, please also express endpoints from studies conducted with Grandidierella japonica (K-CA 8.2.4.2/04; Sutherland & Kruger, 1998) and with Hyallela azteca (K-CA 8.2.4.2/05; Sutherland & Kruger, 1998a) in measured concentrations in overlying water to have a complete overview of these toxicity tests.*

Syngenta response:

The tests conducted with *Hyalella azteca* and *Grandidierella japonica* were designed in order to assess the acute effects of cyprodinil on sediment dwelling species. The tests were conducted according to "OPPTS 850.1740 Whole Sediment Acute Toxicity Invertebrates, Freshwater", in the case of *Grandidierella japonica* and OPPTS 850.1735 Whole Sediment Acute Toxicity Invertebrates, Marine", in the case of *Hyalella azteca.* The sediments used in the tests were directly treated with cyprodinil and hence it is considered that expression of the endpoints in terms of concentrations in the surface water is not appropriate.

In natural habitats *Hyalella azteca* can be considered to be epibenthic, which means that they reside at the water column-sediment interface. However, *H. azteca* demonstrate different behavior in laboratory sediment toxicity studies. As described on page 7 in OPPTS 850.1735, (C) *H. azteca* may be cultured under illumination of 500–1,000 lx. They feed during daylight and avoid bright light by hiding under litter" Given that no cover is provided in laboratory tests, the amphipods would spend the majority of their time in the sediment. Also, in a laboratory experiment with only water and clean reference sediment, Ingersoll *et al*. (2000[3]) noted that *H. azteca* were observed only 11% of the time at the sediment surface but never observed in the water column. Additional studies have made similar qualitative statements concerning the fact that *H. azteca* routinely burrow into the upper layer of sediment during laboratory exposures (Ingersoll *et al*. 1998[4]).

In a review by Wang *et al*. (2004[5]) in which the the natural behaviour of H. Azteca is compared to the harsh laboratory tests, conclusions drawn were as follows: In laboratory sediment toxicity tests H. azteca is deprived of natural food sources such as algal communities on or above the sediments, and is subjected to constant light without any cover except that afforded by burial into the sediments. Under these constraining laboratory conditions, *H. azteca* has been reported to respond to sediment or porewater contamination. Clearly this adds further evidence to the fact that under laboratory test conditions, freshwater and marine amphipods would spend the vast majority of their time in the sediment.

In the 1991 American Society for Testing of Materials (ASTM)[6] document "Guide for conducting 10-day static sediment toxicity test with marine and estuarine amphipods", *Grandidierella japonica* is mentioned as being a suitable species for conducting sediment-dweller toxicity tests. It is clear that the organisms spend their time in the sediment given that in section A.4.3.1 of the ASTM document it is stated that following an overnight equilibriation of the sediment the amphipods are introduced into the test system and are allowed 5 to 10 minutes to burrow into the test substrate. Also, any amphipods which have not burrowed within this time are to be removed, the implication being that they are not exhibiting 'normal' behavior if they have not quickly moved into the sediment.

Grandiddierella japonica survive in brackish water, and burrow in mud bottoms of the bay by building U-shaped tubes in the sediment. In these tube the organisms are often found in pairs (Stephenson, 1938a[7]). Within one study of bays in California the species was found to be most abundant in intertidal

[3] Ingersoll CG, Ivey CD, Brunso EL, Hardesty DK, Kemble NE. 2000. Evaluation of the toxicity: whole-sediment versus overlying-water exposures with amphipod Hyalella azteca. Environ. Toxicol. Chem. 19: 2906-2910.
[4] Ingersoll CG, Brunson EL, Dwyer FJ, Hardesty DK, Kemble NE. 1998. Use of sublethal endpoints in sediment toxicity tests with the amphipod Hyalella azteca. Environ. Toxicol. Chem. 17: 1508-1523.
[5] Wang F, Goulet RR, Champman PM. 2004. Testing sediment biological effects with the freshwater amphipod Hyalella azteca: the gap between laboratory and nature. Chemosphere 57: 1713-1734.
[6] American Society for Testing and Materials. 1991. Guide for conduct- ing 10-day static sediment toxicity test with marine and estuarine amphipods. Pp. 1052- 1075, In: ASTM Standard Methods Series Vol. 11.04. E1367-90. American Society for Testing and Materials, Philadelphia.
[7] Stephensen K. (1938a). Grandidierella japonica n. sp., a new amphipod with stridulating (?) organs from brackish water in Japan. Annotationes Zoologicae Japonenses, 17(2), pp. 179-184.

mud, mud-sand, mud-brick fragments, mud-shell sediments (Chapman and Dorman, 1975[8]). The amphipod feeds on algae and organic detritus present in the sediment (Adams and Stauber, 2008[9]).

Based on ecological observations and habits (feeding and residence) both amphipods can be classed as Benthic. Therefore, contact to contaminants will occur in the sediment phase. The most appropriate test dosing strategy is spiking of the sediment, further the most relevant dosing metric to assess exposure and toxicity levels is to express dose as a function of the mass of sediment (i.e. mg contaminant/kg sediment), not water column for which they do not reside or feed in.

Table 8.2-5: Summary of acute endpoints for aquatic invertebrates for metabolites of cyprodinil

Organism	Test item	Endpoints (mg a.s./L)		Endpoints used for the risk assessment	Reference (author, date, Syngenta File No.)
Daphnia magna	CGA249287	EU	48 h EC_{50} >$100_{(nom)}$ (static test)	48 h EC_{50} >$100_{(nom)}$	***Maetzler (1999) CGA249287/0008***
	CGA275535		48 h EC_{50} = $6.8_{(nom)}$ (static test)	48 h EC_{50} = $6.8_{(nom)}$	***Maetzler (2001) CGA275535/0016***
	CGA321915	New Study	48 h EC_{50} > $98_{(mm)}$ (static test)	48 h EC_{50} > $98_{(mm)}$	***Eckenstein (2015) CGA321915_10005***
	CGA263208 (phenyl guanidine)	Not previously submitted	48 h EC_{50} = $20.6_{(nom)}$ (static test)	48 h EC_{50} = $15.7_{(nom)}$	***Vial (1991) CA1059/0010***
	CA1139A (carbonate salt of phenyl guanidine)		48 h EC_{50} = $15.7_{(nom)}$ (static test)		***Grade (1992) CA1139/0009***
Chironomus riparius	CGA321915	New Study	48 h EC_{50} > $97_{(mm)}$ (static test)	48 h EC_{50} > $97_{(mm)}$	***Tobler (2015) CGA321915_10009***

nom = Endpoint derived using nominal concentration
mm = Endpoint derived using mean measured concentration
~~LIT = endpoint derived from a literature search~~

[8] Chapman J.W., and Dorman J.A. 1975. Diagnosis, systematics, and notes on Grandidierella japonica (Amphipoda: Gammaridea) and its introduction to the Pacific coast of the United States. Bulletin of the Southern California, Academy of Science 74:104-109.

[9] Adams M.S. and Stauber J.L. 2008. Marine whole sediment toxicity tests for use in the temperate and tropical Australian environments: current status. Australian Journal of Ecotoxicology, 14, pp. 155-167.

Table 8.2-6: Summary of chronic endpoints for aquatic invertebrates for cyprodinil

Organism	Test item	Endpoints (mg a.s./L)		Endpoints used for the risk assessment	Reference (author, date, Syngenta File No.)
Daphnia magna	Cyprodinil	EU	21 d NOEC $_{(repro)}$ = 0.0082$_{(mm)}$	-	***Ward et al. (1995) CGA219417/0543 EC_{10}/EC_{20} estimate Taylor & Sanchez (2015) CGA219417_11594***
		EC_{10}/EC_{20} [a]	$EC_{10\ (repro)}$ = 0.00849 [b] $EC_{20\ (repro)}$ = 0.00942 [b] (flow-through test)	-	
		EU	Group A 21 day NOEC $_{(adult\ survival)}$ = 0.0018$_{(im)}$ Group A 21 day $EC_{10\ (repro)}$ = 0.0073$_{(im)}$ Group B 21 day NOEC $_{(repro)}$ = 0.0088$_{(im)}$ (semi-static test)	-	***Rufli (1998) CGA219417/0876 EC_{10}/EC_{20} estimate Taylor & Majdanik (2015) CGA219417_11588***
		EC_{10}/EC_{20} [a]	Group B 14 day EC_{10} = 12.06 Group B 14 day EC_{20} = 16.50 No further ECx values could be reliably determined	-	
M*ysidopsis bahia*		EU	30 day NOEC = 0.0019$_{(mm)}$ (flow-through test)	-	***Drottar & Kreuger (1999) CGA219417/0926 EC_{10}/EC_{20} estimate: Taylor & Sanchez (2015) CGA219417_11596***
		EC_{10}/EC_{20} [a]	$EC_{10\ (adult\ dry\ weight)}$ = 0.00197 [b] $EC_{20\ (repro)}$ = 0.00248 [b]	$EC_{10\ (adult\ dry\ weight)}$ = 0.00197	
Chironomus riparius		EU	27 d NOEC = 80 mg/kg sediment$_{(nom)}$ (static test)	27 d NOEC = 80 mg/kg sediment$_{(nom)}$ (static test)	***Grade (2000) CGA219417/1003 EC_{10}/EC_{20} estimate Taylor & Sanchez (2015) CGA219417_11587***
		EC_{10}/EC_{20}[a]	Could not be estimated	-	
	CGA249287	EU	28 d NOEC = 25.6 mg/kg sediment$_{(nom)}$ (static test)	28 d NOEC = 25.6 mg/kg sediment$_{(nom)}$	***Grade (2001) CGA249217/0024 EC_{10}/EC_{20} estimate Taylor & Pickering (2015) CGA219417_10007***
		EC_{10} and EC_{20}[a]	Could not be estimated	-	

[a] Values estimated in accordance with **Commission Regulation (EU) No 283/2013**
[b] Represents the worst case endpoint
im = Endpoint derived using initial measured concentration
mm = Endpoint derived using mean measured concentration
nom = Endpoint derived using nominal concentration

Table 8.2-7: Summary of endpoints for algae for cyprodinil

Organism	Endpoints (mg a.s./L)		Endpoint used for the risk assessment	Reference (author, date, Syngenta File No.)
Pseudokirkneriella subcapitata	EU	72 h E_rC_{50} = 5.2$_{(nom)}$ 72 h E_bC_{50} = 2.6$_{(nom)}$ 72 h E_rC_{10} = 1.7$_{(nom)}$ 72 h E_bC_{10} = 0.6$_{(nom)}$	-	***Maetzler (2001) CGA219417/1030 EC_{10}/EC_{20} estimate Taylor and Allen (2016) CGA219417_11598***
	EC_{10}/EC_{20} [a]	72 h E_rC_{20} = 4.66 72 h E_yC_{20} = 1.73	-	
Pseudokirkneriella subcapitata	EU	72 h EC_{50} = 2.6$_{(im)}$ 96 h EC_{50} = 2.82$_{(im)}$ 120 h EC_{50} = 2.89$_{(im)}$ 72 h E_rC_{50} = 3.28$_{(mm)}$ 72 h E_yC_{50} = 1.57$_{(mm)}$ 96 h E_rC_{50} = 4.06$_{(mm)}$ 96 h E_yC_{50} = 2.26$_{(mm)}$ 120 h E_yC_{50} = 2.08$_{(mm)}$	72 h E_rC_{50} = 3.28	***Ward et al. (1995) CGA219417/0648 EC_{10}/EC_{20} estimate Taylor et al. ~~and Pickering~~ (2016~~5~~a) CGA219417_11593***
	EC_{10}/EC_{20} [a]	72 h E_rC_{10} = 2.26 72 h E_rC_{20} = 2.70 72 h E_yC_{10} = 0.772 72 h E_yC_{20} = 1.10 96 h E_rC_{10} = 2.74 96 h E_rC_{20} = 3.22 96 h E_yC_{10} = 1.93 96 h E_yC_{20} = 2.20 120 h E_rC_{10} = 3.13 120 h E_rC_{20} = 3.65 120 h E_yC_{10} = 1.36 120 h E_yC_{20} = 1.72 72 h E_rC_{20} = 2.12$_{(mm)}$ 72 h E_rC_{10} = 1.68$_{(mm)}$ 72 h E_yC_{20} = 0.67$_{(mm)}$ 72 h E_yC_{10} = 0.43$_{(mm)}$ 96 h E_rC_{20} = 2.82$_{(mm)}$ 96 h E_rC_{10} = 2.34$_{(mm)}$ 96 h E_yC_{20} = 1.61$_{(mm)}$ 96 h E_yC_{10} = 1.35$_{(mm)}$ 120 h E_yC_{20} = 1.16$_{(mm)}$ 120 h E_yC_{10} = 0.86$_{(mm)}$	-	
Anabaena flos-aquae	EU	72 h EC_{50} = 3.76$_{(mm)}$ 96 h EC_{50} = 2.66$_{(mm)}$ 120 h EC_{50} = 2.25$_{(mm)}$ 96 h E_rC_{50} = 7.55$_{(mm)}$ 120 h E_rC_{50} = 7.64$_{(mm)}$	-	***Ward et al (1995) CGA219417/0647 EC_{10}/EC_{20} estimate Taylor et al. ~~and Blakey~~ (2016~~5~~) CGA219417_11599***

Organism	Endpoints (mg a.s./L)		Endpoint used for the risk assessment	Reference (author, date, Syngenta File No.)
	EC_{10}/EC_{20} [a]	72 h E_yC_{10} = 2.25 72 h E_yC_{20} = 2.69 96 h E_rC_{10} = 3.67 96 h E_rC_{20} = 4.70 96 h E_yC_{10} = 0.413 96 h E_yC_{20} = 0.740 120 h E_rC_{10} = 3.19 120 h E_rC_{20} = 4.31 120 h E_yC_{10} = 0.706 120 h E_yC_{20} = 1.04	-	
Navicula pelliculosa	EU	72 h EC_{50} = 2.11$_{(mm)}$ 96 h EC_{50} = 2.22$_{(mm)}$ 120 h EC_{50} = 2.84$_{(mm)}$ 72 h E_rC_{50} = 3.24	-	***Ward et al. (1995) CGA219417/0646*** ***EC_{10}/EC_{20} estimate Taylor and Radford ~~Pickering~~ (2016~~a~~~~5b~~) CGA219417_11592***
	EC_{10}/EC_{20} [a]	72 h E_rC_{10} = 2.21 72 h E_rC_{20} = 2.52 72 h E_yC_{10} = 0.620 72 h E_yC_{20} = 0.896 96 h E_yC_{10} = 0.928 96 h E_yC_{20} = 1.21	-	
Skeletonema costatum	Not previously submitted	72 h EC_{50} = 1.78$_{(im)}$ 96 h EC_{50} = 1.75$_{(im)}$ 120 h EC_{50} = 1.97$_{(im)}$ 72 h E_yC_{50} 1.10$_{(mm)}$ 96 h E_yC_{50} = 1.14$_{(mm)}$ 120 h E_yC_{50} = 1.33$_{(mm)}$	~~72 h EC_{50} = 1.78$_{(im)}$~~	***Ward et al. (1995) CGA219417/0644*** ***EC_{10}/EC_{20} estimate Taylor et al. ~~and Radford~~ (2016~~5~~) CGA219417_11650***
	EC_{10}/EC_{20} [a]	72 h E_rC_{10} = 1.38 72 h E_rC_{20} = 1.81 72 h E_yC_{10} = 0.890 72 h E_yC_{20} = 1.09 96 h E_rC_{10} = 1.48 96 h E_rC_{20} = 1.87 96 h E_yC_{10} = 0.997 96 h E_yC_{20} = 1.19 120 h E_rC_{10} = 1.65 120 h E_rC_{20} = 2.08 120 h E_yC_{10} = 1.18 120 h E_yC_{20} = 1.32 72 h E_yC_{10} = 0.441$_{(mm)}$ 72 h E_yC_{20} = 0.604$_{(mm)}$ 96 h E_yC_{10} = 0.517$_{(mm)}$ 96 h E_yC_{20} = 0.679$_{(mm)}$ 120 h E_yC_{10} = 0.632$_{(mm)}$ 120 h E_yC_{20} = 0.817$_{(mm)}$	-	

[a] Values estimated in accordance with **Commission Regulation (EU) No 283/2013**
nom = Endpoint derived using nominal concentration
im = Endpoint derived using initial measured concentration
mm = Endpoint derived using mean measured concentration

Table 8.2-8: Summary of endpoints for algae for metabolites

Organism	Test item	Endpoints (mg a.s./L)		Endpoint used for the risk assessment	Reference (author, date, Syngenta File No.)
Psudokirchneriella subcapitata	CGA249287	EU	72h $E_{rb}C_{50}$ >100$_{(nom)}$	72h $E_{rb}C_{50}$ >100$_{(nom)}$	***Maetzler (1999) CGA249287/0006*** ***EC_{10}/EC_{20} estimate*** ***Taylor & Allen (2015) CGA249287_10009***
		EC_{10} and EC_{20}[a]	Could not be estimated		
	CGA275535	EU	72h E_rC_{50} = 18$_{(mm)}$ 72h E_rC_{10} = 6.1$_{(mm)}$ 72h E_bC_{50} = 9.4$_{(mm)}$ 72h E_bC_{10} = 3.9$_{(mm)}$	72h E_rC_{50} = 18$_{(mm)}$	***Maetzler (2001) CGA275535/0015*** ***EC_{10}/EC_{20} estimate*** ***Taylor & Allen (2015) CGA275535_10006***
		EC_{10}/EC_{20}[a]	72 h E_rC_{20} = 10.3 72 h E_yC_{20} = 6.34		
	CGA321915	New study	72 h $EC_{10, 20, 50}$ > 99 96 h $EC_{10, 20, 50}$ > 99	96 h EC_{50} > 99	***Eckenstein (2015) CGA321915_10004***
Desmodesmus subspicatus	CGA263208 (phenyl guanidine)	Not previously submitted	72h E_bC_{50} = 1.86$_{(nom)}$ 72 h E_rC_{50} 5.73$_{(mm)}$ 72 h E_yC_{50} 1.80$_{(mm)}$	~~72h E_bC_{50} = 1.86$_{(nom)}$~~ 72 h E_rC_{50} 4.31$_{(mm)}$	***Vial (1991) CA1059/0012*** ***EC_{10}/EC_{20} estimate*** ***Taylor & Radford (2016) CGA263208_10017***
		EC_{10}/EC_{20}[a]	72 h E_rC_{20} = 1.58$_{(mm)}$ 72 h E_rC_{10} = 0.807$_{(mm)}$ 72 h E_yC_{20} = 0.939$_{(mm)}$ 72 h E_yC_{10} = 0.668$_{(mm)}$		
	CA1139A (carbonate salt of phenyl guanidine)	Not previously submitted	72h E_bC_{50} = 3.80$_{(nom)}$ 72 h E_rC_{50} 4.31$_{(mm)}$ 72 h E_yC_{50} 1.79$_{(mm)}$		***Rufli (1992) CA1139/0010*** ***EC_{10}/EC_{20} estimate*** ***Taylor & Pickering(2016) CA1139_10007***
		EC_{10}/EC_{20}[a]	72 h E_rC_{20} = 1.51$_{(mm)}$ 72 h E_rC_{10} = 0.871$_{(mm)}$ 72 h E_yC_{20} = 1.06$_{(mm)}$ 72 h E_yC_{10} = 0.806$_{(mm)}$		

[a] Values estimated in accordance with **Commission Regulation (EU) No 283/2013**
nom = Endpoint derived using nominal concentration

Table 8.2-9: Summary of endpoints for aquatic macrophytes for cyprodinil

Organism	Endpoints (mg a.s./L)		Endpoint used for the risk assessment	Reference (author, date, Syngenta File No.)
Lemna gibba	EU	14 d EC_{50} = 7.71$_{(im)}$ 14 d E_yC_{50} = 7.42$_{(im)}$	~~14 d EC_{50} = 7.71$_{(im)}$~~ 14 d E_yC_{50} = 7.42$_{(im)}$	***Ward et al. (1995) CGA219417/0645*** ***EC_{10}/EC_{20} estimate*** ***Taylor & Pickering (~~2015~~2016) CGA291417_11589***
	EC_{10}/EC_{20}[a]	14 d EC_{10} = 4.88 14 d EC_{20} = 5.64	-	

[a] Values estimated in accordance with **Commission Regulation (EU) No 283/2013**

Table 8.2-10: Summary of Endpoints from Mesocosm Studies

Organism	Test item	Endpoints (mg a.s./L)		Endpoint used for the risk assessment	Reference (author, date, Syngenta File No.)
Aquatic Ecosystem	Cyprodinil	EU	14 weeks EAC = 0.013	-	***Kennedy & Reed (1995) CGA219417/0636***
	Cyprodinil [a]	New	NOEC = 0.0146; NOEAEC (ERO-RAC, class 3A)	NOEC = 0.0146	***Ashwell et al. (2007) CGA219417/1683*** ***Re-analysis performed by Taylor and Dark (2015) A14325E_10079***

[a] tested as A14325E

CA 8.2.1 Acute toxicity to fish

The studies previously submitted in the EU were summarised in point B.9.2.1 of the DAR. At the request of ANSES summaries have been included in order to aid dossier evaluation. ~~therefore additional study summaries are not repeated here.~~ The endpoints are summarised in Tables 8.2-1 and 8.2-2.

LC_{10} and LC_{20} values have not been calculated since they are not required by the recommended test guidelines set out in Commission Communication 2013/C 95/01 (OECD Test Guideline 203: Fish, Acute Toxicity Test), or by the acute risk assessments according to the current Aquatic Guidance Document (2009)[10].

Report:	Summary submitted at the request of ANSES for ease of dossier evaluation:
	KIIA 8.2.1. Boeri R. L., Magazu J. P. & Ward T. J. (1995). Acute Toxicity of CGA-219417 to the Rainbow Trout, Oncorhyncus mykiss. Report Number 543-CG. T. R. Wilbury Laboraotries, Inc., Massachusettes 01945, USA. (Syngenta File No. CGA219417/0486).

Guidelines

U.S. EPA FIFRA – Guideline No. 72-1.

GLP: Yes

Executive Summary

The toxicity of CGA219417 to the rainbow trout, *Oncorhyncus mykiss*, was determined in a 96-hour acute toxicity test performed under static conditions. Rainbow trout were exposed to nominal CGA219417 concentrations of 0.342, 0.576, 0.972, 1.62 and 2.70 mg/L alongside a dilution medium control (mean measured concentrations 0.280, 0.511, 0.860, 1.48 and 2.72 mg/L). Based on mean measured concentrations, the 96-hour LC_{50} for CGA219417 to *Oncorhyncus mykiss* was 2.41 mg/L with 95% confidence intervals of 1.96 to >2.72 mg/L. The 96-hour no observed effect concentration (NOEC) was determined to be 0.511 mg/L.

Materials

[10] EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2013. Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 2013;11(7):3290, 268 pp. doi:10.2903/j.efsa.2013.3290.

Test Material	CGA219417
Lot/Batch #:	P.012012
Purity:	99.2 %
Description:	Off-white granules
Stability of test compound:	Not reported
Reanalysis/expiry date:	7 April 1995
Treatments	
Test concentrations:	Dilution medium control and nominal concentrations of 0.342, 0.576, 0.972, 1.62 and 2.70 mg/L (measured concentrations 0.280, 0.511, 0.860, 1.48 and 2.72 mg/L)
Solvent:	None
Positive control:	None
Analysis of test concentrations:	Yes, HPLC analysis of CGA219417 at 0 and 96 hours
Test organism	
Species:	*Oncorhyncus mykiss*
Source:	Mount Lassen Trout Farms, Inc., Red Bluff, California
Acclimation period:	56 days
Acclimation medium:	Carbon filtered, deionised water
Feeding:	None during the exposure
Weight and length of dilution water control fish at end of exposure period:	Total length: 38 mm Mean weight: 0.50 g
Test design	
Test vessels:	40 L glass aquaria, containing 30 L test solution
Test medium:	Carbon filtered, deionised water
Replication:	Two replicates for each treatment and control (10 organisms per replicate)
Exposure regime:	Static
Aeration:	No
Duration:	96 hours
Environmental conditions	
Test temperature:	1157 to 12.9 °C
pH:	Test start: 7.8 Test end: 7.3
Dissolved oxygen:	7.1 to 10.2 mg/L
Conductivity:	170 µmhos/cm
Lighting:	16 hour light, 8 hour dark cycle with 15 minute transition period

Study Design and Methods

Experimental dates: 27th to 31st March 1995

A stock solution with a nominal concentration of 5 mg/L was prepared by combining 1.5121 g of the test item with test medium in a 570 L polyethylene tank and adjusting the final volume to 300 L. The stock solution was mixed for 68 hours and filtered through a 5 µm filter prior to use. The concentration of the stock was verified *via* chemical analysis. Appropriate volumes of the stock solution were added directly to dilution medium to prepare the nominal test concentrations.

Twenty rainbow trout were equally distributed among two replicates for each treatment and the control. The test vessels were randomly distributed in a water bath to maintain a temperature of 12 ± 1 °C during the 96-hour exposure. The number of surviving test organisms and the occurrence of sublethal effects was recorded after 24, 48, 72 and 96 hours.

The actual concentrations of the test item were determined at the start and end of the 96-hour exposure period using high performance liquid chromatography (HPLC) with UV detection.

At the start of the test the dissolved oxygen, pH, conductivity and temperature were measured daily during the exposure. The temperature in one test vessel was recorded at least every six hours during the test.

The 24, 48 and 72-hour LC_{50} values could not be calculated due to low toxicity at the highest test concentration. The 96-hour LC_{50} value was estimated using the Probit method.

Results and Discussion

Insoluble test material was observed in the 5 mg/L stock solution and results of chemical analysed indicated the actual concentration with the stock to be 2.7 mg/L. The mean measured concentrations of CGA219417 during the exposure were 0.280, 0.511, 0.860, 1.48 and 2.72 mg/L. The study results were based on the mean measured concentrations.

Table 8.2.1-1: Analytical results

Nominal concentrations (mg/L)	Measured concentration 0-hour (mg/L)	Measured concentration 96-hour (mg/L)	Mean Measured (mg/L)
Control	<LOD	<LOD	-
0.342	0.358	0.201	0.280
0.576	0.618	0.404	0.511
0.972	0.968	0.752	0.860
1.62	1.73	1.22	1.48
2.70	2.75	2.68	2.72

LOD: 0.10 mg/L

Water quality throughout the test was within acceptable limits. During the exposure period the pH ranged from 7.2 to 7.8, the dissolved oxygen ranged from 7.1 to 10.2 mg/L and temperature ranged from 11.5 to 12.9 °C.

Control survival was 100% after 96 hours and no sublethal effects were observed in this treatment group. Mortality of 25% and 5 % was observed in the 1.48 and 2.72 mg/L treatment groups. All other treatments resulted in 0% mortality after 96 hours exposure. Sublethal effects (change in colour) were observed in fish exposed to the 0.860 mg/L treatment after 24 hours. Several fish exposed to this concentration exhibited a change in colouration, loss of equilibrium and erratic swimming after 48, 72 and 96 hours

exposure. All surviving fish in the 1.48 and 2.72 mg/L treatments exhibited a change in colour, loss of equilibrium and erratic swimming after 24 hours exposure.

Table 8.2.1-2: Effects of CGA219417 on survival of *Oncorhyncus mykiss* after 24, 48, 72 and 96 hours exposure

Nominal concentration (mg/L)	Mean measured concentration (mg/L)	Mortality observed (Cumulative number of dead fish) (n = 20)			
		24 hours	48 hours	72 hours	96 hours
Control	-	0	0	0	0
0.342	0.280	0	0	0	0
0.576	0.511	0	0	0	0
0.972	0.860	0	0	0	0
1.62	1.48	0	1	2	5
2.70	2.72	0	3	8	11
LC_{50} mg/L		>2.72	>2.72	>2.72	2.41
95% confidence interval		-	-	-	1.96 – >2.72

Validity Criteria

Not reported.

Conclusions

Exposure of rainbow trout to CGA219417 resulted in a 96-hour LC_{50} of 2.41 mg/L with 95% confidence intervals of 1.96 to >2.72 mg/L. The no observed effect concentration (NOEC) was determined to be 0.511 mg/L.

(Boeri *et al*., 1995)

Report:	Summary submitted at the request of ANSES for ease of dossier evaluation: *KIIA 8.2.1. Boeri R. L., Magazu J. P. & Ward T. J. (1995a). Acute Toxicity of CGA-219417 to Bluegill Sunfish, Lepomis macrochirus. Report Number 542-CG. T. R. Wilbury Laboraotries, Inc., Massachusettes 01945, USA. (Syngenta File No. CGA219417/0507).*

Guidelines

U.S. EPA FIFRA – Guideline No. 72-1.

GLP: Yes

Executive Summary

The toxicity of CGA219417 to the bluegill sunfish, *Lepomis macrochirus*, was determined in a 96-hour acute toxicity test performed under static conditions. Bluegill sunfish were exposed to nominal CGA219417 concentrations of 0.342, 0.576, 0.972, 1.62 and 2.70 mg/L alongside a dilution medium control (mean measured concentrations 0.285, 0.445, 0.922, 1.35 and 2.55 mg/L). Based on mean measured concentrations, the 96-hour LC_{50} for CGA219417 to *Lepomis macrochirus* was 2.17 mg/L with

95% confidence intervals of 1.35 to 2.55 mg/L. The 96-hour no observed effect concentration (NOEC) was determined to be 0.445 mg/L.

Materials

Test Material	CGA219417
Lot/Batch #:	P.012012
Purity:	99.2 %
Description:	Off-white granules
Stability of test compound:	Not reported
Reanalysis/expiry date:	7 April 1995
Treatments	
Test concentrations:	Dilution medium control and nominal concentrations of 0.342, 0.576, 0.972, 1.62 and 2.70 mg/L (measured concentrations 0.285, 0.445, 0.922, 1.35 and 2.55 mg/L)
Solvent:	None
Positive control:	None
Analysis of test concentrations:	Yes, HPLC analysis of CGA219417 at 0 and 96 hours
Test organism	
Species:	*Lepomis macrochirus*
Source:	Northeastern Biologists, Rhinebeck, New York
Acclimation period:	69 days
Acclimation medium:	Carbon filtered, deionised water
Feeding:	None during the exposure
Weight and length of dilution water control fish at end of exposure period:	Total length: 27 mm Mean weight: 0.19 g
Test design	
Test vessels:	20 L glass aquaria, containing 15 L test solution
Test medium:	Carbon filtered, deionised water
Replication:	Two replicates for each treatment and control (10 organisms per replicate)
Exposure regime:	Static
Aeration:	No
Duration:	96 hours
Environmental conditions	
Test temperature:	21.3 to 21.6 °C
pH:	Test start: 7.5 to 7.7 Test end: 7.3
Dissolved oxygen:	7.7 to 9.0 mg/L
Conductivity:	160 to 180 µmhos/cm

Lighting: 16 hour light, 8 hour dark cycle with 15 minute transition period

Study Design and Methods

Experimental dates: 27th to 31st March 1995

A stock solution with a nominal concentration of 5 mg/L was prepared by combining 1.5121 g of the test item with test medium in a 570 L polyethylene tank and adjusting the final volume to 300 L. The stock solution was mixed for 68 hours and filtered through a 5 µm filter prior to use. The concentration of the stock was verified via chemical analysis. Appropriate volumes of the stock solution were added directly to dilution medium to prepare the nominal test concentrations.

Twenty bluegill sunfish were equally distributed among two replicates for each treatment and the control. The test vessels were randomly distributed in a water bath to maintain a temperature of 22 ± 1°C during the hour exposure. The number of surviving test organisms and the occurrence of sublethal effects was recorded after 24, 48, 72 and 96 hours.

The actual concentrations of the test item were determined at the start and end of the 96-hour exposure period using high performance liquid chromatography (HPLC) with UV detection.

At the start of the test the dissolved oxygen, pH, conductivity and temperature were measured daily during the exposure. The temperature in one test vessel was recorded at least every six hours during the test.

The 24, 48 and 72-hour LC_{50} values could not be calculated due to low toxicity at the highest test concentration. The 96-hour LC_{50} value was estimated using binominal/non-linear regression.

Results and Discussion

Insoluble test material was observed in the 5 mg/L stock solution and results of chemical analysed indicated the actual concentration with the stock to be 2.7 mg/L. The mean measured concentrations of CGA219417 during the exposure were 0.285, 0.445, 0.922, 1.35 and 2.55 mg/L. The study results are based on the mean measured concentrations.

Table 8.2.1-3: Analytical results

Nominal concentrations (mg/L)	Measured concentration 0-hour (mg/L)	Measured concentration 96-hour (mg/L)	Mean Measured (mg/L)
Control	<0.100	0.146 (<0.100)[a]	<0.115
0.342	0.311	0.258	0.285
0.576	0.458	0.431	0.445
0.972	1.03	0.813	0.922
1.62	1.64	1.06	1.35
2.70	2.85	2.24	2.55

[a] The sample collected from replicated 2 resulted in a measured value of slightly above the detection limit (0.1 mg/L). The replicate 1 samples was then analyzed resulting in a value of <0.100 mg/L.

Water quality throughout the test was within acceptable limits. During the exposure period the pH ranged from 7.3 to 7.8, the dissolved oxygen ranged from 7.8 to 9.0 mg/L and temperature ranged from 21.3 to 21.6°C.

Control mortality of 5% was observed after 96 hours. Mortality of 65% was observed in the highest test concentration. All other test concentrations resulted in ≤10% mortality after the 96-hour exposure. Sublethal effects (erratic swimming, lethargy and loss of equilibrium) were observed in one fish in the 0.922 mg/L treatment group after 24 hours, and by all surviving fish exposed to 1.35 and 2.55 mg/L after 24 hours.

Table 8.2.1-4: Effects of CGA219417 on survival of *Lepomis macrochirus* after 24, 48, 72 and 96 hours exposure

Nominal concentration (mg/L)	Mean measured concentration (mg/L)	Mortality observed (Cumulative number of dead fish) (n = 20)			
		24 hours	48 hours	72 hours	96 hours
Control	<0.115	1	1	1	1
0.342	0.285	0	0	0	1
0.576	0.445	0	0	0	0
0.972	0.922	0	0	0	0
1.62	1.35	0	0	0	2
2.70	2.55	0	6	9	13
LC_{50} mg/L		>2.55	>2.55	>2.55	2.17
95% confidence interval		-	-	-	1.35 – 2.55

Validity Criteria

Not reported.

Conclusions

Exposure of bluegill sunfish to CGA219417 resulted in a 96-hour LC_{50} of 2.17 mg/L with 95% confidence intervals of 1.35 to 2.55 mg/L.

(Boeri *et al*., 1995a)

Report:	Summary submitted at the request of ANSES for ease of dossier evaluation: *KIIA 8.2.1. Ward T. J., Magazu J. P & Boeri R. L. (1995). Acute Flow-Through Toxicity of CGA-219417 to Bluegill Sunfish, Lepomis macrochirus. Report Number 842-CG. T. R. Wilbury Laboratories, Inc., Massachusetts 01945, USA. (Syngenta File No. CGA219417/0651)*

Guidelines

U.S. EPA FIFRA – Guideline No. 72-1(a).

GLP: Yes

Executive Summary

The toxicity of CGA219417 to the bluegill sunfish, *Lepomis macrochirus*, was determined in a 96-hour acute toxicity test performed under flow-through conditions. Bluegill sunfish were exposed to nominal CGA219417 concentrations of 0.65, 1.1, 1.8, 3.0 and 5.0 mg/L alongside a dilution medium control and

solvent control (mean measured concentrations 0.631, 1.00, 1.64, 2.70 and 4.68 mg/L). Based on mean measured concentrations, the 96-hour LC_{50} for CGA219417 to *Lepomis macrochirus* was 3.20 mg/L with 95 % confidence intervals of 2.70 to 4.68 mg/L. The 96-hour no observed effect concentration (NOEC) was determined to be 0.631 mg/L.

Materials

Test Material	CGA219417
Lot/Batch #:	P.012012
Purity:	99.2 %
Description:	Off-white granules
Stability of test compound:	At least 1 year at room temperature
Reanalysis/expiry date:	7 April 1995
Treatments	
Test concentrations:	Dilution medium control, solvent control and nominal concentrations of 0.65, 1.1, 1.8, 3.0 and 5.0 mg/L (measured concentrations 0.631, 1.00, 1.64, 2.70 and 4.68 mg/L)
Solvent:	Dimethylformamide (100 µL/L)
Positive control:	None
Analysis of test concentrations:	Yes, HPLC analysis of CGA219417 at 0 and 96 hours
Test organism	
Species:	*Lepomis macrochirus*
Source:	Northeastern Biologists, Rhinebeck, New York
Acclimation period:	15 days
Acclimation medium:	Carbon filtered, deionised water
Feeding:	None during the exposure
Weight and length of dilution water control fish at end of exposure period:	Total length: 27 mm Mean weight: 0.21 g
Loading rate	0.14 g/L
Test design	
Test vessels:	20 L glass aquaria, containing 15 L test solution
Test medium:	Carbon filtered, deionised water
Replication:	Two replicates for each treatment and control (10 organisms per replicate)
Exposure regime:	Flow-through
Aeration:	No
Duration:	96 hours
Environmental conditions	
Test temperature:	21.1 to 22.9 °C
pH:	Test start: 7.5 to 7.8

	Test end: 7.5 to 7.6
Dissolved oxygen:	6.6 to 9.2 mg/L
Conductivity:	150 to 200 µmhos/cm
Lighting:	25 footcandles; 16 hour light, 8 hour dark cycle with 15 minute transition period

Study Design and Methods

Experimental dates: 30th November to 4th December 1995

The apparatus used in this test was a continuous flow-through system constructed of glass, stainless steel and Teflon-coated materials.

A solvent stock solution with a nominal concentration of 50 mg/mL was prepared by placing a weighed aliquot of 25.2 g of the test item into a 500 mL volumetric flask and making up to volume with dimethylformamide. Stock solution was added directly to dilution water by a calibrated proportional dilutor (300 µL of solvent stock solution was combined with 3 L of dilution water during each dilutor cycle). During the test the dilutor was activated 729 times, resulting in an average of 6.1 volume additions per 24-hours in each test vessel.

Twenty bluegill sunfish were equally distributed among two replicates for each treatment and the control and solvent control. The test vessels were randomly distributed in a water bath to maintain a temperature of 22 ± 1 °C during the 96-hour exposure. The number of surviving test organisms and the occurrence of sublethal effects was recorded after 0, 24, 48, 72 and 96 hours.

The actual concentrations of the test item were determined at the start and end of the 96-hour exposure period using high performance liquid chromatography (HPLC) with UV detection.

Dissolved oxygen, pH, conductivity and temperature were measured daily during the exposure. The temperature in one test vessel was recorded continuously during the test.

The 24, 48, 72 and 96-hour LC_{50} values was estimated using a binominal method.

Results and Discussion

Insoluble test material was not observed in any test vessel during the test. The mean measured concentrations of CGA219417 during the exposure were 0.631, 1.00, 1.64, 2.70 and 4.68 mg/L and were 90 – 97% of nominal concentrations. The study results are based on the mean measured concentrations.

Table 8.2.1-5: Analytical results

Nominal concentrations (mg/L)	Measured concentration 0-hour (mg/L)	Measured concentration 96-hour (mg/L)	Mean Measured (mg/L)
Control	<LOD	<LOD	<LOD
Solvent Control	<LOD	<LOD	<LOD
0.65	0.602	0.659	0.631
1.1	0.949	1.05	1.00
1.8	1.62	1.65	1.64
3.0	2.68	2.71	2.70
5.0	4.72	4.64	4.68

Water quality throughout the test was within acceptable limits. During the exposure period the pH ranged from 7.5 to 8.1, the dissolved oxygen ranged from 6.6 to 9.2 mg/L and temperature ranged from 21.1 to 22.9°C.

No mortality was observed in either the control or solvent control after 96 hours. Mortality of 100 and 20% was observed in the 2.70 and 4.68 mg/L measured test concentrations, respectively. For all other test concentrations no mortality was observed after the 96-hour exposure. Sublethal effects (erratic swimming, loss of equilibrium and lying at the bottom of the tank) were observed in one fish in the 1.00 mg/L treatment group, and by all surviving fish exposed to 1.64, 2.70 and 4.68 mg/L.

Table 8.2.1-6: Effects of CGA219417 on survival of *Lepomis macrochirus* after 24, 48, 72 and 96 hours exposure

Nominal concentration (mg/L)	Mean measured concentration (mg/L)	Mortality observed (Cumulative number of dead fish) (n = 20)			
		24 hours	48 hours	72 hours	96 hours
Control	<LOD	0	0	0	0
Solvent Control	<LOD	0	0	0	0
0.65	0.631	0	0	0	0
1.1	1.00	0	0	0	0
1.8	1.64	0	0	0	0
3.0	2.70	0	0	0	4
5.0	4.68	14	20	20	20
LC_{50} mg/L		4.14	3.55	3.55	3.20
95% confidence interval		2.70 to >4.68 mg/L	2.70 to 4.68 mg/L	2.70 to 4.68 mg/L	2.70 to 4.68 mg/L

Validity Criteria

Not reported.

Conclusions

Exposure of bluegill sunfish to CGA219417 in a flow-through system resulted in a 96-hour LC_{50} of 3.20 mg/L with 95 % confidence intervals of 2.70 to 4.68 mg/L.

(Ward *et al*., 1995)

Summaries of studies conducted following the original submission of cyprodinil are presented below.

Report:	Submitted for purposes of renewal as conducted on cyprodinil for compliance in Japan: K-CA 8.2.1/01 Liedtke A. (2011) Cyprodinil (CGA219417) - Acute toxicity to carp (*Cyprinus carpio*) in a 96-hour test. Report Number D08015. Harlan Laboratories Ltd., 4452 Itingen, Switzerland. (Syngenta File No. CGA219417_11450)

Guidelines

OECD Guidelines for Testing of Chemicals, Section 2 - Effects on Biotic Systems, Method 203: Fish, Acute Toxicity Test (1992)

Official Journal of the European Communities, Dir 92/69/EEC, O.J. L383A, Part C.1: Acute Toxicity For Fish (1992)

US EPA Ecological Effects Test Guidelines, OPPTS 850.1075: Fish Acute Toxicity Test, Freshwater and Marine (1996)

JMAFF Agchem Test Guidelines 12 Nohsan No. 8147, Effects on Aquatic Organisms, 2-7-1: Acute fish toxicity (2001)

GLP: Yes

Executive Summary

The acute toxicity of cyprodinil (CGA219417) to common carp (*Cyprinus carpio*) was determined under semi-static conditions. Fish were exposed to a range of nominal concentrations of 0.10, 0.22, 0.46, 1.0, 2.2 and 4.6 mg CGA219417/L alongside a dilution water control and a solvent control. Based on geometric mean measured concentrations, the 96 hour LC_{50} was 3.0 mg CGA219417/L.

Materials

Test material	CGA219417
Lot/Batch #:	P.01011
% purity:	99.2%
Description:	Light beige crumbs
Stability of test compound:	Stable under test conditions
Reanalysis/expiry date:	30 April, 2014
Density:	n.a.
Treatments	
Test concentrations:	Dilution water control, solvent control and nominal concentrations of .10, 0.22, 0.46, 1.0, 2.2 and 4.6 mg CGA219417/L
Solvent:	None
Analysis of test concentrations:	Yes, fresh solutions at start of test, fresh and old solutions at each medium renewal, and old solutions at end of test, based on measurement of CGA219417 using HPLC analysis
Test organisms	
Species:	Common carp, *Cyprinus carpio*
Source:	Osage Catfisheries Inc., Osage Beach, Missouri, USA
Acclimatisation period:	One week
Treatment for disease:	None
Weight and length of dilution water control fish at end of exposure period:	Mean length: 4.5 cm (standard deviation 0.08 cm) Mean weight: 1.0 g (standard deviation 0.09 g)
Feeding:	None during test
Test design	
Test vessels:	Glass vessels containing 16 L water
Test medium:	Reconstituted water
Replication:	None
No of fish per tank:	7
Exposure regime:	Semi-static

Duration: 96 hours

Environmental conditions

Test temperature: 21 - 22° C
pH: 7.2 - 7.4
Dissolved oxygen: 8.4 - 8.9 mg/L, gentle aeration provided
Hardness of dilution water: 125 mg/L as $CaCO_3$
Lighting: 16 hours light (140 - 480 lux) and 8 hours dark with 30 minute dawn and dusk transition period

Study Design and Methods

Experimental dates: 22nd November to 18th December 2010

A stock solution with a nominal concentration of 46 mg CGA219417/L was prepared by dissolving 2300.6 mg of the test item in 50 mL of *N*,*N*-dimethylformamide (DMF). Appropriate volumes of the application solutions were prepared by diluting the stock solution with DMF. At test commencement and at each test medium renewal fresh test solutions were prepared by mixing appropriate volumes of the application solutions with 16 L of dilution water in each test vessel to give the test concentrations. All treatments contained 100 µL DMF/L, as did the solvent control. The negative control consisted of dilution water only.

At the start of the test seven fish were randomly allocated to each of the test concentrations and the controls. The test was conducted in a temperature controlled room. Observations for mortalities and symptoms of toxicity were made at 2.5, 24, 48, 72 and 96 hours.

Daily measurements of the test solutions were undertaken throughout the 96 hour period for pH, temperature and dissolved oxygen concentrations.

The test concentrations were verified by chemical analysis of CGA219417 in the fresh solutions at the start of the test, fresh and old solutions at each medium renewal, and the old solutions at the end of the test, based on measurement of CGA219417 using HPLC analysis. Samples for analysis were taken from the centre of the test solutions.

Results and Discussion

At the start of the test and the medium renewal periods, the concentrations of CGA219417 in the fresh media were in the range 97 to 108% of the nominal values. At the end of the test and medium renewal periods the old concentrations of CGA219417 in the old media were in the range 77 to 103% (see table below). The limit of quantification in this study was 0.113 mg CGA219417/L. Geometric mean measured concentrations were used for the calculation and reporting of results.

Table 8.2.1-17: Analytical results

Sampling day/sample age (day/h)	Nominal concentration of CGA219417 (mg/L)	Measured concentration of CGA219417 (mg/L)	Measured concentration as % of nominal (%)
0/0	Control	n.a.	n.a.
	0.46	0.486	106
	1	1.07	107
	2.2	2.36	107
	4.6	4.47	97

1/24	Control	n.a.	n.a.
	0.46	0.356	77
	1	0.873	87
	2.2	1.92	87
	4.6	4.22	92
1/0	Control	n.a.	n.a.
	4.6	4.65	101
2/24	Control	n.a.	n.a.
	4.6	4.73	103
3/0	Control	n.a.	n.a.
	0.46	0.498	108
	1	1.08	108
	2.2	2.27	103
4/24	Control	n.a.	n.a.
	0.46	0.361	79
	1	0.818	82
	2.2	1.72	78

The median lethal concentration (LC_{50}) was defined as the concentration resulting in 50% mortality of the fish in the time period specified and was calculated as the geometric mean of the two consecutive test concentrations with 0 and 100% mortality, and the 95% confidence limits as the test concentrations with 0 and 100% mortality. The NOEC (No Observed Effect Concentration) is defined as the highest tested concentration which did not produce an adverse effect when compared to the control and was determined by visual inspection of the data. Mortalities were observed at the highest geometric mean measured concentration tested (4.5 mg CGA219417/L.

Symptoms of toxicity observed at 2.1 mg/L and above during the study included strong ventilation, tumbling during swimming, apathy, fish mainly on the bottom of the vessel lying on their sides or backs. No mortality or symptoms of toxicity were observed in the negative control or solvent control. The mortality data and estimated LC_{50} values are shown in the table below:

Table 8.2.1-28: Effects of CGA219417 on the survival of carp

Nominal concentrations (mg CGA219417/L)	Mean measured concentrations (mg CGA219417/L)	Mortality observed (cumulative number of dead fish) (n = 7)				
		2.5 hour	24 hours	48 hours	72 hours	96 hours
Dilution water control	Dilution water control	0	0	0	0	0
Solvent control	Solvent control	0	0	0	0	0
0.10	n.a.	0	0	0	0	0
0.22	n.a.	0	0	0	0	0
0.46	0.42	0	0	0	0	0
1.0	0.95	0	0	0	0	0
2.2	2.1	0	0	0	0	0
4.6	4.6	0	4	7	7	7
LC_{50} (mg CGA219417/L)		>4.5	4.1	3.0	3.0	3.0
95% confidence interval		-	n.d.	2.1 – 4.5	2.1 – 4.5	2.1 – 4.5
NOEC		-	2.1	2.1	2.1	2.1

Conclusions

Based on the geometric mean measured concentrations of CGA219417, the 96-hour LC_{50} to rainbow trout (*Oncorhynchus mykiss*) was 3.0 mg CGA219417/L and the 96-hour NOEC was 2.1 mg CGA219417/L.

(Liedtke A, 2011)

Report:	Submitted for purposes of renewal as conducted on cyprodinil for USA compliance: KCA 8.2.1/02 Ward T.J., Magazu J.P. & Boeri R.L. (1995) Acute flow-through toxicity of CGA219417 to the sheepshead minnow (*Cyprinodon variegatus*). Report Number 829-CG. T.R. Wilbury Laboratories, Inc., 40 Doaks Lane, Marblehead, Massachusetts 01945. (Syngenta File No. CGA219417/0652)

Guidelines

US EPA FIFRA Guideline No. 72-3(a)

GLP: Yes

Executive Summary

The acute toxicity of CGA219417 the sheepshead minnow (*Cyprinodon variegatus*) was determined under flow-through conditions. Fish were exposed to a range of nominal concentrations of 0.65, 1.1, 1.8, 3.0 and 5.0 mg/L, alongside a dilution water control and a solvent control. Based on mean measured concentrations, the 96 hour LC_{50} was 1.25 mg/L.

Materials

Test material	CGA219417 technical
Lot/Batch #:	P012012
Purity:	99.2%
Description:	Off-white granules
Stability of test compound:	Stable under test conditions
Reanalysis/expiry date:	April 1995
Treatments	
Test concentrations:	Dilution water control, solvent control and nominal concentrations of 0.65, 1.1, 1.8, 3.0 and 5.0 mg/L.
Solvent:	None
Analysis of test concentrations:	Yes, at 0 and 96 hours using HPLC analysis
Test organisms	
Species:	Sheepshead minnow (*Cyprinodon variegatus*)
Source:	Aquatic Biosystems, Fort Collins, Colorado.
Acclimatisation period:	14 days
Treatment for disease:	None
Weight and length of dilution water control fish	Mean length: 23 mm Mean weight: 0.21 g

at end of exposure period:

Feeding: None during test

Test design

Test vessels: 20 L Glass vessels containing 15 L water
Test medium: Carbon filtered Natural seawater
Replication: None
No of fish per tank: 10
Exposure regime: Flow-through
Duration: 96 hours

Environmental conditions

Test temperature: 21.2 to 23.0° C
pH: 7.8 to 8.2
Dissolved oxygen: 5.0 to 8.1 mg/L
Lighting: 25 footcandles (161 Lux)
16 hours fluorescent light and 8 hours dark with 15 minute dawn and dusk transition periods

Study Design and Methods

Experimental dates: 23rd June to 11th December 1995

A stock solution with a nominal concentration of 50000 mg/L was prepared by dissolving 12.60 g of the test item completely in 250 mL of dimethylformamide. The stock solutions were mixed and appropriate amounts of stock solution were added directly to the dilution water by a proportional diluter.

At the start of the test seven fish were randomly allocated to each of the test concentrations and the dilution water control. The test was conducted in a temperature controlled water-bath. Observations for mortalities and symptoms of toxicity were made at 0, 24, 48, 72 and 96 hours.

Daily measurements of the test solutions were undertaken throughout the 96 hour period for pH, temperature and dissolved oxygen concentration.

The test concentrations were verified by chemical analysis of CGA219417 at 0 and 96 hours using an HPLC method.

Results and Discussion

The measured concentrations are shown in the table below in terms of nominal concentrations. The test concentrations were maintained throughout the study. Mean measured concentrations were used for the calculation and reporting of results.

Table 8.2.1-39: Analytical results

Nominal concentration (mg/L)	% of nominal 0 hours	% of nominal 96 hours	Mean measured concentration (mg a.s./L)
0.65	79	85	0.535
1.1	77	89	0.907
1.8	73	88	1.45
3.0	78	88	2.49

5.0	87	90	4.43

The median lethal concentration (LC_{50}) was defined as the concentration resulting in 50% mortality of the fish in the time period specified and was calculated by the binomial method. The NOEC (No Observed Effect Concentration) is defined as the highest tested concentration which did not produce an adverse effect when compared to the control and was determined by visual inspection of the data. Mortalities were observed at nominal concentrations of 1.45 mg a.s./L and above. Symptoms of toxicity observed included erratic swimming and were observed at concentrations of 1.45 mg/L and above. No mortality or symptoms of toxicity were observed in the control.

The mortality data and estimated LC_{50} values are shown in the table below:

Table 8.2.1-410: Effects of CGA219417 on the survival of *Cyprinodon variegatus*

Mean measured concentration (mg/L)	Mortality observed (Cumulative number of dead fish) (n = 10)			
	24 hours	48 hours	72 hours	96 hours
Dilution water control	0	0	0	0
Solvent control	0	0	0	0
0.535	0	0	0	0
0.907	0	0	0	0
1.45	0	0	7	2
2.49	0	5	10	10
4.43	0	10	10	10
LC_{50} mg/L	>4.33	2.49	1.60	1.25
95% confidence interval	-	1.45 – 4.43	0.907 – 2.49	0.907 – 1.45
NOEC	4.33	1.45	0.907	0.907

Conclusions

Based on mean measured concentrations, the 96-hour LC_{50} for CGA219417 to the sheepshead minnow (*Cyprinodon variegatus*) was 1.25 mg a.s./L and the 96-hour NOEC was 0.907 mg/L.

(Ward *et al*., 1995)

Metabolites of cyprodinil

Report:	Summary submitted at the request of ANSES for ease of dossier evaluation: *KIIA 8.2.1. Maetzler P. (1999). Acute Toxicity of CGA 249287 (Metabolite of CGA 219417) for Rainbow Trout (Determination of the LC-values). Report number 983825. (Syngenta File No. CGA249287/0007).*

Guidelines

OECD Guidelines for Testing of Chemicals. Section 2. No. 203 (1993)

Commission Directive 92 / 69 / EEC C.1 (1992)

GLP: Yes

Executive Summary

The toxicity of CGA 249287 to the rainbow trout, *Oncorhyncus mykiss*, was determined in a 96-hour acute toxicity test performed under static conditions. Rainbow trout were exposed to nominal CGA 249287 concentrations of 4.3, 9.4, 21, 45 and 100 mg/L alongside a dilution medium control. Analytical determinations of CGA 249287 in exposure solution were conducted in the 21 and 45 mg/L test concentrations at 0, 48 and 96 hours and the 100 mg/L test concentration at 0 and 24 hours. Mean measured concentrations were 20.2, 41.7 and 97.3 mg/L, for nominal test concentrations of 21, 45 and 100 mg/L, respectively. As mean measured concentrations were with ±20% of nominal concentrations, the following lethality results were based on nominal concentrations. The 96-hour LC_{50} for CGA249287 to *Oncorhyncus mykiss* was 55 mg/L with 95% confidence intervals of 28 to 107 mg/L.

Materials

Test Material	CGA 249287
Lot/Batch #:	HU-15701
Purity:	99 ± 2%
Description:	Beige, powder
Stability of test compound:	Not reported
Reanalysis/expiry date:	December 2001
Treatments	
Test concentrations:	Dilution medium control and nominal concentrations of 4.3, 9.4, 21, 45 and 100 mg/L (measured concentrations were 20.2, 41.7 and 97.3 mg/L, for nominal test concentrations of 21, 45 and 100 mg/L, respectively; within ±20% of nominal)
Solvent:	None
Positive control:	None
Analysis of test concentrations:	Yes, HPLC analysis of CGA 249287 at 0, 24, 48 and 96 hours
Test organism	
Species:	*Oncorhyncus mykiss*
Source:	P. Hohler, GH-4314 Zeiningen, Switzerland
Acclimation period:	At least 12 days
Feeding:	None during the exposure
Weight and length of dilution water control fish at end of exposure period:	Individual weights not reported, mean length 41 mm
Loading rate:	0.30 – 0.66 g/L
Test design	
Test vessels:	20 L glass aquaria, containing 15 L test solution
Test medium:	Carbon filtered, tap water
Replication:	One replicate vessel for each treatment and control containing 7 fish
Exposure regime:	Static
Aeration:	Slightly aerated
Duration:	96 hours
Environmental conditions	

Test temperature:	14.0 to 14.4 °C
pH:	Test start: 8.0 to 8.2 Test end: 8.2 to 8.3
Dissolved oxygen:	96 to 100 % saturation
Lighting:	Diffuse daylight

Study Design and Methods

Experimental dates: 8th to 17th February 1999

Appropriate weighed aliquots of the test item were added directly to dilution medium followed by high shear mixing to individually prepare the nominal test concentrations. Seven rainbow trout were transferred into each test vessel. The study design consisted of a single replicate test vessel for each test concentration and the dilution medium control.

The number of surviving test organisms and the occurrence of sublethal effects was recorded after 2 – 4, 24, 48, 72 and 96 hours of exposure. Any observed dead fish were removed from the test solutions at least at the above mentioned intervals.

The actual concentrations of the test item were determined in the 21 and 45 mg/L concentrations, at the start, 48 and 96 hours and the 100 mg/L concentration, at the start and 24 hours, using high performance liquid chromatography (HPLC) with UV detection. In concentrations where 100% mortality was observed before the end of the study, analytical samples were collected at the observation interval when all fish were recorded as deceased.

Dissolved oxygen, pH, conductivity and temperature were measured daily during the exposure in all treatments containing live fish.

The 24, 48 and 72-hour LC_{50} values were estimated with a binominal distribution method and the 96-hour LC_{50} value estimated using the Probit method.

Results and Discussion

Measured test item concentrations ranged from 96.2 – 97.7% at the start of the exposure and 93.1 – 96.2% at the end of the exposure. The mean measured CGA 249287 concentrations for the nominal concentration of 21, 45 and 100 mg/L were 20.2, 41.7 and 97. mg/L. The study results were based on the nominal concentrations.

Table 8.2.1-11: Analytical results

Nominal concentrations (mg/L)	Measured concentration (mg/L)				Mean Measured (mg/L)
	0-hour	24-hour	48-hour	96-hour	
Control	<LOD	<LOD	<LOD	<LOD	<LOD
21	20.2	-	20.2	20.2	20.2
45	42.2	-	41.0	41.9	41.7
100	97.7	96.8	-	-	97.3

LOD: 0.06 mg/L

Water quality throughout the test was within acceptable limits. During the exposure period the pH ranged from 8.0 to 8.3, the dissolved oxygen ranged from 96 to 100% saturation and temperature ranged from 14.0 to 14.4°C.

Mortality of 0% after 96-hours exposure was observed in the control treatment group and all CGA 249287 concentrations up to and including 21 mg/L. Mortality of 14% and 100% was observed in the 45 and 100 mg/L treatment groups.

No sublethal effects were observed in the control and test concentrations of 4.3, 9.4 and 21 mg/L. Non-defined severe symptoms were observed in the 45 mg/L test concentration at 24-hours until the end of the test, all fish were dead at the 100 mg/L concentration after 24-hours.

Table 8.2.1-12: Effects of CGA 249287 on survival of *Oncorhyncus mykiss* after 24, 48, 72 and 96 hours exposure

Nominal concentration (mg/L)	Mean measured concentration (mg/L)	Mortality observed (Cumulative number of dead fish) (n = 20)			
		24 hours	48 hours	72 hours	96 hours
Control	-	0	0	0	0
4.3	-	0	0	0	0
9.4	-	0	0	0	0
21	20.2	0	0	0	0
45	41.7	0	0	0	1
100	97.3	7	7	7	7
LC_{50} mg/L		67	67	67	55
95% confidence interval (mg/L)		45 - 100	45 - 100	45 - 100	28 - 107

Validity Criteria

The study was considered valid as the following criteria was met:

- Mortality of ≤ 1 fish was observed in the control treatments
- Dissolved oxygen concentration was >60 % saturation throughout the test

Conclusions

Exposure of rainbow trout to CGA249287 resulted in a 96-hour LC_{50} of 55 mg/L with 95% confidence intervals of 28 to 107 mg/L.

(Maetzler P. 1999)

Report:	Summary submitted at the request of ANSES for ease of dossier evaluation: *KIIA 8.2.1. Pfeifle V. (2001). Acute Toxicity of CGA275535 (Metabolite of CGA219417) to Rainbow Trout (Oncorhyncus mykiss) under static conditions. Report Number 2003592. Solvias AG, CH-4002 Basel, Switzerland. (Syngenta File No. CGA275535/0017).*

Guidelines

OECD Guidelines for Testing of Chemicals. Section 2. No. 203 (1992)

Commission Directive 92 / 69 / EEC C.1

U.S. EPA FIFRA – Guideline No. 72-1.

GLP: Yes

Executive Summary

The toxicity of CGA275535 to the rainbow trout, *Oncorhyncus mykiss*, was determined in a 96-hour acute toxicity test performed under static conditions. Rainbow trout were exposed to nominal CGA275535 concentrations of 0.65, 1.1, 1.8, 3.0 and 5.0 mg/L alongside a dilution medium control and a solvent control (mean measured concentrations 0.46, 0.96, 1.3, 2.7 and 4.5 mg/L). Based on mean measured concentrations, the 96-hour LC_{50} for CGA219417 to *Oncorhyncus mykiss* was 2.1 mg/L with 95 % confidence intervals of 1.6 to 2.7 mg/L.

Materials

Test Material	CGA275535
Lot/Batch #:	KI 6230/3
Purity:	99 ± 2%
Description:	Slight yellow-beige, powder
Reanalysis/expiry date:	31 October 2003
Treatments	
Test concentrations:	Dilution medium control, solvent control and nominal concentrations of 0.65, 1.1, 1.8, 3.0 and 5.0 mg/L (measured concentrations 0.46, 0.96, 1.3, 2.7 and 4.5 mg/L)
Solvent:	Dimethylformamide
Positive control:	None
Analysis of test concentrations:	Yes, HPLC analysis of CGA275535 at 0 and 96 hours
Test organism	
Species:	*Oncorhyncus mykiss*
Source:	P. Hohler, GH-4314 Zeiningen, Switzerland
Acclimation period:	At least 12 days ≤3 % mortality during the 7 days prior to the test
Feeding:	None during the exposure
Weight and length of dilution water control fish at end of exposure period:	Mean length: 51 mm (range 48 – 54 mm) Mean weight: 1.30 g (range 0.97 – 1.59 g)
Loading rate:	0.91 g/L fish biomass to solution
Test design	
Test vessels:	20 L glass aquaria, containing 10 L test solution
Test medium:	Carbon filtered, well water
Replication:	One replicate vessel for each treatment and control containing 7 fish
Exposure regime:	Static
Aeration:	Gentle (oil free air)
Duration:	96 hours
Environmental conditions	
Test temperature:	12.9 to 13.1 °C

pH:	Test start: 8.1 to 8.2 Test end: 7.9 to 8.0
Dissolved oxygen:	96 to 100 % saturation
Lighting:	16 hour light, 8 hour dark cycle with 30 minute transition period

Study Design and Methods

Experimental dates: 12th to 28th March 2001

A stock solution was prepared by dissolving 499.9 mg of the test item in 10 mL dimethylformamide (DMF). Appropriate volumes of the stock solution were added directly to dilution medium to prepare the nominal test concentrations. After an equilibration time of 20 – 30 minutes seven rainbow trout were transferred into each test vessel. The study design consisted of a single replicate test vessel for each test concentration, the dilution medium and the solvent control (0.1 mL/L DMF).

The test vessels were incubated in a water bath to reduce temperature fluctuation during the 96-hour exposure. The number of surviving test organisms and the occurrence of sublethal effects was recorded after 2 – 4, 24, 48, 72 and 96 hours of exposure. Any observed dead fish were removed from the test solutions at least at the above mentioned intervals.

The actual concentrations of the test item were determined at the start and end of the 96-hour exposure period using high performance liquid chromatography (HPLC) with UV detection. In concentrations were 100% mortality was observed before the end of the study, analytical samples were collected at the observation interval when all fish were recorded as deceased.

Dissolved oxygen, pH, conductivity and temperature were measured daily during the exposure in all treatments containing live fish.

The 24 and 48-hour LC_{50} values could not be estimated as less than 50% mortality had occurred in the highest test concentration after these exposure periods. The 72 and 96-hour LC_{50} values were estimated using the Probit method.

Results and Discussion

Measured test item concentrations ranged from 97 – 109% at the start of the exposure and 32 – 83% at the end of the exposure. The mean measured CGA275535 concentrations were 0.46, 0.96, 1.3, 2.7 and 4.5 mg/L. The study results were based on the mean measured concentrations.

Table 8.2.1-13: Analytical results

Nominal concentrations (mg/L)	Measured concentration 0-hour (mg/L)	Measured concentration 96-hour (mg/L)	Mean Measured (mg/L)
Control	<LOD	<LOD	-
Solvent Control	<LOD	<LOD	-
0.65	0.71	0.21	0.46
1.10	1.18	0.73	0.96
1.80	1.87	0.81	1.34
3.00	3.12	2.26	2.69
5.00	4.86	4.13	4.50

Water quality throughout the test was within acceptable limits. During the exposure period the pH ranged from 7.9 to 8.2, the dissolved oxygen ranged from 97 to 100% saturation and temperature ranged from 12.9 to 13.1°C.

Mortality of 0% after, 96-hours exposure, was observed in the control and solvent control treatment groups and all CGA275535 concentrations up to and including 0.96 mg/L. Mortality of 14, 71% and 100 % was observed in the 1.3, 2.7 and 4.5 mg/L treatment groups.

Sublethal effects, such as pigmentation changes, were observed in all treatment levels. Changes of swimming behaviour and respiratory function and loss of equilibrium were observed at test item concentrations of 1.3, 2.7 and 4.5 mg/L.

Table 8.2.1-14: Effects of CGA275535 on survival of *Oncorhyncus mykiss* after 24, 48, 72 and 96 hours exposure

Nominal concentration (mg/L)	Mean measured concentration (mg/L)	Mortality observed (Cumulative number of dead fish) (n = 20)			
		24 hours	48 hours	72 hours	96 hours
Control	-	0	0	0	0
Solvent Control	-	0	0	0	0
0.65	0.46	0	0	0	0
1.10	0.96	0	0	0	0
1.80	1.34	0	0	1	1
3.00	2.69	0	0	4	5
5.00	4.50	0	3	5	7
LC_{50} mg/L		>4.5	>4.5	2.7	2.1
95% confidence interval (mg/L)		-	-	1.7 – 4.2	1.6 – 2.7

Validity Criteria

The study was considered valid as the following criterion was met:

- Mortality of ≤1 fish was observed in the control treatments.

Conclusions

Exposure of rainbow trout to CGA275535 resulted in a 96-hour LC_{50} of 2.1 mg/L with 95% confidence intervals of 1.6 to 2.7 mg/L. Sublethal effects were observed even in the lowest test concentration of 0.46 mg/L.

(Pfeifle, 2001)

Report:	Submitted for purposes of renewal as conducted on relevant aquatic metabolite: K-CA 8.2.1/03 Vial A. (1991). Report on the acute toxicity test of CGA 263208 intermediate to Rainbow Trout (*Salmo gairdneri*). Report Number 918092. Ciba-Geigy Ltd., Product Safety, Ecotoxicology, CH-4002 Basel, Switzerland. (Syngenta File No. CA1059/0009)

Guidelines

OECD Guideline No. 203, Paris, 1984

GLP: Yes

Executive Summary

The acute toxicity of CGA263208 to rainbow trout (*Oncorhynchus mykiss*) was determined. Fish were exposed to nominal concentrations of 10, 18, 32, 58 and 100 mg a.s./L, and a control (dilution water).

The 96 hour LC_{50} (based on nominal concentrations) was >100 mg a.s./L.

Materials

Test Material	CGA263208
Description:	Grey solid
Lot/Batch #:	P.010010
Actual content of a.s.:	72.7%
Stability of test compound:	31/12/92
Test concentrations:	Nominal – 10, 18, 32, 58, 100 mg a.s./L, and a control (dechlorinated tap water). Actual - 10.7, 19.6, 36.5, 64.3, 109.6 mg a.s./L, and a control (dechlorinated tap water)
Vehicle and/or positive control:	Dechlorinated tap water; none
Analysis of test concentrations:	Yes (0 and 96 hours)
Test animals	
Species:	Rainbow trout (*Oncorhynchus mykiss)*
Source:	P. Hohler, CH-4314 Zeiningen
Acclimatisation period:	27 days
Treatment for disease:	None
Weight and length of sub-sample from batch of fish at start of test:	Mean length: 4.6 mm (43-48 mm) Mean weight: 0.81 g (0.65-0.98 g)
Loading:	0.54 g/L
Feeding:	None during test or 24 hours before
Test design	
Exposure regime:	Static
Aeration:	Yes, gentle
Replication:	None
No of fish per tank:	10
Environmental conditions	
Test temperature:	14 °C
pH:	7.8 – 8.2
Dissolved oxygen:	92 to103 %
Hardness:	168 mg/L $CaCO3_3$
Lighting:	16 hours fluorescent light and 8 hours
Aeration:	Gentle aeration during exposure

Length of test: 96 hours

Study Design and Methods

Experimental dates: 22nd April to 7th June 1991

Test procedure and apparatus

A static test system was employed. 20 L glass aquaria were filled with 15 L dilution water control and the test solutions. At the start of the test 10 fish were randomly allocated to the test concentrations and the dilution water control. The fish were not fed during the course of the test.

Preparation of test solutions

Fish were exposed to nominal concentrations of 10, 18, 32, 58, 100 mg a.s./L and a control (dilution water). An aqueous stock solution was prepared by mixing 6.0 g of test material with water and then made up to 2000 mL with test water. Appropriate volumes of the stock were then added to dilution water in each test vessel to give 15 litre volumes of the test concentrations.

Analytical method

The concentrations of CGA263208 in the test solutions were measured at 0 and 96 hours using an HPLC method. Samples for analysis were taken from the centre of the test solutions.

Observations for mortality and symptoms of toxicity

Observations for mortalities and symptoms of toxicity (erratic swimming behaviour, loss of equilibrium, adverse effects on respiratory function, exophthalmus and pigmentation) were made at 24, 48, 72 and 96 hours. The LC_{50} values were estimated from the data obtained.

Physical and chemical parameters

Daily measurements of the test solutions were undertaken throughout the 96 hour period for pH, temperature and dissolved oxygen concentration using calibrated meters.

Results and Discussion

Analytical data

The concentrations of CGA263208 were determined in the test and are shown in the table below. The limit of detection of CGA263208 was 0.1 mg a.s./L. The measured concentrations at the start of the test ranged from 107 to 114% of nominal and at the end of the test ranged from 103 to 111% of nominal. Therefore, nominal concentrations were used for the calculation and reporting of the results.

Table 8.2.1-515: Analytical results

Nominal concentration (mg a.s./L)	Measured concentration at 0 hours (mg a.s./L)	% of nominal 0 hours	Measured concentration at 96 hours (mg a.s./L)	% of nominal 96 hours
Control	<0.1	-	<0.1	-
10	10.70	107	10.30	103
18	19.60	109	19.60	109
32	36.50	114	35.60	111
58	64.30	111	63.80	110

100	109.60	110	107.90	108

Biological data

Mortalities were observed at nominal concentrations of 58 and 100 mg/L. However there was only 1 mortality in the 58 mg/L group at 96 h and 2 mortalities at 100 mg/L by 96 h. No mortality or symptoms of toxicity were observed in the control. No symptoms of toxicity were observed during the study.

Table 8.2.1-616: Effects of CGA263208 on the survival of *Oncorhynchus mykiss*

Mean measured concentrations (mg a.s./L)	Cumulative mortality observed			
	24 hours	48 hours	72 hours	96 hours
Control	0	0	0	0
10	0	0	0	0
18	0	0	0	0
32	0	0	0	0
58	0	0	0	0
100	0	0	1	1

Validity Criteria

The validity criteria were me in that:

- Mortality in the control should be ≤10% (actual recorded was 0%).
- The dissolved oxygen content should be ≥60% of water saturation (actual measured was 92 to 103%).

Conclusions

The 96 hour LC_{50} for CGA263208 to rainbow trout (*Oncorhynchus mykiss*) was >100 mg a.s. based on nominal concentrations.

(Vial A, 1991)

Report:	Submitted for purposes of renewal as conducted on relevant aquatic metabolite: K-CA 8.2.1/04 Grade R. (1992) Report on the acute toxicity test of CA 1139 A to Rainbow Trout (*Oncorhynchus mykiss*). Report Number 928172. Ciba-Geigy Ltd., Product Safety, Ecotoxicology, CH-4002 Basel, Switzerland. (Syngenta File No. CA1139/0008)

Guidelines

OECD Guideline No. 203, Paris, 1984

GLP: Yes

Executive Summary

The 96 hour acute toxicity of CA1139A to rainbow trout (*Oncorhynchus mykiss*) was determined. Fish were exposed to mean measured concentrations of 10, 18, 32, 58 and 100 mg CA1139A/L, and a control (dechlorinated tap water).

The 96 hour LC_{50} (based on nominal concentrations) was >100 mg a.s./L.

Materials

Test Material	CA1139A
Description:	Grey solid
Lot/Batch #:	P.201025
Actual content of a.s.:	76.2 %
Stability of test compound:	12/93
Test concentrations:	Nominal – 10, 18, 32, 58 and 100 mg CA1139A/L, and a control (dechlorinated tap water).
Vehicle and/or positive control:	Dechlorinated tap water; none
Analysis of test concentrations:	Yes (0 and 96 hours)
Test animals	
Species:	Rainbow trout (*Onocorhynchus mykiss)*
Source:	P. Hohler, CH-4314 Zeiningen
Acclimatisation period:	25 days
Treatment for disease:	None
Weight and length of sub-sample from batch of fish at start of test:	Mean length: 52 mm (48-58 mm) Mean weight: 1.26 g (0.95-1.80 g)
Loading:	0.42 g/L
Feeding:	None during test
Test design	
Exposure regime:	Static
Aeration:	Gentle aeration
Replication:	None
No of fish per tank:	10
Environmental conditions	
Test temperature:	14 °C
pH:	7.5-8.4
Dissolved oxygen:	93 to 106 %
Hardness:	155 mg/L $CaCO3_3$
Lighting:	16 hours fluorescent light and 8 hours
Length of test:	96 hours

Study Design and Methods

Experimental dates: 29th July to 29th September 1992

Test procedure and apparatus

A static test system was employed. Glass aquaria (20 L holding capacity) were filled with 15 L dechlorinated tap water for the control and the test solutions. At the start of the test 10 fish were randomly

allocated to the test concentrations and the dechlorinated tap water control. The fish were not fed during the course of the test.

Preparation of test solutions

A stock solution was prepared by dissolving 7.5 g of test material in 2000 mL of water. Appropriate volumes of the stock were then added to dilution water in each test vessel to give the desired nominal test concentrations. Fish were exposed to nominal concentrations of 10, 18, 32, 58 and 100 mg CA1139A/L (nominal) and a control (dechlorinated tap water only). The test substance was well distributed, although in higher concentrations (58 and 100 mg/L) a slight deposit was observed.

Analytical method

The concentrations of CA1139A in the test solutions were measured at 0 and 96 hours using an HPLC method. Samples for analysis were taken from the centre of the test solutions.

Observations for mortality and symptoms of toxicity

Observations for mortalities and symptoms of toxicity were made at 24, 48, 72 and 96 hours. The LC_{50} values were estimated from the data obtained. Fish were examined daily for symptoms such as unusual swimming behaviour, loss of equilibrium, respiratory function, exophthalmus and pigmentation.

Physical and chemical parameters

Daily measurements of the test solutions were undertaken throughout the 96 hour period for pH, temperature and dissolved oxygen concentration using calibrated meters.

Results and Discussion

Analytical data

The concentrations of CA1139A were determined in the test and are shown in the table below. The limit of detection of CA1139A was 1.6 mg a.s./L. The measured concentrations at the start of the test ranged from 95 to 108 % of nominal and at the end of the test ranged from 88 to 100 % of nominal. The nominal concentrations were used for determining the EC_{50}, no effect levels and the reporting of the results.

Table 8.2.1-~~7~~17: Analytical results

Nominal concentration (mg a.s./L)	Measured concentration at 0 hours (mg a.s./L)	% of nominal 0 hours	Measured concentration at 96 hours (mg a.s./L)	% of nominal 96 hours
Control	<1.6	-	<1.6	-
10	8.9	106	8.8	88
18	17.7	95	17.6	8
32	32.0	97	31.8	99
58	58.3	101	57.9	100
100	98.9	108	98.2	98

Biological data

Mortalities and sub-lethal effects were not observed for the duration of the test, in any test concentrations (apart from one incidental mortality at 96 hours within the 32 mg/L group) or the controls.

Table 8.2.1-818: Effects of CA1139A on the survival of *Onocorhynchus mykiss*

Mean measured concentrations (mg a.s./L)	Cumulative mortality observed			
	24 hours	48 hours	72 hours	96 hours
Control	0	0	0	0
10	0	0	0	0
18	0	0	0	0
32	0	0	0	1
58	0	0	0	0
100	0	0	0	0

Validity

Validity criteria as per OECD test guideline 203 were met:

- Control mortality was < 10 %
- Dissolved oxygen was > 60 % for the duration of the test
- Test substance concentrations did not deviate by more than 20 %
- Test conditions were suitably stable for the duration of the test

Conclusions

The 96 hour LC_{50} for CA1139A to rainbow trout (*Onocorhynchus mykiss*) is >100 mg CA1139 A/L based on the nominal concentrations.

(Grade R, 1992)

CA 8.2.2 Long-term and chronic toxicity to fish

Prolonged toxicity studies are no longer a data requirement according to **Commission Regulation (EU) No 283/2013** as a fish ELS test is now the recommended test method. However, the existing data are retained here for completeness. The endpoints are summarised in Table 8.2-3. At the request of ANSES a summary has been included in order to aid dossier evaluation.

Report:	Summary submitted at the request of ANSES for ease of dossier evaluation: *KIIA 8.2.2.1. Vial, A. (1991). Report on the prolonged toxicity test of CGA 219417 technical to rainbow trout. Report Number 901366, CIBA-GEIGY Ltd., Basel, Switzerland. (Syngenta File No. CGA219417/0009)*

Guidelines

OECD Guidelines for Testing of Chemicals. Test Guideline 204, Fish prolonged toxicity test. Adopted 04 April 1984.

GLP: Yes

Executive Summary

The lethal and sublethal effects of prolonged exposure of CGA219417 to juvenile rainbow trout (*Oncorhynchus mykiss*) were evaluated. This study was conducted with nominal concentrations of 0.032,

0.058, 0.1, 0.18 and 0.32 mg CGA219417/L together with a dilution water control (corresponding mean measured concentrations of 0.055, 0.053, 0.083, 0.13, 0.22 mg/L).

Based on growth rate for length and mean measured concentrations the NOEC was determined to be 0.083 mg CGA219417/L; the Lowest Observed Effect Concentration (LOEC) was 0.13 mg CGA219417/L.

Materials

Test Material:	CGA219417
Description:	White solid
Lot/Batch #:	P.912006
Actual content of a.s.:	99.6 %
Stability of test compound:	February 1994
Test concentrations:	Dilution water control and nominal concentrations of 0.032, 0.058, 0.1, 0.18 and 0.32 mg CGA219417/L (corresponding mean measured concentrations of 0.055, 0.053, 0.083, 0.13, 0.22 mg/L).
Vehicle and/or positive control:	Not included
Analysis of test concentrations:	Yes
Test animals	
Species:	Rainbow trout (*Oncorhynchus mykiss*)
Source:	P. Hohler, CH-4314, Switzerland
Acclimatisation period:	22 days (individual fish used in the test were freeze branded 24h before the start of the exposure after light anaesthesia with 0.05 g/L MS222).
Treatment for disease:	
Weight and length of dilution water control fish at start of exposure period:	Weight: range 2.12 – 5.74 g, mean 3.50 g Length: range 63 – 85 mm, mean 70 mm
Feeding:	Commercial fish food (NAFAG No.: 730; Nahr und Fischfutter AG, CH-9202 Gossau, Switzerland) 4 % of initial body weight daily given in two feeds.
Environmental conditions	
Test temperature:	15 ± 1°C (14 to 15°C)
pH range:	8.0 to 8.4
Dissolved oxygen:	85 to 102% saturation
Total hardness of dilution water:	163 - 176 mg/L $CaCO_3$
Conductivity of dilution water	Not reported
Lighting:	16 hours fluorescent light and 8 hours dark
Length of test:	21 days

Study Design and Methods

Exposure dates: 17th October to 12th December 1990

Test procedure and apparatus

The apparatus used in this test was a continuous flow-through system (proportional diluter). A single vessel was prepared for each treatment group. Glass vessels with a maximum capacity of 20 L and a working volume of 15 L were used to hold the test fish. The test solutions were renewed at a nominal rate of 3.75 L/hour (6 volume replacements per day).

At the start of the test, individual fish were randomly selected and anaesthetised using a general anaesthetic (MS222 0.05 g/L) before being weighed and measured. The fish were transferred to a recovery tank for 24 h after which they were transferred to the test vessels. Each test vessel contained 10 fish.

Preparation of test solutions

Five nominal concentrations, 0.032, 0.058, 0.1, 0.18 and 0.32 mg CGA219417/L, together with a dilution water control were used in the study. Corresponding mean measured concentrations were 0.055, 0.053, 0.083, 0.13, 0.22 mg/L.

Stock solutions were prepared by making up 200 mg test substance to 2000 mL with test water. The pH was adjusted to 3 with 2.2 ml 4N HCl followed agitation for approximately 12 hours till the test substance was homogeneously distributed in the vessel. Stock solutions were prepared the day before renewal. The stock solution was metered into water in a proportional diluter which continuously fed into the tanks to prepare all the test concentrations.

The test solutions were gently aerated during the test.

Observations for mortality and symptoms of toxicity

Observations for mortality were made daily (except Sundays).

Fish weights and lengths

Weight and length of fish were measured at the start and at the end of exposure.

Growth rates

For each individual fish, growth rates, based fish weight and length, were calculated as follows, together with change in condition index and food conversion efficiency:

$$r_w = \left(\frac{l}{t}\right) \times log\left(\frac{w_t}{w_q}\right)$$

$$r_x = \left(\frac{l}{t}\right) \times log\left(\frac{l_t}{l_0}\right)$$

$$\Delta K = 100 \times \left(\frac{w_t}{l_t^3}\right) - 100 \times \left(\frac{w_o}{l_0^3}\right)$$

$$c = \frac{100 \times (w_{t_} w_o)}{daily\ ration \times t}$$

where:

r_w: growth rate in terms of weight
r_x: growth rate in terms of length
ΔK: change in condition index
c: food conversion efficiency
r: rate
w: weight in grams
1: length in centimetres
t: the days of the exposure (time t)
0: the start of the exposure (time 0)

Statistical analysis

As no mortality occurred no statistical analysis was appropriate.

For growth parameters, preliminary investigation of the data indicated that the data were non-normal therefore the nonparametric Jonkheere's test (α = 0.05) was used to test for a trend with increasing test item concentration level. The Cochran-Armitage trend test (α = 0.05) was used determine the NOEC.

Analytical method

The concentrations of CGA219417 in the test solutions were measured on days 0, 7, 14 and 21 using a UV high performance liquid chromatography method.

Physical and chemical parameters

The oxygen content, pH, hardness and temperature were measured each Monday, Wednesday and Friday.

Results and Discussion

Analytical data

The measured concentrations in the individual vessels ranged from 76 to 110% of the nominal values throughout the exposure period. Mean measured concentrations have been used for the calculation and reporting of the results.

Table 8.2.2-1: Measured concentrations of CGA219417 for 28 days in a flow-through test

Treatment Group (mg/L)	Measured Concentration									
	Day 0		Day 7		Day 14		Day 21		Mean	
	mg/L	%	mg/L	%	mg/L	%	mg/L	%	mg/L	%
Control	-	-	-	-	-	-	-	-	-	-
0.032	0.08	250	0.04	125	0.05	156	0.05	156.3	0.055	172
0.058	0.06	104	0.04	69.0	0.06	103.5	0.05	86.2	0.053	91.0
0.1	0.10	100	0.08	80.0	0.08	80.0	0.07	70.0	0.083	83.0
0.18	0.19	106	0.11	61.1	0.12	66.7	0.11	61.1	0.13	74.0
0.32	0.26	81.3	0.20	62.5	0.19	59.4	0.22	68.8	0.22	68.0

Observations of mortalities and symptoms of toxicity

No mortalities or symptoms of toxicity were observed in any of the test concentrations except for the highest. No mortality was observed in the dilution water control.

Length and weight data

No significant differences ($\alpha = 0.05$) based on growth rate in terms of weight or food conversion efficiency were observed for any concentration tested compared the control. Significant differences ($\alpha = 0.05$) based on growth rate in terms of length and change in condition index were noted at ≥0.13 and 0.22 mg/L, respectively compared the control.

Based on mean measured concentrations, the No Observed Effect Concentration (NOEC) was 0.083 mg CGA219417/L; the Lowest Observed Effect Concentration (LOEC) was 0.13 mg CGA219417/L.

Growth metrics recorded during the test are given in the table below.

Table 8.2.2-2: Effects of CGA219417 on the length and weight of juvenile *Oncorhynchus mykiss* following exposure for 28 days in a flow-through test

Treatment Group (mg/L)	r_w	r_x	c	ΔK
Control	0.011285	0.0030733	40.1891	0.04675
	0.027128	0.0068143	65.8561	0.1394
	0.013789	0.0037614	45.2550	0.05539
	0.022497	0.0053712	62.8166	0.14690
	0.01074	0.0031547	35.4610	0.02898
	0.025772	0.007739	81.7291	0.05909
	0.017234	0.0054655	64.5052	0.01918
	0.006479	0.0021814	12.1581	-0.00129
	0.016421	0.0046013	44.9173	0.05555
	0.022588	0.0056087	76.6633	0.12887
0.055	0.008438	0.0012871	23.5003	0.10167
	0.0208	0.0056832	60.2968	0.08151
	0.013348	0.003529	27.2109	0.05912
	0.01347	0.0035821	26.2832	0.05838
	0.003786	0.0012871	10.8225	-0.00173
	0.01521	0.0037684	66.7904	0.07986
	0.012837	0.0042491	42.6716	0.00200
	0.007829	0.0014431	14.5331	0.07304
	0.011356	0.0024743	37.7242	0.09246
	0.02075	0.0066837	69.5733	0.01566
0.053	0.015545	0.0050172	40.0000	0.01133
	0.025279	0.0070155	65.9048	0.09183
	0.01153	0.0038631	38.0952	-0.00126

	0.021122	0.0056087	52.9524	0.08966
	0.01766	0.0019983	50.2857	0.23792
	0.00668	0.0020266	19.0476	0.01282
	0.020652	0.0054655	74.6667	0.08327
	0.010135	0.0028019	28.1905	0.04013
	0.015409	0.0032854	48.7619	0.12081
	0.016229	0.005153	56.0000	0.01720

Treatment Group (mg/L)	rw	rx	c	ΔK
0.083	0.010775	0.0027218	27.551	0.05715
	0.011577	0.0033787	30.612	0.03196
	0.016108	0.0054481	43.537	-0.00548
	0.017949	0.006193	60.884	-0.01377
	0.023131	0.005153	67.007	0.16054
	0.014575	0.0053294	55.782	-0.03051
	0.029469	0.008461	118.027	0.09722
	0.018198	0.0049427	43.537	0.07694
	0.010797	0.0031971	33.673	0.02672
	0.007861	0.0013804	19.048	0.08062
0.13	0.016844	0.0042672	40.8671	0.09158
	0.008635	0.0013415	23.8095	0.09988
	0.002513	0.0000	7.8181	0.05429
	0.011606	0.003529	27.3632	0.02197
	0.016952	0.0020858	44.0654	0.24278
	0.00241	0.0000	5.3305	0.04771
	0.022711	0.0047659	89.9076	0.18225
	0.013342	0.0024425	46.1976	0.12329
	0.0074	0.0018201	27.3632	0.04171
	0.034879	0.0013415	81.3788	0.56344
0.22	0.011837	0.0000	34.299	0.27596
	0.01034	0.0007161	24.309	0.19653
	0.014097	0.0000	37.629	0.32945
	0.008254	0.0000	19.98	0.19949
	0.011169	0.0006307	35.298	0.20444
	0.014016	0.0006392	42.624	0.26698
	0.007912	0.0012056	30.636	0.10124
	0.01735	0.0019439	56.9431	0.2852
	0.009877	0.0000	22.644	0.21626
	0.012285	0.0028869	29.304	0.0902

Validity criteria

Not reported.

Conclusions

In a 28-day prolonged exposure test for rainbow trout vs CGA219417, the NOEC was 0.083 mg CGA219417/L; the LOEC was 0.13 mg CGA219417/L.

(Vial, A. 1991)

In accordance with **Commission Regulation (EU) No 283/2013**, the EC_{10} and EC_{20} values should be estimated. However since these studies are no longer data requirements and are only provided for completeness, further calculations of the endpoints have not been carried out.

CA 8.2.2.1 Fish early life stage toxicity test

The study previously submitted in the EU (*Pimephales promelas* - Ward *et al*., 1995, CGA219417/0653) was summarised in point B.9.2.2.2 of the DAR. At the request of ANSES a summary has been included in order to aid dossier evaluation. ~~therefore an additional study summary is not repeated here.~~ The endpoints are summarised in Table 8.2-3.

Report:	Summary submitted at the request of ANSES for ease of dossier evaluation: *KIIA 8.2.2.2. Ward T. J., Magazu J. P & Boeri R. L. (1995). Early-Life-Stage Toxicity of CGA-219417 to the Fathead Minnow, Pimephales Promelas. Report Number 846-CG. T. R. Wilbury Laboratories, Inc., Massachusetts 01945. (Syngenta File No. CGA 219417/0653)*

Guidelines

US EPA FIFRA Guideline No. 72-4(a)

GLP: Yes

Executive Summary

The toxicity of CGA 219417 to early-life stages of the fathead minnow (*Pimephales promelas*) was determined. Fish were exposed to the following range of nominal concentrations of 0.13, 0.26, 0.50, 1.0, and 2.0 mg/L, a solvent control and a dilution water control under flow-through conditions for 36 days (a 4-day hatching period plus a 32-day post-hatch growth period). Results were based on mean measured concentrations which were 0.114, 0.231, 0.455, 0.940, and 1.91 mg/L, respectively.

None of the fish exposed to 1.91 mg/L CGA 219417 survived and survival was significantly reduced at 0.455 and 0.940 mg/L. The time to hatch and feeding were identical for the controls and the highest treatment. The mean total length and dry weight of surviving fish exposed to CGA 219417 were significantly different from the controls at 0.940 mg/L and the wet weight of surviving fish was significantly different from the solvent control at 0.455 mg/L. Consequently, the NOEC (based on mean measured concentrations) was 0.231 mg/L. The LOEC was 0.455 mg/L.

Materials

Test Material	CGA 219417 technical
Description:	Off-white granules
Lot/Batch #:	P012012
Purity:	98.8%
Density:	n/a
Stability of test compound:	Reanalysis date: 31 January 1996; stable for >1 year at room temperature

Treatments

Test concentrations:	Nominal: 0.13, 0.26, 0.50, 1.0, and 2.0 mg/L Mean measured: 0.114, 0.231, 0.455, 0.940, and 1.91 mg/L
Control:	Dilution medium (deionised water adjusted to a hardness of 40 – 48 mg/L $CaCO_3$
Vehicle and/or positive	Dilution medium with dimethylformamide (DMF) at an addition rate of

control:	100 µg/L No positive control
Analysis of test concentrations:	Yes, days 0, 7, 14, 21, 28, 35 and 36

Test animals

Species:	Fathead minnow (*Pimephales promelas*)
Source:	Aquatic Bio Systems, Inc., Fort Collins, Colorado. Embryos were <24 hours post-fertilization at test initiation.
Acclimatisation period:	None
Treatment for disease:	None
Feeding:	Newly-hatched larvae were fed live brine shrimp nauplii (*Artemia salina*) twice per day up to day 36.

Test design

Exposure regime:	Flow-through
Aeration:	None
Replication:	2
Test vessels:	20 L glass tanks with a working volume of 15 L
No of eggs per tank:	20
Duration:	36 days (a 4-day hatching period plus a 32-day post-hatch growth period)

Environmental conditions

Test temperature:	24.1 to 26.0 °C
pH:	7.3 – 7.9
Dissolved oxygen:	6.8 to 8.9 mg/L
Lighting:	16 hours fluorescent light and 8 hours dark with 30 minute dawn and dusk transition periods; light intensity ~20 footcandles

Study Design and Methods

Experimental dates: 12th October to 17th November 1995.

A flow-through test system was employed. At the start of the test 20 eggs were randomly allocated to egg cups and one egg cup suspended in each of four replicate test vessels at each test and control treatment. Hence, 80 eggs were exposed at each treatment. The test was undertaken in a temperature controlled environmental chamber.

Eggs and fry were exposed to nominal concentrations of 0.13, 0.26, 0.50, 1.0, and 2.0 mg/L, a solvent control and a dilution water control. All test solutions and the solvent control contained 100 µL DMF per litre, with the control containing dilution water only.

A primary test substance stock solution was prepared in DMF at a nominal concentration of 20 mg/mL. The stock solution was added directly to dilution water by a calibrated proportional dilutor, which created all the subsequent test item concentrations. During the test the dilutor was activated 7,277 times, resulting in an average of 6.7 volume additions per 24-hours in each test vessel.

The concentrations of CGA 219417 in test solutions were measured at 0, 7, 14, 21, 28, 35 and 36 days using an HPLC method.

Observations for embryo survival, time to hatch, hatching success, larval mortality, deformed larvae and other symptoms of toxicity were made daily during the pre and post-hatch phases, as appropriate. At the

end of the test, total fish lengths and wet/dry weights of the surviving fry were measured. No Observed Effect Concentrations (NOEC) were estimated from the data obtained.

Statistical analysis

Test endpoints analysed statistically for the juvenile fish were % healthy embryos at 48 h, hatching success, larval survival, total length wet weight and dry weight. The results of the statistical analyses were used to aid in the determination of the NOEC and LOEC. .

Data that were statistically analysed included: 1) the percent of healthy and unhealthy embryos at 48 hours and at hatch, 2) mortality and sublethal effects of embryos, larvae, and juveniles 7, 14, 21, 28, and 32 days after hatch, 3) the total length of surviving fish at the end of the test, and 4) the weight (wet and dry) of surviving fish at the end of the test. The time to first hatch and first feeding were not statistically analysed because they were identical at all tested concentrations.

Control and solvent control data were compared with a parametric "t" test and in all cases except wet weight data. No differences were observed ($\alpha = 0.05$) and therefore control and solvent control data were pooled prior to subsequent statistical analyses. In the case of wet weight data, the treatment data were separately compared to the control and solvent control data. Shapiro Wilks test was used to determine if data were normally distributed, and Bartlett's test was used to determine if variances were homogeneous. If data were normally distributed and variances were homogeneous, a parametric one-way analysis of variance (ANOVA) and a Dunnett's test or Bonferroni "t" test were used to compare treatment and control data. If data were not normally distributed or variances were heteroscedastic, a non-parametric analysis of variance (Kruskal and Wallis's test) was used to compare treatment and control data.

All statistical tests were performed using a personal computer with TOXSTAT software.

Results and Discussion

The mean measured concentrations ranged from 88 to 96% of their nominal concentrations. The limit of quantification was 0.100 mg/L. The mean measured concentrations were used for reporting the results.

Table 8.2.2.1-1: Analytical results

Nominal Concentration (mg/L)	Measured Concentration (mg/L)								Percent of Nominal (%)
	Day 0	Day 7	Day 14	Day 21	Day 28	Day 34	Day 36	Mean	
Control	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	na	na
Solvent control	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	na	na
0.13	0.120	0.121	0.113	0.113	0.110	0.111	0.111	0.114	88
0.26	0.247	0.246	0.226	0.232	0.230	0.225	0.213	0.231	89
0.50	0.452	0.460	0.461	0.480	0.445	0.445	0.443	0.455	91
1.0	0.942	0.931	0.942	0.969	0.929	0.950	0.917	0.940	94
2.0	1.89	1.91	1.93	-	-	-	-	1.91	96

na not applicable

- no data as 100% mortality occurred

Time to hatch

Embryos in the controls and all test item treatments hatched on Days 3 to 4. Daily observations of all the embryos indicated that there were no apparent differences in time to hatch between the control groups and any of the CGA 219417 treatment groups.

Hatching success

There were no statistically significant decreases in hatching success at any test item concentration when compared to the pooled controls ($p \leq 0.05$). All of the treatment groups had percent hatching success above the control validity criterion of 75%, minor differences in percent hatching success were not considered to be treatment-related. Therefore, the NOEC for hatching success was 1.91 mg/L and the LOEC was >1.91 mg/L.

Larval survival

All larvae were observed to be dead after 7-days post-hatch in the highest test item concentration of 1.91 mg/L. Statistically significant decreases in larval survival were noted in the next two highest test item concentrations of 0.455 and 0.940 mg/L when compared to the pooled control data ($p \leq 0.05$). Consequently, the NOEC and LOEC based on percent larval survival at test termination were 0.231 and 0.455 mg/L, respectively.

Biological observations

Fish in the control and solvent control and the 0.114, 0.231, 0.455 mg/L treatment groups appeared normal throughout the test. Fish, a maximum of 4 and 2 per replicate, in the 0.940 mg/L treatment group appeared visually smaller, while surviving fish in the 1.91 mg/L treatment group were lethargic before death.

Length and weight

The mean total length and dry weight of surviving fish exposed to CGA 219417 were significantly different from the pooled controls at 0.940 and 1.91 mg/L. Since there were statistically significant differences in wet weight between the control and solvent control groups ($p \leq 0.05$), the treatment data were compared individually to the control and solvent control data. The mean wet weight of surviving fish exposed to CGA219417 was significantly different from the control and solvent control at 0.940 and 1.91 mg/L and the mean wet weight was significantly different from the solvent control at 0.455, 0.940, and 1.91 mg/L. Consequently, the NOEC was 0.231 mg/L and the LOEC was 0.455 mg/L, based on survival or weight. The results are presented in the table below.

Table 8.2.2.1-2: Effects of CGA219417 on the length and weight of sheepshead minnow (*Cyprinodon variegatus*)

Nominal concentration (µg a.s./L)	Hatching success (%) [1]	Percent Survival to Day 32 Post-Hatch	Day 32 post hatch		
			Mean Total Length ± SD (mm)	Mean Wet Weight ± SD (mg)	Mean Dry Weight ± SD (mg)
Control	97.5	91.8	29.7 ± 2.7	0.2742 ± 0.0685	0.0686 ± 0.0178
Solvent control	96.3	93.3	29.7 ± 1.9	0.3012 ± 0.0636	0.0726 ± 0.0164
0.114	100	96.7	29.8 ± 3.0	0.2866 ± 0.0796	0.0690 ± 0.0213
0.231	95.0	90.0	30.0 ± 2.7	0.3094 ± 0.0892	0.0728 ± 0.0207
0.455	97.5	80.0*	27.3 ± 3.3	0.2214 ± 0.0740**	0.0513 ± 0.0184*
0.94	88.8	16.7*	19.5 ± 3.5*	0.0956 ± 0.0409*	0.0182 ± 0.0088*
1.91	77.5	0.0	§	§	§

*: Significantly different from the control and solvent control means ($p \leq 0.05$)
**: Significantly different from the solvent control mean but not the control mean ($p \leq 0.05$)
§: There were no length or weight data for the 1.91 mg/L treatment replicates due to 100 % mortality prior to test termination

Conclusions

For fathead minnows (*Pimephales promelas*) exposed to CGA219417 under flow-through conditions for 36 days (a 4-day hatching period plus a 32-day post-hatch growth period), statistically significant treatment-related effects on survival were noted ≥0.455 mg/L CGA219417. For the length and weight parameters, statistically significant reductions were noted noted at ≥0.455 mg/L CGA 219417. Consequently, the NOEC was 0.231 mg/L and the LOEC was 0.455 mg/L.

(Ward *et al*., 1995)

In accordance with **Commission Regulation (EU) No 283/2013**, EC_{10} and EC_{20} values have been estimated and these statistical re-evaluations are provided below.

Report:	Submitted for purposes of renewal due to change in data requirements guidance: K-CA 8.2.2.1/01 Taylor S. Majdanik V. (2015a) CGA24705 - CGA219417 – Early life stage toxicity of CGA219417 to the Fathead Minnow, *Pimephales promelas*. Statistical Reanalysis. Report Number CEA.1417. Cambridge Environmental Assessments, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN/UK. (Syngenta File No: CGA219417_11595)

Summary

Report number 846-CG (Ward *et al.*, 1995) did not provide estimates of the EC_{10} and EC_{20} for the response variables of surviving fish 7, 14, 21, 28 and 32 days after hatch, length of surviving fish, wet or dry weights of surviving fish. Consequently the data have been re-analysed in order to estimate these values.

Statistical analyses of each parameter revealed significant differences between the control and treatment groups for each parameter. EC_{10}, EC_{20} and 95 % confidence interval values were reliably calculated.

Statistical Analysis

The effective concentrations for post hatch survival and number of healthy fish were assessed. Probit analysis with linear maximum likelihood regression was used to determine the concentration response function. Chi² was used as a goodness of fit measure.

The effective concentrations for larval wet weight, dry weight and length were assessed. Probit analysis with linear maximum likelihood regression was used to determine the concentration response function. Chi² was used as a goodness of fit measure, and the proportion of variance explained by the dose/response function was determined.

Where no meaningful concentration/response was found (p(F) > 0.05) the calculated EC_x's were not valid due to the absence of a concentration/response. All computations were carried out in ToxRat Professional version 2.10 (ToxRat Solutions GmbH, 2001-2010).

Results

There was a significant difference between treatment and control fish in the parameters of larval length (p(F) = 0.003), wet weight of surviving fish (p(F) = 0.002), dry weight of surviving fish (p(F) = 0.002) and fish survival after 7 d (p(F) = 0.031), 14 d (p(F) = 0.031), 21 d (p(F) = 0.031), 28 d (p(F) = 0.031) and 32 d (p(F) = 0.031). Reliable EC_{10} and EC_{20} values and their 95 % confidence intervals were determined.

EC_{10} and EC_{20} Estimates

Fish Survival	7 days	14 days	21 days	28 days	32 days
EC_{20} [mg/L] **95% CL**	0.742 (0.122-1.040)	0.339 (0.053-0.732)	0.334 (0.052-0.555)	0.334 (0.052-0.555)	0.334 (0.052-0.555)
EC_{10} [mg/L] **95% CL**	0.511 (0.030-0.814)	0.243 (0.008-0.449)	0.253 (0.018-0.435)	0.253 (0.018-0.435)	0.253 (0.018-0.435)

CL: Confidence Limits

Parameter	Fish Length	Wet Weight (control comparison)	Wet Weight (solvent control comparison)	Dry Weight
EC_{20} [mg/L] **95% CL**	0.68 (0.62-0.73)	0.47 (0.41-0.52)	0.41 (0.18-0.54)	0.41 (0.29-0.49)
EC_{10} [mg/L] **95% CL**	0.49 (0.41-0.55)	0.36 (0.30-0.42)	0.31 (0.09-0.44)	0.32 (0.20-0.40)

CL: Confidence Limits

Conclusion

Statistical analyses of each parameter revealed significant differences between the control and treatment groups for each parameter. EC_{10}, EC_{20} and 95 % confidence interval values were reliably calculated.

(Taylor & Majdanik, 2015a)

A summary of a study conducted on the sheepshead minnow (*Cyprinodon variegatus*) following the original EU submission of cyprodinil is presented below. The endpoints are summarised in Table 8.2-3.

Report:	Submitted for purposes of renewal as conducted on cyprodinil for USA compliance: K-CA 8.2.2.1/02 Minderhout T., Keller K., Gallagher S.P., & Martin K.H. (2014) Cyprodinil – An Early Life-Stage Toxicity Test with the Sheepshead Minnow (*Cyprinodon variegatus*). Report Number 528A-265. Wildlife International, A Division of EAG Inc. 8598 Commerce Drive Easton, MD 21601 USA. (Syngenta File No. CGA219417_50676)

Guidelines

OECD Guideline 210: Fish, Early-life Stage Toxicity Test (1992)

EPA OPPTS 850.1400: Fish, Early-life Stage Toxicity Test (1996)

GLP: Yes

Executive Summary

The toxicity of CGA219417 to early-life stages of sheepshead minnow (*Cyprinodon variegatus*) was determined. Fish were exposed to the following range of nominal concentrations of 21.9, 43.8, 87.5, 175 and 350 µg a.s./L, a solvent control and a dilution water control under flow-through conditions for 34 days. The mean measured concentrations were 20.0, 40.6, 83.2, 156 and 295 µg a.s./L.

The NOEC was 40.6 µg a.s./L. The LOEC was 83.2 µg a.s./L. The EC_{10} values based on hatching success and survival were 229 and 130 µg a.s./L, respectively. The EC_{20} value based on survival was 164 µg a.s./L. The data were not suitable to calculate EC_{20} value based on hatching success and EC_{10} and EC_{20} values based on growth (length, wet and dry weight).

Materials

Test Material	Cyprodinil Technical CGA219417
Description:	n/a
Lot/Batch #:	P.012011
Actual content of a.s.:	99.2%
Density:	n/a
Stability of test compound:	Reanalysis date: April 2014
Treatments	
Test concentrations:	Nominal: 21.9, 43.8, 87.5, 175 and 350 µg a.s./L Mean measured 20.0, 40.6, 83.2, 156 and 295 µg a.s./L
Control:	Dilution water is dechlorinated tap water
Vehicle and/or positive control:	Dimethylformamide (DMF) Cyprodinil
Analysis of test concentrations:	Yes, at day 0, 6, 14, 21, 28 and 34
Test animals	
Species:	Sheepshead minnow (*Cyprinodon variegate)*
Source:	Embryos were obtained from Aquatic BioSystems, Inc., Fort Collins, Colorado. Embryos were approximately 21 to 26 hours old at test initiation.
Acclimatisation period:	None
Treatment for disease:	None
Feeding:	Newly-hatched larvae were fed an excess amount of live brine shrimp nauplii (*Artemia* sp.) three times per day during the first seven days of post-hatch. Thereafter, they were fed live brine shrimp nauplii three times per day on weekdays and at least two times per day on weekends.
Test design	
Exposure regime:	Flow-through
Aeration:	Gentle
Replication:	4 replicates of 20 embryos
Test vessels:	9 L glass tanks with a working volume of 7 L
Duration:	34 days (6-day incubation period plus a 28-day post-hatch growth period)
Environmental conditions	
Test temperature:	24.2 to 25.2 °C
pH:	7.7 to 7.9
Dissolved oxygen:	7.2 to 7.4 mg/L
Lighting:	16 hours fluorescent light and 8 hours dark with 30 minute dawn and dusk transition periods (537 lux)

Study Design and Methods

Experimental dates: 24th February to 7th April 2014

A flow-through test system was employed. At the start of the test 20 embryos were randomly allocated to egg cups and one egg cup suspended in each of four replicate test vessels at each test and control treatment. Hence, 80 eggs were exposed at each treatment. The test was undertaken in a temperature controlled water-bath.

Eggs and fry were exposed to mean measured concentrations of 20.0, 40.6, 83.2, 156 and 295 µg a.s./L, a solvent control and a dilution water control. All test solutions and the solvent control contained 0.02 mL/L DMF, with the control containing dilution water only.

Stock solutions were prepared once during the definitive study. A primary test substance stock solution was prepared in HPLC-grade DMF at a nominal concentration of 17500 µg a.s./mL. Proportional dilutions of the primary stock were made in DMF to prepare four additional stock solutions at nominal concentrations of 1095, 2190, 4375 and 8750 µg a.s./mL. The primary stock solution was mixed by sonication for approximately 10 minutes followed by inversion at least 20 times. The stock solutions were delivered to the diluter mixing chambers (at a rate of 4.0 µL/minute) where they were mixed with dilution water (at a rate of 200 mL/minute) to achieve the desired test concentrations of 21.9, 43.8, 87.5, 175 and 350 µg a.s./L.

The concentrations of CGA219417 in test solutions were measured at 0, 6, 14, 21, 28 and 32 days using an HPLC method.

Observations for time to hatch, hatching success, larval mortality, deformed larvae and other symptoms of toxicity were made daily during the pre and post-hatch phases, as appropriate. At the end of the test, lengths and dry weights of the surviving fry were measured. No Observed Effect Concentrations (NOEC) was estimated from the data obtained.

Data on time to hatch was evaluated by visual interpretation of the data. Test endpoints analysed statistically for the juvenile fish were hatching success, larval survival and growth of larvae that survived to test end (total length, wet weight and dry weight).

The EC_x values and the 95% confidence intervals based on hatching success and survival were estimated using a linear interpolation method. Since the EC_{20} for survival and EC_{10} and EC_{20} for growth (length, wet and dry weight) values contained zero and/or the 95% confidence intervals were overly wide and the results were estimated by extrapolation, only the NOEC approach indicated in the guideline was used in addition to the EC_{10} for hatching success and EC_{10} and EC_{20} for survival. The results of the statistical analyses were used to aid in the determination of the NOEC, LOEC and MATC. However, scientific judgment was used to determine if statistical differences were biologically meaningful, and if the data followed a concentration-dependent response. The LOEC was defined as the lowest test concentration that produced a significant treatment-related effect on hatching success, survival or growth. The NOEC was the test concentration immediately below the LOEC. The MATC is calculated as the geometric mean of the NOEC and LOEC.

Data from the negative and solvent control groups for each parameter were compared using an appropriate statistical test (t-test). Since no differences were detected in percent hatching success, percent survival or growth (total length, wet weight and dry weights) endpoints between the two control groups ($p > 0.05$), the control data for these parameters were pooled for comparison among the data from the treatment groups.

Hatching success was calculated as the percentage of embryos that hatched successfully. Post-hatch survival was calculated from the number of larvae that survived to test termination as a percentage of the number of embryos that hatched successfully. Hatching success and survival data were considered to be discrete-variable data, while growth data were considered continuous-variable data. Discrete-variable data were analysed using Chi-square and Fisher's Exact test to identify treatment groups that showed a

statistically significant difference ($\alpha = 0.05$) from the pooled controls. All continuous-variable data were evaluated for normality using Shapiro-Wilk's test, and for homogeneity of variance using Levene's tests ($\alpha = 0.01$). Since the data passed the assumptions of normality and homogeneity of variances, those treatments that were significantly different from the pooled control means were identified using Dunnett's one-tailed test ($\alpha = 0.05$).

All statistical tests were performed using a personal computer using TOXSTAT and SAS software.

Results and Discussion

Analytical data

The concentrations of CGA219417 were determined in the test solutions. The mean measured concentrations ranged from 84 to 95% of their nominal concentrations. The limit of quantification was 10.0 µg a.s./L. The mean measured concentrations were used for calculating and reporting the results.

Biological data

Hatching success - Fisher's Exact test detected that there was a statistically significant decrease in hatching success in the 295 µg a.s./L treatment group when compared to the pooled control ($p \leq 0.05$). Therefore, the NOEC for hatching success was 156 µg a.s./L and the LOEC was 295 µg a.s./L. The EC_{10} based on percent hatching success was 229 µg a.s./L with the 95% confidence interval of 161 to 334 µg a.s./L. The EC_{20} based on hatching success was outside of the data range used in the calculation and the 95% confidence intervals were overly wide or contained zero. Therefore, the EC_{20} based on hatching success was not reported.

Larval survival - Fisher's Exact test detected that there were statistically significant decreases in larval survival at the 156 and 295 µg a.s./L treatment groups when compared to the pooled control ($p \leq 0.05$). Therefore, the NOEC for larval survival was 83.2 µg a.s./L and the LOEC was 156 µg a.s./L. The EC_{10} based on survival was 130 µg a.s./L, with the 95% confidence interval of 109 to 159 µg a.s./L. The EC_{20} based on survival was 164 µg a.s./L, with the 95% confidence interval of 154 to 173 µg a.s./L.

Observations - The majority of the fish in the negative control, solvent control and the 20.0, 40.6, 83.2 and 156 µg a.s./L treatment groups appeared normal throughout the test, with occasional observations of fish that were small, weak and noted lying on the bottom of the test chambers for an extended period of time. There was an increased frequency of occurrence of sublethal effects at the 156 and 295 µg a.s./L test concentrations. The sublethal effects noted for the fish in the 156 and 295 µg a.s./L cyprodinil treatment groups were considered to be treatment-related.

Growth - Growth was evaluated at the end of the test by measuring the total length, wet weight and dry weight of each surviving fish. There was a statistically significant reduction in mean total length, wet weight and dry weight in the 83.2 µg a.s./L treatment group in comparison to the pooled control (Dunnett's one-tailed test, $p \leq 0.05$). Consequently, the NOEC was 40.6 µg a.s./L and the LOEC was 83.2 µg a.s./L, based on growth. The EC_{10} and EC_{20} values based on growth (measured as total length, wet weight and dry weight) were not reported since ECx values were outside the data used in calculation and/or the 95% confidence intervals were overly wide.

Table 8.2.2.1-13: Effects of CGA219417 on the growth of Sheepshead minnow *(Cyprinodon variegate)*

Mean measured concentrations (µg a.s./L)	Hatching success (%)	Survival to day 28 post-hatch (%)	Mean length (mm) ± SD	Mean wet weight (mg) ± SD	Mean dry weight (mg) ± SD
Control	93	97	20.3 ± 0.394	105.6 ± 7.27	24.7 ± 2.06
Solvent control	99	99	20.4 ± 0.110	111.3 ± 3.70	24.6 ± 1.21
Pooled control	96	98	20.4 ± 0.269	108.5 ± 6.17	24.7 ± 1.56
20.0	95	99	20.1 ± 0.305	104.3 ± 7.24	24.2 ± 1.32
40.6	96	99	20.2 ± 0.335	107.9 ± 3.42	24.6 ± 0.96
83.2	96	99	19.5 ± 0.391*	97.2 ± 5.18*	21.9 ± 1.14*
156	100	84*	19.7 ± 0.588	105.5 ± 4.98	24.7 ± 1.22
295	83*	0*	-	-	-
EC_{10} (µg a.s./L)	229	130	NA	NA	NA
EC_{20} (µg a.s./L)	NA	164	NA	NA	NA

* Indicates a significant difference ($p<0.05$) from the pooled controls (Fishers Exact test for hatching success and Survival and Dunnett's one-tailed for total length, wet weight and dry weight.)
NA- not applicable
- = no data due to 100% mortality

Conclusions

Sheepshead minnows (*Cyprinodon variegatus*) were exposed to cyprodinil at mean measured concentrations of 20.0, 40.6, 83.2, 156 and 295 µg a.s./L under flow-through conditions for 34 days (a 6-day incubation period plus a 28-day post-hatch growth period). There were statistically significant treatment-related effects on hatching success in the 295 µg a.s./L treatment group and in survival in the 156 and 295 µg a.s./L treatment groups. The most sensitive endpoint for the study was growth. There was a statistically significant treatment related effect on growth (measured as total length, wet and dry weight) in the 83.2 µg a.s./L treatment group. Consequently, the NOEC and LOEC values, based on the growth endpoint, were 40.6 µg a.s./L and 83.2 µg a.s./L, respectively. The MATC was calculated to be 58.1 µg a.s./L. The EC_{10} values based on hatching success and survival were 229 and 130 µg a.s./L, respectively. The EC_{20} value based on survival was 164 µg a.s./L. The calculated EC_{20} values based on hatching success and the calculated EC_{10} and EC_{20} values for growth (length, wet and dry weight) were outside of the data range used in the calculation and the 95% confidence intervals were overly wide or contained zero. Therefore, the ECx for these parameters were not reported.

(Minderhout T, Keller K, Gallagher S, & Martin K, 2014)

Relevant literature on fish embryo toxicity

Scientifically peer-reviewed open literature was found for the potential effects of cyprodinil on fish, and is summarised in the following table. The reports are categorised in accordance with the EFSA Guidance document as:

a. Studies that provide data for establishing or refining risk assessment parameters. These studies should be summarised in detail following the subsequent steps of the OECD Guidance documents (OECD, 2005; 2006) and should be considered for reliability.

b. Studies that are relevant to the data requirement, but in the opinion of the applicant provide only supplementary information that does not alter existing risk assessment parameters. A justification for such a decision should be provided.

c. Studies for which relevance cannot be clearly determined. For each of these studies the applicants should provide an explanation of why the relevance of such studies could not be definitively determined.

Summary of reference

Reference	Categorisation & comments	Summary
Padilla S., Corum D., Padnos B., Hunter D.L., Beam A., Houck K.A., Sipes N., Kleinstreuer N., Knudsen T., Dix D.J. & Reif D.M. (2012) Zebrafish developmental screening of the ToxCast^{TM} Phase I chemical library. *Reproductive Toxicology* **33** 174-187	b). This research provides only supplementary information As the 6d exposure period is for such a short length of time this literature study would be comparable with the acute tests. The regulatory data suggests that zebrafish embryos show similar acute toxicity as the juvenile fish species tested.	A 6 day embryo toxicity test was conducted in which the effects of cyprodinil on zebra fish were evaluated. Test methodology similar to OECD 236 (Fish Embryo Acute Toxicity Test) was used. Embryos were maintained in a 10% Hank's solution in 96-well plates. AC_{50} for cyprodinil was 10.3 µM (2.32 mg/L)

CA 8.2.2.2 Fish full life cycle test

No new data has been generated since the previous submission for cyprodinil.

CA 8.2.2.3 Bioconcentration in fish

No new data has been generated since the previous submission for cyprodinil. The endpoints are summarised in Table 8.1.3-1.

CA 8.2.3 Endocrine disrupting properties

There are no indications of endocrine-disrupting effects from the existing database for this active substance. No conclusions regarding ED effects were made in the EU review.

Report:	K-CA 8.2.3/01 Panter G. (2015) Cyprodinil – Review for Potential for Endocrine Disruption in Ecotoxicology species. Syngenta Ltd. Jealott's Hill International Research Centre Bracknell, Berkshire, RG42 6EY United Kingdom. (Syngenta File No. CGA219417_11639)

Cyprodinil cannot be considered an endocrine disruptor as defined by WHO/IPCS (2002):

"An endocrine disrupter is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations."

An extensive ecotoxicology database exists for cyprodinil. Following evaluation of each of the relevant studies individually and a subsequent weight of evidence evaluation, no concerns for potential endocrine disruption were identified.

Relevant Literature on Endocrine Disrupting Properties

Scientifically peer-reviewed open literature considered relevant for determining effects on endocrine disruption were summarised in the above referenced document. Details of the literature search undertaken can be found in M-CA Section 9 of the current submission.

CA 8.2.4 Acute toxicity to aquatic invertebrates

CA 8.2.4.1 Acute toxicity to *Daphnia magna*

The study previously submitted in the EU were summarised in point B.9.2.4.1 of the DAR. At the request of ANSES a summary has been included in order to aid dossier evaluation~~. therefore additional study summaries are not repeated here~~. The endpoints are summarised in Tables 8.2-4 and 8.2-5.

Report:	Summary submitted at the request of ANSES for ease of dossier evaluation: *KIIA 8.2.4. Boeri R. L., Magazu J.P. & Ward T. J. (1995b). Acute Flow-through Toxicity of CGA-219417 to the Daphnid, Daphnia magna. Report Number 467-CG. T. R. Wilbury Laboratories, Inc. Massachusetts, USA. (Syngenta File No. CGA219417/0461).*

Guidelines

U.S. EPA-FIFRA, Guideline 72-2

GLP: Yes

Executive Summary

The toxicity of CGA219417 to the freshwater invertebrate *Daphnia magna* was determined under flow-through conditions. *Daphnia magna* were exposed to nominal concentrations 23, 38, 60, 90 and 150 µg/L (22.3, 34.0, 60.9, 80.5 and 126 µg/L mean measured concentrations) alongside a dilution medium control and a solvent control. Based on mean measured concentrations, the 48-hour LC_{50} for CGA219417 to *Daphnia magna* was 32.8 µg/L. The 48-hour NOEC was determined to be 22.3 µg/L.

Materials

Test Material	CGA219417
Lot/Batch #:	P012012
Purity:	99.2 %
Description:	Off-white granules
Stability of test compound:	Stable for at least 1 year at room temperature
Reanalysis/expiry date:	7 April 1995
Treatments	
Test concentrations:	Dilution medium control, solvent control and nominal concentrations of 23, 38, 60, 90 and 150 µg/L. Mean measured concentrations of 22.3, 34.0, 60.9, 80.5 and 126 µg/L
Solvent:	Acetone
Positive control:	None
Analysis of test concentrations:	Yes, HPLC analysis of CGA219417 at 0 and 48 hours
Test organism	
Species:	*Daphnia magna* < 24 hours old
Source:	In-house culture, original supplied by Aquatic Research Organisms, Hampton, New Hampshire.

Culture medium:	Carbon filtered, dechlorinated tap water adjusted to a total hardness of approx. 160 – 180 mg as $CaCO_3$
Test design	
Test vessels:	20 litre glass aquaria containing 15 litres of test solution
Test medium:	Carbon filtered, dechlorinated tap water adjusted to a total hardness of approx. 160 – 180 mg as $CaCO_3$
Replication:	Two replicates for each treatment and control (10 organisms per replicate)
Exposure regime:	Flow-through
Aeration:	No
Duration:	48 hours
Environmental conditions	
Test temperature:	19.8 to 20.1 °C
pH:	test start: 8.2 to 8.4 test end: 8.3 to 8.4
Lighting:	16 hour light and 8 our dark with a 15 minute transition. Light intensity of 48 footcandles

Study Design and Methods

Experimental dates: 25th to 27th October 1994

A stock solution with a nominal concentration of 1500 mg/L was prepared by dissolving 0.378 g of the test item in acetone and adjusting the final volume to 250 mL with acetone. Appropriate amounts of stock solution were delivered into the test vessels using a proportional diluter system. The diluter was calibrated before, after and during the test. An average of 5.6 volume additions occurred per 24 hours.

The test was performed in 20 L glass aquaria containing 15 L of test solution. The test organisms were exposed in chambers that consisted of a glass cylinder with a silicone mesh bottom. These chambers were suspended within the test vessels with 20 *D. magna* neonates distributed between two replicate chambers for each treatment group. The test vessels were randomly arranged in a water bath during the 48-hour exposure to maintain a constant temperature.

The number of surviving organisms and the occurrence of sublethal effects was determined visually and recorded after 24 and 48 hours.

Dissolved oxygen, pH, conductivity and temperature was measured daily in each test vessel that contained live animals. The temperature in one test vessel was recorded continuously during the test.

The test concentrations were verified by chemical analysis of CGA219417 at 0 and 48 hours, using high performance liquid chromatography.

The 24 and 48-hour LC_{50} values were calculated using the Probit method based on the number of dead organisms and the mean measured concentrations. The no observed effect concentration was defined as the concentration of the test substance that allowed at least 90 % survival and did not cause any sublethal effects.

Results and Discussion

Mean measured concentrations of 22.3, 34.0, 60.9, 80.5 and 126 µg/L were determined. The measured concentrations at 0 and 48 hours are presented below.

Table 8.2.4.1-1: Analytical results

Nominal concentrations (mg/L)	Measured Concentration of CGA219417 (µg/L)		
	0 hours	48 hours	Mean Measured
0 (control)	<8.0	<8.0	<8.0
0 (solvent control)	<8.0	<8.0	<8.0
23	21.6	22.9	22.3
38	34.0	33.9	34.0
60	45.9	75.9	60.9
90	73.6	87.3	80.5
150	141	111	126

Water quality throughout the test was within acceptable limits. During the exposure period the conductivity ranged from 540 to 570 µmhos/cm, the pH ranged from 8.2 to 8.6, temperature ranged from 19.8 to 20.1 °C and the dissolved oxygen ranged from 8.1 to 9.1 mg/L.

Control and solvent control survival was 95 % and no sublethal effects were observed in these treatments. The survival recorded in each treatment group and the estimated LC_{50} and NOEC values are presented below.

Table 8.2.4.1-2: Effects of CGA219417 on *Daphnia magna* survival after 24 and 48 hours exposure

Mean measured concentration (µg/L)	No. of daphnids tested	Number alive 24 hours		Number alive 48 hours	
		No.	%	No.	%
Control	20	20	100	19	95
Solvent Control	20	19	95	19	95
22.3	20	20	100	18	90
34.0	20	16	80	6	30
60.9	20	16	80	3	15
80.5	20	13	65	0	0
126	20	12	60	0	0
LC_{50} µg/L (95 % Confidence limits)		>126 (n.d.)		32.8 (27.5 – 38.2)	

Conclusions

Based on mean measured concentrations, the 24 and 48-hour LC_{50} for CGA219417 to *Daphnia magna* was >126 and 32.8 µg/L, respectively. The 48-hour NOEC was 22.3 µg/L.

(Boeri *et al.* 1995b)

Metabolites of cyprodinil

At the request of ANSES summaries of studies previously submitted have been included in order to aid dossier evaluation.The endpoints are summarised in Table 8.2-5.

Report:	Summary submitted at the request of ANSES for ease of dossier evaluation: *KIIA 8.2.4. Maetzler P. (1999). Acute Toxicity of CGA 249287 (Metabolite of CGA219417) to Daphnia magna (Immobilisation Test). Report Number 983826. Novartis Services AG, Ecotox Center, CH-4002 Basel, Switzerland. (Syngenta File No. CGA249287/0008).*

Guidelines

OECD Guideline for Testing of Chemicals, Section 2, No. 202 Part I (1993)

Commission Directive 92/69/EEC, Annex L 383 A, C.2 (1992)

GLP: Yes

Executive Summary

The toxicity of CGA249287 to the freshwater invertebrate *Daphnia magna* was determined in a limit test performed under static conditions. *Daphnia magna* were exposed to a single nominal CGA249287 test concentration of 100 mg/L alongside a dilution medium control. No immobilisation or sublethal effects were observed in the control or 100 mg/L treatment group. During the test the measured CGA249287 concentration was 96.9% of nominal at the start of the study and 99.2% at the end of the 48-hour exposure.

Based on the nominal concentration, the 48-hour EC_{50} for CGA249287 to *Daphnia magna* was >100 mg/L. The 48-hour NOEC was determined to be 100 mg/L, the highest concentration tested.

Materials

Test Material	CGA249287 (metabolite of CGA219417)
Lot/Batch #:	HU-15701
Purity:	99 ± 2 %
Description:	Beige powder
Stability of the test compound:	Stable under test conditions
Reanalysis/expiry date:	December 2001
Treatments	
Test concentration:	Dilution medium control and a single CGA249287 concentration of 100 mg/L.
Solvent:	None
Positive control:	Performed every three months using potassium dichromate. Most recent performed 7 January 1999
Analysis of test concentrations:	Yes, LC analysis of CGA249287at 0 and 48 hours

Test organism

Species:	*Daphnia magna* Straus, clone 5 (< 24 hours old)
Source:	In-house culture
Culture medium:	Elendt M4 medium

Test design

Test vessels:	250 mL beaker containing 100 mL test solution
Test medium:	Fortified distilled water with a total hardness of 214 mg/L CaCO
Replication:	Two replicates for each treatment and control (10 organisms per replicate)
Exposure regime:	Static
Aeration:	No
Duration:	48 hours

Environmental conditions

Test temperature:	21 °C
pH:	test start: 7.8 test end: 8.0
Dissolved oxygen	96 - 98 %
Lighting:	14 hour light and 10 our dark

Study Design and Methods

Experimental dates: 18th January to 17th February 1999

The test solution was prepared by dissolving 100.1 mg of the test item with approximately 100 mL test medium in a beaker. The mixture was homogenised by ultrasonic treatment for 18 minutes and made up to 1000 mL with test medium.

Twenty *D. magna* were exposed to the 100 mg/L treatment and control groups with two replicate 250 mL test vessels containing 10 organisms. The test vessels were maintained at 20 ± 2°C with >60% dissolved oxygen. The number of immobilised *Daphnia* in each test vessel was recorded at 24 and 48 hours. Animals not able to swim within 15 seconds after gentle agitation of the test vessel were considered immobile.

Dissolved oxygen, pH and temperature were measured at the start and end of the exposure period. The temperature in one test vessel was recorded continuously during the test.

The test concentrations were verified by chemical analysis of CGA249287 at 0 and 48 hours, using liquid chromatography.

No statistical analysis was performed on the study results as a limit test design was employed and no effects were observed at the tested CGA249287 concentration.

Results and Discussion

Measured concentration of CGA249287 was 96.85 mg/L at the start and 99.2 mg/L at the end of the study. The results of the study are based on the nominal concentration.

Water quality throughout the test was within acceptable limits. During the exposure period the pH ranged from 7.8 to 8.0, temperature was maintained constant at 21°C and the dissolved oxygen ranged from 96 to 98% of saturation.

Control survival was 100% after 48 hours. No immobilisation or sublethal effects were observed in the 100 mg/L treatment and therefore the 48-hour NOEC and EC_{50} values of CGA249287 to *Daphnia magna* were concluded to be 100 mg/L and >100 mg/L, respectively.

Table 8.2.4.1-3: Effects of CGA249287 on *Daphnia magna* survival after 24 and 48 hours exposure

Nominal concentration (mg/L)	No. of daphnids tested	Number immobilised 24 hours		Number immobilised 48 hours	
		No.	%	No.	%
Control	20	0	0	0	0
100	20	0	0	0	0
LC_{50} mg/L (95 % Confidence limits)		>100 (n.d.)		>100 (n.d.)	
NOEC (mg/L)		100		100	

Validity Criteria

The study was considered valid as the following criteria were fulfilled:

- Less than 10 % immobilisation occurred in the control (actual: 0%)
- The dissolved oxygen concentration was ≥60%

Conclusions

Based on nominal concentrations, the 48-hour EC_{50} for CGA249287 to *Daphnia magna* was >100 mg/L. The 48-hour NOEC was concluded to be 100 mg/L.

(Maetzler P. 1999)

Report:	Summary submitted at the request of ANSES for ease of dossier evaluation: *KIIA 8.2.4. Maetzler P. (2001). Acute Toxicity of CGA 275535 (Metabolite of CGA 219417) to Daphnia magna (Immobilisation Test). Report Number 2003590. Solvias AG, Ecotox Center, CH-4002 Basel, Switzerland. (Syngenta File No. CGA275535/0016).*

Guidelines

OECD Guideline for Testing of Chemicals, Section 2, No. 202 Part I (1993)

Commission Directive 92/69/EEC, Annex L 383 A, C.2. (1992)

GLP: Yes

Executive Summary

The toxicity of CGA275535 to the freshwater invertebrate *Daphnia magna* was determined in a 48-hour acute toxicity test performed under static conditions. Young *Daphnia magna* were exposed to nominal CGA275535 concentrations of 0.9, 1.9, 4.3, 9.4 and 21 mg/L and a dilution medium control. The measured concentrations of CGA2755535 were in the range of 83.8 – 117.6% of nominal at the start and 83.9 – 100.6% at the end of the exposure.

No immobilisation was observed in the control or nominal concentrations of 0.9, 1.9 and 4.3 mg/L. Immobilisation of 85 and 100% was observed in the 9.4 and 21 mg/L treatment groups, respectively. Based on nominal concentrations, the 48-hour EC_{50} for CGA275535 to *Daphnia magna* was 6.8 mg/L with 95% confidence limits of 4.3 – 9.4.

Materials

Test Material	CGA275535
Lot/Batch #:	KI 6230/3
Purity:	99 ± 2 %
Description:	Slightly yellow-beige, powder
Reanalysis/expiry date:	31 October 2003
Treatments	
Test concentrations:	Dilution medium control and nominal concentrations of 0.9, 1.9, 4.3, 9.4 and 21 mg/L (mean measured 0.75, 2.02, 4.11, 9.46 and 20.38 mg/L)
Solvent:	None
Positive control:	Sensitivity check performed every three months using potassium dichromate. Most recent performed 11 October 2000
Analysis of test concentrations:	Yes, HPLC analysis of CGA275535 at 0 and 48 hours
Test organism	
Species:	*Daphnia magna* Straus, clone 5 (< 24 hours old)
Source:	In-house culture
Culture medium:	Elendt M4 medium
Test design	
Test vessels:	250 mL beaker containing 100 mL test solution
Test medium:	Fortified distilled water with a total hardness of 178 mg/L CaCO
Replication:	Two replicates for each treatment and control (10 organisms per replicate)
Exposure regime:	Static
Aeration:	No
Duration:	48 hours
Environmental conditions	
Test temperature:	20.9 to 21.8 °C
pH:	test start: 7.7 test end: 7.7 to 7.8

Dissolved oxygen	98 to 100 % saturation
Lighting:	14 hour light and 10 hour dark

Study Design and Methods

Experimental dates: 27th November to 18th December 2000

A stock solution was prepared by mixing 50.2 mg of the test item with approximately 100 mL test medium in a beaker. The mixture was homogenised by ultrasonic treatment for 20 minutes and made up to 1000 mL with test medium. The stock solution was then stirred overnight and was observed to be clear. To prepare the 0.9, 1.9, 4.3, 9.4 and 21 mg/L test solutions, 3.6, 7.8, 17, 38 and 82 mL, respectively, of the stock solution was added to test vessels and made up to 200 mL with dilution medium. The 200 mL solutions were divided between two replicate test vessels for each treatment.

Twenty *D. magna* were exposed to each test concentration and control with two replicate vessels containing ten organisms. The test vessels were maintained at 20 ± 2 °C with ≥60 % dissolved oxygen. The number of immobilised *Daphnia* in each test vessels was recorded at 24 and 48 hours. Animals not able to swim within 15 seconds after gentle agitation of the test vessel were considered immobile.

Dissolved oxygen, pH and temperature was measured at the start and end of the exposure period.

The test concentrations were verified by chemical analysis of CGA275535 at 0 and 48 hours, using high performance liquid chromatography (HPLC) with UV detection.

Statistical analysis of the data was performed with a statistical program and the EC_{50} values and confidence intervals were estimated using the binomial distribution.

Results and Discussion

The mean measured concentrations of CGA275535 during the study were 0.75, 2.02, 4.11, 9.46 and 20.38 mg/L. The test material was stable within the test system and remained with ± 20% of the nominal concentration. Therefore, the results of the study are based on nominal concentrations.

Table 8.2.4.1-4: Analytical results

Nominal concentrations (mg/L)	Measured Concentration of CGA275535				
	0 hours		48 hours		Mean Measured
	mg/L	% of nominal	mg/L	% of nominal	
Control	<0.1	-	<0.1	-	<0.1
0.9	0.75	83.8	0.75	83.9	0.75
1.90	2.24	117.6	1.81	95.0	2.02
4.30	4.12	95.8	4.11	95.5	4.11
9.40	9.46	100.7	9.46	100.6	9.46
21.0	20.24	96.4	20.51	97.7	20.38

Water quality throughout the test was within acceptable limits. During the exposure period the pH ranged from 7.7 to 7.8, temperature ranged from 20.9 to 21.8°C and the dissolved oxygen ranged from 98 to 100% of saturation.

Control survival was 100% after 48 hours. No immobilisation was observed in nominal concentrations of 0.9, 1.9 and 4.3 mg/L. Immobilisation of 85 and 100% was observed in the 9.4 and 21 mg/L treatment

groups after 48-hour exposure. Based on nominal concentrations, the 48-hour EC_{50} for CGA275535 to *Daphnia magna* was 6.8 mg/L with 95% confidence limits of 4.3 – 9.4.

Table 8.2.4.1-5: Effects of CGA275535 on *Daphnia magna* survival after 24 and hours exposure

Nominal concentration (mg/L)	No. of daphnids tested	Number immobilised 24 hours		Number immobilised 48 hours	
		No.	%	No.	%
Control	20	0	0	0	0
0.9	20	0	0	0	0
1.9	20	0	0	0	0
4.3	20	0	0	0	0
9.4	20	6	30	17	85
21.0	20	20	100	20	100
EC_{50} mg/L (95 % Confidence limits)		12 (4.3 – 21)		6.8 (4.3 – 9.4)	

Validity Criteria

The study was considered valid as the following criteria were fulfilled:

- Less than 10% immobilisation occurred in the control (actual: 0%)
- The dissolved oxygen concentration was ≥60%

Conclusions

Based on nominal concentrations, the 48-hour EC_{50} for CGA275535 to *Daphnia magna* was 6.8 mg/L with 95 % confidence limits of 4.3 – 9.4.

(Maetzler P. 2001)

Summaries of studies conducted following the original submission of cyprodinil are presented below.

Report:	Submitted for purposes of renewal as conducted on relevant aquatic metabolite: K-CA 8.2.4.1/01 Eckenstein H. (2015) CGA321915 – Acute Toxicity to *Daphnia magna* in a 48-Hour Immobilization Test. Report number D96733. Harlan Laboratories Ltd., Zelgliweg 1, 4452 Itingen, Switzerland. (Syngenta File No. CGA321915_10005)

Guidelines

OECD Guidelines for Testing of Chemicals, Section 2 - Effects on Biotic Systems, Method 202: *Daphnia* sp., Acute Immobilisation Test (2004)

GLP: Yes

Executive Summary

The acute toxicity of CGA321915 to *Daphnia magna* was determined under static conditions. Daphnids were exposed to a range of nominal concentrations of 6.25, 12.5, 25, 50 and 100 mg CGA321915/L

(mean measured: 6.1, 12, 23, 49 and 98 mg CGA321915/L) alongside a dilution water control. Based on mean measured concentrations, the 48-hour EC_{50} was determined to be > 98 mg CGA321915/L, the highest concentration tested.

Materials

Test Material	CGA321915 CSAA400257
Parent:	CGA219417 (Cyprodinil)
Lot/Batch #:	MES 356/1
Purity:	98 % w/w (estimated error: ± 2 %)
Description:	White solid
Stability of test compound:	Stable under test conditions
Reanalysis/Expiry date:	30 September 2016
Density:	Not applicable
Treatments	
Test concentrations:	Dilution water control and nominal concentrations of 6.25, 12.5, 25, 50 and 100 mg CGA321915/L (mean measured: 6.1, 12, 23, 49 and 98 mg CGA321915/L)
Solvent:	None
Positive control:	Potassium dichromate used twice a year
Analysis of test concentrations:	Yes 0 and 48 hours using HPLC analysis with UV detection
Test organisms	
Species:	*Daphnia magna* Straus, Clone 5
Age:	6 - 24 hours
Source:	Continuous laboratory cultures, originally sourced from University of Sheffield UK in 1992
Feeding:	None during test
Culture medium:	Reconstituted water according to ISO 6341
Test design	
Test vessels:	100 mL glass beakers containing 50 mL test medium, covered with glass plates
Test medium:	Reconstituted test water according to ISO 6341. The test water was aerated prior to the start of the study until oxygen saturation was reached.
Replication:	4 replicates of 5 daphnids
Exposure regime:	Static
Duration:	48 hours
Environmental conditions	
Test temperature:	21 – 22 °C
pH range:	7.9 to 8.1
Dissolved oxygen:	8.2 to 8.4 mg/L
Total hardness of dilution water:	250 mg/L as $CaCO_3$.
Lighting:	16 hours light (800 - 1060 Lux) and 8 hours dark with a 30 minute transition period

Study Design and Methods

Experimental dates: 2nd December 2014 to 6th January 2015

The test medium with the highest nominal concentration of 100 mg CGA321915/L was prepared by dissolving 100.41 mg of the test item in 1000 mL of test water under intense stirring for 15 minutes at

room temperature. Appropriate volumes of this test solution were diluted with test water to prepare the test media of the lower test item concentrations. The control consisted of dilution water only. Test solutions were added to the test vessels and the *Daphnia* added without conscious bias.

The immobility of the daphnids was determined by visual observations after 24 and 48 hours of exposure. Organisms unable to swim within 15 seconds after gentle agitation of the test beaker were considered to be immobile.

The pH, temperature and dissolved oxygen were measured at the start, after 24 hours and at the end of the test in each test concentration and the control.

The test concentrations were verified by chemical analysis of CGA321915 at 0 and 48 hours using HPLC with UV detection. The 48-hour samples were taken from pooled replicates.

Results and Discussion

At the start of the test, the analytically determined concentrations of CGA321915 were in the range 83 to 100 % of the nominal values and at the end of the test were in the range 98 to 102 %, (see table below). The limit of quantification in this study was 1.03 mg CGA321915/L. Arithmetic mean measured concentrations were used for the calculation and reporting of results.

Table 8.2.4.1-16: Analytical results

Nominal concentrations (mg CGA321915/L)	% of nominal measured at 0 hours	% of nominal measured at 48 hours	Mean measured concentrations (mg CGA321915/L)
Control	n.a.	n.a.	n.a.
6.25	94	102	6.1
12.5	98	100	12
25	83	98	23
50	100	98	49
100	97	99	98

n.a. = not applicable
The tabulated values of the samples represent rounded results obtained by calculation using the exact raw data

The median effect concentration (EC_{50}) is defined as the concentration resulting in 50 % immobilisation of the *Daphnia* in the time period specified, and could not be calculated due to the absence of toxicity of the test item. The NOEC (No Observed Effect Concentration) is defined as the highest tested concentration which did not produce an adverse effect when compared to the control and was determined directly from the raw data. There was no immobility observed in the dilution water control. Immobility data and estimated EC_{50} values are shown in the table below:

Table 8.2.4.1-27: Effects of CGA321915 on *Daphnia magna* following exposure for 48-hours in a static test

Mean measured concentration (mg CGA321915/L)	Immobilised daphnids after 24 hours		Immobilised daphnids after 48 hours	
	Number	%	Number	%
Control	0	0	0	0
6.1	0	0	0	0
12	0	0	0	0
23	0 (10 A)	0	0 (8A)	0
49	0 (12 A)	0	0 (12A)	0

Mean measured concentration	Immobilised daphnids after 24 hours		Immobilised daphnids after 48 hours	
98	0 (13A)	0	0 (18A)	0
EC_{50} (mg CGA321915/L)	> 98			
95% Confidence limits	n.d.			
NOEC (mg CGA321915/L)	98			

Values in parenthesis: number of test animals with adverse effects;
A: daphnids trapped at the water surface
n.d. = could not be determined

Validity Criteria

The validity criteria for the test were met:

- Immobilisation or signs of disease or stress in the control ≤10 % (observed 0 %)
- Dissolved oxygen concentration at the end of the test ≥ 3 mg/L in the control and test vessels (measured: 8.2 to 8.4 mg/L)

Conclusions

Based on mean measured concentrations, the 48-hour EC_{50} was determined to be > 98 mg CGA321915/L, the highest concentration tested. The 48-hour NOEC was determined to be 98 mg CGA321915/L.

(Eckenstein H, 2015)

Report:	Submitted for purposes of renewal as conducted on relevant aquatic metabolite: K-CA 8.2.4.1/02 Vial A. (1991a) Report on the acute toxicity test of CGA 263208 intermediate on Daphnia (*Daphnia magna* Straus 1820). Report number 918090. Ciba-Geigy Ltd., Product Safety, Ecotoxicology CH-4002 Basel, Switzerland. (Syngenta File No. CA1059/0010)

Guidelines

OECD Guideline No. 202, Part I, 1984

GLP: Yes

Executive Summary

The 48 h acute toxicity of CGA263208 to *Daphnia magna* was determined under static conditions. Daphnids were exposed to a range of nominal concentrations of 3.2, 5.8, 10, 18 and 32 mg CGA263208/L alongside a dilution water control. Based on nominal concentrations, the 48-hour EC_{50} was 20.6 mg CGA263208/L.

Materials

Test Material CGA263208
Lot/Batch #: P.010010
Purity: 72.7 %
Description: Grey solid

Reanalysis/Expiry date:	31/12/92

Treatments

Test concentrations:	Dilution water control and nominal concentrations 3.2, 5.8, 10, 18, 32 mg CGA 263208/L
Solvent:	None
Positive control:	None
Analysis of test concentrations:	Yes, analysis of CGA 263208 at 0 and 48 hours using HPLC analysis

Test organisms

Species:	*Daphnia magna* Straus 1820
Age:	< 24 hours
Source:	Continuous laboratory cultures
Feeding:	None during test
Culture medium:	Reconstituted water as described below

Test design

Test vessels:	beakers containing 100 mL test medium
Test medium:	Bidistilled water with the following composition: $NaHCO_3$; 65 mg/L, $MgSO_4.7H_2O$; 123 mg/L, KCl; 6 mg/L, $CaCl_2.2H_2O$; 294 mg/L.
Replication:	4 replicates of 5 daphnids
Exposure regime:	Static
Duration:	48 hours

Environmental conditions

Test temperature:	21 °C
pH range:	7.7 – 7.9
Dissolved oxygen:	96 - 98 %
Total hardness of dilution water:	240 mg/L $CaCO_3$.
Lighting:	Fluorescent light, 16 hours light and 8 hours dark, 1500 lux

Study Design and Methods

Experimental dates: 23rd April 1991 to 7th June 1991

A stock solution was prepared by dissolving 200 mg of CGA263208 completely in 2000 mL of test water. This stock solution was used to make nominal testing concentrations of, 3.2, 5.8, 10, 18 and 32 mg CGA263208/L. The control consisted of dilution water only. Test solutions were added to the test vessels and then *Daphnia* added.

The immobilisation of the daphnids was determined by visual observations after 24 and 48 hours of exposure.

The pH, temperature and dissolved oxygen were measured at the start and end of the test in each test concentration and the control.

The test concentrations were verified by chemical analysis of CGA263208 at 0 and 48 hours using high performance liquid chromatography (HPLC).

Results and Discussion

At the start of the test, the analytically determined concentrations of CGA263208 were in the range 102 to 132 % of the nominal values and at the end of the test were in the range 91 to 113 % (see table below). As mean measured concentrations for the duration of the test were between 80-120 % (barring the highest concentration (123%)) nominal concentrations were used for the calculation and reporting of the results.

Table 8.2.4.1-38: Analytical results

Nominal concentrations (mg CGA 263208/L)	% of nominal concentrations measured at 0 hours	% of nominal concentrations measured at 48 hours	Mean % of nominal concentrations measured during test
Blank	-	-	-
3.2	103	91	97
5.8	102	97	100
10	106	106	106
18	123	111	117
32	132	113	123

The median effect concentration (EC_{50}) was defined as the concentration resulting in 50% immobilisation of the *Daphnia* in the time period specified. These values were determined according to Berkson (1953) using gausso-logarithmic paper. There was no immobility observed in the dilution water control. Immobility data and estimated EC_{50} values are shown in the table below:

Table 8.2.4.1-9: Effects of CGA263208 on *Daphnia magna* following exposure for 48 hours in a static test

Nominal concentration (mg CGA 263208/L)	Immobilised daphnids after 24 hours		Immobilised daphnids after 48 hours	
	Number	%	Number	%
Blank	0	0	0	0
3.2	0	0	0	0
5.8	0	0	0	0
10	0	0	0	0
18	1	5	4	20
32	10	50	20	100
EC_{50} mg CGA 263208/L	32.7		20.6	
95% Confidence limits (CGA 263208/L)	26.7 – 48.3		18.06 – 24.7	

Validity Criteria

- Mortality in the control should be ≤10% (actual recorded was 0%).
- The dissolved oxygen content should be ≥3 mg/L in control and test vessels (actual measured was 96 to 98%).

Conclusions

Based on nominal concentrations, the 48-hour EC_{50} for CGA263208 to *Daphnia magna* was 20.6 mg CGA263208/L.

(Vial A, 1991a)

Report:	Submitted for purposes of renewal as conducted on relevant aquatic metabolite: K-CA 8.2.4.1/03 Grade R. (1992a) Report on the acute toxicity test of CA 1139 A on *Daphnia* (*Daphnia magna* Straus 1820). Report number 928173, Ciba-Geigy Ltd., Product Safety, Ecotoxicology CH-4002 Basel, Switzerland. (Syngenta File No. CA1139/0009)

Guidelines

OECD Guideline No. 202, Part I, 1984

GLP: Yes

Executive Summary

The acute toxicity of CA1139A to *Daphnia magna* was determined under static conditions. Daphnids were exposed to a range of nominal concentrations of 5.8, 10, 18, 32, 58 and 100 mg CA1139A/L alongside a dilution water control. Based on nominal concentrations, the 48-hour EC_{50} was 15.7 mg CA1139A/L, the highest concentration tested.

Materials

Test Material	CA1139A
Lot/Batch #:	P.201025
Purity:	76.2 %
Description:	Beige powder
Reanalysis/Expiry date:	02/93
Treatments	
Test concentrations:	Dilution water control and nominal concentrations 5.8, 10, 18, 32, 58 and 100 mg CA1139A/L
Solvent:	None
Positive control:	None
Analysis of test concentrations:	Yes, analysis of CA1139A at 0 and 48 hours using HPLC analysis
Test organisms	
Species:	*Daphnia magna* Straus 1820
Age:	< 24 hours
Source:	CIBA-GEIGY Ltd., testing facility
Feeding:	None during test
Culture medium:	Reconstituted water
Test design	
Test vessels:	beakers containing 100 mL test medium
Test medium:	Bidistilled water with the following composition: $NaHCO_3$; 65 mg/L, $MgSO_4.7H_2O$; 123 mg/L, KCl; 6 mg/L, $CaCl_2.2H_2O$; 294 mg/L.
Replication:	5 replicates of 5 daphnids
Exposure regime:	Static
Duration:	48 hours
Environmental conditions	
Test temperature:	20 – 22 °C

pH range:	7.9 – 8.3
Dissolved oxygen:	101 – 104 %
Total hardness of dilution water:	240 mg/L $CaCO_3$
Lighting:	16 hours light and 8 hours dark, 1500 lux

Study Design and Methods

Experimental dates: 1st September to 16th October 1992

A stock solution was prepared by dissolving 200.2 mg of CA1139A completely in 1000 mL of test water. The stock was used to make nominal test concentrations as follows; 5.8, 10, 18, 32, 58 and 100 mg CA1139A/L. The control consisted of dilution water only. Test solutions were added first to the test vessels and then the *Daphnia* added.

The immobilisation of the daphnids was determined by visual observations after 0, 24 and 48 hours of exposure.

The pH, temperature and dissolved oxygen were measured at the start and end of the test in each test concentration and the control.

The test concentrations were verified by chemical analysis of CA1139A at 0 and 48 hours using high performance liquid chromatography (HPLC).

Results and Discussion

At the start of the test, the analytically determined concentrations of CA1139A were in the range 102 to 104 % of the nominal values and at the end of the test they were in the range 87 to 105 % (see table below). Nominal concentrations were used for the calculation and reporting of results.

Table 8.2.4.1-510: Analytical results

Nominal concentrations (mg CA1139A/L)	% of nominal measured at 0 hours	% of nominal measured at 48 hours
Blank	-	-
5.8	102	102
10	100	101
18	102	87
32	103	90
58	104	101
100	104	104

The median effect concentration (EC_{50}) was defined as the concentration resulting in 50% immobilisation of the *Daphnia* in the time period specified. These were graphically determined on gausso-logarithmic probability paper as described by Berkson (1959) JASA 48, 569-599. There was no immobility observed in the dilution water control. Immobility data and estimated EC_{50} values are shown in the table below:

Table 8.2.4.1-~~6~~11: Effects of CA1139A on *Daphnia magna* following exposure for 48 hours in a static test

Nominal concentration (mg CA1139A/L)	Immobilised daphnids after 24 hours		Immobilised daphnids after 48 hours	
	Number	%	Number	%
Blank	0	0	0	0
5.8	0	0	0	0
10	0	0	0	0
18	0	0	16	80
32	17	85	20	100
58	20	100	20	100
100	20	100	20	100
EC_{50} mg CA1139A/L	27.3		15.7	
95% Confidence limits (CA1139A/L)	22.4-31.4		13.1-17.9	

Validity Criteria

Validity criteria as per OECD guideline 202 were met.

- Control mortality did not exceed 10 % and no abnormal observations were made/ reported
- Dissolved oxygen in all test vessels exceeded 3 mg/L

Conclusions

Based on nominal concentrations, the 48-hour EC_{50} for CA1139A to *Daphnia magna* was 15.7 mg CA1139A/L.

(Grade R, 1992a)

CA 8.2.4.2 Acute toxicity to an additional aquatic invertebrate species

~~The studies previously submitted in the EU are not summarised here.~~ At the request of ANSES summaries have been included in order to aid dossier evaluation. The endpoints are summarised in Tables 8.2-4 and 8.2-5.

Report:	Summary submitted at the request of ANSES for ease of dossier evaluation: *KIIA 8.2.4. Peither A. (2000). Acute Toxicity of CGA 219417 A tech. to Daphnia longispina in a 48-hour Static Immobilisation Test. Report Number 2003727. RCC Ltd, CH-4452 Itingen, Switzerland. (Syngenta File No. CGA219417/0993).*

Guidelines

Based on:

OECD Guideline for Testing of Chemicals, No. 202, *Daphnia* sp., Acute Immobilisation Test and Reproduction Test, Part I (1984)

Commission Directive 92/69/EEC, C.2, Acute Toxicity for *Daphnia* (1992)

US EPA OPPTS No. 850.1010, Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids

US EPA FIFRA – Guideline No. 72-2, Acute Toxicity Test for Freshwater Aquatic Invertebrates

GLP: Yes

Executive Summary

The toxicity of CGA219417 A tech. to the freshwater invertebrate *Daphnia longispina* was determined in a 48-hour acute toxicity test performed under static conditions. *Daphnia longispina* were exposed to mean measured CGA219417 A tech. concentrations of 0.021, 0.044, 0.096, 0.21, 0.46 and 1.1 mg/L and a dilution media control. The measured concentrations at the end of the exposure period ranged from 97 to 107% of the initial measured concentrations, indicating the test material was stable in the test system for the duration of the exposure.

Based on mean measured concentrations, the 48-hour EC_{50} for CGA219417 A tech. to *Daphnia longispina* was 0.22 mg/L with 95 % confidence limits of 0.12 to 0.40 mg/L.

Materials

Test Material	CGA219417 A tech.
Lot/Batch #:	P.012010
Purity:	99.4%
Description:	Beige solid
Stability of the test compound:	Hydrolytically stable: DT_{50} >1 year
Reanalysis/expiry date:	December 2004
Treatments	
Test concentrations:	Dilution medium control and measured concentrations of 0.021, 0.044, 0.096, 0.21, 0.46 and 1.1 mg/L
Solvent:	None
Positive control:	None
Analysis of test concentrations:	Yes, HPLC analysis of CGA219417 A tech. at 0 and 48 hours
Test organism	
Species:	*Daphnia longispina*
Source:	Field collected from Novartis Crop Protection AG, Kanton Aargau, Switzerland.
Acclimation	Collected one day prior to the test start and placed in filtered pond water under similar environmental conditions as those used in the study
Test design	
Test vessels:	100 mL glass beaker containing 50 mL test solution
Test medium:	Filtered natural pond water, collected from same location as test organisms
Replication:	Four replicates for each treatment and control (5 organisms per replicate)
Exposure regime:	Static
Aeration:	No
Duration:	48 hours

Environmental conditions

Test temperature:	20 to 21°C
pH:	8.0 to 8.2
Dissolved oxygen:	8.3 to 8.5 mg/L
Lighting:	16 hour light and 8 hour dark with a 30 minute transition

Study Design and Methods

Experimental dates: 18th July to 9th August 2000

A supersaturated stock dispersion with a loading rate of 100 mg/L was prepared by weighing 300 mg of the test item into 3000 mL test medium. The mixture was homogenised by ultrasonic treatment for 15 minutes and by intense stirring for 3 hours at room temperature in the dark. The saturated solution was then filtered through a 0.45 µm membrane filter before preparation of the test solutions.

The undiluted filtrate of the saturated solution was used as the highest test concentration, and lower concentrations were prepared by dilution of the filtrate with filtered pond water. Dilutions of 1:10, 1:22, 1:46, 1:100, 1:220 and 1:460 were used as the lower test concentrations. The actual concentrations of the test item were determined at the start and end of the 48-hour exposure period using high performance liquid chromatography (HPLC) with UV detection.

Twenty test organisms split between four replicates were exposed to each test concentration and the control. The test vessels were maintained at 20 - 21°C during the exposure period. The number of immobilised or dead *Daphnia longispina* in each test vessels was recorded at 24 and 48 hours. Animals not able to swim within 15 seconds after gentle agitation of the test vessel were considered immobile.

Dissolved oxygen, pH and temperature was measured at the start and end of the exposure period in each test concentration and the control. The total hardness and conductivity of the test medium was measured at the start of the test.

The 24 and 48-hour EC_{50} values were estimated using the Probit method and all endpoints were based on the mean measured concentrations.

Results and Discussion

The mean measured concentrations of CGA219417 A tech. during the study were 0.021, 0.044, 0.096, 0.21, 0.46 and 1.1 mg/L. The test material was stable within the test system during the exposure duration, samples collected from the 48-hour test solutions were within 97 to 107% of the initial measured concentrations.

Table 8.2.4.2-1: Analytical results

Dilution	Measured Concentration 0-hour (mg/L)	Measured Concentration 48-hour (mg/L)	Mean Measured (mg/L)
Control	Not detected	Not detected	-
1:460	0.021	0.021	0.021
1:220	0.044	0.044	0.044
1:100	0.097	0.095	0.096
1:46	0.214	0.208	0.211
1:22	0.450	0.460	0.455
1:10	1.02	1.09	1.05

Water quality throughout the test was within acceptable limits. During the exposure period the pH ranged from 8.0 to 8.2 and the dissolved oxygen ranged from 8.3 to 8.7 mg/L. At the beginning of the study the conductivity of the test water was 216 µS/cm and the hardness was 162 mg/L $CaCO_3$.

No immobility was observed in the control after 48 hours and 5% immobilisation was observed in the 0.021 mg/L treatment group. The immobilisation increased steadily from 40% in the 0.044 mg/L to 70% at the highest test concentration.

Table 8.2.4.2-2: Effects of CGA219417 A tech on *Daphnia longispina* survival after 24 and 48 hours exposure

Mean measured concentration (mg/L)	No. of organisms tested	Number immobilised 24 hours		Number immobilised 48 hours	
		No.	%	No.	%
Control	20	0	0	0	0
0.021	20	0	0	1	5
0.044	20	5	25	8	40
0.096	20	3	15	9	45
0.21	20	4	20	11	55
0.46	20	5	25	11	55
1.1	20	7	35	14	70
EC_{50} mg/L (95 % Confidence limits)		>1.1 (n.d.)		0.22 (0.12 – 0.40)	

Validity Criteria

The study was considered valid as the following criterion was fulfilled:

- Less than 10% immobilisation occurred in the control (actual: 0%)

Conclusions

Based on mean measured concentrations, the 48-hour EC_{50} for CGA219417 A tech. to *Daphnia longispina* was 0.22 mg/L with 95 % confidence limits of 0.12 to 0.40 mg/L.

(Peither A. 2000)

Report:	Summary submitted at the request of ANSES for ease of dossier evaluation: *KIIA 8.2.4. Peither A. (2000a). Acute Toxicity of CGA 219417 A tech. to Daphniopsis sp. in a 24-hour Static Immobilisation Test. Report Number 2003735. RCC Ltd., CH-4452 Itingen, Switzerland. (Syngenta File No. CGA219417/0990)*

Guidelines

Based on:

OECD Guideline for Testing of Chemicals, No. 202, *Daphnia* sp., Acute Immobilisation Test and Reproduction Test, Part I (1984)

Commission Directive 92/69/EEC, C.2, Acute Toxicity for *Daphnia* (1992)

US EPA OPPTS No. 850.1010, Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids

US EPA FIFRA – Guideline No. 72-2, Acute Toxicity Test for Freshwater Aquatic Invertebrates

GLP: Yes

Executive Summary

The toxicity of CGA219417 A tech. to the freshwater invertebrate *Daphniopsis* sp. was determined in a 24-hour acute toxicity test performed under static conditions. *Daphniopsis* sp. were exposed to the 1:10, 1:22, 1:46, 1:100, 1:220, 1:460 and 1:1000 dilutions of a saturated stock solution with a CGA219417 A tech. loading rate of 100 mg/L. The exposure was performed for 48 hours, however due to high mortality in the controls only the 24-hour data were considered valid.

The actual concentration of test item was determined at the start and end of the exposure duration in each test concentration and control, with the exception of the 1:1000 dilution treatment. The mean measured concentrations were 1.1, 0.52, 0.25, 0.12, 0.052 and 0.026 mg/L and the samples collected at the end of test were 93 to 100% of the initially measured concentrations, indicating the test material was stable in the test system for the duration of the exposure.

Based on mean measured concentrations, the 24-hour EC_{50} for CGA219417 A tech. to *Daphniopsis* sp. was 0.21 mg/L with 95% confidence limits of 0.15 to 0.28 mg/L.

Materials

Test Material	CGA219417 A tech.
Lot/Batch #:	P.012010
Purity:	99.4%
Description:	Beige solid
Stability of the test compound:	Hydrolytically stable: DT_{50} >1 year
Reanalysis/expiry date:	December 2004

Treatments

Test concentrations:	Dilution medium control and 1:10, 1:22, 1:46, 1:100, 1:220, 1:460 and 1:1000 dilutions of a saturated solution (100 mg/L loading rate). Mean measured concentrations 1.1, 0.52, 0.25, 0.12, 0.052 and 0.026 mg/L
Solvent:	None
Positive control:	None
Analysis of test concentrations:	Yes, HPLC analysis of CGA219417 A tech. at 0 and 24 hours

Test organism

Species:	*Daphniopsis* sp.
Source:	Laboratory culture, originally sourced from a natural lake (Schöhsee, near Plön, Germany)
Acclimation:	The culture was initiated approximately two weeks before the test start. One day prior to the study the organisms were maintained under similar conditions as those employed in the test
Feeding:	The culture was fed *Scenedesmus subpicatus*. Test organisms were not fed during the exposure
Culture medium:	Filtered (55 µm gaze) natural pond water

Test design

Test vessels:	100 mL glass beaker containing 50 mL test solution
Test medium:	Filtered (0.45 µm) natural pond water, collected from Novartis Crop Protection AG, Kanton Aargau, Switzerland
Replication:	Four replicates for each treatment and control (5 organisms per replicate)
Exposure regime:	Static
Aeration:	No
Duration:	24 hours

Environmental conditions

Test temperature:	19 to 20 °C
pH:	7.8 to 8.1
Dissolved oxygen:	8.4 to 8.8 mg/L
Lighting:	16 hour light and 8 hour dark with a 30 minute transition, light intensity was 200 to 1200 lux

Study Design and Methods

Experimental dates: 28th August to 12th September 2000

A supersaturated stock dispersion with a loading rate of 100 mg/L was prepared by weighing 40 mg of the test item into 400 mL test medium. The mixture was homogenised by ultrasonic treatment for 15 minutes and by intense stirring for 3 hours at room temperature in the dark. The saturated solution was then filtered through a 0.45 µm membrane filter before preparation of the test solutions.

The test solutions were prepared by dilution of the filtrate with filtered pond water. Dilutions of 1:10, 1:22, 1:46, 1:100, 1:220, 1:460 and 1:1000 were used as the test concentrations. In addition, a control group, consisting of filtered pond water with no test item, was including in the study design. The actual

concentrations of the test item were determined at the start and end of the exposure period, in all test concentrations with the exception of the 1:1000 dilution, using high performance liquid chromatography (HPLC) with UV detection.

Twenty test organisms split between four replicates were exposed to each test concentration and the control. The test vessels were maintained at 19 - 20°C during the exposure period. The number of immobilised or dead *Daphniopsis* sp. in each test vessels was recorded after 24 hours. Animals not able to swim within 15 seconds after gentle agitation of the test vessel were considered immobile. The exposure was performed for 48 hours, however due to high mortality in the controls only the 24-hour data were considered valid.

Dissolved oxygen, pH and temperature was measured at the start and end of the exposure period in each test concentration and the control. The total hardness and conductivity of the test medium was measured at the start of the test.

The 24-hour EC_{50} value was estimated using the Probit method and all endpoints were based on the mean measured concentrations.

Results and Discussion

The mean measured concentrations of CGA219417 A tech. during the study were 0.026, 0.052, 0.12, 0.25, 0.52 and 1.1 mg/L. The concentration of the test item was not determined in the 1:1000 treatment group. The test material was stable within the test system during the exposure duration, samples collected at the end of the test were within 93 to 100% of the initial measured concentrations.

Table 8.2.4.2-3: Analytical results

Dilution	Measured Concentration 0-hour (mg/L)	Measured Concentration 48-hour (mg/L)	Mean Measured (mg/L)
Control	Not detected	Not detected	-
1:1000	Not analysed	Not analysed	-
1:460	0.027	0.025	0.026
1:220	0.053	0.051	0.052
1:100	0.117	0.115	0.116
1:46	0.250	0.250	0.250
1:22	0.521	0.517	0.519
1:10	1.13	1.15	1.14

Water quality throughout the test was within acceptable limits. During the study the pH ranged from 7.8 to 8.1 and the dissolved oxygen ranged from 8.4 to 8.8 mg/L. At the beginning of the study the conductivity of the test water was 207 µS/cm and the hardness was 162 mg/L as $CaCO_3$.

During the 24-hour test period 10% immobilisation was observed in the control and the 1:1000 dilution. No immobilisation was observed in the 0.026 mg/L treatment group. From the 0.052 mg/L concentration onwards the immobility rate steadily increased up to 100 % at the highest test concentration of 1.1 mg/L.

Table 8.2.4.2-4: Effects of CGA219417 A tech on *Daphniopsis* sp. survival after 24 and 48 hours exposure

Dilution	Mean measured concentration (mg/L)	No. of organisms tested	Number immobilised 24 hours	
			No.	%
Control	-	20	2	10
1:1000	Not analysed	20	2	10
1:460	0.026	20	0	0
1:220	0.052	20	3	15
1:100	0.12	20	7	35
1:46	0.25	20	12	60
1:22	0.52	20	13	65
1:10	1.1	20	20	100
EC_{50} mg/L (95 % Confidence limits)			0.21 (0.15 – 0.28)	

Validity Criteria

The study was considered valid as the following criterion was fulfilled:

- ≤10 % immobilisation occurred in the control (actual: 10% after 24 hours)

The exposure was performed for 48 hours however >10% immobilisation was observed in the control after 48 hours. Therefore, only the 24-hour endpoint was reported.

Conclusions

Based on mean measured concentrations, the 24-hour EC_{50} for CGA219417 A tech. to *Daphniopsis* sp. was 0.21 mg/L with 95% confidence limits of 0.15 to 0.28 mg/L.

(Peither, 2000a)

Report:	Summary submitted at the request of ANSES for ease of dossier evaluation: *KIIA 8.2.4. Peither A. (2000b). Acute Toxicity of CGA 219417 A tech. to Simocephalus vetulus in a 48-hour Static Immobilisation Test. Report Number 2003726. RCC Ltd, CH-4452 Itingen, Switzerland. (Syngenta File No. CGA219417/0994).*

Guidelines

Based on:

OECD Guideline for Testing of Chemicals, No. 202 Part I (1984)

Commission Directive 92/69/EEC, C.2, Acute Toxicity for Daphnia (1992)

US EPA OPPTS No. 850.1010, Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids

US EPA FIFRA – Guideline No. 72-2, Acute Toxicity Test for Freshwater Aquatic Invertebrates

GLP: Yes

Executive Summary

The toxicity of CGA 219417 A tech. to the freshwater invertebrate *Simocephalus vetulus* was determined in a 48-hour acute toxicity test performed under static conditions. *Simocephalus vetulus* were exposed to mean measured CGA 219417 A tech. concentrations of 0.043, 0.13, 0.40, 1.3 and 3.9 mg/L and a dilution media control. The measured concentrations at the end of the exposure period ranged from 99 to 102% of the initial measured concentrations, indicating the test material was stable in the test system for the duration of the exposure.

Based on mean measured concentrations, the 48-hour EC_{50} for CGA 219417 A tech. to *Simocephalus vetulus* was 0.15 mg/L with 95% confidence limits of 0.095 – 0.22 mg/L.

Materials

Test Material	CGA 219417 A tech.
Lot/Batch #:	P.012010
Purity:	99.4%
Description:	Beige solid
Stability of test compound:	Hydrolytically stable: DT_{50} >1 year
Reanalysis/expiry date:	December 2004
Treatments	
Test concentrations:	Dilution medium control measured concentrations of 0.043, 0.13, 0.40, 1.3 and 3.9 mg/L (prepared from a filtered saturation solution)
Solvent:	None
Positive control:	None
Analysis of test concentrations:	Yes, HPLC analysis of CGA 219417 A tech. at 0 and 48 hours
Test organism	
Species:	*Simocephalus vetulus*
Source:	Field collected from Novartis Crop Protection AG, Kanton Aargau, Switzerland
Acclimation	Collected one week prior to the test start and placed in filtered pond water under similar environmental conditions as those used in the study
Test design	
Test vessels:	100 mL glass beaker containing 50 mL test solution
Test medium:	Filtered natural pond water, collected from same location as test organisms
Replication:	Four replicates for each treatment and control (5 organisms per replicate)
Exposure regime:	Static
Aeration:	No
Duration:	48 hours
Environmental conditions	

Test temperature:	19 to 20°C
pH:	8.1 throughout test
Dissolved oxygen	8.2 to 8.6 mg/L
Lighting:	16 hour light and 8 hour dark with a 30 minute transition (200 – 1200 Lux)

Study Design and Methods

Experimental dates: 25th July to 10th August 2000

A supersaturated stock dispersion with a loading rate of 100 mg/L was prepared by weighing 80 mg of the test item into 800 mL test medium. The mixture was homogenised by ultrasonic treatment for 15 minutes and by intense stirring for 3 hours at room temperature in the dark. The saturated solution was then filtered through a 0.45 µm membrane filter before preparation of the test solutions.

The undiluted filtrate of the saturated solution was used as the highest test concentration, and lower concentrations were prepared by dilution of the filtrate with filtered pond water. Dilutions of 1:3.2, 1:10, 1:32, 1:100 and 1:320 were used as the lower test concentrations. The actual concentrations of the test item were determined at the start and end of the 48-hour exposure period using high performance liquid chromatography (HPLC) with UV detection.

Twenty test organisms split between four replicates were exposed to each test concentration and the control. The test vessels were maintained at 19 - 20°C during the exposure period. The number of immobilised or dead *Simocephalus vetulus* in each test vessels was recorded at 24 and 48 hours. Animals not able to swim within 15 seconds after gentle agitation of the test vessel were considered immobile.

Dissolved oxygen, pH and temperature was measured at the start and end of the exposure period in each test concentration and the control.

The 24-hour EC_{50} was estimated as the geometric mean value of the two consecutive test concentrations with 0 and 100% immobility. The 24-hour EC_{50} was estimated using the Probit method and all endpoints were based on the analysed test concentrations.

Results and Discussion

The mean measured concentrations of CGA 219417 A tech. during the study were 0.043, 0.13, 0.40, 1.3 and 3.9 mg/L. The concentration of CGA 219417 A tech. in the undiluted filtrate was not determined as the same biological response (100% immobility / mortality) was observed in the next lower treatment. The test material was stable within the test system during the exposure duration, samples collected from the 48-hour test solutions were within 99 to 102% of the initial measured concentrations.

Table 8.2.4.2-5: Analytical results

Nominal concentrations	Measured Concentration 0-hour (mg/L)	Measured Concentration 48-hour (mg/L)	Mean Measured (mg/L)*
Control	<0.027	<0.207	-
1:320 dilution	0.043	0.044	0.043
1:100 dilution	0.131	0.130	0.13
1:32 dilution	0.398	0.398	0.40
1:10 dilution	1.25	1.24	1.3
1:3.2 dilution	3.84	3.89	3.9

*: expressed to two significant figures

Water quality throughout the test was within acceptable limits. During the exposure period the pH was maintained at 8.1 and the dissolved oxygen ranged from 8.2 to 8.6 mg/L.

Control immobility was 5% after 48 hours. No immobility was observed in the 0.043 mg/L treatment group after 48 hours of exposure. Immobility steadily increased from 75% at 0.13 mg/L to 100% at 3.9 mg/L. Based on mean measured concentrations, the 48-hour EC_{50} for CGA219417 A tech. to *Simocephalus vetulus* was estimated to be 0.15 mg/L with 95% confidence limits of 0.095 to 0.22 mg/L.

Table 8.2.4.2-6: Effects of CGA 219417 A tech on *Simocephalus vetulus* survival after 24 and 48 hours exposure

Mean measured concentration (mg/L)	No. of organisms tested	Number immobilised 24 hours		Number immobilised 48 hours	
		No.	%	No.	%
Control	20	1	5	1	5
0.043	20	0	0	0	0
0.130	20	1	5	15	75
0.40	20	1	5	16	80
1.3	20	0	0	17	85
3.9	20	20	100	20	100
Undiluted filtrate	20	20	100	20	100
EC_{50} mg/L (95 % Confidence limits)		2.3 (1.3 – 3.9)		0.15 (0.095 – 0.22)	

Validity Criteria

The study was considered valid as the following criterion was fulfilled:

- Less than 10% immobilisation occurred in the control (actual: 5%)

Conclusions

Based on mean measured concentrations, the 48-hour EC_{50} for CGA 219417 A tech to *Simocephalus vetulus* was estimated to be 0.15 mg/L with 95% confidence limits of 0.095 to 0.22 mg/L.

(Peither, 2000b)

Report:	Summary submitted at the request of ANSES for ease of dossier evaluation: *KIIA 8.2.4. Peither A. (2000c). Acute Toxicity of CGA219417 A tech. to Gammarus sp. in a 48-hour Static Immobilisation Test. Report Number 2003731. RCC Ltd, CH-4452 Itingen, Switzerland. (Syngenta File No. CGA219417/0998).*

Guidelines

Based on:

OECD Guideline for Testing of Chemicals, No. 202, *Daphnia* sp., Acute Immobilisation Test and Reproduction Test, Part I (1984)

Commission Directive 92/69/EEC, C.2, Acute Toxicity for *Daphnia* (1992)

US EPA OPPTS No. 850.1010, Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids

US EPA FIFRA – Guideline No. 72-2, Acute Toxicity Test for Freshwater Aquatic Invertebrates

GLP: Yes

Executive Summary

The toxicity of CGA219417 A tech. to the aquatic invertebrate *Gammarus* sp. was determined in a 48-hour acute toxicity test performed under static conditions. The test organisms were exposed to the undiluted filtrate and the following dilutions of a CGA219417 A tech. saturated solution with a loading rate of 100 mg/L: 1:3.2, 1:10, 1:32, 1:100 and 1:320 alongside a dilution medium control.

The actual concentration of test item was determined at the start and end of the exposure duration in the undiluted filtrate, 1:3.2 and 1:10 treatment groups and the mean measured concentrations were 8.8, 3.1 and 1.0 mg/L, respectively. The measured concentrations at the end of the exposure period ranged from 94 to 97% of the initial measured concentrations, indicating the test material was stable in the test system for the duration of the exposure.

Based on mean measured concentrations, the 48-hour EC_{50} for CGA219417 A tech. to *Gammarus* sp. was 1.8 mg/L with 95% confidence limits of 1.0 to 3.1 mg/L.

Materials

Test Material	CGA219417 A tech.
Lot/Batch #:	P.012010
Purity:	99.4%
Description:	Beige solid
Stability of the test compound:	Hydrolytically stable: DT_{50} >1 year
Reanalysis/expiry date:	December 2004
Treatments	
Test concentrations:	Dilution medium control and undiluted filtrate, 1:3.2, 1:10, 1:32, 1:100 and 1:320 dilutions of a saturated solution (100 mg/L loading rate). Mean measured concentrations of 8.8, 3.1 and 1.0 mg/L (undiluted filtrate, 1:3.2 and 1:10 treatment groups)
Solvent:	None
Positive control:	None
Analysis of test concentrations:	Yes, HPLC analysis of CGA219417 A tech. at 0 and 48 hours

Test organism

Species:	*Gammarus* sp. larvae
Source:	Field collected from a natural stream (River Frenke, near Bubendorf, Kanton Baselland, Switzerland)
Acclimation	Collected one day prior to the test start and placed in filtered (55 µm gaze) pond water under similar environmental conditions as those used in the study

Test design

Test vessels:	1 litre petri dish, containing 300 mL test solution
Test medium:	Filtered (0.45 µm) natural pond water, collected from Novartis Crop Protection AG, Kanton Aargau, Switzerland
Replication:	Four replicates for each treatment and control (5 organisms per replicate)
Exposure regime:	Static
Aeration:	No
Duration:	48 hours

Environmental conditions

Test temperature:	15 to 16°C
pH:	8.0 to 8.1
Dissolved oxygen:	8.7 to 8.9 mg/L
Lighting:	16 hour light and 8 hour dark with a 30 minute transition, light intensity 200 to 1200 lux

Study Design and Methods

Experimental dates: 24th July to 9th August 2000

A supersaturated stock dispersion with a loading rate of 100 mg/L was prepared by weighing 210 mg of the test item into 2100 mL test medium. The mixture was homogenised by ultrasonic treatment for 15 minutes and by intense stirring for 3 hours at room temperature in the dark. The saturated solution was then filtered through a 0.45 µm membrane filter before preparation of the test solutions.

The undiluted filtrate of the saturated solution was used as the highest test concentration, and lower concentrations were prepared by dilution of the filtrate with filtered pond water. Dilutions of 1:3.2, 1:10, 1:32, 1:100 and 1:320 were used as the lower test concentrations. The actual concentrations of the test item were determined in the undiluted filtrate, 1:3.2 and 1:10 treatment groups, at the start and end of the 48-hour exposure period, using high performance liquid chromatography (HPLC) with UV detection.

Twenty test organisms split between four replicates were exposed to each test concentration and the control. The test vessels were maintained at 15 to 16°C during the exposure period. The number of immobilised or dead *Gammarus* larvae in each test vessels was recorded at 24 and 48 hours. Animals not able to swim within 15 seconds after gentle agitation of the test vessel were considered immobile.

Dissolved oxygen, pH and temperature was measured at the start and end of the exposure period in each test concentration and the control. The total hardness and conductivity of the test medium was measured at the start of the test.

The 24-hour EC_{50} value was estimated using the Probit analyses. The 48-hour EC_{50} was determined as the geometric mean value of the two consecutive test concentrations with 0% and 100% immobility and the confidence interval was considered to be the test concentrations with 0% and 100% immobility.

Results and Discussion

The mean measured concentrations of CGA219417 A tech during the study were 1.0, 3.1 and 8.8 mg/L in the 1:10, 1:3.2 dilutions and undiluted filtrate treatment groups. The test material was stable within the test system during the exposure duration, samples collected from the 48-hour test solutions were within 94 to 97% of the initially measured concentrations.

Table 8.2.4.2-7: Analytical results

Dilution	Measured Concentration 0-hour (mg/L)	Measured Concentration 48-hour (mg/L)	Mean Measured (mg/L)
Control	Not detected	Not detected	-
1:10	1.039	1.004	1.021
1:3.2	3.203	3.006	3.104
Undiluted filtrate	8.99	8.69	8.84

All test solutions were observed to be clear solutions throughout the study. During the exposure period the pH was of the test solutions ranged from 8.0 to 8.1 and the dissolved oxygen ranged from 8.7 to 8.9 mg/L. At the beginning of the study the conductivity of the test water was 225 µS/cm and the hardness was 162 mg/L as $CaCO_3$.

No immobility or mortality was observed in the control and all test item concentrations up to and including 1.0 mg/L after 48 hours exposure. At the test concentrations of 3.1 and 8.8 mg/L all test organisms were immobile after 48 hours.

Table 8.2.4.2-8: Effects of CGA219417 A tech on *Gammarus* sp. survival after 24 and 48 hours exposure

Dilution	Mean measured concentration (mg/L)	No. of organisms tested	Number immobilised 24 hours		Number immobilised 48 hours	
			No.	%	No.	%
Control	-	20	0	0	0	0
1:320	Not analysed	20	0	0	0	0
1:100	Not analysed	20	0	0	0	0
1:32	Not analysed	20	0	0	0	0
1:10	1.0	20	0	0	0	0
1:3.2	3.1	20	3	15	20	100
Undiluted filtrate	8.8	20	20	100	20	100
EC_{50} mg/L (95 % Confidence limits)			3.9 (Not calculable)		1.8 (1.0 – 3.1)	

Validity Criteria

The study was considered valid as the following criterion was fulfilled:

- Less than 10% immobilisation occurred in the control (actual: 0%).

Conclusions

Based on mean measured concentrations, the 48-hour EC_{50} for CGA219417 A tech. to *Gammarus* sp. was 1.8 mg/L with 95% confidence limits of 1.0 to 3.1 mg/L.

(Peither, 2000c)

Report:	Summary submitted at the request of ANSES for ease of dossier evaluation: *KIIA 8.2.4. Peither A. (2000d). Acute Toxicity of CGA219417 A tech. to Thamnocephalus platyurus in a 24-hour Static Immobilisation Test. Report Number 2003733. RCC Ltd, CH-4452 Itingen, Switzerland. (Syngenta file No. CGA219417/0991)*

Guidelines

Based on:

OECD Guideline for Testing of Chemicals, No. 202, *Daphnia sp.*, Acute Immobilsation Test and Reproduction Test, Part I (1984)

Commission Directive 92/69/EEC, C.2, Acute Toxicity for Daphnia (1992)

US EPA OPPTS No. 850.1010, Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids

US EPA FIFRA – Guideline No. 72-2, Acute Toxicity Test for Freshwater Aquatic Invertebrates

GLP: Yes

Executive Summary

The toxicity of CGA219417 A tech. to the freshwater rotifer *Thamnocephalus platyurus* was determined in a 24-hour acute toxicity test performed under static conditions. The test organisms were exposed to the following dilutions of a CGA219417 A tech. saturated solution with a loading rate of 100 mg/L: 1:40, 1:80, 1:160, 1:320 and 1:640, alongside a dilution medium control.

The actual concentrations were determined in each of the test treatments as at the start and end of the exposure and the mean measured concentrations were 0.29, 0.16, 0.070, 0.034 and 0.016 mg/L. The values determined at the end of the test were 88 and 97% of the initially measured values.

Based on mean measured concentrations, the 24-hour EC_{50} for CGA219417 A tech. to *Thamnocephalus platyurus* was 0.12 mg/L with 95% confidence limits of 0.10 to 0.15 mg/L.

Materials

Test Material	CGA219417 A tech.
Lot/Batch #:	P.012010
Purity:	99.4%
Description:	Beige solid
Stability of test compound:	Hydrolytically stable: DT_{50} >1 year

Reanalysis/expiry date:	December 2004
Treatments	
Test concentrations:	Dilution medium control and 1:40, 1:80, 1:160, 1:320 and 1:640 dilutions of a saturated solution (mean measured concentration 0.29, 0.16, 0.070, 0.034 and 0.016 mg/L)
Solvent:	None
Positive control:	None
Analysis of test concentrations:	Yes, HPLC analysis of 219417 A tech. at 0 and 24 hours
Test organism	
Species:	*Thamnocephalus platyurus* (approximately 4 hours old)
Source:	Bred from cysts in a commercial test kit (THAMNOTOXKIT F), supplied by Creasel, 98000 Deinze, Belgium.
Test design	
Test vessels:	Multiwell test plates. Wells contained 1.0 mL test solution
Test medium:	Reconstituted water according to Commission Directive 92/69/EEC and OECD guideline.
Replication:	Three replicates for each treatment and control (10 organisms per replicate)
Exposure regime:	Static
Aeration:	No
Duration:	24 hours
Environmental conditions	
Test temperature:	24 – 25°C
pH:	7.7 throughout test
Dissolved oxygen	8.4 mg/L throughout test
Lighting:	Test performed under dark conditions

Study Design and Methods

Experimental dates: 7th to 31th August 2000

A supersaturated stock dispersion with a loading rate of 100 mg/L was prepared by weighing 50 mg of the test item into 500 mL test medium. The mixture was homogenised by ultrasonic treatment for 15 minutes and by intense stirring for 3 hours at room temperature in the dark. The saturated solution was then filtered through a 0.45 µm membrane filter before preparation of the test solutions.

The test solutions were prepared by dilution of the filtrate with test medium. Dilutions of 1:40, 1:80, 1:160, 1:320 and 1:640 were employed as the test concentrations, alongside a control treatment consisting of dilution medium without the test item. The actual concentrations of the test item were determined at the start and end of the 24-hour exposure period using high performance liquid chromatography (HPLC) with UV detection.

Thirty test organisms were split between three replicates for each test concentration and the control. The test vessels were maintained at 24 - 25 °C in a temperature controlled water bath for the 24-hour exposure. The number of immobilised *Thamnocephalus platyurus* in each test vessel was recorded after

24 hours. Animals not exhibiting and movement within 10 seconds of observation were considered immobile.

At the start of the test the dissolved oxygen, pH and temperature were measured in the freshly prepared test media of each test concentration and the control. At the end of the test, these parameters were measured in the analytical samples, which were incubated separately (without test organisms) but under identical conditions as the actual test solutions.

The 24-hour EC_{50} value was estimated by Probit analysis and the results were based on mean measured test item concentrations.

Results and Discussion

The measured concentrations of CGA219417 A tech during the study period were 0.016, 0.034, 0.070, 0.16 and 0.29 mg/L. Samples collected at the end of the exposure period were between 88 to 97% of the initially measured concentration. The study results are based on mean measured concentrations.

Table 8.2.4.2-9: Analytical results

Dilution	Measured concentration 0-hour (mg/L)	Measured concentration 24-hour (mg/L)	Mean Measured (mg/L)*
Control	Not detected	Not detected	-
1:640	0.017	0.015	0.016
1:320	0.035	0.033	0.034
1:160	0.072	0.067	0.070
1:80	0.162	0.155	0.16
1:40	0.294	0.285	0.29

*: expressed to two significant figures

Water quality throughout the test was within acceptable limits. During the exposure period the pH was maintained at 7.7 and the dissolved oxygen rat 8.4 mg/L. The conductivity of the test medium was determined at the start of the test to be 320 µS/cm.

No mortality or immobility was observed in the control or 0.016 mg/L treatment group. Immobilisation steadily increased from 10% at 0.034 mg/L to 100% in the highest test item concentration, 0.29 mg/L.

Table 8.2.4.2-10: Effects of CGA219417 A tech. on *Thamnocephalus platyurus* survival after 24 hours exposure

Dilution	Mean Measured Concentrations (mg/L)	No. of organisms tested	Number immobilised 24 hours	
			No.	%
Control	-	30	0	0
1:640	0.016	30	0	0
1:320	0.034	30	3	10
1:160	0.070	30	5	17
1:80	0.16	30	14	47
1:40	0.29	30	30	100

Validity Criteria

The study was considered valid as less than 10 % immobilisation was observed in the control after 24 hours exposure.

Conclusions

The 24-hour EC_{50} of CGA219417 A to *Thamnocephalus platyurus* was estimated to be 0.12 mg/L with 95 % confidence limits of 0.10 to 0.15 mg/L.

(Peither, 2000d)

Report:	Summary submitted at the request of ANSES for ease of dossier evaluation: *KIIA 8.2.4. Peither A. (2000e). Acute Toxicity of CGA 219417 A tech. to Ostracoda in a 48-hour Static Immobilisation Test. Report Number 2003728. RCC Ltd, CH-4452 Itingen, Switzerland. (Syngenta File No. CGA219417/0995).*

Guidelines

Based on:

OECD Guideline for Testing of Chemicals, No. 202, *Daphnia sp*., Acute Immobilisation Test and Reproduction Test, Part I (1984)

Commission Directive 92/69/EEC, C.2, Acute Toxicity for Daphnia (1992)

US EPA OPPTS No. 850.1010, Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids

US EPA FIFRA – Guideline No. 72-2, Acute Toxicity Test for Freshwater Aquatic Invertebrates

GLP: Yes

Executive Summary

The toxicity of CGA 219417 A tech. to Ostracoda was determined in a 48-hour acute toxicity test performed under static conditions. Ostracoda were exposed to the following dilutions of a CGA 219417 A tech. saturated solution with a loading rate of 100 mg/L: 1:100, 1:46, 1:22, 1:10, 1:4.6 and 1:2.2 and a dilution medium control. The analytically determined test item concentrations were 0.096, 0.21, 0.46, 1.1, 2.4 and 5.1 mg/L at the start of the study. At the end of the 48-hour exposure period the measured test item concentrations ranged from 97 to 107% of the initially measured concentrations, indicating the test material was stable in the test system for the duration of the exposure.

Based on mean measured concentrations, the 48-hour EC_{50} for CGA 219417 A tech. to Ostracoda was 1.1 mg/L with 95% confidence limits of 0.94 to 1.4 mg/L.

Materials

Test Material	CGA 219417 A tech.
Lot/Batch #:	P.012010
Purity:	99.4%
Description:	Beige solid
Stability of test compound:	Hydrolytically stable: DT_{50} >1 year
Reanalysis/expiry date:	December 2004
Treatments	
Test concentrations:	Dilution medium control and 1:100, 1:46, 1:22, 1:10, 1:4.6 and 1:2.2 dilutions of a saturated solution (100 mg/L loading rate). Mean measured concentrations 0.097, 0.21, 0.45, 1.1, 2.4 and 5.1 mg/L
Solvent:	None
Positive control:	None
Analysis of test concentrations:	Yes, HPLC analysis of CGA 219417 A tech. at 0 and 48 hours
Test organism	
Species:	Ostracoda
Source:	Field collected from Novartis Crop Protection AG, Kanton Aargau, Switzerland.
Acclimation	Collected one day prior to the test start and placed in filtered pond water under similar environmental conditions as those used in the study
Test design	
Test vessels:	100 mL glass beaker containing 50 mL test solution
Test medium:	Filtered (0.45 µm) natural pond water, collected from same location as test organisms
Replication:	Four replicates for each treatment and control (5 organisms per replicate)
Exposure regime:	Static
Aeration:	No
Duration:	48 hours
Environmental conditions	
Test temperature:	20 to 21°C
pH:	8.1 to 8.2
Dissolved oxygen	8.3 to 8.7 mg/L
Lighting:	16 hour light and 8 hour dark with a 30 minute transition. Intensity 200 to 1200 lux

Study Design and Methods

Experimental dates: 18th July to 8th August 2000

A supersaturated stock dispersion with a loading rate of 100 mg/L was prepared by weighing 300 mg of the test item into 3000 mL test medium. The mixture was homogenised by ultrasonic treatment for 15

minutes and by intense stirring for 3 hours at room temperature in the dark. The saturated solution was then filtered through a 0.45 µm membrane filter before preparation of the test solutions.

Dilutions of 1:100, 1:46, 1:22, 1:10, 1:4.6 and 1:2.2 were used as the test concentrations. The actual concentrations of the test item were determined at the start and end of the 48-hour exposure period using high performance liquid chromatography (HPLC) with UV detection.

Twenty test organisms split between four replicates were exposed to each test concentration and the control. The test vessels were maintained at 20 - 21°C during the exposure period. The number of immobilised Ostracoda in each test vessels was recorded at 24 and 48 hours. Animals not able to swim within 15 seconds after gentle agitation of the test vessel were considered immobile.

Dissolved oxygen, pH and temperature was measured at the start and end of the exposure period in each test concentration and the control. The total hardness and conductivity of the test medium was measured at the start of the test.

The 24 and 48-hour EC_{50} values were estimated using the Probit method and all endpoints were based on the mean measured concentrations.

Results and Discussion

The mean measured concentrations of CGA 219417 A. tech during the study were 0.096, 0.21, 0.46, 1.1, 2.4 and 5.1 mg/L. At the end of the 48-hour exposure period the measured test item concentrations ranged from 97 to 107% of the initially measured concentrations, indicating the test material was stable in the test system for the duration of the exposure.

Table 8.2.4.2-11: Analytical results

Dilution	Measured Concentration 0-hour (mg/L)	Measured Concentration 48-hour (mg/L)	Mean Measured (mg/L)*
Control	Not detected	Not detected	-
1:100	0.097	0.095	0.096
1:46	0.214	0.208	0.21
1:22	0.450	0.460	0.45
1:10	1.02	1.09	1.1
1:4.6	2.32	2.49	2.4
1:2.2	5.14	5.14	5.1

*: expressed to two significant figures

Water quality throughout the test was within acceptable limits. During the exposure period the pH ranged from 8.1 to 8.2 and the dissolved oxygen ranged from 8.3 to 8.7 mg/L. At the beginning of the study the conductivity of the test water was 216 µS/cm and the hardness was 162 mg/L $CaCO_3$.

No immobility or mortality was observed in the control and treatments up to and including 0.46 mg/L, after 48 hours exposure. At 1.1 mg/L CGA 217417 Ostracoda immobilisation of 35% was observed after 48 hours and 100% immobilisation was observed in the 2.41 and 5.14 mg/L treatment groups at the same time point.

Table 8.2.4.2-12: Effects of CGA 219417 A tech on Ostracoda survival after 24 and 48 hours exposure

Dilution	Mean measured concentration (mg/L)	No. of organisms tested	Number immobilised 24 hours		Number immobilised 48 hours	
			No.	%	No.	%
Control	Control	20	0	0	0	0
1:100	0.096	20	0	0	0	0
1:46	0.21	20	0	0	0	0
1:22	0.46	20	0	0	0	0
1:10	1.1	20	0	0	7	35
1:4.6	2.4	20	18	90	20	100
1:2.2	5.1	20	20	100	20	100
EC_{50} mg/L (95 % Confidence limits)			1.9 (1.4 – 2.7)		1.1 (0.94 – 1.4)	

Validity Criteria

The study was considered valid as the following criterion was fulfilled:

- Less than 10 % immobilisation occurred in the control (actual: 0 %)

Conclusions

Based on mean measured concentrations, the 48-hour EC_{50} for CGA 219417 A tech. to Ostracoda was 1.1 mg/L with 95 % confidence limits of 0.94 to 1.4 mg/L.

(Peither A. 2000e)

Report:	Summary submitted at the request of ANSES for ease of dossier evaluation: *KIIA 8.2.4. Peither A. (2000f). Acute Toxicity of CGA219417 A tech. to Brachionus calyciflorus in a 24-hour Static Immobilisation Test. Report Number 2003732. RCC Ltd, CH-4452 Itingen, Switzerland. (Syngenta File No. CGA219417/0992)*

Guidelines

Based on:

OECD Guideline for Testing of Chemicals, No. 202, *Daphnia sp.*, Acute Immobilsation Test and Reproduction Test, Part I (1984)

Commission Directive 92/69/EEC, C.2, Acute Toxicity for Daphnia (1992)

US EPA OPPTS No. 850.1010, Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids

US EPA FIFRA – Guideline No. 72-2, Acute Toxicity Test for Freshwater Aquatic Invertebrates

GLP: Yes

Executive Summary

The toxicity of CGA219417 A tech. to the freshwater rotifer *Brachionus calyciflorus* was determined in a 24-hour acute toxicity test performed under static conditions. *Brachionus calyciflorus* were exposed to dilutions of a CGA219417 A tech. saturated solution. The actual concentrations were determined in two of the test treatments as 4.6 and 9.5 mg/L. The values determined at the end of the test were 102 and 101% of the initially measured values. Concentrations were not analysed in the other treatment groups since these concentrations were below the highest concentration without lethal or sublethal toxic effects.

Based on mean measured concentrations, the 24-hour EC_{50} for CGA219417 A tech. to *Brachionus calyciflorus* was >9.5 mg/L.

Materials

Test Material	CGA219417 A tech.
Lot/Batch #:	P.012010
Purity:	99.4%
Description:	Beige solid
Stability of test compound:	Hydrolytically stable: DT_{50} >1 year
Reanalysis/expiry date:	December 2004
Treatments	
Test concentrations:	Dilution medium control and undiluted filtrate and the following dilutions 1:2, 1:4, 1:8 and 1:16 of a saturated solution (measured concentration 4.7 and 9.5 mg/L for the 1:2 and undiluted filtrate treatments)
Solvent:	None
Positive control:	None
Analysis of test concentrations:	Yes, HPLC analysis of 219417 A tech. at 0 and 24 hours
Test organism	
Species:	*Brachionus calyciflorus* (<2 hours old)
Source:	Bred from cysts in a commercial test kit (ROTOXKIT F), supplied by Creasel, 98000 Deinze, Belgium.
Test design	
Test vessels:	Multiwell test plates. Wells contained 0.3 mL test solution
Test medium:	Reconstituted water according OECD guideline, with hardness reduced by 50%
Replication:	Six replicates for each treatment and control (5 organisms per replicate)
Exposure regime:	Static
Aeration:	No
Duration:	24 hours
Environmental conditions	
Test temperature:	24 – 25°C
pH:	7.9 throughout test
Dissolved oxygen	8.2 to 8.8 mg/L
Lighting:	Test performed under dark conditions

Study Design and Methods

Experimental dates: 7th to 30th August 2000

A supersaturated stock dispersion with a loading rate of 100 mg/L was prepared by weighing 50 mg of the test item into 500 mL test medium. The mixture was homogenised by ultrasonic treatment for 15 minutes and by intense stirring for 3 hours at room temperature in the dark. The saturated solution was then filtered through a 0.45 µm membrane filter before preparation of the test solutions.

The undiluted filtrate of the saturated solution was used as the highest test concentration, and lower concentrations were prepared by dilution of the filtrate with test medium. Dilutions of 1:2, 1:4, 1:8 and 1:16 were used as the lower test concentrations. The actual concentrations of the test item were determined at the start and end of the 24-hour exposure period using high performance liquid chromatography (HPLC) with UV detection.

Thirty test organisms were split between six replicates for each test concentration and the control. The test vessels were maintained at 24 - 25°C in a temperature controlled water bath for the 24-hour exposure. The number of immobilised *Brachionus calyciflorus* in each test vessels was recorded after 24 hours. Animals not exhibiting and movement within 5 seconds of observation were considered immobile.

At the start of the test the dissolved oxygen, pH and temperature were measured in the freshly prepared test media of each test concentration and the control. At the end of the test, these parameters were measured in the analytical samples, which were incubated separately (without test organisms) but under identical conditions as the actual test solutions.

The 24-hour EC_{50} could not be estimated, since only a low toxic effect was observed at the highest test concentration.

Results and Discussion

The measured concentrations of CGA219417 A tech. within samples collected from the 1:2 and undiluted filtrate treatments were 4.6 and 9.5 mg/L, respectively, at the start of the exposure. Samples collected at the end of the exposure period were between 101 – 102% of the initial measured concentration. The concentration of CGA219417 A tech. in the other treatment groups was not determined, since these concentrations were below the highest concentration without lethal or sublethal toxic effects. The study results are based on mean measured concentrations.

Table 8.2.4.2-13: Analytical results

Dilution	Measured concentration 0-hour (mg/L)	Measured concentration 24-hour (mg/L)	Mean Measured (mg/L)*
Control	Not detected	Not detected	-
1:16 dilution	Not analysed	Not analysed	-
1: 8 dilution	Not analysed	Not analysed	-
1:4 dilution	Not analysed	Not analysed	-
1:2 dilution	4.64	4.71	4.7
Undiluted filtrate	9.50	9.57	9.5

*: expressed to two significant figures

Water quality throughout the test was within acceptable limits. During the exposure period the pH was maintained at 7.9 and the dissolved oxygen ranged from 8.2 to 8.8 mg/L.

Control survival was 100% after 24 hours. No mortality or immobility was observed in all treatments up to and including 4.7 mg/L. At the highest test concentration (9.5 mg/L) 17% immobility was observed.

Table 8.2.4.2-14: Effects of CGA219417 A tech on *Brachionus calyciflorus* survival after 24 hours exposure

Dilution (measured concentration)	No. of organisms tested	Number immobilised 24 hours	
		No.	%
Control	30	0	0
1:16	30	0	0
1:8	30	0	0
1:4	30	0	0
1:2 (4.7 mg/L)	30	0	0
Undiluted filtrate (9.5 mg/L)	30	5	17

Validity Criteria

Not reported.

Conclusions

The 24-hour EC_{50} of CGA219417 A tech. could not be determined due to a low toxic effect at the highest treatment concentration (9.5 mg/L).

(Peither, 2000f)

Report:	Summary submitted at the request of ANSES for ease of dossier evaluation: *KIIA 8.2.4. Peither A. (2000g). Acute Toxicity of CGA 219417 A tech. to Cloeon sp.in a 48-hour Static Immobilisation Test. Report Number 2003730. RCC Ltd, CH-4452 Itingen, Switzerland. (Syngenta File No. CGA219417/0996).*

Guidelines

Based on:

OECD Guideline for Testing of Chemicals, No. 202, *Daphnia sp*., Acute Immobilisation Test and Reproduction Test, Part I (1984)

Commission Directive 92/69/EEC, C.2, Acute Toxicity for Daphnia (1992)

US EPA OPPTS No. 850.1010, Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids

US EPA FIFRA – Guideline No. 72-2, Acute Toxicity Test for Freshwater Aquatic Invertebrates

GLP: Yes

Executive Summary

The toxicity of CGA219417 A tech. to the aquatic larvae of *Cloeon* sp. was determined in a 48-hour acute toxicity test performed under static conditions. The test organisms were exposed to the undiluted filtrate and the following dilutions of a CGA219417 A tech. saturated solution with a loading rate of 100 mg/L: 1:2, 1:4, 1:8, 1:16 and 1:32, alongside a dilution medium control. The actual concentration of test item was determined at the start and end of the exposure duration and the mean measured concentrations were 12, 6.0, 3.0, 1.5 and 0.72 mg/L in the undiluted treatment and 1:2, 1:4, 1:8 and 1:16 dilution treatments. The actual concentration of the test item in the 1:32 dilution treatment was not determined. The measured concentrations at the end of the exposure period ranged from 86 to 101% of the initial measured concentrations, indicating the test material was stable in the test system for the duration of the exposure.

Based on mean measured concentrations, the 48-hour EC_{50} for CGA219417 A tech. to *Cloeon* sp. was 3.5 mg/L with 95% confidence limits of 2.8 to 4.4 mg/L.

Materials

Test Material	CGA219417 A tech.
Lot/Batch #:	P.012010
Purity:	99.4%
Description:	Beige solid
Stability of the test compound:	Hydrolytically stable: DT_{50} >1 year
Reanalysis/expiry date:	December 2004
Treatments	
Test concentrations:	Dilution medium control and undiluted filtrate, 1:2, 1:4, 1:8, 1:16 and 1:32 dilutions of a saturated solution (100 mg/L loading rate). Mean measured concentrations of 12, 6.0, 3.0, 1.5 and 0.72 mg/L (1:32 dilution not analysed)
Solvent:	None
Positive control:	None
Analysis of test concentrations:	Yes, HPLC analysis of CGA219417 A tech. at 0 and 48 hours
Test organism	
Species:	*Cloeon* sp. larvae
Source:	Field collected from Novartis Crop Protection AG, Kanton Aargau, Switzerland.
Acclimation	Collected one day prior to the test start and placed in filtered (55 µm gaze) pond water under similar environmental conditions as those used in the study
Test design	
Test vessels:	Petri dish Ø 14 cm, containing 200 mL test solution
Test medium:	Filtered (0.45 µm) natural pond water, collected from same location as test organisms, pH adjusted to 7.9 with HCl
Replication:	Four replicates for each treatment and control (5 organisms per replicate)
Exposure regime:	Static
Aeration:	No

Duration:	48 hours
Environmental conditions	
Test temperature:	20 to 21°C
pH:	7.9
Dissolved oxygen:	8.0 to 8.2 mg/L
Lighting:	16 hour light and 8 hour dark with a 30 minute transition, light intensity 200 to 1200 lux

Study Design and Methods

Experimental dates: 15th to 31th August 2000

A supersaturated stock dispersion with a loading rate of 100 mg/L was prepared by weighing 201 mg of the test item into 2000 mL test medium. The mixture was homogenised by ultrasonic treatment for 15 minutes and by intense stirring for 3 hours at room temperature in the dark. The saturated solution was then filtered through a 0.45 µm membrane filter before preparation of the test solutions.

The undiluted filtrate of the saturated solution was used as the highest test concentration, and lower concentrations were prepared by dilution of the filtrate with filtered pond water. Dilutions of 1:2, 1:4, 1:8, 1:16 and 1:32 were used as the lower test concentrations. The actual concentrations of the test item were determined, in all treatment groups with the exception of the 1:32 dilution, at the start and end of the 48-hour exposure period using high performance liquid chromatography (HPLC) with UV detection.

Twenty test organisms split between four replicates were exposed to each test concentration and the control. The test vessels were maintained at 20 - 21°C during the exposure period. The number of immobilised *Cloeon* larvae in each test vessels was recorded at 24 and 48 hours. Animals not able to swim within 15 seconds after gentle agitation of the test vessel were considered immobile.

Dissolved oxygen, pH and temperature was measured at the start and end of the exposure period in each test concentration and the control. The total hardness and conductivity of the test medium was measured at the start of the test.

The 24 and 48-hour EC_{50} values were estimated using the Probit method and all endpoints were based on the mean measured concentrations.

Results and Discussion

The mean measured concentrations of CGA219417 A tech during the study were 0.72, 1.5, 3.0, 6.0 and 12 mg/L. The test material was stable within the test system during the exposure duration, samples collected from the 48-hour test solutions were within 86 to 101% of the initial measured concentrations.

Table 8.2.4.2-15: Analytical results

Dilution	Measured Concentration 0-hour (mg/L)	Measured Concentration 48-hour (mg/L)	Mean Measured (mg/L)*
Control	Not detected	Not detected	-
1:32	Not analysed	Not analysed	-
1:16	0.771	0.663	0.72
1:8	1.49	1.50	1.5
1:4	3.01	2.97	3.0
1:2	5.99	5.98	6.0
Undiluted filtrate	11.8	11.2	12

*: expressed to two significant figures

All test solutions were observed to be clear solutions throughout the study. During the exposure period the pH was maintained at 7.9 and the dissolved oxygen ranged from 8.0 to 8.2 mg/L. At the beginning of the study the conductivity of the test water was 207 µS/cm and the hardness was 162 mg/L as $CaCO_3$.

No immobility or mortality was observed in the control after 48 hours exposure. A single immobile *Cloeon* sp. larvae was observed in the 1:32 dilution treatment and measured concentration of 0.72 mg/L (1:16 dilution). A steady increase in immobility was observed in the test item concentrations of 1.5 to 12 mg/L with 100 % immobility observed in the highest test concentration.

Table 8.2.4.2-16: Effects of CGA219417 A tech on *Cloeon* sp. survival after 24 and 48 hours exposure

Dilution	Mean measured concentration (mg/L)	No. of organisms tested	Number immobilised 24 hours		Number immobilised 48 hours	
			No.	%	No.	%
Control	-	20	0	0	0	0
1:32	Not analysed	20	0	0	1	5
1:16	0.72	20	0	0	1	5
1:8	1.5	20	0	0	3	15
1:4	3.0	20	1	5	7	35
1:2	6.0	20	7	35	15	75
Undiluted filtrate	12	20	20	100	20	100
EC_{50} mg/L (95 % Confidence limits)			6.4 (5.5 – 7.4)		3.5 (2.8 – 4.4)	

Validity Criteria

The study was considered valid as the following criterion was fulfilled:

- Less than 10 % immobilisation occurred in the control (actual: 0 %)

Conclusions

Based on mean measured concentrations, the 48-hour EC_{50} for CGA219417 A tech. to *Cloeon* sp. was 3.5 mg/L with 95 % confidence limits of 2.8 to 4.4 mg/L.

(Peither, 2000g)

Report:	Summary submitted at the request of ANSES for ease of dossier evaluation: *KIIA 8.2.4. Peither A. (2000h). Acute Toxicity of CGA 219417 A tech. to Chaoborus sp.in a 48-hour Static Immobilisation Test. Report Number 2003729. RCC Ltd, CH-4452 Itingen, Switzerland. (Syngenta file No. CGA219417/0999).*

Guidelines

Based on:

OECD Guideline for Testing of Chemicals, No. 202, *Daphnia sp*., Acute Immobilisation Test and Reproduction Test, Part I (1984)

Commission Directive 92/69/EEC, C.2, Acute Toxicity for Daphnia (1992)

US EPA OPPTS No. 850.1010, Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids

US EPA FIFRA – Guideline No. 72-2, Acute Toxicity Test for Freshwater Aquatic Invertebrates

GLP: Yes

Executive Summary

The toxicity of CGA 219417 A tech. to the aquatic larvae of *Chaoborus* sp. was determined in a 48-hour acute toxicity test performed under static conditions. The test organisms were exposed to mean measured CGA 219417 A tech. concentrations of 0.21, 0.46, 1.1, 2.4, 5.1 and 11 mg/L and a dilution media control. The measured concentrations at the end of the exposure period ranged from 97 to 107% of the initial measured concentrations, indicating the test material was stable in the test system for the duration of the exposure.

Based on mean measured concentrations, the 48-hour EC_{50} for CGA 219417 A tech. to *Chaoborus* sp. was 4.0 mg/L with 95% confidence limits of 3.1 to 5.1 mg/L.

Materials

Test Material	CGA 219417 A tech.
Lot/Batch #:	P.012010
Purity:	99.4%
Description:	Beige solid
Stability of the test compound:	Hydrolytically stable: DT_{50} >1 year
Reanalysis/expiry date:	December 2004

Treatments

Test concentrations:	Dilution medium control and 1:100, 1:46, 1:22, 1:10, 1:4.6 and 1:2.2 dilutions and an undiluted filtrate of a saturated solution (100 mg/L loading rate). Mean measured concentrations of 0.21, 0.46, 1.1, 2.4, 5.1 and 11 mg/L
Solvent:	None
Positive control:	None
Analysis of test concentrations:	Yes, HPLC analysis of CGA 219417 A tech. at 0 and 48 hours

Test organism

Species:	*Chaoborus* sp. larvae
Source:	Field collected from Novartis Crop Protection AG, Kanton Aargau, Switzerland
Acclimation	Collected one day prior to the test start and placed in filtered (55 µm gaze) pond water under similar environmental conditions as those used in the study

Test design

Test vessels:	250 mL glass beaker containing 200 mL test solution
Test medium:	Filtered (0.45 µm) natural pond water, collected from same location as test organisms
Replication:	Four replicates for each treatment and control (5 organisms per replicate)
Exposure regime:	Static
Aeration:	No
Duration:	48 hours

Environmental conditions

Test temperature:	20 to 21°C
pH:	8.0 to 8.2
Dissolved oxygen:	7.6 to 8.7 mg/L
Lighting:	16 hour light and 8 hour dark with a 30 minute transition, light intensity 200 to 1200 lux

Study Design and Methods

Experimental dates: 18th July to 9th August 2000

A supersaturated stock dispersion with a loading rate of 100 mg/L was prepared by weighing 300 mg of the test item into 3000 mL test medium. The mixture was homogenised by ultrasonic treatment for 15 minutes and by intense stirring for 3 hours at room temperature in the dark. The saturated solution was then filtered through a 0.45 µm membrane filter before preparation of the test solutions.

The undiluted filtrate of the saturated solution was used as the highest test concentration, and lower concentrations were prepared by dilution of the filtrate with filtered pond water. Dilutions of 1:2.2, 1:4.6, 1:10, 1:22, 1:220 and 1:46 were used as the lower test concentrations. The actual concentrations of the test item were determined at the start and end of the 48-hour exposure period using high performance liquid chromatography (HPLC) with UV detection. The 48-hour analytical samples were collected from separate test vessel containing the appropriate test solutions without test organisms and incubated under the same conditions as the other test vessels.

Twenty test organisms split between four replicates were exposed to each test concentration and the

control. The test vessels were maintained at 20 - 21°C during the exposure period. The number of immobilised *Chaoborus* larvae in each test vessels was recorded at 24 and 48 hours. Animals not able to swim within 15 seconds after gentle agitation of the test vessel were considered immobile.

Dissolved oxygen, pH and temperature was measured at the start and end of the exposure period in each test concentration and the control. The total hardness and conductivity of the test medium was measured at the start of the test.

The 24 and 48-hour EC_{50} values were estimated using the Probit method and all endpoints were based on the mean measured concentrations.

Results and Discussion

The mean measured concentrations of CGA 219417 A tech during the study were 0.21, 0.46, 1.1, 2.4, 5.1 and 11 mg/L. The test material was stable within the test system during the exposure duration, samples collected from the 48-hour test solutions were within 97 to 107% of the initial measured concentrations.

Table 8.2.4.2-17: Analytical results

Dilution	Measured Concentration 0-hour (mg/L)	Measured Concentration 48-hour (mg/L)	Mean Measured (mg/L)*
Control	Not detected	Not detected	-
1:46	0.214	0.208	0.21
1:22	0.450	0.460	0.46
1:10	1.02	1.09	1.1
1:4.6	2.32	2.49	2.4
1:2.2	5.14	5.14	5.1
Undiluted filtrate	10.7	10.8	11

*: expressed to two significant figures

All test solutions were observed to be clear solutions throughout the study. During the exposure period the pH ranged from 8.0 to 8.2 and the dissolved oxygen ranged from 7.6 to 8.7 mg/L. At the beginning of the study the conductivity of the test water was 216 µS/cm and the hardness was 162 mg/L $CaCO_3$.

No immobility was observed in the control and the test item concentrations of 0.21 and 1.1 mg/L, after 48 hours exposure. A single immobile *Chaoborus* sp. larvae was observed in the 0.46 mg/L treatment group, however this was not considered to be a significant toxic effect since 10% immobilisation is tolerated in the control by the test guidelines. A steady increase in immobility was observed in the test item concentrations of 2.4 to 11 mg/L with 90% immobility observed in the highest test concentration.

Table 8.2.4.2-18: Effects of CGA 219417 A tech on *Chaoborus* sp. survival after 24 and 48 hours exposure

Mean measured concentration (mg/L)	No. of organisms tested	Number immobilised 24 hours		Number immobilised 48 hours	
		No.	%	No.	%
Control	20	0	0	0	0
0.21	20	0	0	0	0
0.46	20	0	0	1	5
1.1	20	0	0	0	0
2.4	20	4	20	6	30
5.1	20	8	40	13	65
11	20	16	80	18	90
EC_{50} mg/L (95 % Confidence limits)		5.7 (4.3 – 7.6)		4.0 (3.1 – 5.1)	

Validity Criteria

The study was considered valid as the following criterion was fulfilled:

- Less than 10 % immobilisation occurred in the control (actual: 0 %)

Conclusions

Based on mean measured concentrations, the 48-hour EC_{50} for CGA 219417 A tech. to *Chaoborus* sp. was 4.0 mg/L with 95 % confidence limits of 3.1 to 5.1 mg/L.

(Peither, 2000h)

Report:	Summary submitted at the request of ANSES for ease of dossier evaluation: *KIIA 8.2.4. Peither A. (2000i). Acute Toxicity of CGA 219417 A tech. to Lymnea stagnalis in a 48-hour Static Immobilisation Test. Report Number 2003734. RCC Ltd, CH-4452 Itingen, Switzerland. (Syngenta file No. CGA219417/0997)*

Guidelines

Based on:

OECD Guideline for Testing of Chemicals, No. 202, *Daphnia* sp., Acute Immobilisation Test and Reproduction Test, Part I (1984)

Commission Directive 92/69/EEC, C.2, Acute Toxicity for *Daphnia* (1992)

US EPA OPPTS No. 850.1010, Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids

US EPA FIFRA – Guideline No. 72-2, Acute Toxicity Test for Freshwater Aquatic Invertebrates

GLP: Yes

Executive Summary

The toxicity of CGA219417 A tech. to the freshwater invertebrate *Lymnea stagnalis* was determined in a 48-hour acute toxicity test performed under static conditions. The test organisms were exposed to the undiluted filtrate and the following dilutions of a CGA219417 A tech. saturated solution with a loading rate of 100 mg/L: 1:2, 1:4, 1:8, 1:16 and 1:32 alongside a dilution medium control.

The actual concentration of test item was determined at the start and end of the exposure duration in the undiluted filtrate, 1:2, 1:4 and 1:8 treatment groups and the mean measured concentrations were 11, 5.6, 2.8 and 1.4 mg/L, respectively. The measured concentrations at the end of the exposure period ranged from 90 to 103% of the initial measured concentrations, indicating the test material was stable in the test system for the duration of the exposure.

Based on mean measured concentrations, the 48-hour EC_{50} for CGA219417 A tech. to *Lymnea stagnalis* was 2.9 mg/L with 95% confidence limits of 2.5 to 3.4 mg/L.

Materials

Test Material	CGA219417 A tech.
Lot/Batch #:	P.012010
Purity:	99.4%
Description:	Beige solid
Stability of the test compound:	Hydrolytically stable: DT_{50} >1 year
Reanalysis/expiry date:	December 2004
Treatments	
Test concentrations:	Dilution medium control and undiluted filtrate, 1:2, 1:4, 1:8, 1:16 and 1:32 dilutions of a saturated solution (100 mg/L loading rate). Mean measured concentrations of 11, 5.6, 2.8 and 1.4 mg/L (undiluted filtrate, 1:2, 1:4 and 1:8 treatment groups)
Solvent:	None
Positive control:	None
Analysis of test concentrations:	Yes, HPLC analysis of CGA219417 A tech. at 0 and 48 hours
Test organism	
Species:	*Lymnea stagnalis* (approximately 5 weeks old)
Source:	Continuous laboratory culture
Acclimation	One day prior to the study test organisms were placed in filtered (55 µm gaze) pond water under similar environmental conditions as those used in the study
Test design	
Test vessels:	Petri dish Ø 7 cm, containing 110 mL test solution
Test medium:	Filtered (0.45 µm) natural pond water
Replication:	Four replicates for each treatment and control (5 organisms per replicate)
Exposure regime:	Static

Aeration:	Not reported
Duration:	48 hours
Environmental conditions	
Test temperature:	20 to 21°C
pH:	7.8 to 8.1
Dissolved oxygen:	8.1 to 8.4 mg/L
Lighting:	16 hour light and 8 hour dark with a 30 minute transition, light intensity was 200 to 1200 lux

Study Design and Methods

Experimental dates: 21st to 31st August 2000

A supersaturated stock dispersion with a loading rate of 100 mg/L was prepared by weighing 141 mg of the test item into 1400 mL test medium. The mixture was homogenised by ultrasonic treatment for 15 minutes and by intense stirring for 3 hours at room temperature in the dark. The saturated solution was then filtered through a 0.45 µm membrane filter before preparation of the test solutions.

The undiluted filtrate of the saturated solution was used as the highest test concentration, and lower concentrations were prepared by dilution of the filtrate with filtered pond water. Dilutions of 1:2, 1:4, 1:8, 1:16 and 1:32 were used as the lower test concentrations. A control treatment, consisting of natural pond water without test material, was included in the study design. The actual concentrations of the test item were determined, in the undiluted filtrate, 1:2, 1:4 and 1:8 treatment groups, at the start and end of the 48-hour exposure period using high performance liquid chromatography (HPLC) with UV detection.

Twenty test organisms, split between four replicates, were exposed to each test concentration and the control. The test vessels were maintained at 20 - 21°C during the exposure period. The number of immobilised or dead *Lymnea stagnalis* in each test vessels was recorded at 24 and 48 hours. Animals not able to move after mechanical stimulus were considered immobile.

Dissolved oxygen, pH and temperature was measured at the start and end of the exposure period in each test concentration and the control. The total hardness and conductivity of the test medium was measured at the start of the test.

The 24 and 48-hour EC_{50} values were estimated using the Probit method and all endpoints were based on the mean measured concentrations.

Results and Discussion

The mean measured concentrations of CGA219417 A tech. during the study were 11, 5.6, 2.8 and 1.4 mg/L, within the undiluted filtrate, 1:2, 1:4 and 1:8 treatment groups. The test material was stable within the test system during the exposure duration, samples collected from the 48-hour test solutions were within 90 to 103% of the initial measured concentrations.

Table 8.2.4.2-19: Analytical results

Dilution	Measured Concentration 0-hour (mg/L)	Measured Concentration 48-hour (mg/L)	Mean Measured (mg/L)*
Control	Not detected	Not detected	-
1:8	1.45	1.30	1.4
1:4	2.88	2.67	2.8
1:2	5.64	5.64	5.6
Undiluted filtrate	11.2	11.5	11

*: expressed to two significant figures

During the exposure period the pH ranged from 7.8 to 8.1 and the dissolved oxygen ranged from 8.1 to 8.4 mg/L. At the beginning of the study the conductivity of the test water was 202 µS/cm and the hardness was 144 mg/L $CaCO_3$.

No immobility was observed in the control and concentrations up to 1.4 mg/L after 48 hours. The immobilisation increased from 50 % in the 2.8 mg/L to 100% at the highest test concentration.

Table 8.2.4.2-20: Effects of CGA219417 A tech on *Lymnea stagnalis* survival after 24 and 48 hours exposure

Dilution	Mean measured concentration (mg/L)	No. of organisms tested	Number immobilised 24 hours		Number immobilised 48 hours	
			No.	%	No.	%
Control	-	20	0	0	0	0
1:32	Not analysed	20	0	0	0	0
1:16	Not analysed	20	0	0	0	0
1:8	1.4	20	0	0	0	0
1:4	2.8	20	0	0	10	50
1:2	5.6	20	13	65	19	95
Undiluted filtrate	11	20	20	100	20	100
EC_{50} mg/L (95 % Confidence limits)			5.3 (4.5 – 6.2)		2.9 (2.5 – 3.4)	

Validity Criteria

The study was considered valid as the following criterion was fulfilled:

- Less than 10% immobilisation occurred in the control (actual: 0%)

Conclusions

Based on mean measured concentrations, the 48-hour EC_{50} for CGA219417 A tech. to *Lymnea stagnalis* was 2.9 mg/L with 95% confidence limits of 2.5 to 3.4 mg/L.

(Peither, 2000i)

Summaries of studies conducted since the previous EU review are presented below:

Report:	Study was conducted in order to refine the aquatic invertebrate risk assessment K-CA 8.2.4.2/01 Maynard S.K. (2011) CGA219417 – 96 hour acute toxicity to juvenile *Asellus aquaticus*. Report number CEMR-5069. CEMAS, North Ascot, Berkshire, UK. (Syngenta File No. CGA219417_11453)

Guidelines

OECD Guidelines for Testing of Chemicals, Section 2 - Effects on Biotic Systems, Method 202: *Daphnia* sp., Acute Immobilisation Test (2004)

GLP: Yes

Executive Summary

The acute toxicity of CGA219417 to juvenile *Asellus aquaticus* was determined under static conditions over 96 hours. Juvenile *Asellus* were exposed to a range of nominal concentrations of 0.116, 0.256, 0.563, 1.24, 2.73 and 6.00 mg CGA219417/L, alongside a dilution water control. Based on nominal concentrations, the 96-hour EC_{50} was 2.64 mg CGA219417/L. Control mortality at this time point had reached 35%. At 48-hours the EC_{50} was 2.35 mg CGA219417/L, with 5% control mortality.

Materials

Test Material	CGA219417
Lot/Batch #:	P.012011
Actual content of active ingredients:	99.2%
Description:	Light beige crumbs
Stability of test compound:	Stable under standard conditions.
Reanalysis/Expiry date:	30 April, 2014
Density:	n.a.
Treatments	
Test concentrations:	Dilution water control and nominal formulation concentrations of 0.116, 0.256, 0.563, 1.24, 2.73 and 6.00 mg CGA219417/L
Solvent:	None
Positive control:	n.a.
Analysis of test concentrations:	Yes 0 and 96 hours (based on measurement of CGA219417) using LC analysis
Test organisms	
Species:	*Asellus aquaticus*
Age:	Juveniles, <5mm
Source:	Commercial supplier, Mesocosm GmbH, Homberg, Germany
Feeding:	None during test
Culture medium:	Elendt M4
Test design	
Test vessels:	300 mL crystalising dishes with ~ 5 g fine quartz sand substrate
Test medium:	Elendt M4
Replication:	4 replicates, each containing 5 Asellus (one replicate at 2.73 mg/L contained 6

	animals)
Exposure regime:	Static
Duration:	96 hours
Environmental conditions	
Test temperature:	15.1 to 16.0°C
pH range:	7.00 to 7.20
Dissolved oxygen:	84 to 98 % air saturation value (ASV), no aeration
Total hardness of dilution water:	246 mg/L as CaCO3.
Lighting:	Photoperiod - 16 hours light and 8 hours dark

Study Design and Methods

Experimental dates: 21st to 25th June, 2011

The day before the test a stock solution with a nominal concentration of 6 mg/L was prepared by dissolving CGA219417 in dilution water using ultrasonication for 30 minutes, then one hour of stirring followed by one further minute of ultrasonication. Using this stock solution the test concentrations were prepared on the day of the test by dilution. The control consisted of dilution water. Test solutions were added to the test vessels, together with ~ 5 g fine quartz sand substrate, then five juvenile *Asellus* were added to each of the four replicates at each treatment. An additional animal was exposed at 2.73 mg/L. Preliminary work had identified the need for an inert substrate to provide the test animals some purchase on the base of the vessels.

The immobility of the *Asellus* was determined by visual observations after 24, 48, 72 and 96 hours of exposure. Organisms unable to move within 15 seconds after gentle agitation of the test beaker were considered to be immobile. Abnormal behaviour, such as lethargy, was also noted.

The pH, temperature and dissolved oxygen were measured at the start and end of the test in each test concentration and the control.

The test concentrations were verified by chemical analysis of CGA219417 at 0 and 96 hours using liquid chromatography with ultra violet-visible detection. In addition, the 6.00 mg/L treatment was sampled for analysis at 48 hours, as all the test organisms were immobile at this time.

Results and Discussion

At the start of the test, the concentrations of CGA219417 were in the range 82 to 92% of the nominal values, and at the end of the test were in the range 88 to 100% (see table below). The limit of quantification in this study was 0.01 mg CGA219417/L. Nominal concentrations were used for the calculation and reporting of results.

Table 8.2.4.2-421: Analytical results

Nominal concentrations of CGA219417 (mg/L)	% of nominal measured at 0 hours	% of nominal measured at 96 hours
0 (control)	n.a.	n.a.
0.116	91	88
0.256	89	91
0.563	89	90
1.24	88	90
2.73	82	90
6.00	92	100 (96)*

n.a. = not applicable, *= 48-h sample

The median effect concentrations (EC_{50}) were calculated using maximum likelihood-probit analysis. Where control mortality was >5%, Abbott's correction was applied before conducting Maximum likelihood-Probit analysis. Abbott's correction was not applied to 96-hour data before the linear interpolation analysis. The 24 and 48-h NOECs were determined using Steel's Many-One Rank Test. At 72 & 96-h Dunnett's test was used. Immobility data and estimated EC_{50} values are shown in the table below:

Table 8.2.4.2-222: Effects of CGA219417 on juvenile *Asellus aquaticus* following exposure for 96-hours in a static test

Nominal concentration of CGA219417 (mg/L)	Cumulative immobilisation 24 hours		Cumulative immobilisation 48 hours		Cumulative immobilisation 72 hours		Cumulative immobilisation 96 hours	
	Number	%	Number	%	Number	%	Number	%
0 (control)	0	0	1	5	3	15	7	35
0.116	0	0	1	5	3	15	7	35
0.256	0	0	0	0	3	15	4	20
0.563	0	0	0	0	5	25	6	30
1.24	0	0	3	15	7	35	8	40
2.73*	2	9	12	56	13	62	14	67
6.00	16	80	20	100	20	100	20	100
EC_{50} (mg CGA219417/L)	4.41		2.35		2.27**		2.64**	
95% Confidence limits	3.65 – 5.39		1.81 – 2.92		1.38 – 3.02		0.289 – 4.42	
NOEC	2.73		1.24		1.24		2.73	

*= 11 animals tested at this treatment, **= Control immobility at 72 and 96 hours was outside the validity criteria for the study

Conclusions

Based on nominal concentrations, the 96-hour EC_{50} was 2.64 mg CGA219417/L. Control mortality at this time point had reached 35%. At 48-hours the EC_{50} was 2.35 mg CGA219417/L, with 5% control mortality.

(Maynard S, 2011)

Report:	Study was conducted in order to refine the aquatic invertebrate risk assessment K-CA 8.2.4.2/02 Maynard S.K. (2011a) CGA219417 – 96 hour acute toxicity to adult *Asellus aquaticus*. Report number CEMR-5070. CEMAS, North Ascot, Berkshire, UK. (Syngenta File No. CGA219417_11454)

Guidelines

OECD Guidelines for Testing of Chemicals, Section 2 - Effects on Biotic Systems, Method 202: *Daphnia* sp., Acute Immobilisation Test (2004)

OECD Guidelines for Testing of Chemicals, Section 2 - Effects on Biotic Systems, Method 211: *Daphnia magna* reproduction test (2008)

GLP: Yes

Executive Summary

The acute toxicity of CGA219417 to adult *Asellus aquaticus* was determined under static conditions over 96 hours. Adult *Asellus* were exposed to a range of nominal concentrations of 0.116, 0.256, 0.563, 1.24, 2.73 and 6.00 mg CGA219417/L, alongside a dilution water control. Based on nominal concentrations, the 96-hour EC_{50} was 1.96 mg CGA219417/L.

Materials

Test Material	CGA219417
Lot/Batch #:	P.012011
Actual content of active ingredients:	99.2%
Description:	Light beige crumbs
Stability of test compound:	Stable under standard conditions.
Reanalysis/Expiry date:	30 April, 2014
Density:	n.a.
Treatments	
Test concentrations:	Dilution water control and nominal formulation concentrations of 0.116, 0.256, 0.563, 1.24, 2.73 and 6.00 mg CGA219417/L
Solvent:	None
Positive control:	n.a.
Analysis of test concentrations:	Yes 0 and 96 hours (based on measurement of CGA219417) using LC analysis
Test organisms	
Species:	*Asellus aquaticus*
Age:	Adults, >5mm
Source:	Commercial supplier, Mesocosm GmbH, Homberg, Germany
Feeding:	None during test
Culture medium:	Elendt M4
Test design	
Test vessels:	250 mL beakers with ~ 5 g fine quartz sand substrate
Test medium:	Elendt M4

Replication:	10 replicates, each containing 1 *Asellus* (one replicate at 6.00 mg/L contained 2 animals)
Exposure regime:	Static
Duration:	96 hours

Environmental conditions

Test temperature:	14.6 to 16.7°C
pH range:	7.06 to 7.38
Dissolved oxygen:	89 to 97 % air saturation value (ASV), no aeration
Total hardness of dilution water:	242 to 244 mg/L as $CaCO_3$.
Lighting:	Photoperiod - 16 hours light and 8 hours dark

Study Design and Methods

Experimental dates: 19th to 23rd July 2011

The day before the test a stock solution with a nominal concentration of 6 mg/L was prepared by dissolving CGA219417 in dilution water using ultrasonication for 30 minutes, then one hour of stirring followed by one further minute of ultrasonication. Using this stock solution the test concentrations were prepared on the day of the test by dilution. The control consisted of dilution water. Test solutions were added to the test vessels, together with ~ 5 g fine quartz sand substrate, then one *Asellus* was added to each of the ten replicates at each treatment. One replicate at 6.00 mg/L contained 2 animals. Preliminary work had identified the need for an inert substrate to provide the test animals some purchase on the base of the vessels.

The immobility of the *Asellus* was determined by visual observations after 24, 48 72 and 96 hours of exposure. Organisms unable to move within 15 seconds after gentle agitation of the test beaker were considered to be immobile. Abnormal behaviour, such as lethargy, was also noted.

The pH, temperature and dissolved oxygen were measured at the start and end of the test in each test concentration and the control.

The test concentrations were verified by chemical analysis of CGA219417 at 0 and 96 hours using liquid chromatography with ultra violet-visible detection. In addition, the 2.73 mg/L treatment was sampled for analysis at 72 hours and the 6.00 mg/L treatment at 24 hours, as all the test organisms were immobile at these times.

Results and Discussion

At the start of the test, the concentrations of CGA219417 were in the range 91 to 107% of the nominal values, and at the end of the test were in the range 94 to 104% (see table below). The limit of quantification in this study was 0.01 mg CGA219417/L. Nominal concentrations were used for the calculation and reporting of results.

Table 8.2.4.2-323: Analytical results

Nominal concentrations of CGA219417 (mg/L)	% of nominal measured at 0 hours	% of nominal measured at 96 hours
0 (control)	n.a.	n.a.
0.116	98	97
0.256	96	94
0.563	107	104
1.24	98	98
2.73	100	100 (98)*
6.00	91	99 (95)**

n.a. = not applicable, *= 72-h sample, **= 24-h sample

The median effect concentrations (EC_{50}), defined as the concentration resulting in 50% immobilisation of the *Asellus* in the time period specified, were calculated by linear interpolation. The NOEC (No Observed Effect Concentration) is defined as the highest tested concentration which did not produce an adverse effect when compared to the control and was determined using Steel's Many-One Rank Test. There was 20% immobility observed in the dilution water control. Immobility data and estimated EC_{50} values are shown in the table below:

Table 8.2.4.2-24: Effects of CGA219417 on adult *Asellus aquaticus* following exposure for 96-hours in a static test

Nominal concentration of CGA219417 (mg/L)	Cumulative immobilisation 24 hours		Cumulative immobilisation 48 hours		Cumulative immobilisation 72 hours		Cumulative immobilisation 96 hours	
	Number	%	Number	%	Number	%	Number	%
0 (control)	1	10	2	20	2	20	2	20
0.116	1	10	2	20	2	20	2	20
0.256	0	0	0	0	0	0	2	20
0.563	0	0	0	0	0	0	1	10
1.24	1	10	1	10	1	10	2	20
2.73	5	50	9	90	10	100	10	100
6.00	11	100	11	100	11	100	11	100
EC_{50} (mg CGA219417/L)	2.89		2.08		1.99		1.96	
95% Confidence limits	2.09 – 3.90		1.75 – 2.38		1.75 – 1.99		1.49 – 1.99	
NOEC	2.73		1.24		1.24		1.24	

Conclusions

Based on nominal concentrations, the 96-hour EC_{50} was 1.96 mg CGA219417/L and the NOEC was 1.24 mg CGA219417/L.

(Maynard S, 2011a)

Report:	Submitted for purposes of renewal as conducted on cyprodinil for USA compliance: K-CA 8.2.4.2/03 Ward T.J., Magazu J.P. & Borei R.L. (1995a) Acute flow-through toxicity of CGA219417 to the mysid (*Mysidopsis bahia*). Report Number 827-CG. T. R. Wilbury Laboratories, Inc. 40 Doaks Lane, Marblehead, Massachusetts. (Syngenta File No. CGA219417/0649)

Guidelines

US EPA FIFRA Guideline No. 72-3(c)

GLP: Yes

Executive Summary

The acute toxicity of CGA219417 to the saltwater mysid (*Mysidopsis bahia*) was determined under flow-through conditions. This study was run with mean measured concentrations of 4.90, 6.25, 8.94, 14.5, and 23.5 µg a.s./L together with negative and solvent controls.

The LC_{50} was 8.05 µg a.s./L based on mean measured concentrations.

Materials

Test material	CGA219417
Description:	Off-white granules
Lot/Batch #:	P012012
Purity:	99.2%
Recertification date:	April 1995
Treatments	
Test concentrations:	3.9, 6.6, 11, 18 and 30 µg/L nominal (4.90, 6.25, 8.94, 14.5, and 23.5 µg a.s./L mean measured)
Dilution water:	Carbon filtered, natural seawater
Vehicle and/or positive control:	None
Analysis of test concentrations:	Yes at 0and 96 hours using HPLC analysis
Test organisms	
Species:	Saltwater mysid (*Mysidopsis bahia*)
Source:	Test facility (originally: Aquatic Biosystems, Inc. Fort Collins, Colorado.)
Acclimatisation period:	Adults acclimated 10 days before collection of juveniles
Treatment for disease:	None
Life stage of test organism:	Juvenile (<24 hours)
Feeding:	Live brine shrimp (*Artemia salina* nauplii) daily during test
Test design	
Test vessels:	20L glass aquaria containing 15 L test solution.
Replication:	2 replicates, 10 mysids per replicate
Exposure regime:	Flow-through
Duration:	96 hours
Environmental conditions	

Test temperature:	21.4 to 22.5 at start and 21.4 to 22.6 at test end
pH range:	8.2 to 8.5 measured daily
Dissolved oxygen:	5.5 to 8.3 mg/L measured daily
Salinity of dilution water:	15‰ at test start
Lighting:	16 hours fluorescent light and 8 hours dark daily, with 30 minute dawn and dusk transition periods. Light intensity ~ 215 lux at water surface.

Study Design and Methods

Experimental dates: 14th to 18th December 1995

Test chambers were suspended in the aquaria which were filled with 15 L test water. A continuous flow diluter was used to deliver each concentration of the test substance, solvent control and negative control. A syringe pump was used to deliver the five test substance stocks and the solvent control stock into the mixing chambers assigned to each treatment group.

The test chambers were impartially positioned within a water bath to maintain temperature. Two replicate tanks were prepared for the controls and each test solution. Ten mysids were randomly allocated to each prepared test vessel.

A primary stock of 300 mg a.s./mL test material was prepared by combining 0.151 g of test substance with dimethylformamide in a 500 ml volumetric flask. Appropriate amounts of the stock solution were added directly to the dilution water to formulate test media. Nominal concentrations were 3.9, 6.6, 11, 18 and 30 µg/L.

The concentrations of test material in the test solutions were measured at the beginning, and at 96 hours using HLPC analysis.

Observations were made for mortality and clinical symptoms of toxicity at approximately 6, 24, 48, 72 and 96 hours.

Results and Discussion

The measured concentrations are shown in the table below in terms of nominal concentrations. The test concentrations were maintained throughout the study. Mean measured concentrations were used for the calculation and reporting of results.

Table 8.2.4.2-525: Analytical results

Nominal concentration (µg a.s./L)	% of nominal 0 hours	% of nominal 96 hours	Mean measured concentration (µg a.s./L)
3.9	142	108	4.90
6.6	96	95	6.25
11	80	81	8.94
18	81	81	14.5
30	82	78	23.5

Mysids in the controls the three lowest treatment groups appeared normal throughout the test. Lethargy was observed in the 14.5 µg/L treatment after 48 hours.

Table 8.2.4.2-626: Effects of test material on the survival of saltwater mysids (*Mysidopsis bahia*) following exposure for 96 hours in a flow-through test

Mean measured concentration (mg a.s./L)	Mean number alive (n = 10)			
	24 hour	48 hour	72 hour	96 hour
Dilution water control	10	10	10	10
Solvent control	9.5	9.5	9.5	9.5
4.90	10	10	9.5	9
6.25	10	10	9	8.5
8.94	10	10	5.5	3.5
14.5	10	4.5	0	0
23.5	10	0	0	0
LC_{50} (µg a.s./L)	>23.4	14.0	8.85	8.05
95% confidence limits	n.d.	8.94 – 23.5	7.96 - 9.99	7.22 – 9.05
Method	-	Binomial	Probit	Probit

a Ten mysids were exposed in each test vessel, two replicates per treatment.
n.d. –not determined

Conclusions

The 96 hour LC_{50} for test material to the saltwater mysid (*Mysidopsis bahia*) was calculated to be 8.05 µg a.s./L, based on mean measured concentrations.

(Ward *et al*., 1995a)

Report:	Submitted for purposes of renewal as conducted on cyprodinil for USA compliance: K-CA 8.2.4.2/04 Sutherland C.A. & Kruger H.O. (1998) CGA219417 A 10 day sediment toxicity test with the marine amphipod (*Grandidierella japonica*). Report Number 108A-203. Wildlife International Ltd, Easton, MD, USA. (Syngenta File No. CGA219417/0893)

Guidelines

OPPTS 850.1740

ASTM Designation E 1367-92

GLP: Yes

Executive Summary

Toxicity to the marine amphipod (*Grandidierella japonica*) was determined in a 10 day sediment toxicity test. Nominal concentrations of 0.25, 0.50, 1.0, 2.0 and 4.0 mg/kg sediment d.w. were tested alongside a negative control and a solvent control.

The 10 day LC_{50} was 0.42 mg a.s./kg, the no observed effect concentration (NOEC) was 0.53 mg a.s./kg and the Maximum acceptable toxicant concentration (MATC) was 0.36 mg a.s./kg.

Materials

Test material	CGA219417 technical
Description:	Off-white granules
Lot/Batch #:	P012012
Purity:	99.2%
Stability of test compound:	Not given
Treatments	
Test concentrations:	0.25, 0.50, 1.0, 2.0 and 4.0 mg a.s./kg sediment d.w
Dilution water:	Saltwater (0.25 µm filtered seawater)
Vehicle and/or positive control:	Dimethylformamide (DMF)
Analysis of test concentrations:	Yes at test start and test end using HPLC analysis
Test organisms	
Species:	Marine amphipod (*Grandidierella japonica*)
Source:	David Gutoff San Diego, California
Acclimatisation period:	3 days
Treatment for disease:	None
Feeding:	None during test
Test design	
Test vessels:	1000 mL glass beakers containing 2cm of sediment and 500 mL of overlying water (depth 8.4 cm)
Test sediment:	0.2% organic content, 0.8% moisture, cation exchange 8.9, particle distribution of 98%, 2% silt, 0% clay.
Replication:	4 replicates of 10 amphipods
Exposure regime:	Static
Duration:	10 days
Environmental conditions	
Test temperature:	17 ± 1 °C
pH range:	Water: 7.6 to 7.9 Sediment: 7.2 to 7.9
Dissolved oxygen:	6.2 to 7.8 mg/L
Lighting:	16 hours fluorescent light and 8 hours dark daily, with 30 minute dawn and dusk transition periods. Light intensity 308 lux at water surface.

Study Design and Methods

Experimental dates: 30th October to 17th November 1998

Test chambers were 1000 mL glass beakers containing 2 cm of sediment and 500 mL of overlying water (saltwater). The depth of overlying water in a representative test chamber was 8.4 cm. Test chambers were covered to minimise evaporation and were randomly positioned in an environmental chamber designed to maintain a temperature of 17 ± 1 °C.

A primary stock of 40 mg a.s./mL test material was prepared in DMF, and then serial diluted with DMF to create four additional stock solutions. Dry soil was weighed into 2 L Nalgene bottles and the

appropriate aliquots of stock solution were added. Nominal concentrations of 0.25, 0.50, 1.0, 2.0 and 4.0 mg a.s./kg sediment d.w were produced by serial dilution. Salt water was added to facilitate mixing. 500 mL test solution was added to each replicate test chamber.

The concentrations of test material in the test solutions were measured at the beginning, and at test termination using HLPC analysis.

Observations were made at test termination to determine mortalities and the number of individuals exhibiting clinical signs of toxicity or abnormal behaviour.

Results and Discussion

The measured concentrations are shown in the table below in terms of nominal concentrations for sediment and the overlying water. Mean measured concentrations were used for the calculation and reporting of results.

Table 8.2.4.2-727: Analytical results in sediment

Nominal concentration (mg a.s./kg)	% of nominal 0 days	% of nominal 10 days	Mean measured concentration (mg a.s./kg)
0.25	50	49	0.13
0.50	56	41	0.24
1.0	57	50	0.53
2.0	47	50	0.98
4.0	50	51	2.0

Table 8.2.4.2-828: Analytical results in overlying water

Nominal concentration (mg a.s./kg)	% of nominal 0 days	% of nominal 10 days	Mean measured concentration (mg a.s./kg)
0.25	8	11	0.245
0.50	10	12	0.056
1.0	8	11	0.094
2.0	7	9	0.163
4.0	8	11	0.382

Surviving amphipods in the negative and solvent controls appeared normal and healthy at the end of the test. Surviving amphipods in the 0.13 and 0.24 mg a.s./kg treatment groups also appeared normal and healthy a test termination. Several mortalities were observed in the 0.53 mg a.s./kg treatment group, while erratic swimming, lethargy and mortality were observed in the 0.98 and 2.0 mg a.s./kg treatment groups. LC_{50} values and 95% confidence intervals were calculated using the probit method from the mortality data. Bonferonni's test was used to see if the results were significantly reduced compared with the control.

Table 8.2.4.2-929: Effects of test material on the survival of marine amphipod (*Grandidierella japonica*) following a 10 day test

Mean measured sediment concentration (mg a.s./L)	Percent dead after 10 days (%)
Negative control	18
Solvent control	13
0.13	13
0.24	25
0.53	60*
0.98	80*
2.0	98*
LC_{50} (mg a.s./kg)	0.42
95% confidence limits	0.34 – 0.51

* Statistically significant difference for survival determined using Bonferonni's test when compared with the pooled control group.

Conclusions

The 10 day LC_{50} value for the marine amphipod (*Grandidierella japonica*) exposed to CGA219417 was 0.42 mg a.s./kg sediment. The 95% confidence limits were 0.34 to 0.51 mg a.s./kg sediment. The LOEC was 0.53 mg a.s./kg sediment, the NOEC was 0.24 mg a.s./kg sediment and the MATC, calculated as the geometric mean of the LOEC and NOEC was 0.36 mg a.s./kg sediment.

(Sutherland C & Kruger H, 1998)

Report:	Submitted for purposes of renewal as conducted on cyprodinil for USA compliance: K-CA 8.2.4.2/05 Ward T.J., Magazu J.P. & Boeri R.L. (1995b) Acute flow through mollusc shell deposition test with CGA219417. Report Number 828-CG. T.R Wilbury Laboratories Inc, 40 Doaks Lane, Marblehead, Massachusetts. (Syngenta File No. CGA219417/0650)

Guidelines

US EPA FIFRA Guideline No. 72-3 (c)

GLP: Yes

Executive Summary

The acute toxicity of CGA219417 to the eastern oyster (*Crassostrea virginica*) was determined under flow-through conditions. This study was run with mean measured concentrations of 0.078, 0.13, 0.22, 0.36 and 0.60 mg/L together with negative and solvent controls.

The 96 hour EC_{50} value was 0.360 mg a.s./L the 96 hour NOEC was 0.213 mg/L.

Materials

Test material	CGA219417
Description:	Off-white powder
Lot/Batch #:	P012012
Purity:	99.2%
Stability of test compound:	April 1995
Treatments	
Test concentrations:	Negative (saltwater) control, solvent control and nominal concentrations of 0.078, 0.13, 0.22, 0.36 and 0.60 mg/L (mean measured 0.0812, 0.137, 0.213, 0.368, and 0.601 mg a.s./L)
Dilution water:	Natural seawater
Vehicle and/or positive control:	None
Analysis of test concentrations:	Yes at 0 and 96 hours using HPLC analysis
Test organisms	
Species:	Eastern oyster (*Crassostrea virginica*)
Source:	P. Cummins Oyster Company, Pasadena, Maryland
Acclimatisation period:	Acclimatised 10 days before test initiation
Treatment for disease:	None
Length of oysters at test start:	Length: range 25 to 50 mm
Feeding:	Marine phytoplankton
Test design	
Test vessels:	20 L glass aquaria containing 15 L of test solution
Replication:	2 replicates of 10 oysters
Exposure regime:	Flow-through
Duration	96 hours
Environmental conditions	
Test temperature:	19.1 to 20.8 °C in test chambers and measured continuously in the negative control
pH range:	7.9 to 8.4 measured daily
Dissolved oxygen:	6.3 to 8.1 mg/L value measured daily
Lighting:	16 hours fluorescent light and 8 hours dark daily, with 15 minute dawn and dusk transition periods. Light intensity = 15 footcandles ≈ 161 Lux at water surface.

Study Design and Methods

Experimental dates: 23rd June to 13th December 1995

A stock solution with nominal concentration of 6000 mg/L was prepared by combining 6.048 g of test substance with dimethylformamide volume to 1000 ml. Appropriate amounts of stock solution were added directly to dilution water by a proportional diluter.

The concentrations of CGA219417 in the test solutions were measured at 0, 24, 48, 72 and 96 hours using high performance liquid chromatography with UV detection.

Observations were made daily for mortality and clinical symptoms of toxicity at approximately 24, 48, 72 and 96 hours. At the end of the exposure period, mean shell deposition was calculated for each control and treatment group by measuring the longest finger of growth for each oyster. Shell deposition was expressed as percent inhibition of mean shell growth for each treatment relative to the control.

Results and Discussion

Mean measured concentrations, calculated from the average of all samples, ranged from 97 to 105% of nominal concentrations. Mean measured concentrations were used for the reporting of the results.

100% survival occurred in the control and at all test concentrations, and no sublethal effects were noted during the exposure period. There was inhibition of shell deposition at 0.368 and 0.601 mg/L in comparisons with the pooled controls. The NOEC was 0.213 mg a.s./L, based on lethal and sublethal effects.

Table 8.2.4.2-~~1030~~: Effects of test material on the shell deposition of the eastern oyster *Crassostrea virginica* following exposure for 96 hours in a flow-through test

Mean measured concentration (mg a.s./L)	Shell deposition (mm)		Percent of control %	Number of dead oysters after 96-hours
	Mean	Standard deviation		
Negative control	2.46	1.13	-	0
Solvent control	2.30	0.99	93	0
0.0812	2.57	0.80	104	0
0.137	1.94	0.96	79	0
0.213	2.47	1.03	100	0
0.368	1.15*	0.99	47	0
0.601	0.82*	0.78	33	0
EC_{50} (mg a.s./L)	0.360			>0.601
95% confidence limits	0.213 – 0.601			n.d.

* - statistically significant com pared with the control and solvent control

Conclusions

The 96 hour LC_{50} for CGA219417 to the eastern oyster (*Crassostrea virginica*) was calculated to be 0.360 mg a.s./L, based on mean measured concentrations.

(Ward *et al*.1995b)

Report:	Submitted for purposes of renewal as conducted on cyprodinil for USA compliance: K-CA 8.2.4.2/06 Sutherland C. & Kruger H. (1998a) CGA219417 A 10 day sediment toxicity test with the marine amphipod (*Hyalella azteca*). Report Number 108A-202. Wildlife International Ltd, Easton, MD, USA. (Syngenta File No. CGA219417/0892)

Guidelines

OPPTS 850.1740

ASTM Designation E 1367-92

GLP: Yes

Executive Summary

A 10 day sediment toxicity test to the marine amphipod (*Hyalella azteca*) was determined. Nominal concentrations of 0.25, 0.50, 1.0, 2.0 and 4.0 mg/kg sediment d.w were tested alongside a negative control and a solvent control.

The 10 day LC_{50} was 0.73 mg a.s./kg, the no observed effect concentration (NOEC) was 0.57 mg a.s./kg and the Maximum acceptable toxicant concentration (MATC) was 0.79 mg a.s./kg.

Materials

Test material	CGA219417 technical
Description:	Off-white granules
Lot/Batch #:	P012012
Purity:	99.2%
Stability of test compound:	Not given
Treatments	
Test concentrations:	0.25, 0.50, 1.0, 2.0 and 4.0 mg a.s./kg sediment d.w
Dilution water:	Saltwater (0.25 µm filtered seawater)
Vehicle and/or positive control:	Dimethylformamide (DMF)
Analysis of test concentrations:	Yes at test start and test end using HPLC analysis
Test organisms	
Species:	Marine amphipod (*Hyalella azteca*)
Source:	Aquatic Research organisms, Hampton, New Hampshire
Acclimatisation period:	2 days
Treatment for disease:	None
Feeding:	None during test
Test design	
Test vessels:	300 mL glass beakers containing 100 mL of overlying water (depth 8.5 cm)
Test sediment:	1.2% organic content, 32% water content, 13% sand, 13% silt, 61% clay.
Replication:	4 replicates of 10 amphipods
Exposure regime:	Static
Duration:	10 days
Environmental conditions	
Test temperature:	22.2 to 23.2 °C
pH range:	Water: 7.5 to 7.9 Sediment: 6.1 to 6.9
Dissolved oxygen:	7.4 to 8.2 mg/L
Lighting:	16 hours fluorescent light and 8 hours dark daily, with 30 minute dawn and dusk transition periods. Light intensity 289 lux at water surface.

Study Design and Methods

Experimental dates: 29th October to 12th November 1998

Test chambers were 300 mL glass beakers containing 100 mL of overlying water (saltwater). The depth of overlying water in a representative test chamber was 8.5 cm. Test chambers were covered to minimise evaporation and were randomly positioned in an environmental chamber designed to maintain a temperature of 23 ± 1°C.

A primary stock of 40 mg a.s./mL test material was prepared in DMF, and then serial diluted with DMF to create four additional stock solutions. Dry soil was weighed into 2L Nalgene bottles and the appropriate aliquots of stock solution were added. Nominal concentrations of 0.25, 0.50, 1.0, 2.0 and 4.0 mg a.s./kg sediment d.w. were produced by serial dilution. Salt water was added to facilitate mixing. 100 mL test solution was added to each replicate test chamber.

The concentrations of test material in the test solutions were measured at the beginning, and at test termination using HLPC analysis.

Observations were made at test termination to determine mortalities and the number of individuals exhibiting clinical signs of toxicity or abnormal behaviour.

Results and Discussion

The measured concentrations are shown in the table below in terms of nominal concentrations for sediment and the overlying water. Mean measured concentrations were used for the calculation and reporting of results.

Table 8.2.4.2-~~1~~1131: Analytical results in sediment

Nominal concentration (mg a.s./kg)	% of nominal 0 days	% of nominal 10 days	Mean measured concentration (mg a.s./kg)
0.25	64	51	0.14
0.50	61	56	0.29
1.0	58	55	0.57
2.0	59	55	1.1
4.0	63	58	2.4

Table 8.2.4.2-~~2~~1232: Analytical results in overlying water

Nominal concentration (mg a.s./kg)	% of nominal 0 days	% of nominal 10 days	Mean measured concentration (mg a.s./kg)
0.25	0.40	0.80	0.002
0.50	0.20	1.00	0.003
1.0	0.30	0.60	0.005
2.0	0.20	0.95	0.012
4.0	0.25	1.38	0.033

Surviving amphipods in the negative and solvent controls appeared normal and healthy at the end of the test.

Amphipods in the 0.14, 0.29 and 0.57 mg a.s./kg treatment groups also appeared normal and healthy a test termination, erratic swimming and lethargy were observed in the 1.1 and 2.4 mg a.s./kg treatment groups.

LC_{50} values and 95 % confidence intervals were calculated using the probit method from the mortality data. Bonferonni's test was used to see if the results were significantly reduced compared with the control.

Table 8.2.4.2-~~1~~1333: Effects of test material on the survival of marine amphipod (*Hyalella azteca*) following a 10 day soil test

Mean measured sediment concentration (mg a.s./kg)	Percent dead after 10 days (%)
Negative control	23
Solvent control	10
0.14	25
0.29	23
0.57	35
1.1	65*
2.4	78*
LC_{50} (mg a.s./kg)	0.73
95% confidence limits	0.53 - 1.06

* Statistically significant difference for survival determined using Bonferonni's test when compared with the pooled control group.

Conclusions

The 10 day LC_{50} value for the marine amphipod (*Hyalella azteca*) exposed to CGA219417 was 0.73 mg a.s./kg sediment. The 95% confidence limits were 0.53 to 1.06 mg a.s./kg sediment. The LOEC was 1.1 mg a.s./kg sediment, the NOEC was 0.57 mg a.s./kg sediment and the MATC, calculated as the geometric mean of the LOEC and NOEC was 0.79 mg a.s./kg sediment.

(Sutherland C & Kruger H, 1998)

Metabolites of cyprodinil

Report:	Submitted for purposes of renewal as conducted on relevant aquatic metabolite: K-CA 8.2.4.2/07 Tobler N. (2015) CGA321915 – Acute Toxicity to First-Instar Larvae of *Chironomus riparius*. Report Number D96744. Harlan Laboratories Ltd, Zelgliweg 1, 4452 Itingen/Switzerland. (Syngenta File No. CGA321915_10009)

Guidelines

OECD Guidelines for Testing of Chemicals, Section 2 - Effects on Biotic Systems, Method 235: *Chironomus sp*., Acute Immobilisation Test (2011)

GLP: Yes

Executive Summary

The acute toxicity of CGA321915 to *Chironomus riparius* was determined under static conditions. First instar larvae were exposed to a range of nominal concentrations of 0.32, 1.0, 3.2, 10, 32 and 100 mg CGA321915/L (mean measured: 0.34, 1.0, 3.2, 10, 32 and 97 mg CGA321915/L) alongside a dilution water control. Based on mean measured concentrations, the 48-hour EC_{50} was > 97 mg CGA321915/L, the highest concentration tested.

Materials

Test Material:	CGA321915
Parent:	CGA219417 (Cyprodinil)
Lot/Batch #:	MES 356/1
Purity:	98 % w/w (estimated error: ± 2 %)
Description:	White solid
Stability of test compound:	Stable under test conditions
Reanalysis/Expiry date:	30 September 2016
Density:	Not applicable

Treatments

Test concentrations:	Dilution water control and nominal concentrations of 0.32, 1.0, 3.2, 10, 32 and 100 mg CGA321915/L (mean measured concentrations: 0.34, 1.0, 3.2, 10, 32 and 97 mg CGA321915/L)
Solvent:	None
Positive control:	Potassium dichromate was tested in parallel to this study in laboratory study number D97778, demonstrating the sensitivity of the test organisms, and satisfactory test conditions
Analysis of test concentrations:	Yes, 0 and 48 hours (based on measurement of CGA321915) using HPLC with UV detection

Test organisms:

Species:	*Chironomus riparius*
Age:	First instar (2 days post hatching)
Source:	Continuous laboratory cultures
Feeding*:	Tetra Min® fish-food, finely ground and suspended in purified water, was added at the start of the test. The total food concentration was 10 mg carbon/L test water.
Culture medium:	Reconstituted water (Elendt M7 medium)

Test design:

Test vessels:	60 mL glass dishes containing 50 mL of media
Test medium:	Elendt M7
Replication:	4 replicates of 5 chironomids
Exposure regime:	Static
Duration:	48 hours

Environmental conditions

Test temperature:	20 to 21 °C
pH:	7.8
Dissolved oxygen:	8.1 to 8.2 mg/L (no aeration)
Total hardness of dilution water:	250 mg/L as $CaCO_3$
Lighting:	16 hours light (approximately 970 Lux) and 8 hours dark, with a 30 minute transition period

*The test guideline states that the test organisms should not be fed during the test. This deviation was considered necessary to ensure sufficient survival of the test animals during the test period, based on previous tests.

Study Design and Methods

Experimental dates: 27th November 2014 to 14th February 2015

The test medium of the highest test concentration of 100 mg CGA321915/L was prepared by dissolving 100.1 mg of the test item in 1000 mL of dilution water by intense stirring for 15 minutes at room temperature. Adequate volumes of this test medium were used in a series of dilutions to prepare the test media of all test item concentrations. The control consisted of dilution water only. Test solutions were added to the test vessels and the chironomid larvae added without conscious bias.

The immobility of the chironomids was determined by visual observations after 24 and 48 hours of exposure. Organisms which were motionless after gentle agitation of the test vessel were considered to be immobile.

The pH, temperature and dissolved oxygen were measured at the start and end of the test in each test concentration and the control.

The test concentrations were verified by chemical analysis of CGA321915 at 0 and 48 hours using high performance liquid chromatography with ultra violet detection.

Results and Discussion

At the start of the test, the concentrations of CGA321915 were in the range 95 to 103 % of the nominal values and at the end of the test were in the range 98 to 106 % (see table below). The limit of quantification in this study was 0.102 mg CGA321915/L. Geometric mean measured concentrations were used for the calculation and reporting of results.

Table 8.2.4.2-1434: Analytical results

Nominal concentrations of CGA321915 (mg/L)	% of nominal measured at 0 hours	% of nominal measured at 48 hours	Mean measured concentration (mg CGA321915/L)
0.32	103	106	0.34
1.0	100	102	1.0
3.2	100	102	3.2
10	98	102	10
32	98	101	32
100	95	98	97

The tabulated values of the samples represent rounded results obtained by calculation using the exact raw data

The median effect concentration (EC_{50}) was defined as the concentration resulting in 50 % immobilisation of the chironomids in the time period specified and could not be calculated since sufficient toxicity was not observed. The NOEC (No Observed Effect Concentration) is defined as the highest tested concentration which did not produce an adverse effect when compared to the control and was determined by visual inspection of the data. There was no immobility observed in the dilution water control. Immobility data and estimated EC_{50} values are shown in the table below:

Table 8.2.4.2-~~1~~1535: Effects of CGA321915 on the response of *Chironomus riparius* following exposure for 48-hours in a static test

Mean measured concentration (mg CGA321915/L)	Immobilised chironomids after 24 hours		Immobilised chironomids after 48 hours	
	Number	%	Number	%
Dilution water control	0	0	0	0
0.34	0	0	0	0
1.0	0	0	0	0
3.2	0	0	0	0
10	0	0	0	0
32	0	0	0	0
97	0	0	8	40
EC_{50}	> 97 mg CGA321915/L		> 97 mg CGA321915/L	
95% Confidence limits	n.d.		n.d.	
NOEC	97 mg CGA321915/L		32 mg CGA321915/L	

n.d. not determined

Validity Criteria

The test was considered to be valid, as in the control no test organisms showed immobilisation.

Conclusions

Based on mean measured concentrations, the 48-hour EC_{50} for CGA321915 to *Chironomus riparius* was > 97 mg CGA321915/L, the highest concentration tested. The 48-hour NOEC was 32 mg CGA321915/L.

(Tobler N, 2015)

Relevant Literature on Toxicity of Cyprodinil to Additional Invertebrate Species

Scientifically peer-reviewed open literature that was possibly relevant was found for the potential effects of cyprodinil on fish, and is summarised in the following table. The reports are categorised in accordance with the EFSA Guidance document as:

a. Studies that provide data for establishing or refining risk assessment parameters. These studies should be summarised in detail following the subsequent steps of the OECD Guidance documents (OECD, 2005; 2006) and should be considered for reliability.

b. Studies that are relevant to the data requirement, but in the opinion of the applicant provide only supplementary information that does not alter existing risk assessment parameters. A justification for such a decision should be provided.

c. Studies for which relevance cannot be clearly determined. For each of these studies the applicants should provide an explanation of why the relevance of such studies could not be definitively determined.

Summary of references

Reference	Categorisation & comments	Summary
Padilla S., Corum D., Padnos B., Hunter D.L., Beam A., Houck K.A., Sipes N., Kleinstreuer N., Knudsen T., Dix D.J. & Reif D.M. (2012) Zebrafish developmental screening of the ToxCast™ Phase I chemical library. *Reproductive Toxicology* **33** 174-187	b). This research provides only supplementary information As the 6d exposure period is for such a short length of time this literature study would be comparable with the acute tests. The regulatory data suggests that zebrafish embryos show similar acute toxicity as the juvenile fish species tested.	A 6 day embryo toxicity test was conducted in which the effects of cyprodinil on zebra fish were evaluated. Test methodology similar to OECD 236 (Fish Embryo Acute Toxicity Test) was used. Embryos were maintained in a 10% Hank's solution in 96-well plates. AC_{50} for cyprodinil was 10.3 µM (2.32 mg/L)
Beketov M.A. and Liess M. (2008) Potential of 11 pesticides to initiate downstream drift of stream macroinvertebrates. *Archives of Environmental Contamination and Toxicology*, 55: 247-253	~~a~~) b) ~~Reliable with restrictions,~~ This research provides only supplementary information. Control mortality was not reported. Non-GLP, and concentrations were not confirmed within the acute toxicity studies, nor was control survival. Also no information was provided on the acclimatisation period or feeding during this time. Although, the study was conducted near guideline study published in peer-reviewed literature. Methodology is reasonably well documented.	Organisms were exposed individually to a range of concentrations for 96 hours. Mortality within the control and at each concentration was monitored daily. The death of the organisms was defined as the absence of movement of the gammarids. The 96 hour LC_{50} for *Gammarus pulex* when exposed to cyprodinil was determined to be 690 µg/L (95% C.I. 460-1040 µg/L).

~~A summary of the potentially relevant literature article is presented below:~~

~~Report:~~	~~K-CA 8.2.4.2/08 Beketov M.A. and Liess M. (2008) Potential of 11 pesticides to initiate downstream drift of stream macroinvertabrates. *Archives of Environmental Contamination and Toxicology*, 55: 247-253 (Syngenta File No: CGA219417_11653)~~

~~Guidelines~~

~~The study does not allude to a particular guideline that was used but the study design was well reported.~~

~~GLP:~~ ~~No.~~

~~Executive Summary~~

~~Organisms were exposed individually to a range of concentrations for 96 hours. Mortality within the control and at each concentration was monitored daily. The death of the organisms was defined as the absence of movement of the gammarids.~~

~~The 96 hour LC_{50} for *Gammarus pulex* when exposed to cyprodinil was determined to be 690 µg/L (95% C.I. 460-1040 µg/L).~~

~~Materials~~

~~Test Material~~	~~Cyprodinil~~
~~Purity~~	~~Analytical grade~~
~~Source:~~	~~Sigma-Aldrich Laborchemikalien, Seelze, Germany~~
~~Test concentrations:~~	~~Not stated~~
~~Vehicle and/or positive control:~~	~~None~~

Analysis of test concentrations:	No.

Test animals

Species:	*Gammarus pulex*
Source:	Small uncontaminated stream (near Pulsnitz city, Saxony, Germany)
Acclimatisation period:	Several days
Treatment for disease:	Not stated
Weight and length of sub-sample from batch of fish at start of test:	Not reported
Feeding:	None during test

Test design

Exposure regime:	Static
Aeration:	Not stated

Environmental conditions

Medium:	Elendt M7
Test temperature:	15 ± 2°C
pH:	7.4
Dissolved oxygen:	Not stated
Hardness of dilution water and highest test concentration:	180mg/L $CaCO_3$
Conductivity:	600 µS/cm
Lighting:	10 hours per day

Study Design and Methods

The study tested several pesticides and their effects to *Gammarus pulex*, and two fly larvae. The main aim of the study was to establish if the addition of pesticides caused abnormal behaviour within the organisms which resulted in migration downstream. This is not a quantifiable and usable endpoint for the risk assessment. However to establish suitable concentrations for the sub-lethal test, an acute test was initially conducted to derive the EC_{50}.

Cyprodinil stock solutions were made up in DMSO and subsequently added to the test vessels. Ten animals were exposed in individual beakers (100 mL filled with 60 mL holding water) per treatment concentration and the control. Test duration was 96 hours and the solutions were not renewed for the course of the study (i.e. static test) and animals were not fed.

Mortality within the control and at each concentration was monitored daily. The death of the organisms was defined as the absence of movement of the gammarids.

Results and Discussion

The LC_{50} was determined to be 690 (95% C.I. 460-1040) µg/L.

Conclusions

The LC_{50} falls within the range of acute toxicities observed for aquatic invertebrates within the EFSA scientific report (2005; 51, 1-78, Conclusion on the peer review of cyprodinil), but in this instance the LC_{50} is lower than that of the suggested endpoint for *Gammarus* sp. (i.e. appears more toxic in this study).

However the study does not report the concentration range used or the number of concentrations tested which may be the cause of the result, furthermore the response was not plotted but just the end result reported, nor the control survival. More detail would be helpful in ascertaining the validity of this study

Study Reliability Evaluation	**Yes**	**No**	**Not reported**	**Not applicable**	**Comments**
Standardised test procedure followed			X		The study looked at the *Gammarus* sp. LC_{50}. The methodology that was provided did not appear to deviate greatly from USEPA methods or OECD 202 (2004) testing guidelines.
Appropriate test procedure followed	X				
Data quality assured (GLP or equivalent)		X			
Controls appropriate			X		
Control response acceptable, or accounted for statistically			X		However trimmed Spearman-Karber method was used to derive EC_{50}, the model "trims" values at the extremes of the sigmoidal response, thus controls should not affect the concentration response.
Temperature, pH & dissolved oxygen reported	X				
Alkalinity and hardness reported (metals)	X				Hardness was reported but not alkalinity, full medium as reported/ referenced to.
Statistics appropriate	X				
Effect levels above analytical limit of detection/quantification		X			
Material tested within limits of solubility, or effects above the limits of solubility sufficiently explained	X				Test substance was dissolved in DMSO to be used as carrier, and is a suitable method to increase solubility of the substance without inducing toxic responses at the levels used.
Analytical verification of test concentrations/doses		X			
Measurement of precipitate or undissolved material		X			
Appropriate dilution water used (e.g. not chlorinated tap, rain water etc)	X				Elendt M7 medium

Study assessment	**Score**	**Rationale**
Reliability/Repeatability	Klimisch 2	Reliable with restrictions, Non-GLP, and concentrations were not confirmed within the acute toxicity studies, nor was control survival. Although, the study was conducted near guideline study published in peer-reviewed literature. Methodology is reasonably well documented and end point would be useful.
Limitations		No raw data presented, health of control in question and measured concentrations not reported.
Relevance	Lethal median	LC_{50} was reported, but no lower or higher than that currently

~~Study assessment~~	~~Score~~	~~Rationale~~
	~~concentration (i.e. that causing 50 % mortality of the population) results are relevant for ecotoxicity RA.~~	~~reported by the EFSA conclusions.~~
~~Significance~~	~~LC_{50} results suitable for use in risk assessment~~	~~The LC50 reported here may consolidate those already reported by EFSA, however the study is poorly reported.~~

CA 8.2.5 Long-term and chronic toxicity to aquatic invertebrates

CA 8.2.5.1 Reproductive and development toxicity to *Daphnia magna*

The studies previously submitted in the EU were summarised in point B.9.2.5 of the DAR. At the request of ANSES summaries have been included in order to aid dossier evaluation. ~~therefore additional study summaries are not repeated here~~. The endpoints are summarised in Table 8.2-6.

Report:	Summary submitted at the request of ANSES for ease of dossier evaluation: *KIIA 8.2.5. Ward T. J., Magazu J.P. & Boeri R. L. (1995c). Chronic Toxicity of CGA-219417 to Daphnid, Daphnia magna. Report Number 468-CG. T. R. Wilbury Laboratories, Inc. Massachusetts 01945, USA. (Syngenta File No. CGA219417/0543).*

Guidelines

U. S. EPA-FIFRA, Guideline 72-4(b)

GLP: Yes

Executive Summary

The chronic toxicity of CGA219417 to the freshwater invertebrate *Daphnia magna* was determined in a 21-day study under flow-through conditions. *D. magna* were exposed to nominal CGA219417 concentrations of 9.6, 21, 40, 80 and 160 µg/L (8.16, 19.3, 40.2, 79.2 and 147 µg/L mean measured concentrations) alongside a dilution medium control and a solvent control. Based on mean measured concentrations, the 21-day EC_{50} for CGA219417 to *Daphnia magna* was 25.3 µg/L. The 21-day NOEC was determined to be 8.16 µg/L.

Materials

Test Material	CGA219417
Lot/Batch #:	P012012
Purity:	99.2%
Description:	Off-white granules
Stability of test compound:	Not reported
Reanalysis/expiry date:	7 April 1995

Treatments

Test concentrations:	Dilution medium control, solvent control and nominal concentrations of 9.6, 21, 40, 80 and 160 µg/L. Mean measured concentrations of 8.16, 19.3, 40.2, 79.2 and 147 µg/L
Solvent:	Dimethylformamide
Positive control:	None
Analysis of test concentrations:	Yes, HPLC analysis of CGA219417 in samples collected on days 0, 7, 14 and 21.

Test organism

Species:	*Daphnia magna* (< 24 hours old)
Source:	In-house culture, original supplied by Aquatic Research Organisms, Hampton, New Hampshire.
Feeding	Twice daily with *Selenastrum capricornutum* and yeast/trout chow suspension
Culture medium:	Deionized water adjusted to a total hardness of 160 – 180 mg as $CaCO_3$

Test design

Test vessels:	1 L glass beakers containing 850 mL test solution
Test medium:	Deionized water adjusted to a total hardness of 160 – 180 mg as $CaCO_3$
Replication:	Four replicates for each treatment and controls (10 organisms per replicate)
Exposure regime:	Flow-through
Aeration:	No
Duration:	21 days

Environmental conditions

Test temperature:	19.4 to 21.6 °C
pH:	7.7 to 8.2
Dissolved oxygen	6.2 to 8.7 mg/L
Conductivity	590 to 750 µmhos/cm
Lighting:	16 hour light and 8 our dark with a 15 minute transition. Light intensity of 28 footcandles

Study Design and Methods

Experimental dates: 10th to 31th August 1995

A stock solution with a nominal concentration of 16 g/L was prepared three times during the study by dissolving either 4.049 g or 1.619 g of the test substance in 250 or 100 mL dimethylformamide. Appropriate amounts of stock solution were transferred into the test vessels using a proportional diluter system. The diluter was calibrated before and after the test and during the test an average of 9.5 volume additions occurred per 24 hours in each test vessel.

Forty *D. magna* were indiscriminately and equally distributed between four replicates for each treatment. *Daphnia* were exposed in 1 L glass beakers containing 850 mL test solution. The test vessels were randomly distributed within a water bath during the 21-day exposure to maintain a constant temperature.

The number of surviving adult *D. magna* and the occurrence of sublethal effects was determined daily during the test. Dead test organisms were removed when first observed. The number of neonates produced in each replicate was recorded daily, after the onset of reproduction, and the young were removed from the test vessels. At the termination of the test the surviving adult *Daphnia* were measured and dried at 62°C for 91 hours and individually weighed.

Dissolved oxygen, pH, conductivity and temperature was measured daily in each test vessel that contained live animals. The temperature in one test vessel was recorded continuously during the test. Hardness and alkalinity were measured in the dilution water weekly during the test.

The test concentrations were verified by chemical analysis of CGA219417 in samples collected on days 0, 7, 14 and 21 using high performance liquid chromatography with a UV detector.

Survival of parental *D. magna* on Day 21, the time to first brood, the number of neonates produced per female and the mean length and dry weight of parental *D. magna* were statistically analysed. Normality and homogeneity of variance of the dataset were investigated using a chi-square test and Bartlett's test. The data concerning neonate production, dry weight and length fulfilled the criteria of equal variance and normality and therefore a parametric one-way analysis of variance (ANOVA) and Bonferroni's test were used to compare treatments to the pooled control and solvent control means. The survival and day to first brood data did not meet the assumptions of normality or homogeneity of variance. A Kruskal-Wallis test was used to compare the treatment means to the pooled control mean for these datasets. The 21-day median effective concentration (EC_{50}) was estimated by binomial / nonlinear interpolation.

Results and Discussion

Mean measured concentrations of the test item ranged from 85 to 101% of nominal and were stable during the test (see table below). Mean measured concentrations were used for the calculation and reporting of the results.

Table 8.2.5.1-1: Analytical results

Nominal concentration (µg/L)	Replicate	Measured concentration (µg/L)					Percent of nominal (%)
		Day 0	Day 7	Day 14[a]	Day 21[a]	Mean measured concentration (µg/L)	
Control	1	<0.200	0.449	0.381	<0.200	-	-
	2	0.240	0.375	0.247	<0.200		
Solvent control	1	<0.200	0.562	0.226	<0.200	-	-
	2	0.284	0.430	0.263	<0.200		
9.6	1	9.47	8.78	5.98	8.29	8.16	85
	2	9.05	8.81	7.33	7.52		
	3	N/A	N/A	N/A	8.24		
21	1	18.6	20.4	20.3	16.9	19.3	92
	2	18.4	22.0	20.8	17.2		
40	1	39.0	39.1	-	-	40.2	101
	2	36.2	46.5	-	-		
80	1	81.0	78.7	-	-	79.2	99
	2	77.1	79.9	-	-		
160	1	141	146	-	-	147	92
	2	151	148	-	-		

[a] Samples not collected from 40 µg/L

Water quality throughout the test was within acceptable limits. During the exposure period the conductivity ranged from 590 to 750 µmhos/cm, the pH ranged from 7.7 to 8.2, temperature ranged from 19.4 to 21.6 °C and the dissolved oxygen ranged from 6.2 to 8.7 mg/L.

Control and solvent control survival averaged 95% after 21-days exposure. Production of the first brood occurred on Day 8 in the control and solvent control. At the end of the test the control and solvent control parental daphnids had an average dry weight of 1.13 and 1.14 mg, respectively and an average length of 4.4 mm.

The average survival, dry weight, length and neonate production is presented in the table below for each test concentration.

Table 8.2.5.1-2: Effects of CGA219417 on *Daphnia magna* survival, reproduction and growth after 21-days exposure

Mean measured concentration (µg/L)	Biological Endpoint			
	Parental survival (%)	Neonates produced per female	Parental dry weight (mg)	Parental length (mm)
Control	95	89.1	1.14	4.4
Solvent Control	95	86.5	1.13	4.4
8.16	100	82.3	1.16	4.4
19.3	80*	1.1*	0.48*	3.5*
40.2	0*	0*	-	-
79.2	0*	0*	-	-
147	0*	0*	-	-
EC_{50} µg/L (95 % Confidence limits)	25.3 (19.3 – 40.2)	Not reported	Not reported	Not reported
NOEC (µg/L)	8.16	8.16	8.16	8.16
LOEC (µg/L)	19.3	19.3	19.3	19.3

* Statistical significant reduction compared to control

The survival of adult *D. magna* was significantly reduced at 40.2, 79.2 and 147 µg/L CGA219417 in comparison to the control. The mean number of young produced, the time to first brood, the mean length and dry weight of surviving *D. magna* were significantly different than the controls at 19.3 µg/L CGA219417.

Validity criteria

The study was considered valid as the following validity criteria were met:

- ≥70 % average control survival (95 % observed in both solvent and dilution media control) with <30 % immobilised, stressed or diseased parental control *Daphnia magna*
- ≥40 neonates were produced per control *Daphnia magna* (89.1 and 86.5 for control and solvent control)
- No ephippa were reported in controls
- Dissolved oxygen remained above 50 % saturation and pH variation did not exceed 1

Conclusions

Based on mean measured concentrations, the 21-day NOEC for the effect CGA219417 to *Daphnia magna* was 8.16 µg/L. The 21-day EC_{50} based on survival was estimated to be 25.3 µg/L.

(Ward *et al*. 1995c)

~~For the studies conducted by Ward *et al*., 1995 (CGA219417/0543) and Rufli, 1998 (CGA219417/0876),~~
In accordance with **Commission Regulation (EU) No 283/2013**, EC_{10} and EC_{20} values have been estimated and a summary of this statistical re-evaluation is provided below.

Report:	Submitted for purposes of renewal due to change in data requirements guidance: K-CA 8.2.5.1/01 Taylor S., Sanchez A. (2015) Cyprodinil (CGA219417) – Chronic toxicity of CGA219417 to the daphnid *Daphnia magna*. Statistical Re-analysis. Report Number CEA.1415. Cambridge Environmental Assessments, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK. (Syngenta File No: CGA219417_11594)

Summary

Report number 468-CG (Ward *et al.*, 1995) did not provide estimates of the EC_{10} and EC_{20} for the total number of surviving adults after 21 days and the total number of offspring produced per surviving parent animal. Consequently the data generated in this study have been re-analysed in order to provide these values.

Treatment related effects were observed and the relationship between dose and response was significant for number of surviving adults and mean number of offspring per surviving adult. EC_x values and the 95% and 99% confidence limits could be reliably determined.

Statistical Analysis

Probit analysis with linear maximum likelihood regression was used to determine the concentration response function. Chi² was used as a goodness of fit measure. All computations were carried out in the Statistical program: ToxRat Professional 2.10.05 (ToxRat Solutions GmbH, 2001-2010).

Results

Number of surviving adults

There was a significant relationship between dose and mortality (p(F) = 0.017). $EC_{10, 20, 50}$ values and the 95% and 99% confidence limits were reliably determined.

Mean number of offspring per surviving adult

There was a significant relationship between dose and reproduction (p(F) = 0.000). $EC_{10, 20, 50}$ values and the 95% and 99% confidence limits were reliably determined.

EC_{10}, EC_{20} and EC_{50} Estimates

Estimates of the EC_{10} and EC_{20} can be summarized as follows:

Parameter	Reproduction	Adult Mortality
EC_{50} (mg/L) **95% CL**	11.47 (11.46-11.49)	25.33 (21.42-1647.29)
EC_{20} (mg/L) **95% CL**	9.42 (9.41-9.42)	19.28 (11.49-27.14)
EC_{10} (mg/L) **95% CL**	8.49 (8.49-8.50)	16.71 (1.36-19.28)

CL: Confidence Limits
n.d.: Not Determined

Conclusion

Treatment related effects were observed and the relationship between dose and response was significant for reproduction and adult mortality. EC_x values and the 95% and 99% confidence limits could be reliably determined.

(Taylor S & Sanchez A, 2015)

Report:	Summary submitted at the request of ANSES for ease of dossier evaluation: *KIIA 8.2.5. Rufli H. (1998). Daphnia magna Reproduction Test: Effects of CGA 219417 (Cyprodinil) on the Reproduction of the Cladoceran Daphnia magna Straus in a semi-static Laboratory Test Under Realistic Conditions. Report Number 973606. Novartis Crop Protection AG, CH-4002 Basel, Switzerland. (Syngenta File No. CGA219417/0876).*

Guidelines

OECD Guideline No. 202, Part 11, including revised draft US EPA FIFRA-Guideline No. 72-4. Modified to mimic realistic exposure in the aquatic system

GLP: Yes

Executive Summary

The chronic toxicity of CGA219417 to the freshwater invertebrate *Daphnia magna* was determined under realistic exposure conditions. The initial test concentrations were 0.20, 0.78, 3.1, 13 and 50 µg/L administered in a single pulse-dose. Exposure concentrations were altered to achieve approximately a 5-day half-life throughout the duration of the study. The test was performed using *Daphnia magna* of two different age groups: less than 24 hours old (Group A) and 8 – 9 days old (Group B). The test was performed in two phases; initially the first generation *D. magna* were exposed for 21 days to CGA219417 (Phase 1), the second phase involved exposing second generation daphnids to the test medium without test medium (Phase 2).

D. magna were exposed to initial nominal CGA219417 concentrations of 0.20, 0.78, 3.1, 13 and 50 µg/L a dilution medium control. Measured test concentrations of CGA219417 at the beginning of the test were 0.12, 0.50, 1.8, 8.8 and 32 µg/L and 0.11, 0.45, 1.9, 10 and 32 µg/L in the 1-day old test solutions, respectively.

First generation control daphnids of age group A and B produced an average of 73 and 112 neonates after 21 days, respectively. The corresponding values for the second generation (Phase 2) were 75 and 77 neonates, respectively. A significant reduction in the survival of the first generation, group A, *D. magna* was observed at the initial concentration of 8.8 µg/L. For group B no mortality was observed at this concentration however 80 % mortality was observed in the next higher treatment of 32 µg/L.

For age group A and B the EC_{50} values based on survival were estimated to be 9.2 µg/L and 28 µg/L. The corresponding EC_{10} values were estimated to be 7.3 and 23 µg/L for age group A and B, respectively. The surviving *D. magna* in the 8.8 µg/L (Group A) and 32 µg/L (Group B) showed no significant difference in the reproductive capacity or other sublethal effects, compared to the control group. The NOEC for sublethal effects was concluded to be 8.8 µg/L for the most sensitive age group.

No lethal or sublethal effects were observed during the exposure of second generation *D. magna* to test medium without the test substance (Phase 2).

Materials

Test Material	CGA219417
Lot/Batch #:	P012011
Purity:	99.2%
Description:	Beige solid
Stability of test compound:	Not reported
Reanalysis/expiry date:	January 1999
Treatments	
Test concentrations:	Dilution medium control and initial nominal concentrations of 0.20, 0.78, 3.1, 13 and 50 µg/L. Initial measured concentrations of 0.12, 0.50, 1.8, 8.8 and 32 µg/L
Solvent:	None
Positive control:	None
Analysis of test concentrations:	Yes, HPLC analysis of CGA219417 in samples collected on days 0, 7, 14 and 21.
Test organism	
Species:	*Daphnia magna* Straus 1820, clone 5
Source:	In-house culture
Feeding:	Daily during the exposure (exception of Saturday and Sunday) with 0.025 – 0.075 mL *Scenedesmus subspicatus* per animal and 0.025 – 0.050 mL Tetramin-extract per animal
Culture medium:	Elendt M4 medium with a hardness of 256 – 288 $CaCO_3$/L
Test design	
Test vessels:	100 mL glass beakers containing 50 mL test medium
Test medium:	Elendt M4 medium
Replication:	Twelve replicates for each treatment and controls (1 organism per replicate). Reduced to ten randomly selected replicates on Day 14
Exposure regime:	Semi-static
Aeration:	No
Duration:	21 days (Phase 1) and 21 days (Phase 2)
Environmental conditions	
Test temperature:	21°C

pH:	Fresh solution: 8.2 to 8.6 Aged solutions: 7.8 to 8.6
Dissolved oxygen:	>95% saturation
Conductivity:	651 to 748 µS/cm
Lighting:	16 hour light and 8 our dark with a 30 minute transition. Light intensity of 1300-1500 (not: <1200 Lux)

Study Design and Methods

Experimental dates: 28th January to 22th April 1998

A stock solution was prepared every working day by mixing 1.02 – 1.09 mg CGA219417 with M4 medium and making up to 2000 mL. The solution was stirred for approximately 2 – 3 hours. At the start of the test and at each renewal, calculated amounts of the stock solution were diluted in 2000 mL test medium to produce final test solutions. The concentration of CGA219417 was gradually reduced by approximately 10% daily in order to simulate the continuous degradation of cyprodinil.

The test was performed using *Daphnia magna* of two different age groups: less than 24 hours old (Group A) and 8 – 9 days olds (Group B). Furthermore, the test was performed in two phases; initially the first generation *D. magna* were exposed for 21 days to CGA219417 (Phase 1), the second phase involved exposing second generation daphnids to the test medium without test item (Phase 2).

At the start of the test, twelve age group A and age group B animals were used for each of the initial nominal concentrations of 0.20, 0.78, 3.1, 13 and 50 µg/L and the dilution medium control. Each organism was individual exposed in a 100 mL beaker containing 50 mL test solution which was renewed five times a week. On Day 14, ten of the replicates of each age group were randomly selected for further testing and only data from these replicates was used for the determination of the study endpoints. The last brood of neonates produced from each age group was used as the test organisms for the second phase of the study, where neonates were exposed to test medium without the addition of CGA219417.

During both phases of the study the number of immobilised adult *D. magna* and the occurrence of sublethal effects was determined daily (with exception of Sunday) during the test and the number of neonates produced in each replicate was recorded every working day. The length of the parental daphnids was determined at the end of the exposure periods.

Dissolved oxygen, pH and temperature was measured at the start of the test and in the aged solution on Day 1 in one representative vessel per treatment and control. In addition, these parameters were measured at each renewal (except Day 15) in one vessel of the control and highest test concentration during phase 1 and the test medium during phase 2. Hardness and conductivity of the freshly prepared M4 media were measured at test start and at each renewal.

The initial test concentrations were verified by chemical analysis of CGA219417 in samples collected at the start of the study from each test concentration, as well as from the aged test solutions on Day 1. Samples were also collected from the stock solutions at the start of the study and on Day 0, 1, 2, 5, 6, 7 and 8. Samples were analysed using high performance liquid chromatography with a UV detector.

The total number of living offspring and dead offspring per parent animal alive at 14 days of exposure and at test end were calculated for each replicate. Replicates in which the parent died before the end of the test were excluded and the analysis was based on a reduced number of replicates. EC_x values were estimated according to the maximum likelihood method, Probit model. Dunnett's multiple means comparison test was used to determine the reproduction and length NOEC values.

Results and Discussion

The initial measured concentrations were 0.12, 0.50, 1.8, 8.8 and 32 µg/L. In aged solutions collected on Day 1 the measured concentrations were 0.11, 0.45, 1.9, 10 and 32 µg/L. The concentrations in the stock solutions on day 0, 1, 2, 5, 6, 7 and 8 were on average 93% of the nominal concentration and within 68 – 118% of nominal. The study results are based on initial measured concentrations.

Table 8.2.5.1-3: Analytical results

Nominal concentration (µg/L)	Measured concentration (µg/L)				Standard deviation
	0-hour fresh		24-hour aged		
	µg/L	%	µg/L	%	
Control	<0.2	-	<0.2	-	-
0.20	0.12	60	0.11	55	0.007
0.78	0.50	64	0.45	58	0.035
3.1	1.8	58	1.9	61	0.071
13	8.8	68	10	77	0.849
50	32	64	32	64	0.000

Water quality throughout the test was within acceptable limits. During the exposure period the conductivity ranged from 651 - 748 µS/cm, the pH ranged from 8.2 to 8.6 and the dissolved oxygen was maintained above 95% saturation. The temperature of the test solutions was maintained constant at 21.0°C.

Control survival averaged 100% after 21-days exposure in phase 1 and phase 2 of the study. First generation control daphnids of age group A and B produced an average of 73 and 112 neonates after 21 days, respectively. The corresponding values for the second generation (Phase 2) were 75 and 77 neonates, respectively.

The average survival, length and neonate production is presented in the table below for each test concentration.

Table 8.2.5.1-4: Effects of CGA219417 on the survival, reproduction and growth of Age Group A *Daphnia magna* after 21-days exposure

Initial measured concentration (µg/L)	Biological Endpoint		
	Parental survival (%)	Neonates produced per female	Ave. Parental length (µm)
Control	100	72.6	4281
0.12	100	73.4	4244
0.50	100	59.1	4190
1.8	100	67.0	4270
8.8	60	60.2	4392
32	0	N/A	N/A
EC_{10} µg/L (95 % Confidence limits)	7.3 (Not reported)	Not reported	Not reported
EC_{50} µg/L (95 % Confidence limits)	9.2 (Not reported)	Not reported	Not reported
NOEC (µg/L)	1.8	>8.8	8.8
LOEC (µg/L)	8.8	>8.8	>8.8

A significant reduction in the survival of the first generation, group A, *D. magna* was observed at the initial concentration of 8.8 µg/L. For group B no mortality was observed at this concentration, however 80% mortality was observed at the next highest concentration of 32 µg/L.

In both age groups statistical analysis showed no significant difference in either the time to first brood or the number of live neonates produced in any test concentration compared to the control. The average size of parental daphnids at concentration 8.8 µg/L of age group A, as well as at all concentrations of age group B were larger than in the control. In both age groups no significant reduction in size was observed in any test concentration compared to the control groups.

Table 8.2.5.1-5: Effects of CGA219417 on the survival, reproduction and growth of Age Group B *Daphnia magna* after 21-days exposure

Initial measured concentration (µg/L)	Biological Endpoint		
	Parental survival (%)	Neonates produced per female	Ave. Parental length (µm)
Control	100	112.1	4333
0.12	100	148.2	4524
0.50	90	140.4	4352
1.8	100	133.2	4441
8.8	100	142.0	4534
32	20	89.0	4401
EC_{10} µg/L (95 % Confidence limits)	23 (Not reported)	Not reported	Not reported
EC_{50} µg/L (95 % Confidence limits)	28 (Not reported)	Not reported	Not reported
NOEC (µg/L)	8.8	32	32
LOEC (µg/L)	32	>32	>32

In the second phase of the study, where second generation *Daphnia* were exposed to medium without CGA219417 no lethal or sublethal effects were observed.

Validity criteria

The study was considered valid as the following protocol defined validity criteria were meet:

- Mortality of the parent animals and the presence of males did not exceed 20% (actual: 0%)
- An average of ≥60 neonates were produced per control *Daphnia magna* (Phase 1: 72.6 and 112.1 for age group A and B. Phase 2: 75.3 and 76.8 for age group A and B for control and solvent control)

Conclusions

Based on initial measured concentrations, the 21-day CGA219417 NOEC for sublethal effect (time to first brood, reproduction and length) to *Daphnia magna* was 8.8 µg/L for the most sensitive age group. No significant adverse effects on the reproductive capacity of the surviving daphnids <24 hours old at the start of the test were observed at 8.8 µg/L, and no adverse effects on the older age group were observed at this concentration.

(Rufli, 1998)

In accordance with **Commission Regulation (EU) No 283/2013**, EC_{10} and EC_{20} values have been estimated and a summary of this statistical re-evaluation is provided below.

Report:	Submitted for purposes of renewal due to change in data requirements guidance: K-CA 8.2.5.1/02 Taylor S., Majdanik V. (2015b) Cyprodinil (CGA219417) – Effects of CGA219417 (Cyprodinil) on the reproduction of the cladoceran *Daphnia magna* in a semi-static laboratory test under realistic conditions. Statistical Re-analysis. Report Number CEA.1416. Cambridge Environmental Assessments, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK. (Syngenta File No: CGA219417_11588)

Summary

Report number 973606 (Rufli, 1998) did not provide estimates of the EC_{10} and EC_{20} for the total number of cumulative adult immobilization after 14 and 21 days, cumulative number of live and dead young per female after 14 and 21 days, time to first brood or length of parent daphnids. Consequently the data generated in this study have been re-analysed in order to provide these values.

There was a significant dose response for cumulative number of live young per female after 14 days, therefore EC_x values and the 95% and 99% confidence limits could be reliably determined. There were no significant dose responses in the remaining data and therefore no EC_x values could be reliably determined.

Statistical Analysis

The test was run with two different age groups of daphnids: 24 h (group A) and 8-9 days old (group B). There were also two phases: exposure to a geometric series of five test concentrations and a water control over 21 days (phase 1) and 12 offspring per treatment from groups A and B were monitored for reproduction without exposure to the test item for 21 days (phase 2).

The effective concentrations for cumulative number of live offspring were assessed. Probit analysis with linear maximum likelihood regression was used to determine the concentration response function. Chi² was used as a goodness of fit measure, and the proportion of variance explained by the dose/response function was determined.

The effective concentrations for adult immobility and cumulative number of dead young per female were assessed. Probit analysis with linear maximum likelihood regression was used to determine the concentration response function. Chi² was used as a goodness of fit measure.

Where no meaningful concentration/response was found (p(F) > 0.05) the calculated EC_x's were not valid due to the absence of a concentration/response. All computations were carried out in ToxRat Professional version 2.10 (ToxRat Solutions GmbH, 2001-2010).

Results

<u>Parental immobility after 14 days</u>

Group A Phase 1: There was a significant dose response (p(F) = 0.000), however, the slope function = 0.000 and therefore no reliable EC_x were determined.

Group B Phase 1: There was no significant dose response relationship (p(F) = 0.248) and therefore no reliable EC_x were determined.

Cumulative number of live young per surviving female after 14 days

Group A Phase 1: There was no significant dose response relationship (p(F) = 0.059) and therefore no reliable EC_x were determined.

Group A Phase 2: The relationship between dose and response could not be determined due to a lack of available responses and therefore no reliable EC_x were determined.

Group B Phase 1: There was a significant relationship between dose and reproduction (p(F) = 0.000). EC_{10} and EC_{20} values and the 95% and 99% confidence limits were reliably determined.

Group B Phase 2: There was no significant dose response relationship (p(F) = 0.193) and therefore no reliable EC_x were determined.

Dead offspring per surviving female after 14 days

Group B Phase 1: There was no significant dose response relationship (p(F) = 0.099) and therefore no reliable EC_x were determined.

Parental immobility after 21 days

Group A Phase 1: The relationship between dose and response could not be determined due to a lack of available responses and therefore no reliable EC_x were determined.

Group B Phase 1: There was no significant dose response relationship (p(F) = 0.265) and therefore no reliable EC_x were determined.

Cumulative number of live young per surviving female after 21 days

Group A Phase 1: There was no significant dose response relationship (p(F) = 0.529) and therefore no reliable EC_x were determined.

Group A Phase 2: There was no significant dose response relationship (p(F) = 0.399) and therefore no reliable EC_x were determined.

Group B Phase 1: The relationship between dose and response could not be determined due to a lack of treatment related effect and therefore no reliable EC_x were determined.

Group B Phase 2: There was no significant dose response relationship (p(F) = 0.069) and therefore no reliable EC_x were determined.

Dead offspring per surviving female after 21 days

Group B Phase 1: There was no significant dose response relationship (p(F) = 0.084) and therefore no reliable EC_x were determined.

EC_{10} and EC_{20} Estimates

Estimates of the EC_{10} and EC_{20} can be summarized as follows:

Parameter	Cumulative number of live young per female after 14 days (Group B Phase 1)
EC_{20} (mg/L) **95% CL**	16.50 (16.43-16.57)
EC_{10} (mg/L) **95% CL**	12.06 (11.99-12.13)

CL: Confidence Limits

Conclusion

There was a significant dose response for cumulative number of live young per female after 14 days, therefore EC_x values and the 95% and 99% confidence limits could be reliably determined. There were no significant dose responses in the remaining data and therefore no EC_x values could be reliably determined.

(Taylor S & Majdanik V, 2015b)

CA 8.2.5.2 Reproductive and development toxicity to an additional aquatic invertebrate species

Summaries of studies conducted since the previous submission of cyprodinil are presented below:

Report:	Submitted for purposes of renewal as conducted on cyprodinil for USA compliance: K-CA 8.2.5.2/01 Drottar K.R. & Krueger H.O. (1999) CGA219417: A flow-through life cycle toxicity test with the saltwater mysid (*Mysidopsis bahia*). Report Number 108A-205. Wildlife International Ltd, Easton, MD, USA. (Syngenta File No. CGA219417/0926)

Guidelines

EPA Guideline No. 72-4

OPPTS No. 850.1350 Draft

GLP: Yes

Executive Summary

The chronic toxicity of CGA219417 to the saltwater mysid (*Mysidopsis bahia*) was determined under flow-through conditions in a life-cycle test. This study was run with nominal concentrations of 0.5, 1.0, 2.0, 4.0, and 8.0 µg a.s./L together with negative and solvent controls.

There were no statistically significant effects on survival, reproduction and growth of mysid shrimp exposed to CGA219417 at concentrations of 1.9 µg/L and above after 30 days. The No observed effect concentration (NOEC) was 1.9 µg /L, the Lowest observed effect concentration (LOEC) based on survival was 3.7 µg /L and the Maximum acceptable toxicant concentration (MATC) was 2.7 µg /L.

Materials

Test material CGA219417
Description: White powder
Lot/Batch #: P012012

Purity:	99.2%
Stability of test compound:	Not given

Treatments

Test concentrations:	Nominal concentrations of 0.5, 1.0, 2.0, 4.0, and 8.0 µg a.s./L
Dilution water:	Saltwater (0.25 µm filtered seawater)
Vehicle and/or positive control:	Dimethylformamide (DMF)
Analysis of test concentrations:	Yes at test start and end using HPLC analysis

Test organisms

Species:	Saltwater mysid (*Mysidopsis bahia*)
Source:	Test facility
Acclimatisation period:	Adults acclimated 14 days before collection of juveniles
Treatment for disease:	None
Life stage at test start:	Juvenile < 24 hours
Feeding:	Live brine shrimp (*Artemia* sp.) 3 – 4 times a day

Test design

Test vessels:	Before maturity: 2 L glass beakers covered with a nylon mesh After maturity: 5 cm diameter glass petri dishes with sides of nylon mesh attached with silicon adhesive, placed in 9L glass aquaria test chambers containing 5L of test solution.
Replication:	Before maturity: 4 replicates of 15 mysids After maturity: 4 replicates containing differing amounts of mysids (5-14) dependant on juvenile survival.
Exposure regime:	Flow-through
Duration:	96 hours

Environmental conditions

Test temperature:	25 ± 2 °C
pH range:	8.0 to 8.2 measured daily
Dissolved oxygen:	> 5.9 mg/L
Salinity of dilution water:	20 % throughout test

Study Design and Methods

Experimental dates: 24th February to 27th March 1999

Mysids less than 24 hours old were exposed to a series of five test concentrations, a solvent control and a negative control for 30 days. Four replicate test chambers, each containing 15 mysids, were maintained for each treatment and control group.

A continuous flow through diluter was used to deliver each concentration of the test substance. The diluter was adjusted so that each test chamber received approximately 14 volume additions of test water every 24 hours. Prior to sexual maturity, mysids were held in 2 L glass beakers covered with a nylon mesh for 15 days. After sexual maturity, reproductive pairs were placed in reproductive compartments (one pair per compartment). The reproductive compartments were 5 cm diameter glass petri dishes with sides of nylon mesh attached with silicon adhesive. The compartments were placed in 9 L glass aquaria test chambers containing 5 L of test solution.

The test was undertaken in a temperature controlled environmental chamber.

A primary stock of 8.0 µg a.s./mL test material was prepared by mixing 0.1614 g of the test substance with DMF, bringing the final volume to 1000 mL. This stock solution was then used to make up the test concentrations by serial dilution.

The concentrations of test material in the test solutions were measured at the beginning, and at the end of the test using HPLC analysis.

Observations were made for mortality and clinical symptoms of toxicity at test start and day 7, 14, 21, 28 and 30.

Results and Discussion

The measured concentrations are shown in the table below in terms of nominal concentrations. The test concentrations were maintained throughout the study. The limit of quantification in this study was 0.20 µg a.s./L. Mean measured concentrations were used for the calculation and reporting of results.

Table 8.2.5.2-1: Analytical results

Nominal concentration (µg a.s./L)	% of nominal 0 days	% of nominal 30 days	Mean measured concentration (µg a.s./L)
0.50	109	103	0.51
1.0	106	96	0.99
2.0	95	99	1.9
4.0	95	94	3.7
8.0	93	98	7.5

Negative and solvent control data was compared to the mortality, reproductive output per day, dry weight and survival of first brood data using 2 x 2 contingency tables and the chi squared or student's t-test. Survival was evaluated before pairing (day 0 – 15) and after pairing (day 15 - 30). Reproduction and growth data were analysed by evaluating homogeneity and assessing normality using Sharpio-Wilk's test. Treatments that were statistically significant from controls were identified using Bonferroni t-test. The results of these tests were used to determine the LOEC and NOEC. The MATC was calculated as the geometric mean of the NOEC and LOEC. All statistical tests were performed using computer software SPSS/PC Version 2.0 or TOXSTAT Release 3.5.

Table 8.2.5.2-2: Effects of test material on the survival of saltwater mysids (*Mysidopsis bahia*) following exposure for 96 hours in a flow-through test

Mean measured concentration (µg a.s./L)	Mean juvenile survival (%) [a]	Mean adult survival (%) [b]	Mean no. young produced per reproductive day	Mean dry weight of adult mysids at end of test (mg)	Mean total length of adult mysids at test end (mm)
Negative control	90	92	0.308	0.595	6.35
Solvent control	97	98	0.275	0.589	6.32
Pooled control	93	96	0.291	0.592	6.33
0.51	92	93	0.321	0.585	6.35
0.99	95	100	0.280	0.603	6.31
1.9	97	98	0.287	0.573	6.28
3.7	78*	84	0.0391[1]	0.397[1]	6.00[1]

Mean measured concentration (µg a.s./L)	Mean juvenile survival (%) [a]	Mean adult survival (%) [b]	Mean no. young produced per reproductive day	Mean dry weight of adult mysids at end of test (mg)	Mean total length of adult mysids at test end (mm)
7.5	38*	57*	n/a[2]	0.303[1]	4.93[1]

[a] (*) indicates a significant difference from the pooled control using 2 x 2 contingency tables ($p \leq 0.05$)
[b] (*)indicates a significant difference from the pooled control using 2 x 2 contingency tables ($p \leq 0.05$)
[1] this treatment group was not included in the statistical analysis of reproduction due to a statistically significant difference in survival from test initiation to pairing
[2] the treatment was not mature enough to sex on day 15 and was not paired

Conclusions

There were no statically significant effects on survival, reproduction or growth of mysid shrimp (*Mysidopsis bahia*) exposed to CGA219417 at concentrations of ≤ 1.9 µg a.s./L for 30 days. The NOEC was 1.99 µg a.s./L, the LOEC for survival was 3.79 µg a.s./L and the MATC was 2.79 µg a.s./L

(Drottar K & Krueger H, 1999)

In accordance with **Commission Regulation (EU) No 283/2013**, estimation of EC_{10} and EC_{20} values was conducted.

Report:	Submitted for purposes of renewal due to change in data requirements guidance: K-CA 8.2.5.2/02 Taylor S., Sanchez A. (2015a) Cyprodinil (CGA219417) – A flow through life cycle toxicity test with the saltwater mysid (*Mysidopsis bahia*). Statistical Re-analysis. Report Number CEA.1421. Cambridge Environmental Assessments, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK. (Syngenta File No: CGA219417_11596)

Summary

Report number 108A-205 (Drottar and Krueger, 1999) did not provide estimates of the EC_{10}, EC_{20} or EC_{50} for the survival from day 0 to day 15; survival from day 15 to day 30; reproductive rate; mean adult dry weight or mean adult length. Consequently the data generated in this study have been re-analysed in order to provide these values.

Statistical analyses of the parameters revealed that reliable $EC_{10,20,50}$ values could be reliably determined for reproductive rate and adult dry weight. Reliable estimates of the EC_{10} and EC_{20} were calculated for adult total length. No reliable dose response was identified in the survival parameters.

Statistical Analysis

A Fisher's Exact Binomial test was used to determine a difference between the negative and solvent controls for survival from Day 0 to Day 15 and from Day 15 to Day 30 using pair-wise comparisons. For the parameters of reproduction rate, final adult length and dry weight a Student's t-test was used to determine a significant difference between the negative and solvent control using pair-wise comparisons. Where there was no significant difference the data were pooled.

The effective concentrations for survival from Day 0 to Day 15 and from Day 15 to Day 30 were assessed. Probit analysis with linear maximum likelihood regression was used to determine the concentration response function. Chi² was used as a goodness of fit measure.

The effective concentrations for reproduction rate, final adult length and dry weight were assessed. Probit analysis with linear maximum likelihood regression was used to determine the concentration response function. Chi² was used as a goodness of fit measure, and the proportion of variance explained by the dose/response function was determined.

Results

Survival

There was no significant difference between treatment and control mysids from Day 0 to Day 15 (p(F) = 0.094) or Day 15 to Day 30 (p(F) = 0.166) and as such, no EC_{10}, EC_{20} or EC_{50} values could be reliably determined.

Reproduction Rate

There was a significant difference between treatment and control mysids (p(F) = 0.046) and the EC_{10}, EC_{20} and EC_{50} values were reliably determined.

Mean Adult Dry Weight

There was a significant difference between treatment and control mysids (p(F) = 0.021) and the EC_{10}, EC_{20} and EC_{50} values were reliably determined.

Mean Adult Length

There was a significant difference between treatment and control mysids (p(F) = 0.024) and the EC_{10}, EC_{20} and EC_{50} values were reliably determined.

EC_{10}, EC_{20} and EC_{50} Estimates

Fish Survival	Reproductive Rate	Adult Dry Weight	Adult Total Length
EC_{50} (µg/L) **95% CL**	2.95 (0.04-3.37)	7.15 (5.02-21.58)	n.d.
EC_{20} (µg/L) **95% CL**	2.48 (0.00-3.04)	3.07 (0.76-4.42)	8.92 (4.19-439.18)
EC_{10} [(µg/L) **95% CL**	2.27 (0.00-2.89)	1.97 (0.17-3.18)	3.37 (1.48-13.47)

CL: Confidence Limits
n.d.: Not Determined

Conclusion

Statistical analyses of the parameters revealed that reliable $EC_{10,20,50}$ values could be reliably determined for reproductive rate and adult dry weight. Reliable estimates of the EC_{10} and EC_{20} were calculated for adult total length. No reliable dose response was identified in the survival parameters.

(Taylor S & Sanchez A, 2015a)

CA 8.2.5.3 Development and emergence in *Chironomus riparius*

The study previously submitted in the EU for cyprodinil (Grade, 2000, Syngenta File No. CGA219417/1003) was summarised in point B.9.2.7 of the DAR. At the request of ANSES a summary has been included in order to aid dossier evaluation. ~~therefore an additional study summary is not repeated here.~~ The endpoints are summarised in Table 8.2-6.

Report:	Summary submitted at the request of ANSES for ease of dossier evaluation: *KIIA 8.2.7. Grade R. (2000). Toxicity Test of CGA219417 A on Sediment-Dwelling Chironomus riparius (syn. Chironomus thummi) under Static Conditions. Report Number 2003518. Novartis Crop Protection AG, CH-4002 Basel, Switzerland. (Syngenta File No. CGA219417/1003).*

Guidelines

Effects of Plant Protection Products on the Development of Sediment-Dwelling Larvae of Chironomus riparius in a Water-Sediment System (BBA Guideline Proposal 1995)

OECD Guideline for testing of chemicals, Proposal for Toxicity Test with Chironomidae (1998)

GLP: Yes

Executive Summary

Larvae of the insect *Chironomus riparius* were exposed to a control, solvent control and nominal CGA219417 concentrations of 20, 40, 80, 160 and 320 mg/kg sediment (dry weight) for 27 days and the effects on adult emergence and development rate were investigated.

Based nominal concentrations the estimated EC_{50} values for the effects of CGA219417 on *Chironomus riparius* emergence rate and development rate were 162 and 363 mg/kg dry sediment, respectively. The corresponding NOEC values were 80 and 160 mg/kg dry weight.

Materials

Test Material	CGA219417
Lot/Batch #:	P.012010
Purity:	99.4%
Description:	Beige solid
Stability of test compound:	Stable under test conditions
Reanalysis/expiry date:	31 December 2004
Treatments	
Test concentrations:	Aged sediment control, solvent control and nominal concentrations of 20, 40, 80, 160 and 320 mg/kg dry sediment
Solvent:	Acetone
Positive control:	None
Analysis of test concentrations:	Concentration of CGA219417 measured in sediment samples collected on Day 0, 7 and 27 in the 320 and 160 mg/kg treatment groups Concentration of CGA219417 measured in water samples collected on Day 0, 2, 7, 14 and 27 from each test concentration and control
Test organism	
Species:	*Chironomus riparius*, 1st instar larvae, 2 – 3 days old
Source:	Continuous laboratory culture
Feeding:	During the exposure test organisms were feed a fish food suspension (TETRAMIN) at a rate of 1 mg per larvae per day

Test design

Test vessels:	1 L glass beakers
Test medium:	Elendt M4 medium. Water column approximate depth of 8 cm
Sediment:	Artificial sediment; 5.5 % peat, 20 % kaolin clay, 74.5 industrial sand, pH adjusted to 6.9. Organic carbon content around 1.7 %. Sediment layer approximately 1.6 cm deep
Replication:	Three replicates for each treatment and control, 20 larvae per replicate
Exposure regime:	Static
Aeration:	Gentle, provided through a glass Pasteur pipette
Duration:	27 days

Environmental conditions

Test temperature:	20.8 to 21.5°C
pH:	Test start: 8.1 to 8.3 Test end: 9.0 to 10.0
Dissolved oxygen:	7.0 to 8.8 mg/L
Hardness:	254 to 272 mg/L as $CaCO_3$
Conductivity:	687 to 715 µS/cm
Lighting:	16 hour light and 8 our dark with a 30 minute transition. Light intensity of 800 to 1000 Lux

Study Design and Methods

Experimental dates: 30th August to 26th September 2000

A series of stock solutions were prepared by mixing the test item with acetone. Appropriate amounts of the stock solutions were then applied to 17.2 g of sand and the acetone was allowed to evaporate off. The artificial sediment was conditioned for 7 days in flowing water prior to being dosed. The treated sand was then combined with the conditioned artificial soil (860 g dry sediment) to achieve the nominal test concentrations and thoroughly mixed with a Hobart Mixer for approximately 30 minutes. Two control treatments were included in the study design, aged sediment containing no test material with and without acetone treated sand.

Each beaker was filled with a layer of 1.6 cm of spiked artificial sediment and covered with M4 medium to an approximate depth of 8 cm. The test vessels were covered with parafilm with small holes to prevent escape of emerging adult *Chironomus riparius*. The test vessels were prepared 48 hours prior to the introduction of the test organisms to allow stabilisation of the system, following this 20 first instar *Chironomus riparius* larvae were added to each test vessel. During the 27-day exposure the test vessels were incubated in a temperature controlled room at 20 ± 2 °C under a 16-hour light, 8-hour dark cycle.

Aqueous samples of the test solutions were collected from each beaker per test concentration and control at day 0, 2, 7, 14 and 27. At the beginning, after 7 days and at the end of the exposure the concentration of the test item in the sediment and interstitial water was measured in separate analytical vessels prepared at the two highest test item concentrations.

Visual assessments (behaviour, mortality and emergence) were made daily, with the exception of exposure days 3, 4, 10, 11, 17, 18, 24 and 25. The number, time and sex of emerged adults was recorded and they were then removed from the test vessels. The weight of larvae exposed for 9 days was measured after sieving the sediment from each test beaker using a 250 µm sieve. The dry weight of larvae was

measured after drying at 60°C.

The water temperature was continuously measured in the test room and in a test vessel on different days. Oxygen content and pH were measured once weekly with appropriate electrodes directly in the test vessels. The water conductivity and hardness were measured after preparing the M4 medium.

The emergence rates and the gender ratio data were transformed using an arcsine-squareroot transformation prior to statistical analysis. Dunnett tests was used to calculate NOEC and LOEC values for these parameters. EC_x and 95% confidence limits were calculated by logit analysis for emergence rate and development rate.

Results and Discussion

The mass balance of the test system for day 0, 7 and 27 for the 160 mg/kg treatment was 94.9, 100.5 and 91.5% of the nominal concentration and for the 320 mg/kg treatment was 95.0, 91.4 and 88.1%. Indicating that the desired amount of test item was present in the test systems over the whole exposure period and was mainly located in the sediment. The study endpoints are based on the nominal sediment concentrations.

Table 8.2.5.3-1: Analytical results from separate analytical vessels

Nominal Concentration (mg/kg)		Absolute Amounts Measured Based on Whole Test Vessel (mg)				
		Overlying Water	Sediment	Interstitial Water	Total measured	Mass Balance (%)
160	Day 0	0.40	17.07	0.04	17.51	94.9
	Day 7	1.00	17.41	0.13	18.54	100.5
	Day 27	0.90	15.96	0.06	16.92	91.7
	Day 27	0.90	15.91	0.04	16.85	91.3
320	Day 0	0.55	34.43	0.07	35.05	95.0
	Day 7	1.55	31.99	0.18	33.72	91.4
	Day 27	1.35	30.62	0.10	32.07	86.9
	Day 27	1.35	31.52	0.11	32.98	89.4

[a] Based on theoretical 18.45 and 36.90 mg in the 160 and 320 mg/kg treatment groups, respectively.

Water quality throughout the test was within acceptable limits. Water temperature ranged from 20.8 to 21.5°C. Oxygen content ranged from 7.9 to 8.2 mg/L at the start of the exposure and 7.7 to 8.8 mg/L at the end of the exposure period.

There was no indication of different sensitivities of sexes at all test concentrations therefore male and female results were pooled for statistical analyses. The test material had no influence on the weight of larvae collected after 9 days incubation in the 20, 40 and 80 mg/kg treatment groups. Visible smaller larvae were observed in the 160 and 320 mg/kg. Significant reduction of adult emergence was observed at CGA219417 concentrations of 160 mg/kg and above.

The average emergence, development rate and larvae dry weight at Day 9 is presented in the table below for each test concentration and the pooled controls.

Table 8.2.5.3-2: Effects of CGA219417 A on the emergence, development rate and weight of *Chironomus riparius*

Nominal Concentration (mg/kg)	Biological Endpoint		
	Average Emergence Rate	Average Development Rate (1/day)	Larvae Weight at Day 9
Pooled Controls	0.94	0.068	0.76
20	0.88	0.068	0.75
40	0.90	0.069	0.77
80	0.92	0.068	0.79
160	0.52	0.065	0.70
320	0.00	-	0.48
EC_{50} µg/L (95 % Confidence limits)	162 (n.d.)	363 (n.d.)	Not reported
NOEC (µg/L)	80	160	Not reported
LOEC (µg/L)	160	320	Not reported

Validity criteria

The study was considered valid as the following protocol defined validity criteria were met:

- Mortality in the controls did not exceed 20 % (actual: 6 %)
- Chironomus hatching occurred between 12 and 23 days (actual: 14 to 19 days)
- The oxygen concentration remained above 3 mg/L

Conclusions

Based on nominal concentrations, the 27-day EC_{50} for the effects of CGA219417 on *Chironomus riparius* emergence and development rate were 162 and 363 mg/kg dry sediment, respectively. The corresponding NOEC values were 80 and 160 mg/kg dry sediment for emergence and development rate, respectively.

(Grade, 2000)

In accordance with Regulation 283/2013, the study data have been re-evaluated for the purposes of calculating EC_{10} and EC_{20} values. The outcome of the re-evaluation is provided below.

Report:	Submitted for purposes of renewal due to change in data requirements guidance: K-CA 8.2.5.3/01 Taylor S., & Alba A. (2015) Cyprodinil (CGA219417) - Toxicity test of CGA219417A on the sediment-dwelling *Chironomus riparius* (syn. *Chironomus thummi*) under static conditions. Statistical Re-analysis. Report Number: CEA.1414. Cambridge Environmental Assessments, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK. (Syngenta File No: CGA219417_11587)

Summary

Report number 2003518 (Grade, 2000) did not provide estimates of the EC_{10} and EC_{20} for the response variables of emergence rate, development rate or sex ratio of cyprodinil to *Chironomus riparius* in a

water-sediment system. Consequently the data generated in this study have been re-analysed in order to provide these values.

EC_x could not be reliably calculated for any measured parameter due to a lack of dose response.

Statistical Analysis

Students-t-tests with pairwise comparisons were to determine whether there were significant differences between negative and solvent controls. Where no difference existed the data were pooled.

Probit analysis with linear maximum likelihood regression was used to determine the concentration response function. Chi² was used as a goodness of fit measure. All computations were carried out in ToxRat Professional version 2.10 (ToxRat Solutions GmbH, 2001-2010).

Results

There was no significant relationship between dose and response for emergence rate (p(F) = 0.219), development rate (p(F) = 0.096) or gender rate (p(F) = 0.284) and as a result no EC_{10} or EC_{20} values were reliably determined.

Conclusion

EC_x could not be reliably calculated for any measured parameter due to a lack of dose response.

(Taylor S., Alba A. 2015)

Metabolites of cyprodinil

The study previously submitted in the EU for CGA249287 (Grade, 2001, Syngenta File No. CGA249287/0024) was summarised in point B.9.2.7 of the DAR. At the request of ANSES a summary has been included in order to aid dossier evaluation. ~~therefore an additional study summary is not repeated here.~~ The endpoints are summarised in Table 8.2-6.

Report:	Summary submitted at the request of ANSES for ease of dossier evaluation: *KIIA 8.2.7. Grade R. (2001). Toxicity Test of CGA2 49287 (Metabolite of CGA 219417) on Sediment-Dwelling Chironomus riparius (syn. Chironomus thummi) under Static Conditions. Report Number 2003768. Syngenta Crop Protection AG, CH-4002 Basel, Switzerland. (Syngenta File No. CGA249287/0024)*

Guidelines

Effects of Plant Protection Products on the Development of Sediment-Dwelling Larvae of Chironomus riparius in a Water-Sediment System (BBA Guideline Proposal 1995)

OECD Guideline for testing of chemicals, Proposal for Toxicity Test with Chironomidae (1998)

GLP: Yes

Executive Summary

Larvae of the insect *Chironomus riparius* were exposed to a control, solvent control and nominal CGA249287 concentrations of 0.4, 0.8, 1.6, 3.2, 6.4, 12.8 and 25.6 mg/kg dry sediment for 28 days and

the effects on adult emergence and development rate were investigated. The test item was dosed onto sand and mixed with aged artificial sediment to prepare the test treatments. The spiked sediments were covered with a layer of water (8 cm depth) and the test system was equilibrated for 48 hours prior to the introduction of the test organisms.

Based nominal concentrations the estimated EC_{50} values for the effects of CGA249287 on *Chironomus riparius* emergence rate and development rate were >25.6 mg/kg dry sediment. The overall NOEC value was 12.8 mg/kg dry weight, the highest concentration tested.

Materials

Test Material	CGA249287
Lot/Batch #:	KI4014/11
Purity:	100 ± 2%
Description:	White solid
Stability of test compound:	Not reported
Reanalysis/expiry date:	20th October 2002
Treatments	
Test concentrations:	Aged sediment control, solvent control and nominal concentrations of 0.4, 0.8, 1.6, 3.2, 6.4, 12.8 and 25.6 mg/kg dry sediment
Solvent:	Acetone
Positive control:	None
Analysis of test concentrations:	Concentration of CGA249287 measured in sediment, water and interstitial water samples collected on Day 0, 7 and 28 in the 12.8 and 25.6 mg/kg treatment groups Concentration of CGA249287 measured in water samples collected on Day 0, 2, 7, 14 and 28 from each test concentration and control
Test organism	
Species:	*Chironomus riparius*, 1st instar larvae, 2 – 3 days old
Source:	Continuous laboratory culture
Feeding:	During the exposure test organisms were feed a fish food suspension (TETRAMIN) at a rate of 1 mg per larvae per day
Culture medium:	Elendt M4 medium
Test design	
Test vessels:	1 L glass beakers. Approximately 160 g wet weight sediment (1.6 cm layer) covered with 8 cm deep water layer
Test medium:	Elendt M4 medium.
Sediment:	Artificial sediment; 5.5 % peat, 20 % kaolin clay, 74.5 industrial sand, pH adjusted to 6.6. Organic carbon content around 1.6 %.
Replication:	Three replicates for each treatment and control, 20 larvae per replicate
Exposure regime:	Static
Aeration:	Gentle, provided through a glass Pasteur pipette
Duration:	28 days

Environmental conditions

Test temperature:	21.3 to 21.8°C
pH:	Test start: 7.9 to 8.0 Test end: 9.0 to 9.9
Dissolved oxygen:	7.4 to 8.9 mg/L
Hardness:	296 to 309 mg/L as $CaCO_3$
Conductivity:	760 to 780 µS/cm
Lighting:	16 hour light and 8 our dark with a 30 minute transition. Light intensity of 800 to 1000 Lux

Study Design and Methods

Experimental dates: 7th February to 27th March 2001

A stock solution was prepared by mixing 230.8 mg the test item with acetone and making up to 10 mL with acetone. Several application solutions were prepared by diluting aliquots of the initial stock. Appropriate amounts of the application solutions were then applied to 17.7 g of sand and the acetone was allowed to evaporate off. The artificial sediment was conditioned for 7 days in flowing water prior to spiking. The treated sand was then combined with the conditioned artificial soil (884 g dry sediment) to achieve the nominal test concentrations and thoroughly mixed with a Hobart Mixer for approximately 30 minutes. Two control treatments were included in the study design, aged sediment containing no test material with and without acetone treated sand.

Each test beaker was filled with a 1.6 cm layer of spiked artificial sediment and covered with Elendt M4 medium to an approximate depth of 8 cm. The test vessels were prepared 48 hours prior to the introduction of the test organisms to allow stabilisation of the system, following this 20 first instar *Chironomus riparius* larvae were added to each test vessel. The test vessels were covered with parafilm with small holes to prevent escape of emerging adult *Chironomus riparius*. During the 28-day exposure the test vessels were incubated in a temperature controlled room at 20 ± 2 °C under a 16-hour light, 8-hour dark cycle.

Aqueous samples of the test solutions were collected from each beaker per test concentration and control at day 0, 2, 7, 14 and 28. At the beginning, after 7 days and at the end of the exposure the concentration of the test item in the sediment, water and interstitial water was measured in separate analytical vessels prepared at the two highest test item concentrations.

Visual assessments (behaviour, mortality and emergence) were made daily, with the exception of exposure days 1, 3, 4, 6, 8, 10, 11, 17, 18, 24, 25 and 26. The number, time and sex of emerged adults was recorded and were removed from the test vessels. The weight of larvae exposed for 9 days was measured after sieving the sediment from each test beaker using a 250 µm sieve. The dry weight of larvae was measured after drying at 60°C.

The water temperature was continuously measured in the test room and in a test vessel at different days. Oxygen content and pH were measured once weekly with appropriate electrodes directly in the test vessels. The water conductivity and hardness were measured after preparing the Elendt M4 medium.

The emergence rate was calculated by dividing the number of emerged adults by the number introduced. The development rate was calculated as the reciprocal of the development time (time span between addition of larvae and emergence of experiment cohort). Data concern emergence rates and the gender ratio were transformed using an arcsine-squareroot transformation prior to statistical analysis. Dunnett

tests was used to calculate NOEC and LOEC values for these parameters. EC_x and 95 % confidence limits were estimated by logit analysis for emergence rate and development rate.

Results and Discussion

The mass balance of the test system for day 0, 7 and 28 for the 12.8 mg/kg treatment was 103.8, 98.0 and 90.9% of the nominal concentration and for the 25.6 mg/kg treatment was 95.8, 98.9 and 87.5%, indicating that the desired amount of test item was present in the test systems over the whole exposure period. The study endpoints are based on the nominal sediment concentrations.

Table 8.2.5.3-3: Analytical results from overlying water samples

Nominal Concentration (mg/kg)	Measured Concentrations in Overlying Water Samples (mg/L)				
	Day 0	Day 2	Day 7	Day 14	Day 28
Control	<LOD	<LOD	<LOD	<LOD	<LOD
Solvent control	<LOD	<LOD	<LOD	<LOD	<LOD
0.4	0.009	0.012	0.015	0.013	0.017
0.8	0.021	0.029	0.036	0.037	0.048
1.6	0.044	0.059	0.078	0.081	0.10
3.2	0.12	0.16	0.20	0.22	0.24
6.4	0.24	0.34	0.46	0.51	0.61
12.8	0.60	0.79	1.03	1.17	1.38
25.6	1.09	1.50	2.0	2.22	2.61

LOD = 0.002 mg/L

Table 8.2.5.3-4: Analytical results from separate analytical vessels

Nominal Concentration (mg/kg)		Absolute Amounts Measured Based on Whole Test Vessel (mg)				
		Overlying Water	Sediment	Interstitial Water	Total measured	% Mass Balance[a]
12.8	Day 0	0.30	1.24	0.038	1.58	103.8
	Day 7	0.52	0.93	0.039	1.49	98.0
	Day 28	0.69	0.66	0.031	1.38	90.9
25.6	Day 0	0.55	2.29	0.073	2.91	95.8
	Day 7	1.00	1.94	0.068	3.01	98.9
	Day 28	1.31	1.30	0.049	2.66	87.5

[a] Based on theoretical 1.52 and 3.04 mg in the 12.8 and 25.6 mg/kg treatment groups, respectively.

Water temperature ranged from 21.3 to 21.8°C during the exposure period. The pH ranged from 7.9 to 8.0, immediately after the addition of the test organisms to values of 8.4 to 8.9 at test termination. The dissolved oxygen content ranged from 7.4 to 8.9 mg/L during the exposure period.

There was no indication of different sensitivities of sexes at all test concentrations therefore male and female results were pooled for statistical analyses. The test material had no influence on the weight of larvae collected after 9 days incubation in all treatment groups. No significant effects on larvae development rate or adult emergence was observed at any of the CGA249287 tested.

The average emergence, development rate and larvae dry weight at Day 9 is presented in the table below for each test concentration and the pooled controls.

Table 8.2.5.3-5: Effects of CGA249287 on the emergence, development rate and weight of *Chironomus riparius*

Nominal Concentration (mg/kg)	Biological Endpoint		
	Average Emergence Rate	Average Development Rate (1/day)	Larvae Weight at Day 9
Pooled Controls	0.91	0.0606	0.68
0.4	0.90	0.0612	0.40
0.8	0.93	0.0585	0.71
1.6	0.93	0.0583	0.48
3.2	0.82	0.0634	0.92
6.4	0.93	0.0619	0.67
12.8	0.95	0.0631	0.78
25.6	0.73	0.0643	0.94
EC_{50} µg/L (95 % Confidence limits)	>25.6 (not calculable)	>25.6 (not calculable)	Not determined
NOEC (µg/L)	25.6	25.6	Not determined
LOEC (µg/L)	>25.6	>25.6	Not determined

Validity criteria

The study was considered valid as the following validity criteria were met:

- Mortality in the controls did not exceed 20 % (actual: 9 %)
- Mean development time for control larvae was not more than 23 days (actual: 21 days for pooled controls)
- The oxygen concentration in the overlying water was >3 mg/L

Conclusions

Based on nominal concentrations, the 28-day EC_{50} value for the effects of CGA249287 on *Chironomus riparius* emergence and development rate was >25.6 mg/kg. No significant effects were observed in the study at any of the CGA249287 concentrations tested, therefore the overall NOEC was 25.6 mg/kg, the highest concentration tested.

(Grade, 2001)

In accordance with Regulation 283/2013, the study data have been re-evaluated for the purposes of calculating EC_{10} and EC_{20} values. The outcome of the re-evaluation is provided below.

Report:	Submitted for purposes of renewal due to change in data requirements guidance: K-CA 8.2.5.3/02 Taylor S. & Pickering F. (2015) CGA249287 – Toxicity test of CGA249287 on sediment-dwelling *Chironomus riparius* (syn. *Chironomus thummi*) under static conditions. Statistical Re-analysis. Report Number: CEA.1426. Cambridge Environmental Assessments, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK. (Syngenta File No: CGA249287_10007)

Summary

Report number 2003768 (Grade, 2001) did not provide estimates of the EC_{10} and EC_{20} for the response variables of emergence, development or gender rate of CGA249287 to *Chironomus riparius* in a water-sediment system. Consequently the data generated in this study have been re-analysed in order to provide these values.

Statistical analyses of the available data revealed that no reliable EC_x values were able to be calculated for emergence, development or gender rate.

Statistical Analysis

A Student's t-test was used to determine whether there was any significant difference between the negative and solvent control using pairwise comparisons. Where there was no difference the data were pooled. Probit analysis with linear maximum likelihood regression was used to determine the concentration response function. Chi² was used as a goodness of fit measure. All computations were carried out in ToxRat Professional version 2.10 (ToxRat Solutions GmbH, 2001-2010).

Results

There was no significant difference in the dose response relationship for either emergence rate (p(F) = 0.077) or development rate (p(F) = 0.114). As a result EC_x and confidence limits could not be reliably determined. There was a clear absence of dose response in the ratio of male and female emerged midges so gender rate was not analysed.

Conclusion

Statistical analyses of the available data revealed that no reliable EC_x values were able to be calculated for emergence, development or gender rate.

(Taylor S., Pickering F. 2015)

CA 8.2.5.4 Sediment dwelling organisms

No new data has been generated since the previous submission for cyprodinil.

CA 8.2.6 Effects on algal growth

CA 8.2.6.1 Effects on growth of green algae

Studies previously submitted in the EU for cyprodinil were summarised in point B. 9.2.6 of the DAR. At the request of ANSES a summary has been included in order to aid dossier evaluation. ~~therefore additional study summaries are not repeated here.~~ The endpoints are summarised in Table 8.2-7.

Report:	Summary submitted at the request of ANSES for ease of dossier evaluation: *KIIA 8.2.6. Ward T. J., Magazu J.P. & Boeri R. L. (1995). Growth and Reproduction Toxicity Test with CGA-219417 and the Freshwater Alga, Selenastrum capricornutum. Report Number 791-CG. T. R. Wilbury Laboratories, Inc. Massachusetts, USA. (Syngenta File No. CGA219417/0648).*

Guidelines

FIFRA Subdivision J, 123-2

GLP: Yes

Executive Summary

The toxicity of CGA219417 to the freshwater green alga *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*) was determined. Algae were exposed to nominal concentrations 0.6, 1.3, 2.5, 5.0 and 10 mg/L (0.303, 0.773, 1.769, 4.441 and 8.851 mg/L mean measured) alongside a culture medium control.

Based on initial measured concentrations, the 72-hour and 96-hour EC_{50} for CGA219417 to *Pseudokirchneriella subcapitata* was 2.60 mg/L and 2.82 mg/L, respectively.

Based on mean measured concentrations, The 72-hour E_rC_{50} and E_vC_{50} values were 3.28 and 1.57 mg/L, respectively. The 96-hour E_rC_{50} and E_yC_{50} values were 4.06 and 2.26 mg/L, respectively. The 120 hour E_yC_{50} value was 2.08 mg/L.

Materials

Test Material	CGA219417
Lot/Batch #:	P012012
Purity:	98.8%
Description:	Off-white granules
Stability of test compound:	Stable for at least 1 year at room temperature
Reanalysis/expiry date:	31 January 1996
Treatments	
Test concentrations:	Culture medium control and nominal concentrations of 0.6, 1.3, 2.5, 5.0 and 10 mg/L. (mean measured: 0.303, 0.773, 1.769, 4.441 and 8.851mg/L).
Solvent:	Not applicable
Positive control:	None
Analysis of test concentrations:	Yes, HPLC analysis
Test organism	
Species:	*Selenastrum capricornutum* (UTEX 1648)
Source:	Culture Collection of Algae at the University of Texas
Test design	
Test vessels:	250 mL glass Erlenmeyer flasks containing 50 mL of media
Test medium:	Sterile enriched media (U.S. EPA, 1978)
Replication:	Three replicates for each treatment
Starting cell density:	10,000 cells/mL
Exposure regime:	Static
Aeration:	No

Duration:	120 hours
Environmental conditions	
Test temperature:	23.6 to 24.0°C
pH:	test start: 7.4 to 7.5 test end: 7.8 to 10.2
Lighting:	Constant light, 3600 lux

Study Design and Methods

Experimental dates: 6th to 11th October 1995

A stock solution with a nominal concentration of 10 mg/L was prepared by dissolving 0.0101 g of the test item completely in sterile media and adjusting the final volume to 1000 mL. The stock solution was mixed for approximately 48 hours to aid dissolution. The test solutions were prepared by diluting appropriate aliquots of the stock solution in sterile enriched media.

An aliquot of test solution was dispensed into each test vessel and the test started by inoculation of 10,000 algal cells per mL of test medium. Test solutions were constantly shaken and were held in a temperature controlled incubator under continuous illumination. Small volumes of all test concentrations and controls were taken from all test flasks after 24, 48, 72, 96 and 120 hours of exposure. The algal cell densities in these samples were determined by counting using a microscope and haemocytometer.

The pH was measured at the start and at the end of the test. The test system temperature within the incubator was continuously measured using a representative flask of water incubated among the test vessels.

The test concentrations were verified by chemical analysis of CGA219417 at 0 and 120 hours, using high performance liquid chromatography. At the beginning of the experiment 40 mL samples were collected from each test solution prior to its addition into replicate test vessels. Replicate test vessels were pooled prior to collection of the analytical sample at 120 hours and passed through a 0.45 µm filter.

The algal cell densities were measured at 24, 48, 72, 96 and 120 hours. The 72-hour, 96-hour and 120 hour EC_{25} and EC_{50} values were calculated using weighted least squares non-linear regression analysis. Determination of the NOEC (No Observed Effect Concentration) values a parametric one-way analysis of variance (ANOVA) and Dunnett's t-test were used to compare treatment and control data.

The data were re-analysed in order to determine statistics in terms of yield and growth rate. For this E_rCx and E_vCx values were estimated using Probit analysis with linear maximum likelihood regression determine the concentration response function. Chi^2 was used as a goodness of fit measure. If the probability, $p(Chi^2)$, was lower than or equal to 0.100, the data were scattered around the computed dose/response function. In this case, the confidence limits were corrected for heterogeneity (i.e. they were made wider).

Results and Discussion

At the start of the test, the measured concentrations were in the range 93 to 96% of the nominal values and at the end of the test were in the range 27 to 85% (see table below).

Table 8.2.6.1-1: Analytical results

Nominal concentrations (mg/L)	Measured Concentration of CGA219417 (mg/L)	
	0 hours	120 hours
0 (control)	<0.100	<0.100
0.6	0.575	0.160
1.3	1.23	0.486
2.5	2.37	1.32
5.0	4.74	4.16
10	9.27	8.45

Water quality throughout the test was within acceptable limits. The incubator temperature ranged from 23.6 to 24.0 ºC during test period and the measured pH ranged from 7.4 at test initiation to 10.2 at 120 hours.

The algae population in the control vessels grew well, resulting in an average cell density of 3,831,000 cells/mL after 120 hours.

Results presented in the report

The mean cell densities and $EC_{25/50}$ values are presented in the table below.

Table 8.2.6.1-2: Mean cell densities at each concentration of CGA219417 at 24, 48, 72, 96 and 120 hours and the estimated EC_x and NOEC values

Initial Measured concentrations (mg/L)	Mean Cell Density (cells/mL x 10^3)				
	24 hours	48 hours	72 hours	96 hours	120 hours
Control	20	78	737	1,913	3,831
0.575	21	79	622	1,859	3,672
1.23	20	76	530	1,960	3,050
2.37	19	63	442	1,394	2,632
4.74	14	41	34	96	250
9.27	15	<10	<10	<10	<10
EC_{25} mg/L (95% confidence limits)	Not reported		1.89 (1.46 – 2.44)	2.23 (1.88 – 2.64)	2.28 (1.98 – 2.62)
EC_{50} mg/L (95% confidence limits)	Not reported		2.60 (2.17 – 3.11)	2.82 (2.49 – 3.19)	2.89 (2.61 – 3.20)
NOEC (mg/L)	Not reported		<0.575	1.23	0.575

Re-estimated endpoints based on mean measured concentrations

Mean measured concentrations of 0.303, 0.773, 1.769, 4.441 and 8.851mg/L were used for the calculation and reporting of results.

Growth rates

The growth rate was calculated for each replicate culture and the means are shown below, alongside the estimated EC_x values.

Table 8.2.6.1-3: Mean values at each concentration of CGA219417 for growth rate for *Pseudokirchneriella subcapitata*

Mean measured concentrations (mg/L)	Mean growth rate (day^{-1}) 0 – 72 hrs	Percentage inhibition (%)	Mean growth rate (day^{-1}) 0 – 96 hrs	Percentage inhibition (%)	Mean growth rate (day^{-1}) 0 – 120 hrs	Percentage inhibition (%)
Control	1.432	-	1.313	-	1.189	-
0.303	1.377	3.9	1.306	0.5	1.180	0.8
0.773	1.323	7.6	1.319	-0.5	1.144	3.8
1.769	1.262	11.9	1.234	6.0	1.114	6.3
4.441	0.411	71.3	0.564	57.1	0.644	45.9
8.851	0.000	100.0	0.000	100.0	0.000	100.0
E_rC_{10}	1.682 (1.646 – 1.718)		2.336 (2.245 – 2.422)		n.d.	
E_rC_{20}	2.115 (2.079 – 2.150)		2.824 (2.742 – 2.900)		n.d.	
E_rC_{50}	3.276 (3.240 – 3.311)		4.058 (4.008 – 4.107)		n.d.	

Numbers in brackets represent 95% confidence intervals
n.d. = values could not be determined

Yield

The yield was calculated for each replicate culture and the means are shown below, together with the estimated EC_x values.

Table 8.2.6.1-4: Mean values at each concentration of CGA219417 for yield for *Pseudokirchneriella subcapitata*

Mean measured concentrations (mg/L)	Mean cells/mL ($x10^4$) 0 – 72 hrs	Percentage inhibition (%)	Mean cells/mL ($x10^4$) 0 – 96 hrs	Percentage inhibition (%)	Mean cells/mL ($x10^4$) 0 – 120 hrs	Percentage inhibition (%)
Control	72.7	-	190.3	-	382.1	-
0.303	61.2	15.9	184.9	2.8	366.2	4.2
0.773	52.0	28.5	195.0	-2.5	304.0	20.4
1.769	43.2	40.6	138.4	27.3	262.2	31.4
4.441	2.4	96.7	8.6	95.5	24.0	93.7
8.851	0.0	100.0	0.0	100.0	0.0	100.0
E_yC_{10}	0.432 (0.410 – 0.455)		1.345 (1.274 – 1.407)		0.859 (0.747- 0.960)	
E_yC_{20}	0.673 (0.646 – 0.699)		1.606 (1.547 – 1.660)		1.164 (1.050 – 1.268)	
E_yC_{50}	1.568 (1.529 – 1.608)		2.256 (2.182 – 2.342)		2.083 (1.957 – 2.220)	

Numbers in brackets represent 95% confidence intervals

Validity criteria

Not reported.

Conclusions

The toxicity of CGA219417 to the green alga *Pseudokirchneriella subcapitata* was determined.

Based on initial measured concentrations, the 72-hour and 96-hour EC_{50} for CGA219417 to *Pseudokirchneriella subcapitata* was 2.60 mg/L and 2.82 mg/L, respectively. The 72 and 96-hour NOEC of CGA219417 were <0.575 mg/L and 1.23 mg/L, respectively.

Based on mean measured concentrations, The 72-hour E_rC_{50} and E_yC_{50} values were 3.28 and 1.57 mg/L, respectively. The 96-hour E_rC_{50} and E_yC_{50} values were 4.06 and 2.26 mg/L, respectively. The 120 hour E_yC_{50} value was 2.08 mg/L.

(Ward *et al.* 1995)

For Ward *et al*. 1995 (Syngenta File No. CGA219417/0648) the EC_{10} and EC_{20} values have been estimated in the following report:

Report:	Submitted for purposes of renewal due to change in data requirements guidance: K-CA 8.2.6.1/~~02~~01 Taylor S., Pickering F. & Allen (201~~5a6~~) Cyprodinil (CGA219417) – Growth and reproduction toxicity test with CGA219417 and the freshwater alga, *Selenastrum capricornutum*. Statistical Re-analysis. Report Number: CEA.142~~3~~4. Cambridge Environmental Assessments, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK. Amended (Syngenta File No: CGA219417_11593)

Report number 791-CG (Ward *et al.*, 1995) did not provide EC_{10} or EC_{20} estimates relating to yield or average specific growth rate for *Pseudokirchneriella subcapitata*. Consequently the data generated in this study have been re-analysed in order to provide these values.

A significant dose response relationship was observed between treatment and algal growth rate ~~and yield~~ at 24, 72 and 96 ~~and 120 h~~, and for yield at 24, 48, 72, 96 and 120 h, therefore EC_{10}, EC_{20} and EC_{50} values and the 95% and 99% confidence limits could be reliably determined. There was no significant relationship between dose and response in the remaining parameters and therefore no EC_x could be reliably calculated.

Statistical Analysis

The algal yield represents the cell number/biomass generated during the test or evaluation period minus the cell number/biomass at the start of the exposure or evaluation period. The average specific growth rate represents the slope of the growth curve. All computations were carried out in ToxRat Professional version 2.10 (ToxRat Solutions GmbH, 2001-2010). Probit analysis with linear maximum likelihood regression was used to determine the concentration response function. Chi² was used as a goodness of fit measure and the proportion of variance explained by the dose/response relationship was determined.

Data were re-evaluated using mean measured concentrations.

Results

Average Specific Growth Rate

Treatment related effects were observed and the relationship between dose and response was significant at 24 h (p(F) = 0.01~~54~~), 72 h (p(F) ~~= 0.000~~ <0.001) and 96 h (p(F) ~~= 0.000~~ <0.001) ~~and 120 h (p(F) = 0.000)~~. E_rC_x values and the 95% and 99% confidence limits were reliably determined for these time periods. There was no significant relationship at 48 h (p(F) = 0.590) and therefore no reliable EC_x could be calculated for this time point.

Yield

Treatment related effects were observed and the relationship between dose and response was significant at 24 h (p(F) = 0.00~~98~~), 48 h (p(F) ~~= 0.000~~ <0.001), 72 h (p(F) ~~= 0.000~~ <0.001), 96 h (p(F) ~~= 0.000~~

<0.001) and 120 h (p(F) ~~= 0.000~~ <0.001). E_yC_x values and the 95% and 99% confidence limits were reliably determined for these time periods.

EC_{10}, EC_{20} and EC_{50} estimates

Estimates of the EC_{10}, EC_{20} and EC_{50} can be summarized as follows:

Parameter	24 hours			48 hours			72 hours		
	E_rC_{10}	E_rC_{20}	E_rC_{50}	E_rC_{10}	E_rC_{20}	ErC_{50}	E_rC_{10}	E_rC_{20}	E_rC_{50}
Value [mg/L]	0.814	1.900	9.620	1.682	2.115	3.276	2.336	2.824	4.058
lower 95%-cl	0.105	0.613	5.931	1.646	2.079	3.240	2.245	2.742	4.008
upper 95%-cl	1.608	3.055	31.353	1.718	2.150	3.311	2.422	2.900	4.107

Parameter	24 hours			48 hours			72 hours		
	E_yC_{10}	E_yC_{20}	E_yC_{50}	E_yC_{10}	E_yC_{20}	E_yC_{50}	E_yC_{10}	E_yC_{20}	E_yC_{50}
Value [mg/L]	0.688	1.478	6.377	1.405	1.931	3.548	0.432	0.673	1.568
lower 95%-cl	0.060	0.335	3.950	1.254	1.772	3.374	0.410	0.646	1.529
upper 95%-cl	1.443	2.528	16.915	1.547	2.080	3.728	0.455	0.699	1.608

Parameter	96 hours			120 hours		
	E_yC_{10}	E_yC_{20}	E_yC_{50}	E_yC_{10}	E_yC_{20}	E_yC_{50}
Value [mg/L]	1.345	1.606	2.256	0.859	1.164	2.083
lower 95%-cl	1.274	1.547	2.182	0.747	1.050	1.957
upper 95%-cl	1.407	1.660	2.342	0.960	1.268	2.220

~~Parameter~~	~~After 24 h~~		~~After 48 h~~		~~After 72 h~~	
	~~Growth rate~~	~~Yield~~	~~Growth rate~~	~~Yield~~	~~Growth rate~~	~~Yield~~
~~EC_{20} (mg/L) 95% CL~~	~~2.445 (0.206-4.259)~~	~~1.976 (0.232-3.386)~~	~~n.d.~~	~~2.493 (2.077-2.831)~~	~~2.696 (2.447-2.903)~~	~~1.096 (0.708-1.401)~~
~~EC_{10} (mg/L) 95% CL~~	~~1.173 (0.009-2.456)~~	~~1.025 (0.026-2.110)~~	~~n.d.~~	~~1.925 (1.502-2.271)~~	~~2.259 (1.990-2.484)~~	~~0.772 (0.421-1.059)~~

~~CL: Confidence Limits~~
~~n.d.: Not determined~~

~~Parameter~~	~~After 96 h~~		~~After 120 h~~	
	~~Growth rate~~	~~Yield~~	~~Growth rate~~	~~Yield~~
~~EC_{20} (mg/L) 95% CL~~	~~3.224 (3.078-3.350)~~	~~2.204 (2.132-2.269)~~	~~3.645 (3.270-3.895)~~	~~1.720 (1.232-2.053)~~
~~EC_{10} [(mg/L) 95% CL~~	~~2.737 (2.568-2.884)~~	~~1.929 (1.836-2.007)~~	~~3.134 (2.668-3.447)~~	~~1.363 (0.854-1.708)~~

~~CL: Confidence Limits~~

Conclusion

A significant dose response relationship was observed between treatment and algal growth rate ~~and yield~~ at 24, 72 and 96 ~~and 120 h~~, and for yield at 24, 48, 72, 96 and 120 h, therefore EC_{10}, EC_{20} and EC_{50} values

and the 95% and 99% confidence limits could be reliably determined. There was no significant relationship between dose and response in the remaining parameters and therefore no EC_x could be reliably calculated.

(Taylor S. et al, 2016, ~~Pickering F. 2015a~~)

Report:	Summary submitted at the request of ANSES for ease of dossier evaluation: *KIIA 8.2.6. Maetzler P. (2001). Toxicity of CGA 219417 tech. to Green Algae (Growth Inhibition Test). Report Number 2013515. Solvias AG, Basel, Switzerland. (Syngenta File No. CGA219417/1030).*

Guidelines

OECD Guidelines for Testing of Chemicals, Section 2, Number 201 (1993)

Commission Directive 92/69/EEC, Annex L 383 A, C.3 (1992)

GLP: Yes

Executive Summary

The toxicity of CGA219417 to the green alga *Pseudokirchneriella subcapitata* was determined. Algae were exposed to nominal concentrations of 0.4, 0.9, 1.9, 4.3 and 9.4 mg/L (mean measured: 0.303, 0.773, 1.769, 4.441 and 8.851) alongside a culture medium control. The results were based on mean measured concentrations. The 72-hour E_rC_{50} and E_yC_{50} values were 3.28 and 1.57 mg/L, respectively. The 96-hour E_rC_{50} and E_yC_{50} values were 4.06 and 2.26 mg/L, respectively.

Materials

Test Material	CGA219417
Batch #:	P.012010
Purity:	99.4%
Description:	Beige solid
Stability of test compound:	Not reported
Recertification Date:	31 December 2004
Treatments	
Test concentrations:	Culture medium control and nominal concentrations of 0.4, 0.9, 1.9, 4.3 and 9.4 mg/L
Solvent:	Not applicable
Positive control:	Potassium dichromate is used within every 3 months
Analysis of test concentrations:	Yes, analysis of CGA 219417 at 0 and 72 hours
Test organism	
Species:	*Pseudokirchneriella subcapitata,* strain 61.81 SAG (*Selenastrum capricornutum*, ATCC 22662)

Source:	Continuous laboratory cultures, originally obtained by the Sammlung von Algenkulturen, Pflanzenphysiologisches Institut der Universität Göttingen, D-37073 Göttingen
Test design	
Test vessels:	100 mL glass flasks containing 50 mL of media and sealed with a plug
Test medium:	OECD test medium
Replication:	Six vessels for the control and three vessels for each test concentration
Starting cell density:	1.0×10^4 cells/mL
Exposure regime:	Static
Aeration:	No
Duration:	72 hours
Environmental conditions	
Test temperature:	22.5 – 23.5°C
pH:	test start: 7.8 to 7.9 test end: 6.9 to 10.0
Lighting:	Continuous illumination at 79 µE/m^{2}s (between the wavelengths of 400 -700 nm)

Study Design and Methods

Experimental dates: 28th May to 19th June 2001

A primary stock solution was prepared by dissolving 9.9 mg of CGA219417 in approximately 200 mL test medium in a beaker and then making up to 1000 mL with test medium. The stock solution was stirred overnight. The exposure solutions were prepared by dilution of appropriate aliquots of the stock solution in 200 mL test medium containing 4 mL algae inoculum. These solutions were divided between three replicate flasks for each concentration.

A 1 mL aliquot of solution was taken from all test concentration and control flasks after 24, 48 and 72 hours of exposure. The algal cell densities in these samples were determined by counting with an electronic particle counter. In addition, after 72 hours exposure observation of the health and appearance of the algal cells in the highest concentration still showing growth and the control were recorded using a microscope.

The pH was measured at the start and at the end of the test in each test concentration and the control. The water temperature was recorded continuously in a flask incubated under the same conditions as the test flasks.

The test concentrations were verified by chemical analysis of CGA219417 at 0 and 72 hours, using high performance liquid chromatography (HPLC).

The algal cell densities were measured at 24, 48 and 72 hours and the mean biomass and growth rate calculated. The 72-hour E_bC_{50} and E_rC_{50} values were calculated using non-linear regression. For determination of the NOEC values, Dunnett's Test was used to identify significant differences in the calculated mean biomass and growth rate at the test item treatments compared to the control.

Results and Discussion

The limit of quantification (LOQ) for this study was 0.07 mg/L. The test concentrations remained stable over the 72 hour test period and were within ± 20% of the nominal. However, the results were based on mean measured concentrations.

Table 8.2.6.1-5: Analytical results

Nominal concentrations (mg/L)	0 Hour Measured Concentrations		72 Hour Measured Concentrations	
	mg/L	% of nominal	mg/L	% of nominal
Control	<0.07	-	<0.07	-
0.4	0.42	105	0.40	100
0.9	0.98	109	0.91	101
1.9	2.04	107	1.97	104
4.3	4.33	101	4.48	104
9.4	9.70	103	9.24	98

Cells were observed to appear normal after the 72 hour exposure period.

Growth rates

The growth rates for each replicate culture and the means are shown below, alongside the estimated EC_{50} values.

Table 8.2.6.1-6: Mean values at each concentration of CGA219417 for growth rate for *Pseudokirchneriella subcapitata*

Mean measured concentrations (mg/L)	Mean growth rate (day^{-1}) 0 – 72 hrs	Percentage inhibition (%)	Mean growth rate (day^{-1}) 0 – 96 hrs	Percentage inhibition (%)	Mean growth rate (day^{-1}) 0 – 120 hrs	Percentage inhibition (%)
Control	1.432	-	1.313	-	1.189	-
0.303	1.377	3.9	1.306	0.5	1.180	0.8
0.773	1.323	7.6	1.319	-0.5	1.144	3.8
1.769	1.262	11.9	1.234	6.0	1.114	6.3
4.441	0.411	71.3	0.564	57.1	0.644	45.9
8.851	0.000	100.0	0.000	100.0	0.000	100.0
E_rC_{10}	1.682 (1.646 – 1.718)		2.336 (2.245 – 2.422)		n.d.	
E_rC_{20}	2.115 (2.079 – 2.150)		2.824 (2.742 – 2.900)		n.d.	
E_rC_{50}	3.276 (3.240 – 3.311)		4.058 (4.008 – 4.107)		n.d.	

Numbers in brackets represent 95% confidence intervals
n.d. = values could not be determined

Yield

The yield was calculated for each replicate culture and the means are shown below, together with the estimated EC_x values.

Table 8.2.6.1-7: Mean values at each concentration of CGA219417 for yield for *Pseudokirchneriella subcapitata*

Nominal concentrations (mg/L)	Mean cells/mL ($x10^4$) 0 – 72 hrs	Percentage inhibition (%)	Mean cells/mL ($x10^4$) 0 – 96 hrs	Percentage inhibition (%)	Mean cells/mL ($x10^4$) 0 – 120 hrs	Percentage inhibition (%)
Mean measured concentrations (mg/L)	72.7	-	190.3	-	382.1	-
Control	61.2	15.9	184.9	2.8	366.2	4.2
0.303	52.0	28.5	195.0	-2.5	304.0	20.4
0.773	43.2	40.6	138.4	27.3	262.2	31.4
1.769	2.4	96.7	8.6	95.5	24.0	93.7
4.441	0.0	100.0	0.0	100.0	0.0	100.0
E_yC_{10}	0.432 (0.410 – 0.455)		1.345 (1.274 – 1.407)		0.859 (0.747- 0.960)	
E_yC_{20}	0.673 (0.646 – 0.699)		1.606 (1.547 – 1.660)		1.164 (1.050 – 1.268)	
E_yC_{50}	1.568 (1.529 – 1.608)		2.256 (2.182 – 2.342)		2.083 (1.957 – 2.220)	

Numbers in brackets represent 95% confidence intervals

Validity criteria

The test was regarded as valid, because the cell concentration in the control cultures increased by a factor of more than 16.

Conclusions

The toxicity of CGA 219417 to the green alga *Pseudokirchneriella subcapitata* was determined. Based on mean measured concentrations, The 72-hour E_rC_{50} and E_vC_{50} values were 3.28 and 1.57 mg/L, respectively. The 96-hour E_rC_{50} and E_yC_{50} values were 4.06 and 2.26 mg/L, respectively.

(Maetzler P. 2001)

For Maetzler 2001 (Syngenta File No. CGA219417/1030), the EC_{10} and EC_{20} values have been estimated in the following report:

Report:	Submitted for purposes of renewal due to change in data requirements guidance: K-CA 8.2.6.1/~~0~~102 Taylor S. & Allen M. (~~2015a~~2016) Cyprodinil (CGA219417) – Toxicity of CGA219417 tech to green algae (growth inhibition test). Statistical Re-analysis. Report Number: CEA.1423. Cambridge Environmental Assessments, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK. Amended (Syngenta File No: CGA219417_11598)

Summary

Report number L01-002763 (Maetzler, 2001) did not provide EC_{20} estimates relating to yield or average specific growth rate for *Pseudokirchneriella subcapitata*. Consequently, the data generated in this study have been re-analysed in order to provide these values.

A dose response relationship was observed between treatment and algal growth rate and yield at 24, 48, and 72 h, therefore EC_{20} values and the 95% and 99% confidence limits could be reliably determined.

Statistical Analysis

The algal yield represents the cell number/biomass generated during the test or evaluation period minus the cell number/biomass at the start of the exposure or evaluation period. The average specific growth rate represents the slope of the growth curve. All computations were carried out in ToxRat Professional version 2.10 (ToxRat Solutions GmbH, 2001-2010). Probit analysis with linear maximum likelihood regression was used to determine the concentration response function. Chi² was used as a goodness of fit measure and the proportion of variance explained by the dose/response relationship was determined.

Results

Average Specific Growth Rate

Treatment related effects were observed and the relationship between dose and response was significant at 24 h (p(F) = 0.000), 48 h (p(F) = 0.000) and 72 h (p(F) = 0.000). E_rC_{20} values and the 95% and 99% confidence limits were reliably determined for these time periods.

Yield

Treatment related effects were observed and the relationship between dose and response was significant at 24 h (p(F) = 0.000), 48 h (p(F) = 0.000) and 72 h (p(F) = 0.000). E_yC_{20} values and the 95% and 99% confidence limits were reliably determined for these time periods.

EC_{10} and EC_{20} Estimates

Estimates of the EC_{10} and EC_{20} can be summarized as follows:

Parameter	After 24 h		After 48 h		After 72 h	
	Growth rate	Yield	Growth rate	Yield	Growth rate	Yield
EC_{20} (mg/L)	2.790	2.062	3.954	1.282	4.013	1.738
95% CL	(2.294-3.227)	(1.699-2.390)	(3.747-4.145)	(1.046-1.503)	(3.904-4.117)	(1.587-1.877)

CL: Confidence Limits

Conclusion

A dose response relationship was observed between treatment and algal growth rate and yield at 24, 48, and 72 h, therefore EC_{20} values and the 95% and 99% confidence limits could be reliably determined.

(Taylor S and Allen M, 2015~~a6~~)

Metabolites of cyprodinil

Studies previously submitted in the EU for metabolites of cyprodinil were summarised in point B. 9.2.6 of the DAR. At the request of ANSES a summary has been included in order to aid dossier evaluation. ~~therefore additional study summaries are not repeated here.~~ The endpoints are summarised in Table 8.2-7.

Report:	Submitted for purposes of renewal as conducted on relevant aquatic metabolite: *KIIA 8.2.6. Maetzler P. (1999). Toxicity of CGA 249287 (Metabolite of CGA 219417) to Green Algae (Growth Inhibition Test). Report Number 983827. Novartis Services AG, Basel, Switzerland. (Syngenta File No. CGA249287/0006).*

Guidelines

OECD Guidelines for Testing of Chemicals, Section 2, Number 201 (1993)

Commission Directive 92/69/EEC, Annex L 383 A, C.3 (1992)

GLP: Yes

Executive Summary

The toxicity of CGA249287 to the green alga *Pseudokirchneriella subcapitata* was determined. Algae were exposed to nominal concentrations of 4.3, 9.4, 21, 45 and 100 mg/L alongside a culture medium control. CGA249287 was stable in the test system over the 72 hour exposure duration and therefore the results were based on nominal concentrations. The 72 hour E_rC_{50} and E_bC_{50} values were >100 mg/L. No significant inhibition was observed at any concentration after 72 hours, therefore the 72 hour NOEC for both growth rate and biomass was 100 mg/L, the highest concentration tested.

Materials

Test Material	CGA 249287
Batch #:	HU-15701
Purity:	99 ± 2%
Description:	Beige powder
Stability of test compound:	Not reported
Recertification Date:	December 2001
Treatments	
Test concentrations:	Culture medium control and nominal concentrations of 4.3, 9.4, 21, 45 and 100 mg/L.
Solvent:	Not applicable
Positive control:	Potassium dichromate is used within every 3 months
Analysis of test concentrations:	Yes, analysis of CGA249287 at 0 and 72 hours
Test organism	
Species:	*Pseudokirchneriella subcapitata,* strain 61.81 SAG (*Selenastrum capricornutum*, ATCC 22662)
Source:	Continuous laboratory cultures, originally supplied by the Sammlung von Algenkulturen, Pflanzenphysiologisches Institut der Universität Göttingen, D-37073 Göttingen
Test design	
Test vessels:	100 mL glass flasks containing 50 mL of media and sealed with a plug
Test medium:	OECD test medium
Replication:	Six vessels for the control and three vessels for each test concentration
Starting cell density:	1.0×10^4 cells/mL
Exposure regime:	Static
Aeration:	No
Duration:	72 hours
Environmental conditions	

Test temperature:	23 ± 2°C
pH:	test start: 7.4 to 7.8
	test end: 7.5 to 9.9
Lighting:	Continuous illumination at 79 µE/m^{2}s

Study Design and Methods

Experimental dates: 18th January 1999 to 17th February 1999

A primary stock solution was prepared by mixing 200.1 mg of CGA 249287 in approximately 200 mL test medium in a beaker. The mixture was homogenised for 15 minutes and then made up to 1000 mL with test medium. The exposure solutions were prepared by dilution of appropriate aliquots of the stock solution. The test was started by spiking an algae inoculum into the test solutions. The initial algae density was 1.0×10^4 cells/mL and the test vessels were sealed with plugs and continuously shaken during the exposure duration.

A 1 mL aliquot of solution was taken from all test concentration and control flasks after 24, 48 and 72 hours of exposure. The algal cell densities in these samples were determined by counting with an electronic particle counter. In addition, after 72 hours exposure observation of the health and appearance of the algal cells in the highest concentration still showing growth and the control were recorded using a microscope.

The pH was measured at the start and at the end of the test in each test concentration and the control. The water temperature was recorded continuously in a flask incubated under the same conditions as the test flasks.

The test concentrations were verified by chemical analysis of CGA 249287 in the top concentration at 0 and 72 hours, using high performance liquid chromatography (HPLC).

The algal cell densities were measured at 24, 48 and 72 hours and the mean biomass and growth rate calculated. The 72 hour E_bC_{50} and E_rC_{50} values (defined as the concentration resulting in 50% reduction of each parameter) were calculated using non-linear regression. For determination of the NOEC values, Dunnett's Test was used to identify significant differences in the calculated mean biomass and growth rate at the test item treatments compared to the control.

Results and Discussion

The limit of detection (LOD) for this study was 0.06 mg/L. The test concentrations remained stable over the 72 hour test period and were within ± 20% of the nominal. Therefore, the results were based on nominal concentrations.

Table 8.2.6.1-9: Analytical results

Nominal concentrations (mg/L)	0 Hour Measured Concentrations		72 Hour Measured Concentrations	
	mg/L	% of nominal	mg/L	% of nominal
Control	<0.06	-	<0.06	-
100	95.86	95.9	96.99	97.0

Cells were observed to appear normal after the 72 hour exposure period.

Growth rates

The growth rate 0 to 72 hours was calculated for each replicate culture and the means are shown below, alongside the estimated EC_{50} values.

Table 8.2.6.1-10: Mean values at each concentration of CGA249287 for the growth rate at 72 hours for *Pseudokirchneriella subcapitata*

Nominal concentrations (mg/L)	Mean growth rate (day^{-1}) 0 – 72 hrs	Percentage inhibition (%)
Control	1.44	-
4.3	1.41	2.0
9.4	1.48	-2.7
21.0	1.41	2.3
45.0	1.43	0.5
100.0	1.41	2.3
E_rC_{10} mg /L (95% confidence limits)	>100 (n.d.)	
E_rC_{50} mg /L (95% confidence limits)	>100 (n.d.)	
NOEC	100 mg/L	
LOEC	>100 mg/L	

Biomass (area under the growth curve)

The areas under the growth curve for 0 to 72 hours were calculated for each replicate culture and the means are shown below, alongside the estimated EC_{50} values.

Table 8.2.6.1-11: Mean values at each concentration of CGA249287 for the biomass integral (area under the growth curve) at 72 hours for *Pseudokirchneriella subcapitata*

Nominal concentrations (mg/L)	Mean Area Under Curve 0 – 72 hrs	Percentage inhibition (%)
Control	15875	-
4.3	14965	5.7
9.4	17487	-10.2
21.0	15309	3.6
45.0	15460	2.6
100.0	13971	12.0
E_bC_{10} mg /L (95% confidence limits)	>100 (n.d.)	
E_bC_{50} mg /L (95% confidence limits)	>100 (n.d.)	
NOEC	100 mg/L	
LOEC	>100 mg/L	

Validity criteria

The test was regarded as valid because the cell concentration in the control cultures increased by a factor of more than 16.

Conclusions

The toxicity of CGA249287 to the green alga *Pseudokirchneriella subcapitata* was determined. Based on nominal concentrations, the 72-hour E_rC_{50} and E_bC_{50} value was >100 mg/L. No significant inhibition was observed at any concentration after 72 hours, therefore the 72 hour NOEC for both growth rate and biomass was 100 mg/L, the highest concentration tested.

(Maetzler P. 1999)

For Maetzler 1999 (Syngenta File No. CGA249287/0006), the EC_{10} and EC_{20} values have been estimated in the following report:

Report:	Submitted for purposes of renewal due to change in data requirements guidance: K-CA 8.2.6.1/~~0~~603 Taylor S., Allen M. (2015~~b~~a) CGA249287 – Toxicity test of CGA249287 to green algae (growth inhibition test). Statistical Re-analysis. Report Number: CEA.1428. Cambridge Environmental Assessments, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK. (Syngenta File No: CGA249287_10009)

Summary

Report number G 571 17 (Maetzler, 1999) did not provide EC_{20} estimates relating to yield or average specific growth rate for *Pseudokirchneriella subcapitata*. Consequently the data generated in this study have been re-analysed in order to provide these values.

The EC_{20} could not be reliably determined as the EC_{10} was > 100 mg/L, the highest concentration tested.

Statistical Analysis

The test design consisted of five nominal test concentrations: 4.3, 9.4, 21, 45 and 100 mg/L and a control. Nominal concentrations were confirmed by chemical analysis.

Results

The EC_{20} could not be reliably calculated as the EC_{10} and NOEC values were originally reported as > 100 mg/L and 100 mg/L respectively, the highest concentration tested.

Conclusion

The EC_{20} could not be reliably determined as the EC_{10} was > 100 mg/L, the highest concentration tested.

(Taylor S and Allen M, 2015~~b~~a)

For Maetzler 2001 (Syngenta File No. CGA275535/0015), the EC_{10} and EC_{20} values have been estimated in the following report:

Report:	Submitted for purposes of renewal due to change in data requirements guidance: K-CA 8.2.6.1/~~0~~704 Taylor S., Allen M. (2015~~a~~b) CGA275535 – Toxicity of CGA275535 to green algae (growth inhibition test). Statistical Re-analysis. Report Number: CEA.1412. Cambridge Environmental Assessments, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK. (Syngenta File No: CGA275535_10006)

Summary

Report number G 655 17 (Maetzler, 2001) did not provide EC_{20} estimates relating to yield or average specific growth rate for *Pseudokirchneriella subcapitata*. Consequently the data generated in this study have been re-analysed in order to provide these values.

A dose response relationship was observed between treatment and algal growth rate and yield at 24, 48 and 72 h. EC_{20} values and the 95% and 99% confidence limits could therefore be reliably determined.

Statistical Analysis

The algal yield represents the cell number/biomass generated during the test or evaluation period minus the cell number/biomass at the start of the exposure or evaluation period. The average specific growth rate represents the slope of the growth curve. All computations were carried out in ToxRat Professional version 2.10 (ToxRat Solutions GmbH, 2001-2010). Probit analysis with linear maximum likelihood regression was used to determine the concentration response function. Chi² was used as a goodness of fit measure and the proportion of variance explained by the dose/response function as determined.

Results

Average Specific Growth Rate

Treatment related effects were observed and the relationship between dose and response was significant at 24 h (p(F) = 0.000), 48 h (p(F) = 0.000) and 72 h (p(F) = 0.000). The E_rC_x values and the 95% and 99% confidence limits were reliably determined for these time periods.

Yield

Treatment related effects were observed and the relationship between dose and response was significant at 24 h (p(F) = 0.000), 48 h (p(F) = 0.000) and 72 h (p(F) = 0.000). The E_yC_x values and the 95% and 99% confidence limits were reliably determined for all time periods.

EC_{10} and EC_{20} Estimates

Estimates of the EC_{10} and EC_{20} can be summarized as follows:

Parameter	After 24 h		After 48 h		After 72 h	
	Growth rate	Yield	Growth rate	Yield	Growth rate	Yield
EC_{20} (mg/L)	21.694	4.117	10.537	5.523	10.342	6.342
95% CL	(13.048-31.085)	(1.837-6.516)	(5.323-15.343)	(4.504-6.392)	(5.457-14.773)	(5.595-6.936)

CL: Confidence Limits
n.d.: Not Determined

Conclusion

A dose response relationship was observed between treatment and algal growth rate and yield at 24, 48 and 72 h. EC_{20} values and the 95% and 99% confidence limits could therefore be reliably determined.

(Taylor S and Allen M, 2015eb)

Report:	Submitted for purposes of renewal as conducted on relevant aquatic metabolite: *KIIA 8.2.6. Maetzler P. (2001). Toxicity of CGA 275535 (Metabolite of CGA 219417) to Green Algae (Growth Inhibition Test). Report Number 2003593. Solvias AG, Basel, Switzerland. (Syngenta File No. CGA275535/0015).*

Guidelines

OECD Guidelines for Testing of Chemicals, Section 2, Number 201 (1993)

Commission Directive 92/69/EEC, Annex L 383 A, C.3 (1992)

GLP: Yes

Executive Summary

The toxicity of CGA275535 to the green alga *Pseudokirchneriella subcapitata* was determined. Algae were exposed to nominal concentrations of 4.3, 9.4, 21, 45 and 100 mg/L (3.59, 8.99, 20.41, 38.82 and 72.52 mg/L as mean measured concentrations) alongside a culture medium control. Based on mean measured concentrations, the 72-hour E_rC_{50} and E_bC_{50} values were 18 and 9.4 mg/L, respectively. The 72 hour NOEC of CGA275535 based on growth rate was 3.6 mg/L. For biomass a significant effect was observed at 3.6 mg/L and therefore the NOEC could not be accurately determined but can be concluded to be <3.6 mg/L.

Materials

Test Material	CGA275535
Batch #:	KI6230/3
Purity:	99%
Description:	Slightly yellow-beige powder
Stability of test compound:	Not reported
Recertification Date:	31 October 2003
Treatments	
Test concentrations:	Culture medium control and nominal concentrations of 4.3, 9.4, 21, 45 and 100 mg/L (3.59, 8.99, 20.41, 38.82 and 72.52 mg/L as mean measured concentrations)
Solvent:	Not applicable
Positive control:	Potassium dichromate is used within every 3 months
Analysis of test concentrations:	Yes, analysis of CGA275535 at 0 and 72 hours
Test organism	
Species:	*Pseudokirchneriella subcapitata,* strain 61.81 SAG (*Selenastrum capricornutum*, ATCC 22662)
Source:	Continuous laboratory cultures, originally obtained by the Sammlung von Algenkulturen, Pflanzenphysiologisches Institut der Universität Göttingen, D-37073 Göttingen

Test design

Test vessels:	100 mL glass flasks containing 50 mL of media and sealed with a plug
Test medium:	OECD test medium
Replication:	Six vessels for the control and three vessels for each test concentration
Starting cell density:	1.0×10^4 cells/mL
Exposure regime:	Static
Aeration:	No
Duration:	72 hours
Environmental conditions	
Test temperature:	22.5°C
pH:	test start: 7.2 to 7.8
	test end: 7.0 to 9.2
Lighting:	Continuous illumination at 80 µE/m^{2}s (between wavelengths 400 – 700 nm)

Study Design and Methods

Experimental dates: 18th December 2000 to 30th January 2001

A primary stock solution was prepared by mixing 99.9 mg of CGA275535 in approximately 200 mL test medium in a beaker. The mixture was homogenising by ultrasonic treatment for 15 minutes and then made up to 1000 mL with test medium. The stock solution was stirred overnight. The exposure solutions were prepared by dilution of appropriate aliquots of the stock solution in 200 mL test medium containing 4 mL algae inoculum. These solutions were divided between three replicate flasks for each concentration.

A 1 mL aliquot of solution was taken from all test concentration and control flasks after 24, 48 and 72 hours of exposure. The algal cell densities in these samples were determined by counting with an electronic particle counter. In addition, after 72 hours exposure observation of the health and appearance of the algal cells in the highest concentration still showing growth and the control were recorded using a microscope.

The pH was measured at the start and at the end of the test in each test concentration and the control. The water temperature was recorded continuously in a flask incubated under the same conditions as the test flasks.

The test concentrations were verified by chemical analysis of CGA275535 at 0 and 72 hours, using high performance liquid chromatography (HPLC).

The algal cell densities were measured at 24, 48 and 72 hours and the mean biomass and growth rate calculated. The 72-hour and 96-hour E_bC_{50} and E_rC_{50} values (defined as the concentration resulting in 50% reduction of each parameter) were calculated using non-linear regression. For determination of the NOEC values, Dunnett's Test was used to identify significant differences in the calculated mean biomass and growth rate at the test item treatments compared to the control.

Results and Discussion

The limit of quantification (LOQ) for this study was 0.1 mg/L. Mean measured concentrations were used for the calculation and reporting of results.

Table 8.2.6.1-12: Analytical results

Nominal concentrations (mg/L)	0 Hour Measured Concentrations		72 Hour Measured Concentrations		Mean measured concentration (mg/L)
	mg/L	% of nominal	mg/L	% of nominal	
Control	<0.1	-	<0.1	-	<0.1
4.3	4.01	93.2	3.17	73.6	3.59
9.4	9.65	102.6	8.33	88.6	8.99
21	20.93	99.7	19.88	94.7	20.41
45	42.04	93.4	35.60	79.1	38.82
100	94.52	94.5	50.52	50.5	72.52

Cells were observed to appear normal after the 72 hour exposure period.

Growth rates

The growth rate 0 to 72 hours was calculated for each replicate culture and the means are shown below, alongside the estimated E_rC_x values.

Table 8.2.6.1-13: Mean values at each concentration of CGA275535 for the growth rate at 72 hours for *Pseudokirchneriella subcapitata*

Mean measured concentrations (mg/L)	Mean growth rate (day^{-1}) 0 – 72 hrs	Percentage inhibition (%)
Control	1.35	-
3.59	1.33	1.3
8.99	1.17	13.8[a]
20.41	0.41	69.6 [a]
38.82	0.10	92.6 [a]
72.52	0.19	86.3 [a]
E_rC_{10} mg /L (95% confidence limits)	6.1 (0.02 – 12.90)	
E_rC_{50} mg /L (95% confidence limits)	18 (4.34 – 48.87)	
NOEC	3.6	
LOEC	9.0	

[a] Significantly reduced compared to the pooled control, based on Dunnett's t-test.

Biomass (area under the growth curve)

The areas under the growth curve for 0 to 72 hours were calculated for each replicate culture and the means are shown below, alongside the estimated E_bC_x values.

Table 8.2.6.1-14: Mean values at each concentration of CGA275535 for the biomass integral (area under the growth curve) at 72 hours for *Pseudokirchneriella subcapitata*

Nominal concentrations (mg/L)	Mean biomass integral (x 10^4) 0 – 72 hrs	Percentage inhibition (%)
Control	10727	-
3.59	9965	7.1[a]
8.99	6032	43.8[a]
20.41	608	94.3[a]
38.82	101	99.1[a]
72.52	263	97.6[a]
E_bC_{10} mg /L (95% confidence limits)	3.9 (0.0 – 8.18)	
E_bC_{50} mg /L (95% confidence limits)	9.4 (0.23 – 37.59)	
NOEC	<3.6	
LOEC	3.6	

[a] Significantly reduced compared to the pooled control, based on Dunnett's t-test.

Validity criteria

The test was regarded as valid, because the cell concentration in the control cultures increased by a factor of more than 16.

Conclusions

The toxicity of CGA275535 to the green alga *Pseudokirchneriella subcapitata* was determined. Based on mean measured concentrations, the 72-hour E_rC_{50} and E_bC_{50} values were 18 and 9.4 mg/L, respectively. The 72 hour NOEC of CGA275535 based on growth rate was 3.6 mg/L. For biomass a significant effect was observed at 3.6 mg/L and therefore the NOEC can not be accurately determined but can be concluded to be <3.6 mg/L.

(Maetzler P. 2001)

Summaries of studies conducted following the original EU submission of cyprodinil are presented below. The endpoints are summarised in Table 8.2-8.

Report:	Submitted for purposes of renewal as conducted on relevant aquatic metabolite: K-CA 8.2.6.1/~~03~~05 Eckenstein H., (2015a) CGA321915 - Toxicity to *Pseudokirchneriella subcapitata* in a 96-Hour Algal Growth Inhibition Test. Report Number D96711. Harlan Laboratories Ltd., Zelgliweg 1, 4452 Itingen, Switzerland, (Syngenta File No. CGA321915_10004)

Guidelines

OECD Guidelines for Testing of Chemicals, Section 2 - Effects on Biotic Systems, Method 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test (2011)

GLP: Yes

Executive Summary

The toxicity of CGA321915 to the green alga *Pseudokirchneriella subcapitata* was determined. Algae were exposed to nominal concentrations of 2.2, 4.6, 10, 22, 46 and 100 mg CGA321915/L (mean measured: 2.1, 4.6, 9.8, 21, 45 and 99 mg CGA321915/L) alongside a culture medium control. Based on the mean measured concentrations the 72- and 96-hour E_rC_{50}, E_yC_{50} and E_bC_{50} were > 99 mg CGA321915/L, the highest concentration tested.

Materials

Test Material	CGA321915 CSAA400257
Parent:	CGA219417 (Cyprodinil)
Lot/Batch #:	MES 356/1
Purity:	98 % w/w (estimated error: ± 2 %)
Description:	White solid
Stability of test compound:	Stable under test conditions
Reanalysis/Expiry date:	30 September 2016
Density:	Not applicable
Test concentrations:	Culture medium control and nominal concentrations of 2.2, 4.6, 10, 22, 46 and 100 mg CGA321915/L (mean measured concentrations: 2.1, 4.6, 9.8, 21, 45 and 99 mg CGA321915/L)
Solvent:	None
Positive control:	Potassium dichromate is used twice a year (last study – D94966, August 2014)
Analysis of test concentrations:	Yes, 0 and 96 hours using HPLC with UV detection
Test organism	
Species:	*Pseudokirchneriella subcapitata*, Strain No. 61.81 SAG
Source:	Laboratory cultures, originally obtained from Collection of Algal Cultures (SAG, Institute for Plant Physiology, University of Göttingen, 37073 Göttingen, Germany)
Test design	
Test vessels:	50-mL plastic Erlenmeyer flasks containing 15 mL of media covered with glass dishes
Test medium:	Reconstituted test water (AAP medium) prepared according to OECD guideline 201
Replication:	Six vessels for the control and three vessels for each test concentration
Starting cell density:	0.5×10^4 cells/mL
Exposure regime:	Static
Aeration:	None reported
Duration:	96 hours
Environmental conditions	
Test temperature:	22 °C
pH:	test start: 7.2 test end: 8.6 to 8.7
Lighting:	Continuous illumination at an approximately 4900 Lux (range: 4360 to 5290 Lux)

Study Design and Methods

Experimental dates: 19th November 2014 to 6th January 2015

A stock solution of the highest nominal concentration of 100 mg CGA321915/L was prepared by mixing 50.18 mg of the test item completely in 500 mL of test water using intense stirring for 15 minutes at room temperature. This stock solution was used in a series of dilutions with test water to prepare the test media of the lower test concentrations. The control consisted of culture medium only.

An aliquot of test solution was placed into each test vessel and the test was started by inoculation of 5000 algal cells per mL of test medium. Test solutions were continuously stirred with magnetic stirrers and were held in a temperature controlled water bath under continuous illumination.

Small volumes of all test concentrations and controls were taken from all test flasks after 24, 48, 72 and 96 hours of exposure. The algal cell densities in these samples were determined by fluorescence measurement. In addition, after 96 hours exposure, a sample was taken from the control and from the nominal 100 mg CGA321915/L test concentration. The shape and size of the algal cells was examined microscopically in these samples.

The pH was measured at the start and at the end of the test. The water temperature was measured daily in a flask incubated under the same conditions as the test flasks. The appearance of the test media was also recorded daily.

The test concentrations were verified by chemical analysis of CGA321915 at 0 (without algae) and 96 hours (containing algae), using HPLC with UV detection. For sampling at the end of the test, the test medium of the treatment replicates were pooled.

Results and Discussion

At the start of the test, the analytically determined concentrations of CGA321915 were in the range 98 to 100 % of the nominal values and at the end of the test were in the range 91 to 101 % (see table below). The limit of quantification in this study was 1.03 mg CGA321915/L. Arithmetic mean measured concentrations were used for the calculation and reporting of results.

Table 8.2.6.1-15: Analytical results

Nominal concentrations (mg CGA321915/L)	% of nominal measured at 0 hours	% of nominal measured at 96 hours*	Mean measured concentrations (mg CGA321915/L)
Control	n.a.	n.a.	n.a.
2.2	99	95	2.1
4.6	98	101	4.6
10	100	95	9.8
22	98	91	21
46	99	97	45
100	98	99	99

* Pooled treatment replicates samples were centrifuged
n.a. = not applicable
The tabulated values of the samples represent rounded results obtained by calculation using the exact raw data

The algal cell densities were measured at 24, 48, 72 and 96 hours and the mean biomass, growth rate and yield calculated. The 72-hour E_bC_{10} was calculated by Probit Analysis using linear maximum likelihood regression. No further EC_x values could be determined because none of the responses exceeded 50 %. Dunnett's t-test (one-sided smaller, $\alpha = 0.05$) was used to identify significant differences in the calculated biomass, growth rate and yield of test item treatments compared to the control.

There were no abnormalities, observed microscopically, in the control or the nominal 100 mg CGA321915/L test culture at 96 hours.

Growth rates

The growth rate 0 to 72 hours and 0 to 96 hours were calculated for each replicate culture and the means are shown below.

Table 8.2.6.1-216: Mean values at each concentration of CGA321915 for the growth rate at 72 and 96 hours for *Pseudokirchneriella subcapitata*

Mean measured concentrations (mg CGA321915/L)	Mean growth rate (1/day) 0 – 72 hrs	Percentage inhibition	Mean growth rate (1/day) 0 – 96 hrs	Percentage inhibition
Control	1.515	0.0	1.427	0.0
2.1	1.521	-0.5	1.418	0.6
4.6	1.521	-0.4	1.423	0.3
9.8	1.492	1.5	1.399	1.9
21	1.495	1.3	1.411	1.1
45	1.503	0.8	1.411	1.1
99	1.469	3.0	1.402	1.7

No statistically significant effects compared to control (according to Dunnett t-test, one-sided smaller, $\alpha = 0.05$)

Yield

The yield 0 to 72 hours and 0 to 96 hours were calculated for each replicate culture and the means are shown below.

Table 8.2.6.1-317: Mean values at each concentration of CGA321915 for the yield at 72 and 96 hours for *Pseudokirchneriella subcapitata*

Mean measured concentrations (mg CGA321915/L)	Mean yield (x 10^3 cells/mL) 0 – 72 hrs	Percentage inhibition	Mean yield (x 10^3 cells/mL) 0 – 96 hrs	Percentage inhibition
Control	54.5	0.0	175.6	0.0
2.1	55.7	-2.2	169.2	3.6
4.6	55.7	-2.3	172.7	1.6
9.8	51.0	6.5	157.9	10.1
21	51.4	5.6	165.0	6.0
45	52.7	3.4	165.4	5.8
99	47.8	12.3	159.2	9.3

No statistically significant effects compared to control (according to Dunnett t-test, one-sided smaller, $\alpha = 0.05$)

Biomass (area under the growth curve)

The areas under the growth curve for 0 to 72 hours and 0 to 96 hours were calculated for each replicate culture and the means are shown below.

Table 8.2.6.1-418: Mean values at each concentration of CGA321915 for the biomass integral (area under the growth curve) at 72 and 96 hours for *Pseudokirchneriella subcapitata*

Mean measured concentrations (mg CGA321915/L)	Mean biomass integral (x 10^3*day) 0 – 72 hrs	Percentage inhibition	Mean biomass integral (x 10^3*day) 0 – 96 hrs	Percentage inhibition
Control	44.2	0.0	159.2	0.0
2.1	44.1	0.2	156.6	1.7
4.6	43.8	1.0	158.0	0.8
9.8	40.7	7.9	145.1	8.9
21	41.7	5.8	149.9	5.9
45	41.8	5.4	150.9	5.3
99	38.4*	13.1	141.9*	10.9

*: mean value statistically significantly lower than in the control (according to Dunnett t-test, one-sided smaller, $\alpha = 0.05$)

Validity criteria

The algal biomass in the control increased by a factor of 94 over 72 hours (must be least 16). The mean coefficient of variation of the daily growth rates in the control cultures was 13 % and 17 % over 72 and 96 hours, respectively (must be ≤ 35 %). The coefficient of variation of average specific growth rates in the control cultures was 0.8 % and 0.5 % over 72 and 96 hours, respectively (must be ≤ 7 %). Therefore, all validity criteria were met.

Conclusions

Based on the mean measured concentrations the 72- and 96-hour E_rC_{50}, E_yC_{50} and E_bC_{50} were > 99 mg CGA321915/L, the highest concentration tested.

The LOEC at 72 and 96 hours, based on biomass, was 99 mg CGA321915/L and the corresponding NOEC was 45 mg CGA321915/L. The LOEC at 72 and 96 hours, based on growth rate and yield, could not be determined. The NOEC at 72 and 96 hours, based on growth rate and yield, was 99 mg CGA321915/L.

(Eckenstein H, 2015a)

Report:	Submitted for purposes of renewal as conducted on relevant aquatic metabolite: K-CA 8.2.6.1/~~04~~06 Vial A. (1991b) Report on the growth inhibition test of CGA 263208 intermediate to green algae. Report Number 918088. Ciba-Geigy Ltd., Product Safety, Ecotoxicology, CH-4002 Basel, Switzerland. (Syngenta File No. C1059/0012)

Guidelines

OECD Guideline No. 201, Paris 1984

GLP: Yes

Executive Summary

The toxicity of CGA263208 to the green alga (*Pseudokirchneriella subcapitata)* was determined under static conditions.

Algae were exposed to a range of nominal concentrations of 0.123, 0.37, 1.11, 3.3, 10 mg a.s./L alongside a dilution water control.

Based on nominal concentrations of CGA263208 the E_bC_{50} (72 h) was determined to be 1.86 mg a.s./L and the NOE_bC (72 h) was 0.123 mg a.s./L.

Materials

Test Material	CGA263208
Lot/Batch #:	P.010010
Content of a.s.:	72.7 %
Description:	Grey solid
Reanalysis/Expiry date:	31/12/92
Treatments	
Test concentrations:	Culture medium control and nominal concentrations of 2, 4, 8, 16, 32, 64 and 128 µg a.s./L
Solvent:	None
Positive control:	None
Analysis of test concentrations:	0 and 96 h
Test organism	
Species:	Green Algae (*Pseudokirchneriella subcapitata*)
Source:	Ciba-Geigy Ltd., testing facility
Culture medium:	Synthetic test water prepared according to the test guidelines.
Test design	
Test vessels:	100 mL Erlenmeyer flasks containing 50 mL of test solution per flask
Test medium:	Synthetic test water prepared according to the test guidelines.
Replication:	Three vessels for each test concentration and 6 for the control
Starting cell density:	5100 cells/mL
Exposure regime:	Static
Aeration:	No
Duration:	72 hours
Environmental conditions	
Test temperature:	23 °C ± 1 °C
pH:	test start: 7.7–7.8 test end: 7.6-7.9
Lighting:	Continuous illumination at 128 µE/m^2

Study Design and Methods

Experimental dates: 22nd April to 7th June, 1991

The study comprised a 72 h exposure period.

A stock solution was prepared by dissolving 20.4 mg of the test item completely in 2000 mL of test water. This stock solution was used in a series of dilutions to prepare the test media for all test concentrations. The test media were prepared just before the addition of the algae.

The test was started (0 hours) by transfer of 5100 algal cells/mL to the test medium. These cells were taken from an exponentially growing pre-culture which was set up three days prior to the test under the same conditions as in the test.

Samples from each replicate of the test groups were collected at 24, 48 and 72 hours exposure and cell counts were conducted using a "TOA" cell counter.

Temperature was measured continuously throughout the exposure period. pH was recorded at the start of the test and at the end of the exposure period. Samples of test medium were collected from each treatment and control group at the beginning and the end of the test for analytical determination (HPLC) of test concentrations.

Results and Discussion

At the start of the test the concentrations of CGA263208 were in the range 106 to 123% of the nominal values. Over the 96 h test period the concentrations of the test item were 81-141% of the nominal concentrations. Nominal concentrations were used to derive all algal endpoints within this study.

The measured cell densities during the study are shown in the table below:

Table 8.2.6.1-519: Measured cell densities during study

Nominal concentration (µg/L)	Measured Density (cells/mL*10000)		
	24 h	48 h	72 h
Control	2.2	9.8	221.9
0.123	2.6	17.0	207.5
0.37	2.1	14.4	167.7
1.11	2.5	16.1	179.0
3.33	1.9	2.2	6.8
10	0.7	0.6	5.8

Inhibition (area under growth curves)

The areas under the growth curve for 0 to 72 hours were calculated for each treatment and are shown in the table below:

Table 8.2.6.1-620: Mean values at each concentration of CGA263208 for the biomass (area under the growth curve) for *Desmodesmus subspicatus*

Conc. Nominal (mg/L)	Areas under Growth Curves (A) * 10000						Mean A	Inhibition* IA 0-72h (%)
	1	2	3	4	5	6		
Blank	2648	2968	2978	2878	2911	3139	2920	0.0
0.123	2987	2170	3625	-	-	-	2927	0.0
0.37	2823	2211	2095	-	-	-	2376	18.6
1.11	2464	2960	2264	-	-	-	2563	12.2
3.33	190	131	123	-	-	-	148	94.9
10	50	41	119	-	-	-	70	97.6

* promotion of algal growth compared to control is indicated as 0

The 72 h E_bC_{50} was determined to be 1.86 mg CGA263208/L.

The 72 h NOEC was determined to be 0.123 µg CGA263208/L, since up to and including this test concentration the mean biomass of the algae was not statistically significantly lower than in the control.

Validity Criteria

Validity criteria were met:

- Coefficient of variation was < 7 % and was under 35 % for section by section growth rates.
- Growth rates were sufficient within the controls

Conclusions

Based on nominal concentrations of CGA263208, the E_bC_{50} (72 h) was determined to be 1.86 mg a.s./L. The NOE_bC (72 h) was 0.123 mg a.s./L.

(Vial A, 1991b)

In accordance with Regulation 283/2013, the study data have been re-evaluated for the purposes of calculating EC_{10} and EC_{20} values. The outcome of the re-evaluation is provided below.

Report:	Submitted for purposes of renewal due to change in data requirements guidance: K-CA 8.2.6.1/07 Taylor S. & Radford F. (2016) Cyprodinil CGA263208 – Report on the Growth Inhibition Test of CGA263208 Intermediate to Green Algae (*Scenedesmus subspicatus*). Report Number: CEA.1714. Cambridge Environmental Assessments, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK. (Syngenta File No: CGA263208_10017)

Summary

The report from Ciba-Geigy Ltd. test number 918088 (Vial, 1991) for the toxicity of CGA263208 to the green alga *Scenedesmus subspicatus* did not provide estimates of the EC_{10}, EC_{20} and EC_{50} for the response variables of yield and average specific growth rate. Consequently, the data generated in this study have been re-analysed in an attempt to provide these values.

A significant dose response relationship was observed between treatment and algal growth rate at 24, 48 and 72 h, and for yield at 24 and 72 h, therefore EC_{10} and EC_{20} values and the 95% and 99% confidence limits could be reliably determined. There was no significant relationship between dose and response in the remaining parameters and therefore no EC_x could be reliably calculated.

Statistical Analysis

The algal yield represents the cell number/biomass generated during the test or evaluation period minus the cell number/biomass at the start of the exposure or evaluation period. The average specific growth rate represents the slope of the growth curve. All computations were carried out in ToxRat Professional version 2.10 (ToxRat Solutions GmbH, 2001-2010). Probit analysis with linear maximum likelihood regression was used to determine the concentration response function. Chi² was used as a goodness of fit measure and the proportion of variance explained by the dose/response relationship was determined.

Results

Average Specific Growth Rate

Treatment related effects were observed and the relationship between dose and response was significant at 24 h (p(F) <0.001), 48 h (p(F) <0.001) and 72 h (p(F) <0.001). E_rC_x values and the 95% and 99% confidence limits were reliably determined for these time periods. There was no significant relationship at 48 h (p(F) = 0.590) and therefore no reliable EC_x could be calculated for this time point.

Yield

Treatment related effects were observed and the relationship between dose and response was significant at 24 h (p(F) <0.001) and 48 h (p(F) = 0.002). E_yC_x values and the 95% and 99% confidence limits were reliably determined for these time periods.

EC_{10} and EC_{20} Estimates

Estimates of the EC_{10}, EC_{20} and EC_{50} values can be summarized as follows:

Parameter	24 hours			48 hours			72 hours		
	E_rC_{10}	E_rC_{20}	E_rC_{50}	E_rC_{10}	E_rC_{20}	E_rC_{50}	E_rC_{10}	E_rC_{20}	E_rC_{50}
Value [mg/L]	4.228	5.441	8.815	1.922	2.526	4.260	0.807	1.582	5.725
lower 95%-cl	2.835	4.006	7.452	1.137	1.758	3.793	0.559	1.221	4.967
upper 95%-cl	5.343	6.576	10.191	2.452	3.010	4.748	1.065	1.936	6.654

Parameter	24 hours			72 hours		
	E_yC_{10}	E_yC_{20}	E_yC_{50}	E_yC_{10}	E_yC_{20}	E_yC_{50}
Value [mg/L]	3.459	4.418	7.055	0.668	0.939	1.797
lower 95%-cl	1.956	2.894	5.518	0.433	0.681	1.485
upper 95%-cl	4.582	5.634	9.279	0.872	1.164	2.203

Conclusion

A significant dose response relationship was observed between treatment and algal growth rate at 24, 48 and 72 h, and for yield at 24 and 72 h, therefore EC_{10} and EC_{20} values and the 95% and 99% confidence limits could be reliably determined. There was no significant relationship between dose and response in the remaining parameters and therefore no EC_x could be reliably calculated.

(Taylor S. and Radford F., 2016)

Report:	Submitted for purposes of renewal as conducted on relevant aquatic metabolite: K-CA 8.2.6.1/~~0~~508 Grade R. (1993) Report on the growth inhibition test of CA 1139 A to green algae (*Pseudokirchneriella subcapitata*), Report Number 928174, /Ciba-Geigy Ltd., Product Safety, Ecotoxicology, CH-4002 Basel, Switzerland. (Syngenta File No. CA1139A/0010)

Guidelines

OECD Guideline No. 201, Paris 1984

GLP: Yes

Executive Summary

The toxicity of CA1139A to *Pseudokirchneriella subcapitata* was determined.

Algae were exposed to a range of nominal concentrations of 0.041, 0.123, 0.37, 1.1 3.3 and 10 mg CA1139A/L alongside a dilution water control.

Based on nominal concentrations of CA1139A the E_bC_{50} (72 h) was determined to be 3.8 mg CA1139A/L and the NOE_bC (72 h) was 1.1 mg CA1139A/L.

Materials

Test Material	CA1139A
Lot/Batch #:	P.201025
Content of a.s.:	76.2 %
Description:	-
Reanalysis/Expiry date:	02/93

Treatments

Test concentrations:	Culture medium control and nominal concentrations of 0.041, 0.123, 0.37, 1.1 3.3 and 10 mg CA1139A/L
Solvent:	Test water
Analysis of test concentrations:	0 and 96 h

Test organism

Species:	Green Algae (*Pseudokirchneriella subcapitata*)
Source:	Ciba-Geigy Ltd., testing facility

Test design

Test vessels:	100 mL Erlenmeyer flasks containing 50 mL of test solution per flask
Test medium:	Synthetic test water prepared according to the test guidelines.
Replication:	Three vessels for each test concentration and 6 for the control
Starting cell density:	5,100 cells/mL
Exposure regime:	Static
Aeration:	No
Duration:	72 hours

Environmental conditions

Test temperature:	23°C ± 2 °C	
pH:	test start:	7.5–8.2
	test end:	7.8 – 8.0
Lighting:	Continuous illumination at 122 µE/m^2	

Study Design and Methods

Experimental dates: 19th October to 13th November, 1992

The study comprised a 72 h exposure period.

A stock solution was prepared by dissolving 20.2 mg of the test item completely in 1000 mL of test water. This stock solution was used in a series of dilution to prepare the test media to make nominal concentrations of 0.041, 0.123, 0.37, 1.1 3.3 and 10 mg CA1139A/L. The test media were prepared just before the addition of the algae.

The test was started (0 hours) with initial cell densities of 10 200 algal cells/mL of test medium.

These cells were taken from an exponentially growing pre-culture which was set up under the same conditions as in the test (the assumption is that although not stated in the report, the report does state that this is in-line with guideline, therefore the statement is assumed to be true). The cells had been under test conditions for 3 days.

Samples from each replicate of the test groups were collected at 24, 48 and 72 hours exposure and cell counts were conducted using a "TOA" cell counter.

Temperature was measured continuously throughout the period. pH was recorded at the start of the test and at the end of the exposure period. Samples of test media were collected from each treatment and control group at the beginning and the end of the test for analytical determination (HPLC) of test concentrations.

Results and Discussion

At the start of the test the concentrations of CA1139A were in the range of 91 to 191% of the nominal values. Over the 96 h test period the concentrations of the test item were 73-184% of the nominal concentrations.

The measured cell densities during the study are shown in the table below.

Table 8.2.6.1-~~7~~21: Measured cell densities during study

Nominal concentration (mg/L)	Measured Density (cells/mL*10000)		
	24 h	48 h	72 h
Control	4.6	27.6	110.9
0.041	5.0	30.5	104.8
0.123	6.2	31.9	133.5
0.37	4.4	29.6	143.8
1.1	4.8	27.4	124.7
3.3	4.1	8.6	13.0
10	1.7	1.6	2.5

Validity:

Validity criteria as per the OECD were generally met:

- Growth in the controls was over a factor of
- The coefficient of variation in growth between the controls on each day is < 35 %
- The coefficient of variation between the replicate controls was below 7 % (actual CV within these controls is 3 %)

Conclusions

Based on nominal concentrations of CA1139A the E_bC_{50} (72 h) was determined to be 3.8 mg CA1139A/L and the NOE_bC (72 h) was 1.1 mg CA1139A/L.

(Grade R, 1993)

In accordance with Regulation 283/2013, the study data have been re-evaluated for the purposes of calculating EC_{10} and EC_{20} values. The outcome of the re-evaluation is provided below.

Report:	Submitted for purposes of renewal due to change in data requirements guidance: K-CA 8.2.6.1/09 Taylor S. & Pickering F. (2016a) CA1139A – Report on the Growth Inhibition Test of CA1139A to Green Algae (*Scenedesmus subspicatus*). Report Number: CEA.1715. Cambridge Environmental Assessments, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK. (Syngenta File No: CA1139_10007)

Summary

The report from CIBA-GEIGY Ltd. Test number 928174 (Grade, 1993), for the algal growth inhibition test of CA1139A to *Scenedesmus subspicatus,* did not provide estimates of the EC_{10}, EC_{20} and EC_{50} for yield and average specific growth rate. Consequently the data generated in this study have been re-analysed in an attempt to provide these values.

A significant dose response relationship was observed between treatment and algal growth rate and yield at 24, 48 and 72 h, therefore EC_{10} and EC_{20} values and the 95% and 99% confidence limits could be reliably determined. There was no significant relationship between dose and response in the remaining parameters and therefore no EC_x could be reliably calculated.

Statistical Analysis

The algal yield represents the cell number/biomass generated during the test or evaluation period minus the cell number/biomass at the start of the exposure or evaluation period. The average specific growth rate represents the slope of the growth curve. All computations were carried out in ToxRat Professional version 2.10 (ToxRat Solutions GmbH, 2001-2010). Probit analysis with linear maximum likelihood regression was used to determine the concentration response function. Chi² was used as a goodness of fit measure and the proportion of variance explained by the dose/response relationship was determined.

Results

Average Specific Growth Rate

Treatment related effects were observed and the relationship between dose and response was significant at 24 h ($p(F) < 0.001$), 48 h ($p(F) < 0.001$) and 72 h ($p(F) < 0.001$). E_rC_x values and the 95% and 99% confidence limits were reliably determined for these time periods. There was no significant relationship at 48 h ($p(F) = 0.590$) and therefore no reliable EC_x could be calculated for this time point.

Yield

Treatment related effects were observed and the relationship between dose and response was significant at 24 h ($p(F) < 0.001$), 48 h ($p(F) < 0.001$), and 72 h ($p(F) < 0.001$). E_yC_x values and the 95% and 99% confidence limits were reliably determined for these time periods.

EC_{10}, EC_{20} and EC_{50} estimates

Estimates of the EC_{10}, EC_{20} and EC_{50} values can be summarized as follows:

Parameter	24 hours			48 hours			72 hours		
	E_rC_{10}	E_rC_{20}	E_rC_{50}	E_rC_{10}	E_rC_{20}	E_rC_{50}	E_rC_{10}	E_rC_{20}	E_rC_{50}
Value [mg/L]	4.140	6.058	12.547	1.163	1.873	4.664	0.871	1.508	4.308
lower 95%-cl	1.802	3.256	9.703	1.013	1.691	4.297	0.706	1.294	3.851
upper 95%-cl	6.174	8.230	14.825	1.310	2.054	5.091	1.035	1.721	4.862

Parameter	24 hours			48 hours			72 hours		
	E_yC_{10}	E_yC_{20}	E_yC_{50}	E_yC_{10}	E_yC_{20}	E_yC_{50}	E_yC_{10}	E_yC_{20}	E_yC_{50}
Value [mg/L]	3.103	4.441	8.817	1.232	1.493	2.157	0.806	1.060	1.787
lower 95%-cl	0.879	1.658	5.133	0.715	0.988	1.795	0.696	0.939	1.621
upper 95%-cl	5.281	6.970	12.894	1.559	1.794	2.396	0.912	1.178	1.978

Conclusion

A significant dose response relationship was observed between treatment and algal growth rate and yield at 24, 48 and 72 h, therefore EC_{10} and EC_{20} values and the 95% and 99% confidence limits could be reliably determined. There was no significant relationship between dose and response in the remaining parameters and therefore no EC_x could be reliably calculated.

(Taylor S. and Pickering F., 2016a)

CA 8.2.6.2 Effects on growth of an Additional Algal Species

The studies previously submitted in the EU were summarised in point B.9.2.6 of the DAR. At the request of ANSES a summary has been included in order to aid dossier evaluation. ~~therefore additional study summaries are not repeated here.~~ The endpoints are summarised in Table 8.2-7.

Report:	Summary submitted at the request of ANSES for ease of dossier evaluation: *KIIA 8.2.6. Ward T. J., Magazu J.P. & Boeri R. L. (1995). Growth and Reproduction Toxicity Test with CGA-219417 and the Freshwater Alga, Anabaena flos-aquae. Report Number 793-CG. T. R. Wilbury Laboratories, Inc. Massachusetts 01945, USA. (Syngenta File No. CGA219417/0647).*

Guidelines

FIFRA Subdivision J, 123-2

GLP: Yes

Executive Summary

The toxicity of CGA219417 to the freshwater alga *Anabaena flos-aquae* was determined. Algae were exposed to nominal concentrations 0.6, 1.3, 2.5, 5.0 and 10 mg/L (0.538, 1.26, 2.46, 4.81 and 9.44 mg/L mean measured concentrations) alongside a culture medium control.

Based on mean measured concentrations, 72, 96 and 120 hour E_bC_{50} values for CGA 219417 to *Anabaena flos-aquae* were 3.76, 3.81 and 2.25 mg/L, respectively.

The 96 and 120 hour E_rC_{50} values were 3.67 and 3.19 mg/L, respectively.

Materials

Test Material	CGA219417
Lot/Batch #:	P012012
Purity:	98.8%
Description:	Off-white granules
Stability of test compound:	Stable for at least 1 year at room temperature
Reanalysis/expiry date:	31 January 1996
Treatments	
Test concentrations:	Culture medium control and nominal concentrations of 0.6, 1.3, 2.5, 5.0 and 10 mg/L. Mean measured concentrations of 0.538, 1.26, 2.46, 4.81 and 9.44 mg/L
Solvent:	Not applicable
Positive control:	None
Analysis of test concentrations:	Yes, HPLC analysis of CGA 219417
Test organism	
Species:	*Anabaena flos-aquae* (UTEX 1444)
Source:	Culture Collection of Algae at the University of Texas, maintained in test media for more than 14 days prior to definitive test
Test design	
Test vessels:	250 mL glass Erlenmeyer flasks containing 50 mL of media
Test medium:	Sterile enriched media (U.S. EPA, 1978)
Replication:	Three replicates for each treatment
Starting cell density:	3000 cells/mL
Exposure regime:	Static
Aeration:	No
Duration:	120 hours
Environmental conditions	
Test temperature:	23.6 to 24.5°C
pH:	test start: 7.4 to 7.4 test end: 7.7 to 7.8
Lighting:	Constant light, 2100 ± 325 lux

Study Design and Methods

Experimental dates: 13th to 18th October 1995

A stock solution with a nominal concentration of 10 mg/L was prepared by dissolving 0.0101 g of the test item completely in sterile media and adjusting the final volume to 1000 mL. The stock solution was

mixed for approximately 48 hours to aid dissolution. The test solutions were prepared by diluting appropriate aliquots of the stock solution in sterile enriched media.

An aliquot of test solution was dispensed into each test vessel and the test started by inoculation of 3000 algal cells per mL of test medium. Test solutions were constantly shaken and were held in a temperature controlled incubator under continuous illumination. Small volumes of all test concentrations and controls were taken from all test flasks after 24, 48, 72, 96 and 120 hours of exposure. The algal cell densities in these samples were determined by counting using a microscope and haemocytometer.

The pH was measured at the start and at the end of the test. The test system temperature within the incubator was continuously measured using a representative flask of water incubated among the test vessels.

The test concentrations were verified by chemical analysis of CGA219417 at 0 and 120 hours, using high performance liquid chromatography. At the beginning of the experiment 40 mL samples were collected from each test solution prior to its addition into replicate test vessels. Replicate test vessels were pooled prior to collection of the analytical sample at 120 hours.

The algal cell densities were measured at 24, 48, 72, 96 and 120 hours and the mean biomass determined. The 72 hour, 96 hour and 120 hour EC_{25} and EC_{50} values were calculated using weighted least squares non-linear regression analysis. The NOEC values were determined using a parametric one-way analysis of variance (ANOVA) and Dunnett's t-test to compare treatment and control data.

The data were re-analysed in order to determine statistics in terms of yield and growth rate. For this E_rCx and E_vCx values were estimated using Probit analysis with linear maximum likelihood regression was used to determine the concentration response function. Chi^2 was used as a goodness of fit measure. If the probability, $p(Chi^2)$, was lower than or equal to 0.100, the data were scattered around the computed dose/response function. In this case, the confidence limits were corrected for heterogeneity (i.e. they were made wider).

Results and Discussion

At the start of the test, the measured concentrations were in the range 99 to 103% of the nominal values and at the end of the test were in the range 78 to 94 % (see table below). Mean measured concentrations of 0.538, 1.26, 2.46, 4.81 and 9.44 mg/L were used for the calculation and reporting of results.

Table 8.2.6.2-1: Analytical results

Nominal concentrations (mg/L)	Measured Concentration of CGA 219417 (mg/L)		
	0 hours	120 hours	Mean
0 (control)	<0.100	<0.100	<0.100
0.6	0.607	0.468	0.538
1.3	1.34	1.18	1.26
2.5	2.57	2.34	2.46
5.0	4.93	4.68	4.81
10	9.96	8.92	9.44

Water quality throughout the test was within acceptable limits. The incubator temperature ranged from 23.6 to 24.5ºC during test period and the measured pH ranged from 7.4 at test initiation to 7.8 at 120 hours.

The algae population in the control vessels grew well, resulting in an average cell density of 3,733,000 cells/mL after 120 hours. The mean cell densities and $EC_{25/50}$ values are presented in the table below.

Table 8.2.6.2-2: Mean cell densities at each concentration of CGA219417 at 24, 48, 72, 96 and 120 hours and the estimated EC_x and NOEC values

Initial Measured concentrations (mg/L)	Mean Cell Density (cells/mL x 10^3)				
	24 hours	48 hours	72 hours	96 hours	120 hours
Control	20	78	737	1,913	3,831
0.575	21	79	622	1,859	3,672
1.23	20	76	530	1,960	3,050
2.37	19	63	442	1,394	2,632
4.74	14	41	34	96	250
9.27	15	<10	<10	<10	<10
EC_{25} mg/L (95% confidence limits)	Not reported		1.89 (1.46 – 2.44)	2.23 (1.88 – 2.64)	2.28 (1.98 – 2.62)
EC_{50} mg/L (95% confidence limits)	Not reported		2.60 (2.17 – 3.11)	2.82 (2.49 – 3.19)	2.89 (2.61 – 3.20)
NOEC (mg/L)	Not reported		<0.575	1.23	0.575

Re-estimated endpoints for growth rate and yield

Growth rate

Table 8.2.6.2-3: Mean values at each concentration of CGA219417 for growth rate for *Anabaena flos-aquae*

Mean measured concentrations (mg/L)	Mean growth rate (day^{-1}) 0 – 72 hrs	Percentage inhibition (%)	Mean growth rate (day^{-1}) 0 – 96 hrs	Percentage inhibition (%)	Mean growth rate (day^{-1}) 0 – 120 hrs	Percentage inhibition (%)
Control	1.599	0.0	1.515	0.0	1.425	0.0
0.538	1.640	-2.6	1.466	3.2	1.419	0.4
1.26	1.625	-1.6	1.388	8.3	1.376	3.4
2.46	1.561	2.4	1.383	8.7	1.214	14.8
4.81	1.189	25.6	1.234	18.5	1.165	18.2
9.44	0.000	100.0	0.508	66.5	0.499	65.0
E_rC_{10}	n.d.		3.667 (2.864 – 4.288)		3.193 (2.324 – 3.866)	
E_rC_{20}	n.d.		4.699 (3.948 – 5.281)		4.308 (3.468 – 4.954)	
E_rC_{50}	n.d.		7.551 (6.915 – 8.301)		7.641 (6.865 – 8.656)	

Numbers in brackets represent 95% confidence intervals
n.d. = values could not be determined

Table 8.2.6.2-4: Mean values at each concentration of CGA219417 for yield for *Anabaena flos-aquae*

Nominal concentrations (mg/L)	Mean cells/mL ($x10^3$) 0 – 72 hrs	Percentage inhibition (%)	Mean cells/mL ($x10^3$) 0 – 96 hrs	Percentage inhibition (%)	Mean cells/mL ($x10^3$) 0 – 120 hrs	Percentage inhibition (%)
Control	363.667	0.0	1283.667	0.0	3730.333	0.0
0.538	410.333	-12.8	1057.000	17.7	3630.333	2.7
1.26	397.000	-9.2	777.000	39.5	2930.333	21.4
2.46	327.000	10.1	757.000	41.0	1317.000	64.7
4.81	103.667	71.5	417.000	67.5	1023.667	72.6
9.44	0.000	100.0	20.333	98.4	33.667	99.1
E_yC_{10}	2.249 (1.715 – 2.630)		0.413 (0.171 – 0.667)		0.706 (0.444 – 0.938)	
E_yC_{20}	2.694 (2.202 – 3.050)		0.740 (0.395 – 1.062)		1.040 (0.742 – 1.293)	
E_yC_{50}	n.d.		n.d.		n.d.	

Numbers in brackets represent 95% confidence intervals
n.d. = values could not be determined

Validity criteria

Not reported.

Conclusions

Based on mean measured concentrations, the 96 hour and 120 hour E_rC_{50} values for CGA219417 to *Anabaena flos-aquae* were 3.67 and 3.19 mg/L, respectively.

The 96 and 120 hour E_rC_{50} values were 3.67 and 3.19 mg/L, respectively.

(Ward *et al*., 1995)

In accordance with **Commission Regulation (EU) No 283/2013**, EC_{10} and EC_{20} values have been estimated for previously submitted studies, and these statistical re-evaluations are provided below.

Report:	Submitted for purposes of renewal due to change in data requirements guidance: K-CA 8.2.6.2/01 Taylor S., Blakey A. & Howells A. (20156) Cyprodinil (CGA219417) – Growth and reproduction toxicity test with CGA219417 and the freshwater algae *Anabaena flos-aquae*. Statistical Re-analysis. Report Number: CEA.1412. Cambridge Environmental Assessments, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK. Amended (Syngenta File No: CGA219417_11599)

Summary

Report number 793-CG (Ward *et al.*, 1995) did not provide EC_{10} and EC_{20} estimates relating to yield or average specific growth rate for *Anabaena flos-aquae*. Consequently the data generated in this study have been re-analysed in order to provide these values.

A dose response relationship was observed between treatment and algal growth rate and yield at 24, 48, 96 and 120 h; yield was significant at 72 h. EC_{10} and EC_{20} values and the 95% and 99% confidence limits could be reliably determined.

Statistical Analysis

The algal yield represents the cell number/biomass generated during the test or evaluation period minus the cell number/biomass at the start of the exposure or evaluation period. The average specific growth rate represents the slope of the growth curve. All computations were carried out in ToxRat Professional version 2.10 (ToxRat Solutions GmbH, 2001-2010). Probit analysis with linear maximum likelihood regression was used to determine the concentration response function. Chi² was used as a goodness of fit measure.

Results

Average Specific Growth Rate

Treatment related effects were observed and the relationship between dose and response was significant at 24 h (p(F) = 0.014), 48 h (p(F) = 0.004),96 h (p(F) = 0.000) and 120 h (p(F) = 0.000). The E_rC_x values and the 95% and 99% confidence limits were reliably determined for these time periods. There was no significant relationship at 72 h (p(F) = 0.303) and therefore E_rC_x could not be determined for this time point.

Yield

Treatment related effects were observed and the relationship between dose and response was significant at 24 h (p(F) = 0.026), 48 h (p(F) = 0.012), 72 h (p(F) = 0.000), 96 h (p(F) = 0.000) and 120 h (p(F) = 0.000). The E_yC_x values and the 95% and 99% confidence limits were reliably determined for all time periods.

EC_{10} and EC_{20} Estimates

Estimates of the EC_{10}, EC_{20} and EC_{50} values can be summarized as follows:

Parameter	After 24 h		After 48 h		After 72 h	
	Growth rate	Yield	Growth rate	Yield	Growth rate	Yield
EC_{50} (mg/L) 95% CL	6.146 (3.883 – 11.937)	n.d.	7.528 (5.864 – 10.098)	n.d.	n.d.	n.d.
EC_{20} (mg/L) 95% CL	3.268 (0.471-4.769)	1.687 (0.007-3.116)	4.798 (2.253-6.089)	1.286 (0.055-2.465)	n.d.	2.694 (2.202-3.050)
EC_{10} (mg/L) 95% CL	2.349 (0.122-3.772)	1.011 (0.000-2.185)	3.792 (1.245-5.130)	0.629 (0.004-1.507)	n.d.	2.249 (2.202-3.050)

CL: Confidence Limits
n.d.: Not Determined

Parameter	After 96 h		After 120 h	
	Growth rate	Yield	Growth rate	Yield
EC_{50} (mg/L) 95% CL	7.551 (6.915 – 8.301)	n.d.	7.641 (6.865 – 8.656)	n.d.
EC_{20} (mg/L) 95% CL	4.699 (3.948-5.281)	0.740 (0.395-1.062)	4.308 (3.468-4.954)	1.040 (0.742-1.293)
EC_{10} (mg/L) 95% CL	3.667 (2.864-4.288)	0.413 (0.171-0.667)	3.193 (2.324-3.866)	0.706 (0.444-0.938)

CL: Confidence Limits
n.d.: Not Determined

Conclusion

A dose response relationship was observed between treatment and algal growth rate and yield at 24, 48, 96 and 120 h; yield was significant at 72 h. EC_{10} and EC_{20} values and the 95% and 99% confidence limits could be reliably determined.

(Taylor S Blakey A & Howells A, 2015~~6~~)

Report:	Summary submitted at the request of ANSES for ease of dossier evaluation: *KIIA 8.2.6. Ward T. J., Magazu J.P. & Boeri R. L. (1995). Growth and Reproduction Toxicity Test with CGA-219417 and the Freshwater Alga, Navicula pelliculosa. Report Number 794-CG. T. R. Wilbury Laboratories, Inc. Massachusetts 01945, USA. (Syngenta File No. CGA219417/0646).*

Guidelines

FIFRA Subdivision J, 123-2

GLP: Yes

Executive Summary

The toxicity of CGA219417 to the freshwater alga *Navicula pelliculosa* was determined. Algae were exposed to nominal concentrations 0.6, 1.3, 2.5, 5.0 and 10 mg/L (0.574, 1.23, 2.49, 5.00 and 8.77 mg/L mean measured concentrations) alongside a culture medium control. Based on mean measured concentrations, the 72 hour, 96 hour and 120 hour E_bC_{50} values for CGA219417 to *Navicula pelliculosa* were 2.11, 2.22 and 2.84 mg/L, respectively.

The 72 hour E_rC_{50} value was 2.21 mg/L.

Materials

Test Material	CGA219417
Lot/Batch #:	P012012
Purity:	98.8%
Description:	Off-white granules
Stability of test compound:	Stable for at least 1 year at room temperature
Reanalysis/expiry date:	31 January 1996
Treatments	
Test concentrations:	Culture medium control and nominal concentrations of 0.6, 1.3, 2.5, 5.0 and 10 mg/L. Mean measured concentrations of 0.574, 1.23, 2.49, 5.00 and 8.77 mg/L
Solvent:	Not applicable
Positive control:	None
Analysis of test concentrations:	Yes, HPLC analysis of CGA219417
Test organism	

Species:	*Navicula pelliculosa* (UTEX 661)
Source:	Culture Collection of Algae at the University of Texas, maintained in under test conditions for more than 14 days prior to definitive test
Test design	
Test vessels:	250 mL glass Erlenmeyer flasks containing 50 mL of media
Test medium:	Sterile enriched media (U.S. EPA, 1978)
Replication:	Three replicates for each treatment
Starting cell density:	3000 cells/mL
Exposure regime:	Static
Aeration:	No
Duration:	120 hours
Environmental conditions	
Test temperature:	24.0 to 24.6°C
pH:	test start: 7.5 test end: 8.1 to 9.9
Lighting:	Constant light, 4100 lux

Study Design and Methods

Experimental dates: 20th to 25th October 1995

A stock solution with a nominal concentration of 10 mg/L was prepared by dissolving 0.0101 g of the test item completely in sterile media and adjusting the final volume to 1000 mL. The test solutions were prepared by diluting appropriate aliquots of the stock solution in sterile enriched media.

An aliquot of test solution was dispensed into each test vessel and the test started by inoculation of 3000 algal cells per mL of test medium. Test solutions were constantly shaken and were held in a temperature controlled incubator under continuous illumination. Small volumes of all test concentrations and controls were taken from all test flasks after 24, 48, 72, 96 and 120 hours of exposure. The algal cell densities in these samples were determined by counting using a microscope and haemocytometer.

The pH was measured at the start and at the end of the test. The temperature within the incubator was continuously measured using a representative flask of water incubated among the test vessels.

The test concentrations were verified by chemical analysis of CGA219417 at 0 and 120 hours, using high performance liquid chromatography. At the beginning of the experiment 40 mL samples were collected from each test solution prior to its addition into replicate test vessels. Replicate test vessels were pooled prior to collection of the analytical sample at 120 hours and passed through a 0.45 µm filter.

The cell densities determined at 24, 48, 72, 96 and 120 hours were used to calculate the mean biomass in each treatment at each time point. The 72 hour, 96 hour and 120 hour EC_{25} and EC_{50} values were calculated using weighted least squares non-linear regression analysis. The NOEC values were determined using a parametric one-way analysis of variance (ANOVA) and Dunnett's t-test to compare treatment and control data.

The data were re-analysed in order to determine statistics in terms of yield and growth rate. For this E_rCx and E_vCx values were estimated using Probit analysis with linear maximum likelihood regression was used to determine the concentration response function. Chi² was used as a goodness of fit measure. If the

probability, p(Chi²), was lower than or equal to 0.100, the data were scattered around the computed dose/response function. In this case, the confidence limits were corrected for heterogeneity (i.e. they were made wider).

Results and Discussion

At the start of the test, the measured concentrations were in the range 101 to 105% of the nominal values and at the end of the test were in the range 74 to 96 % (see table below). Mean measured concentrations of 0.574, 1.23, 2.49, 5.00 and 8.77 mg/L were used for the calculation and reporting of results.

Table 8.2.6.2-5: Analytical results

Nominal concentrations (mg/L)	Measured Concentration of CGA219417 (mg/L)		
	0 hours	120 hours	Mean
0 (control)	<0.100	<0.100	<0.100
0.6	0.617	0.531	0.574
1.3	1.33	1.12	1.23
2.5	2.62	2.35	2.49
5.0	5.17	4.82	5.00
10	10.1	7.43	8.77

Water quality throughout the test was within acceptable limits. The incubator temperature ranged from 24.0 to 24.6 ºC during test period and the measured pH ranged from 7.5 at test initiation to 9.9 at 120 hours.

The algae population in the control vessels grew well, resulting in an average cell density of 2,439,000 cells/mL after 120 hours.

Results presented in the report

The mean cell densities and $EC_{25/50}$ values are presented in the table below.

Table 8.2.6.2-6: Mean cell densities at each concentration of CGA219417 at 24, 48, 72, 96 and 120 hours and the estimated EC_x and NOEC values

Mean Measured concentrations (mg/L)	Mean Cell Density (cells/mL x 10^3)				
	24 hours	48 hours	72 hours	96 hours	120 hours
Control	5	33	183	1110	2439
0.574	6	29	171	1087	2333
1.23	5	25	123	860	2217
2.49	<4	19	84	442	1717
5.00	5	<4	4	<3	<3
8.77	<3	<3	<3	<3	<3
EC_{25} mg/L (95% confidence limits)	Not reported		1.42 (0.994 – 2.03)	1.72 (1.39 – 2.12)	2.44 (0.892 – 6.70)
EC_{50} mg/L (95% confidence limits)	Not reported		2.11 (1.63 – 2.72)	2.22 (1.92 – 2.57)	2.84 (1.20 – 6.71)
NOEC (mg/L)	Not reported		0.574	0.574	0.574

Re-estimated endpoints based on growth rate and yield

Growth rate

The growth rate was calculated for each replicate culture and the means are shown below, together with the estimated E_rC_x values.

Table 8.2.6.2-7: Mean values at each concentration of CGA219417 for growth rate for *Navicula pelliculosa*

Mean measured concentrations (mg/L)	Mean growth rate (day^{-1}) 0 – 72 hrs	Percentage inhibition (%)	Mean growth rate (day^{-1}) 0 – 96 hrs	Percentage inhibition (%)	Mean growth rate (day^{-1}) 0 – 120 hrs	Percentage inhibition (%)
Control	1.365	0.0	1.477	0.0	1.340	0.0
0.574	1.339	1.9	1.472	0.4	1.331	0.6
1.23	1.237	9.4	1.413	4.3	1.321	1.4
2.49	1.110	18.7	1.247	15.6	1.270	5.2
5.00	0.096	93.0	0.000	100.0	0.000	100.0
8.77	0.000	100.0	0.000	100.0	0.000	100.0
E_rC_{10}	2.213 (1.998 – 2.386)		n.d.		n.d.	
E_rC_{20}	2.522 (2.332 – 2.690)		n.d.		n.d.	
E_rC_{50}	3.238 (3.037 – 3.496)		n.d.		n.d.	

Numbers in brackets represent 95% confidence intervals
n.d. = values could not be determined

Yield

The yield was calculated for each replicate culture and the means are shown below, together with the estimated E_yC_x values.

Table 8.2.6.2-8: Mean values at each concentration of CGA219417 for yield for *Navicula pelliculosa*

Mean measured concentrations (mg/L)	Mean cells/mL ($x10^3$) 0 – 72 hrs	Percentage inhibition (%)	Mean cells/mL ($x10^3$) 0 – 96 hrs	Percentage inhibition (%)	Mean cells/mL ($x10^3$) 0 – 120 hrs	Percentage inhibition (%)
Control	180.333	0.0	Control	0.0	2435.667	0.0
0.574	167.667	7.0	0.574	2.1	2330.333	4.3
1.23	119.667	33.6	1.23	22.6	2213.667	9.1
2.49	81.000	55.1	2.49	60.3	1713.667	29.6
5.00	1.000	99.4	5.00	100.0	0.000	100.0
8.77	0.000	100.0	8.77	100.0	0.000	100.0
E_yC_{10}	0.620 (0.369 – 0.836)		0.928 (0.690 – 1.118)		n.d.	
E_yC_{20}	0.896 (0.613 – 1.130)		1.208 (0.972 – 1.395)		n.d.	
E_yC_{50}	n.d.		n.d.		n.d.	

Numbers in brackets represent 95% confidence intervals
n.d. = values could not be determined

Conclusions

Based on mean measured concentrations, the 72 hour, 96 hour and 120 hour E_bC_{50} values for CGA219417 to *Navicula pelliculosa* were 2.11, 2.22 and 2.84 mg/L, respectively. The 72 hour E_rC_{50} value was 2.21 mg/L.

(Ward *et al*., 1995)

For Ward *et al*. 1995 (Syngenta File No. CGA219417/0646) the EC_{10} and EC_{20} values have been estimated in the following report:

Report:	Submitted for purposes of renewal due to change in data requirements guidance: K-CA 8.2.6.2/02Taylor S. & Radford F. ~~Pickering F.~~ (2015~~6ba~~) Cyprodinil (CGA219417) – Growth and reproduction toxicity test with CGA219417 and the freshwater algae *Navicula pelliculosa*. Statistical Re-analysis. Report Number: CEA.1422. Cambridge Environmental Assessments, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK. Amended (Syngenta File No: CGA219417_11592)

Summary

Report number 794-CG (Ward *et al.*, 1995) did not provide EC_{10} and EC_{20} estimates relating to yield or average specific growth rate for *Navicula pelliculosa*. Consequently the data generated in this study have been re-analysed in order to provide these values.

A dose response relationship was observed between treatment and algal growth rate and yield at 48, and 72 h; yield was also significant at 96 h. EC_{10} and EC_{20} values and the 95% and 99% confidence limits could be reliably determined for these time points.

Statistical Analysis

The algal yield represents the cell number/biomass generated during the test or evaluation period minus the cell number/biomass at the start of the exposure or evaluation period. The average specific growth rate represents the slope of the growth curve. All computations were carried out in ToxRat Professional version 2.10 (ToxRat Solutions GmbH, 2001-2010). Probit analysis with linear maximum likelihood regression was used to determine the concentration response function. Chi² was used as a goodness of fit measure.

Results

Average Specific Growth Rate

Treatment related effects were observed and the relationship between dose and response was significant at 48 h (p(F) = 0.000) and 72 h (p(F) = 0.000). E_rC_x values and the 95% and 99% confidence limits were reliably determined for these time periods. There was no significant dose response relationship at 96 h (p(F) = 0.176) or 120 h (p(F) = 0.165) and therefore E_rC_x could not be determined for these time points.

Yield

Treatment related effects were observed and the relationship between dose and response was significant at 48 h (p(F) = 0.000), 72 h (p(F) = 0.000) and 96 h (p(F) = 0.000). E_yC_x values and the 95% and 99% confidence limits were reliably determined for these time periods. There was no significant dose response relationship at 120 h (p(F) = 0.489) and therefore E_yC_x could not be determined for this time point.

EC_{10} and EC_{20} Estimates

Estimates of the EC_{10}, EC_{20} and E_rC_{50} values can be summarized as follows:

Parameter	After 48 h		After 72 h		After 96 h	
	Growth rate	Yield	Growth rate	Yield	Growth rate	Yield
EC_{50} (mg/L) 95% CL	n.d.	n.d.	3.238 (3.037 – 3.496)	n.d.	n.d.	n.d.
EC_{20} (mg/L) 95% CL	2.375 (2.048-2.624)	1.088 (0.665-1.416)	2.522 (2.332-2.690)	0.896 (0.613-1.130)	n.d.	1.208 (0.972-1.395)
EC_{10} (mg/L) 95% CL	2.047 (1.665-2.308)	0.771 (0.391-1.079)	2.213 (1.998-2.386)	0.620 (0.369-0.836)	n.d.	0.928 (0.690-1.118)

CL: Confidence Limits
n.d.: Not Determined

Conclusion

A dose response relationship was observed between treatment and algal growth rate and yield at 48, and 72 h; yield was also significant at 96 h. EC_{10} and EC_{20} values and the 95% and 99% confidence limits could be reliably determined for these time points.

(Taylor S and ~~Pickering~~ ~~F~~ Radford F., 201~~5b6a~~)

Report:	Submitted for purposes of renewal as conducted on cyprodinil for USA compliance: K-CA 8.2.6.2/03-Ward T.J., Magazu J.P. & Boeri R.L. (1995c) Growth and reproduction toxicity test with CGA219417 and the marine alga (*Skeletonema costatum*). Report Number 795-CG. T.R. Wilbury Laboratories Inc, 40 Doaks Lane, Marblehead, Massachusetts. (Syngenta File No. CGA219417/0644)

Guidelines

US EPA FIFRA Guideline No. 123-2

GLP: Yes

Executive Summary

The toxicity of CGA219417 to the marine alga *Skeletonema costatum* was determined. Algae were exposed to nominal concentrations of 0.60, 1.3, 2.5, 5.0 and 10 mg/L alongside a culture medium control. Based on initial measured concentrations, the 120 hour EC_{50} was 1.97 mg/L with 95% confidence intervals of 1.81 to 2.15 mg/L. the 120 hour No Observed Effect Concentration (NOEC) was 1.05 mg/L.

Based on mean measured concentrations, the 72, 96 and 120 hour E_yC_{50} values were 1.10, 1.14 and 1.33 mg/L, respectively. Estimation of the E_rC_x values was not possible.

Materials

Test Material	CGA219417
Lot/Batch #:	Po12012
Purity:	99.2%
Description:	Off-white granules

Stability of test compound:	Stable under standard conditions
Reanalysis/expiry date:	July 1995

Treatments

Test concentrations:	Culture medium control and nominal concentrations of 0.60, 1.3, 2.5, 5.0 and 10 mg/L
Solvent:	None
Positive control:	None
Analysis of test concentrations:	Yes, analysis at 0 and 120 hours

Test organism

Species:	*Skeletonema costatum*
Source:	Originally from: Culture Collection of Algae, University of Texas, Austin.

Test design

Test vessels:	250 mL glass Erlenmeyer flasks containing 50 mL of media
Test medium:	OECD algal medium
Replication:	3
Starting cell density:	1.0×10^4 cells/mL
Exposure regime:	Static
Aeration:	No
Duration:	120 hours

Environmental conditions

Test temperature:	19.6 to 19.9 °C	
pH:	test start:	8.1 to 8.2
	test end:	8.2 to 9.6
Lighting:	16 hour photoperiod (4000 Lux)	

Study Design and Methods

Experimental dates: 3rd to 8th November 2015

A stock solution with a nominal concentration of 10 mg/L was prepared by dissolving 0.0101 g of the test item completely in 1000 mL of test medium. Appropriate volumes of the stock solution were diluted to give the test concentration series. The control consisted of culture medium only.

An aliquot of test solution was placed into each test vessel and the test was started by inoculation of 10000 algal cells per mL of test medium. Test solutions were constantly shaken and were held in a temperature controlled room under continuous illumination.

Small volumes of all test concentrations and controls were taken from all test flasks after 24, 48, 72 and 96 hours of exposure. The algal cell densities in these samples were determined by counting with a microscope and hemacytometer. In addition, after 120 hours exposure, a sample was taken from the control and from a test concentration with reduced algal growth. The shape of the algal cells was examined microscopically in these samples.

The pH was measured at the start and at the end of the test. The water temperature was measured daily in a flask incubated under the same conditions as the test flasks.

The test concentrations were verified by chemical analysis of CGA219417 at 0 and 120 hours, using high performance liquid chromatography.

The algal cell densities were measured at 72, 96 and 120 hours and the mean cell density. The 72-hour, 96-hour and 120 hour EC_{25} and EC_{50}, (defined as the concentration resulting in 25 and 50% reduction of each parameter) were calculated using the weighted least squares non-linear regression method. The NOEC for algal growth was statistically calculated (TOXSTAT). A parametric one-way analysis or variance (ANOVA) and Dunnett's t-test were used to compare treatment and control data. The slope of the 120 dose response curve was calculated using the probit method.

E_yC_x values for 72, 96 and 120 h have been estimated based on mean measured concentrations. It was not possible to determine E_rC_x values based on growth rates for these assessment times. E_yCx values were estimated using Probit analysis with linear maximum likelihood regression was used to determine the concentration response function. Chi^2 was used as a goodness of fit measure. If the probability, $p(Chi^2)$, was lower than or equal to 0.100, the data were scattered around the computed dose/response function. In this case, the confidence limits were corrected for heterogeneity (i.e. they were made wider).

Results and Discussion

At the start of the test, the measured concentrations were in the range 72 to 90% of the nominal values and at the end of the test were in the range 17 to 78% (see table below). Initial measured concentrations were used for the calculation and reporting of results.

Table 8.2.6.2-19: Analytical results

Nominal concentrations (mg/L)	% of nominal measured at 0 hours	% of nominal measured at 120 hours	Initial measured concentration (mg a.s./L)
0.60	90	17	0.541
1.3	81	22	1.05
2.5	84	54	2.09
5.0	84	78	4.19
10	72	58	7.23

The growth data at 72 hours, 96 hours and 120 hours were calculated for each replicate culture and the means are shown below, alongside the estimated EC_{50} values.

Table 8.2.6.2-210: Mean values at each concentration of CGA219417 for the growth at 72, 96 and 120 hours for *Skeletonema costatum* and relevant endpoints

Initial measured concentrations (mg/L)	Mean number of cells/ml x 10^3 at 72 hours	Percentage of control (%)	Mean number of cells/ml x 10^3 at 96 hours	Percentage of control (%)	Mean number of cells/ml x 10^3 at 120 hours	Percentage of control (%)
Control	1022	-	1341	-	1533	-
0.541	995	97	1338	100	1571	102
1.05	848	83	1175	88	1415	92
2.09	305	30	374	28	554	36
4.19	<10	<1	<10	<1	<10	<1
7.23	<10	<1	<10	<1	<10	<1
EC_{25} mg /L (95% confidence limits)	1.45 (1.24 – 1.69)		1.44 (1.25 – 1.65)		1.75 (1.36 – 2.24)	

EC_{50} mg /L (95% confidence limits)	1.78 (1.62 – 1.96)	1.75 (1.62 – 1.91)	1.97 (1.81 – 2.15)
NOEC	0.541	1.05	1.05

The data were reanalysed using mean measured concentrations of 0.164, 0.554, 1.673, 4.032, and 6.453 mg a.s./L. The results are summaried in the tables below.

Table 8.2.6.2-11: Mean values at each concentration of CGA219417 for growth rate for *Skeletonema costatum*

Mean measured concentrations (mg/L)	Mean growth rate (day^{-1}) 0 – 72 hrs	Percentage inhibition (%)	Mean growth rate (day^{-1}) 0 – 96 hrs	Percentage inhibition (%)	Mean growth rate (day^{-1}) 0 – 120 hrs	Percentage inhibition (%)
Control	1.542	-	1.224	-	1.006	-
0.164	1.532	0.7	1.223	0.0	1.011	-0.5
0.554	1.480	4.1	1.191	2.7	0.990	1.6
1.673	1.121	27.3	0.901	26.3	0.802	20.3
4.032	0.000	100.0	0.000	100.0	0.000	100.0
6.453	0.000	100.0	0.000	100.0	0.000	100.0
E_rC_{10}	n.d.		n.d.		n.d.	
E_rC_{20}	n.d.		n.d.		n.d.	
E_rC_{50}	n.d.		n.d.		n.d.	

Numbers in brackets represent 95% confidence intervals
n.d. = values could not be determined

Table 8.2.6.2-12: Mean values at each concentration of CGA219417 for yield for *Skeletonema costatum*

Mean measured concentrations (mg/L)	Mean cells/mL ($x10^3$) 0 – 72 hrs	Percentage inhibition (%)	Mean cells/mL ($x10^3$) 0 – 96 hrs	Percentage inhibition (%)	Mean cells/mL ($x10^3$) 0 – 120 hrs	Percentage inhibition (%)
Control	1012.0	-	1330.7	-	1523.3	-
0.164	984.7	2.7	1328.0	0.2	1560.7	-2.5
0.554	838.0	17.2	1165.3	12.4	1405.3	7.7
1.673	295.3	70.8	364.0	72.6	544.0	64.3
4.032	0.0	100.0	0.0	100.0	0.0	100.0
6.453	0.0	100.0	0.0	100.0	0.0	100.0
E_yC_{10}	0.441 (0.377 – 0.501)		0.517 (0.448 – 0.580)		0.632 (0.571 – 0.689)	
E_yC_{20}	0.604 (0.535 – 0.667)		0.679 (0.608 – 0.743)		0.817 (0.756 – 0.872)	
E_yC_{50}	1.101 (1.023 – 1.182)		1.142 (1.068 – 1.216)		1.333 (1.280 – 1.384)	

Numbers in brackets represent 95% confidence intervals

Conclusions

Based on initial measured concentrations, the 72-hour EC_{50} for CGA219417 to *Skeletonema costatum* was 1.78 mg/L. The 96 hour EC_{50} was 1.75 mg/L and the 120 hour EC_{50} was 1.97 mg/L.

Based on mean measured concentrations, the 72, 96 and 120 hour E_yC_{50} values were 1.10, 1.14 and 1.33 mg/L, respectively. Estimation of the E_rC_x values was not possible.

The Lowest Observed Effect Concentration at 120 hours was 2.09 mg/L, and the No Observed Effect Concentration at 120 hours was 1.05 mg/L.

(Ward *et al.* 1995c)

In accordance with **Commission Regulation (EU) No 283/2013**, EC_{10} and EC_{20} values have been estimated for previously submitted studies, and these statistical re-evaluations are provided below.

Report:	Submitted for purposes of renewal due to change in data requirements guidance: K-CA 8.2.6.2/04 Taylor, S., Radford F. & Allen M. (2015~~6~~) Cyprodinil (CGA219417) – Growth inhibition test to marine algae (*Skeletonema costatum*) statistical re-analysis. Report Number: CCE.1430, Reference number CCE2046_021. Cambridge Environmental Assessments, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK. Amended (Syngenta File No: CGA219417_11650)

Summary

The report from Ciba-Geigy study number 795-CG (Ward et al, 1995) for the toxicity of CGA219417 to the marine alga, Skeletonema costatum, did not provide estimates of the EC_{10} and EC_{20} for the response variables evaluated as part of the original study. Consequently the data generated in this study have been re-analysed in an attempt to provide these values.

Statistical analyses of the available data for yield revealed that the following E_yC_{10} and E_yC_{20} values were reliably calculated:

Parameter	24 hours		48 hours		72 hours		96 hours		120 hours	
	E_yC_{10}	E_yC_{20}	E_yC_{10}	E_yC_{20}	E_yC_{10}	E_yC_{20}	E_yC_{10}	E_yC_{20}	E_yC_{10}	E_yC_{20}
Value [mg/L]	1.405	1.727	0.717	0.929	0.890	1.092	0.997	1.185	1.117	1.320
lower 95%-cl	0.597	0.949	0.423	0.636	0.729	0.941	0.838	1.039	0.995	1.211
upper 95%-cl	1.842	2.154	0.926	1.137	1.018	1.213	1.121	1.300	1.217	1.408
lower 99%-cl	0.477	0.807	0.363	0.567	0.682	0.895	0.792	0.994	0.956	1.175
upper 99%-cl	2.302	2.534	1.081	1.275	1.087	1.275	1.187	1.359	1.266	1.451

cl: confidence limits

Statistical analyses of the available data for average specific growth rate revealed that the following E_rC_{10} and E_rC_{20} values were reliably calculated:

Parameter	24 hours		48 hours		72 hours		96 hours		120 hours	
	E_rC_{10}	E_rC_{20}	E_rC_{10}	E_rC_{20}	E_rC_{10}	E_rC_{20}	E_rC_{10}	E_rC_{20}	E_rC_{10}	E_rC_{20}
Value [mg/L]	1.613	2.045	1.140	1.614	1.375	1.805	1.480	1.870	1.651	2.078
lower 95%-cl	1.216	1.649	1.051	1.545	1.304	1.760	1.460	1.859	1.626	2.066
upper 95%-cl	1.935	2.362	1.217	1.678	1.435	1.847	1.498	1.880	1.674	2.090

lower 99%-cl	1.001	1.419	0.989	1.493	1.253	1.726	1.445	1.850	1.607	2.056
upper 99%-cl	2.349	2.744	1.293	1.736	1.494	1.884	1.514	1.889	1.694	2.100

cl: confidence limits

Statistical analyses of the available data when using the geometric mean measured concentrations revealed that the following $E_{yr}C_{10}$, $E_{yr}C_{20}$ and $E_{yr}C_{50}$ values were reliably calculated:

Parameter	24 hours			48 hours			72 hours		
	E_yC_{10}	E_yC_{20}	E_yC_{50}	E_yC_{10}	E_yC_{20}	E_yC_{50}	E_yC_{10}	E_yC_{20}	E_yC_{50}
Value [mg/L]	0.999	1.301	2.160	0.319	0.471	0.996	0.441	0.604	1.101
lower 95%-cl	0.400	0.675	1.634	0.234	0.374	0.874	0.377	0.535	1.023
upper 95%-cl	1.393	1.701	2.803	0.398	0.560	1.129	0.501	0.667	1.182

Parameter	96 hours			120 hours		
	E_yC_{10}	E_yC_{20}	E_yC_{50}	E_yC_{10}	E_yC_{20}	E_yC_{50}
Value [mg/L]	0.517	0.679	1.142	0.632	0.817	1.333
lower 95%-cl	0.448	0.608	1.068	0.571	0.756	1.280
upper 95%-cl	0.580	0.743	1.216	0.689	0.872	1.384

Parameter	24 hours		
	E_rC_{10}	E_rC_{20}	E_rC_{50}
Value [mg/L]	1.199	1.596	2.759
lower 95%-cl	0.713	1.081	2.266
upper 95%-cl	1.584	1.993	3.274

Statistical Analysis

The algal yield represents the cell number/biomass generated during the test or evaluation period minus the cell number/biomass at the start of the exposure or evaluation period. The average specific growth rate represents the slope of the growth curve.

All computations were carried out in ToxRat Professional version 2.10 (ToxRat Solutions GmbH, 2001-2010). For yield (Y) Probit analysis with linear maximum likelihood regression was used to determine the concentration response function. For growth rate (μ), linear maximum likelihood regression did not derive reliable ECx values therefore, Probit analysis with simple weighted linear regression was used. Chi² was used as a goodness of fit measure.

Where no meaningful concentration/response was found (p(F) > 0.05; i.e. the slope of the relationship was not significantly different from zero) the calculated ECx's were not valid due to the absence of a concentration/response.

Results

Average Specific Growth Rate

A treatment related effect was observed and the relationship between dose and response was significant at 24 h (p(F) = 0.010), 48 h (p(F) = 0.000), 72 h (p(F) = 0.000), 96 h (p(F)= 0.000), and 120 h p(F)=0.000). E_rC_x values and the 95% and 99% confidence limits were reliably determined for these time points.

Yield

A treatment related effect was observed and the relationship between dose and response was significant at 24 h (p(F) = 0.002), 48 h (p(F) = 0.000), 72 h (p(F) = 0.000), 96 h (p(F)= 0.000), and 120 h p(F)=0.000). E_yC_x values and the 95% and 99% confidence limits were reliably determined for these time points.

EC_{10} and EC_{20} Estimates

Statistical analyses of the available data for yield revealed that the following E_yC_{10} and E_yC_{20} values were reliably calculated:

Parameter	24 hours		48 hours		72 hours		96 hours		120 hours	
	E_yC_{10}	E_yC_{20}	E_yC_{10}	E_yC_{20}	E_yC_{10}	E_yC_{20}	E_yC_{10}	E_yC_{20}	E_yC_{10}	E_yC_{20}
Value [mg/L]	1.405	1.727	0.717	0.929	0.890	1.092	0.997	1.185	1.117	1.320
lower 95%-cl	0.597	0.949	0.423	0.636	0.729	0.941	0.838	1.039	0.995	1.211
upper 95%-cl	1.842	2.154	0.926	1.137	1.018	1.213	1.121	1.300	1.217	1.408
lower 99%-cl	0.477	0.807	0.363	0.567	0.682	0.895	0.792	0.994	0.956	1.175
upper 99%-cl	2.302	2.534	1.081	1.275	1.087	1.275	1.187	1.359	1.266	1.451

cl: confidence limits

Statistical analyses of the available data for average specific growth rate revealed that the following E_rC_{10} and E_rC_{20} values were reliably calculated:

Parameter	24 hours		48 hours		72 hours		96 hours		120 hours	
	E_rC_{10}	E_rC_{20}	E_rC_{10}	E_rC_{20}	E_rC_{10}	E_rC_{20}	E_rC_{10}	E_rC_{20}	E_rC_{10}	E_rC_{20}
Value [mg/L]	1.613	2.045	1.140	1.614	1.375	1.805	1.480	1.870	1.651	2.078
lower 95%-cl	1.216	1.649	1.051	1.545	1.304	1.760	1.460	1.859	1.626	2.066
upper 95%-cl	1.935	2.362	1.217	1.678	1.435	1.847	1.498	1.880	1.674	2.090
lower 99%-cl	1.001	1.419	0.989	1.493	1.253	1.726	1.445	1.850	1.607	2.056
upper 99%-cl	2.349	2.744	1.293	1.736	1.494	1.884	1.514	1.889	1.694	2.100

cl: confidence limits

The data were re-analysed using geometric mean measured concentrations of 0.164, 0.554, 1.673, 4.032 and 6.453 mg/L. The results are presented below.

Parameter	24 hours			48 hours			72 hours		
	E_yC_{10}	E_yC_{20}	E_yC_{50}	E_yC_{10}	E_yC_{20}	E_yC_{50}	E_yC_{10}	E_yC_{20}	E_yC_{50}
Value [mg/L]	0.999	1.301	2.160	0.319	0.471	0.996	0.441	0.604	1.101
lower 95%-cl	0.400	0.675	1.634	0.234	0.374	0.874	0.377	0.535	1.023
upper 95%-cl	1.393	1.701	2.803	0.398	0.560	1.129	0.501	0.667	1.182

Parameter	96 hours			120 hours		
	E_yC_{10}	E_yC_{20}	E_yC_{50}	E_yC_{10}	E_yC_{20}	E_yC_{50}
Value [mg/L]	0.517	0.679	1.142	0.632	0.817	1.333

lower 95%-cl	0.448	0.608	1.068	0.571	0.756	1.280
upper 95%-cl	0.580	0.743	1.216	0.689	0.872	1.384

Parameter	24 hours		
	E_rC_{10}	E_rC_{20}	E_rC_{50}
Value [mg/L]	1.199	1.596	2.759
lower 95%-cl	0.713	1.081	2.266
upper 95%-cl	1.584	1.993	3.274

Conclusion

Statistical analyses of the available data for yield revealed that the following E_yC_{10} and E_yC_{20} values were reliably calculated:

Parameter	24 hours		48 hours		72 hours		96 hours		120 hours	
	E_yC_{10}	E_yC_{20}	E_yC_{10}	E_yC_{20}	E_yC_{10}	E_yC_{20}	E_yC_{10}	E_yC_{20}	E_yC_{10}	E_yC_{20}
Value [mg/L]	1.405	1.727	0.717	0.929	0.890	1.092	0.997	1.185	1.117	1.320
lower 95%-cl	0.597	0.949	0.423	0.636	0.729	0.941	0.838	1.039	0.995	1.211
upper 95%-cl	1.842	2.154	0.926	1.137	1.018	1.213	1.121	1.300	1.217	1.408
lower 99%-cl	0.477	0.807	0.363	0.567	0.682	0.895	0.792	0.994	0.956	1.175
upper 99%-cl	2.302	2.534	1.081	1.275	1.087	1.275	1.187	1.359	1.266	1.451

cl: confidence limits

Statistical analyses of the available data for average specific growth rate revealed that the following E_rC_{10} and E_rC_{20} values were reliably calculated:

Parameter	24 hours		48 hours		72 hours		96 hours		120 hours	
	E_rC_{10}	E_rC_{20}	E_rC_{10}	E_rC_{20}	E_rC_{10}	E_rC_{20}	E_rC_{10}	E_rC_{20}	E_rC_{10}	E_rC_{20}
Value [mg/L]	1.613	2.045	1.140	1.614	1.375	1.805	1.480	1.870	1.651	2.078
lower 95%-cl	1.216	1.649	1.051	1.545	1.304	1.760	1.460	1.859	1.626	2.066
upper 95%-cl	1.935	2.362	1.217	1.678	1.435	1.847	1.498	1.880	1.674	2.090
lower 99%-cl	1.001	1.419	0.989	1.493	1.253	1.726	1.445	1.850	1.607	2.056
upper 99%-cl	2.349	2.744	1.293	1.736	1.494	1.884	1.514	1.889	1.694	2.100

cl: confidence limits

Statistical analyses of the available data when using the geometric mean measured concentrations revealed that the following $E_{yr}C_{10}$, $E_{yr}C_{20}$ and $E_{yr}C_{50}$ values were reliably calculated:

Parameter	24 hours			48 hours			72 hours		
	E_yC_{10}	E_yC_{20}	E_yC_{50}	E_yC_{10}	E_yC_{20}	E_yC_{50}	E_yC_{10}	E_yC_{20}	E_yC_{50}
Value [mg/L]	0.999	1.301	2.160	0.319	0.471	0.996	0.441	0.604	1.101
lower 95%-cl	0.400	0.675	1.634	0.234	0.374	0.874	0.377	0.535	1.023
upper 95%-cl	1.393	1.701	2.803	0.398	0.560	1.129	0.501	0.667	1.182

Parameter	96 hours			120 hours		
	E_yC_{10}	E_yC_{20}	E_yC_{50}	E_yC_{10}	E_yC_{20}	E_yC_{50}

Value [mg/L]	0.517	0.679	1.142	0.632	0.817	1.333
lower 95%-cl	0.448	0.608	1.068	0.571	0.756	1.280
upper 95%-cl	0.580	0.743	1.216	0.689	0.872	1.384

Parameter	**24 hours**		
	E_rC_{10}	**E_rC_{20}**	**E_rC_{50}**
Value [mg/L]	1.199	1.596	2.759
lower 95%-cl	0.713	1.081	2.266
upper 95%-cl	1.584	1.993	3.274

(Taylor, S., Radford F. & Allen M., 2015~~6~~)

Relevant Literature on Algae

No relevant scientifically peer-reviewed open literature could be found on cyprodinil or its major metabolites. Details of the literature search undertaken can be found in M-CA Section 9 of the current submission.

CA 8.2.7 Effects on aquatic macrophytes

The study previously submitted in the EU (Ward *et al*. 2001, CGA219417/0645) was summarised in point B.9.2.8 of the DAR. At the request of ANSES a summary has been included in order to aid dossier evaluation.~~therefore an additional study summary is not repeated here.~~ The endpoints are summarised in Table 8.2-9.

Report:	Summary submitted at the request of ANSES for ease of dossier evaluation: *KIIA 8.2.8. Ward T. J., Magazu J.P. & Boeri R. L. (1995). Growth and Reproduction Toxicity Test with CGA-219417 and the duckweed, Lemna gibba G3. Report Number 792-CG. T. R. Wilbury Laboratories, Inc. Massachusetts 01945. (Syngenta File No. CGA219417/0645).*

Guidelines

FIFRA Subdivision J, 123-2

GLP: Yes

Executive Summary

The toxicity of CGA219417 to the aquatic plant *Lemna gibba* was determined in a 14 day static test. *Lemna gibba* were exposed to nominal concentrations 0.6, 1.3, 2.5, 5.0 and 10 mg/L (0.542, 1.17, 2.21, 4.42 and 9.38 mg/L initial measured concentrations) alongside a culture medium control. Based on initial measured concentrations and measurement of frond number, the 14 day E_yC_{50} of CGA219417 to *Lemna gibba* was 7.42 mg/L.

Materials

Test Material CGA219417

Lot/Batch #:	P012012
Purity:	98.8%
Description:	Off-white granules
Stability of test compound:	Stable for at least 1 year at room temperature
Reanalysis/expiry date:	31 January 1996
Treatments	
Test concentrations:	Culture medium control and nominal concentrations of 0.6, 1.3, 2.5, 5.0 and 10 mg/L. Initial measured concentrations of 0.542, 1.17, 2.21, 4.42 and 9.38 mg/L
Solvent:	Not applicable
Positive control:	None
Analysis of test concentrations:	Yes, HPLC analysis of CGA 219417 on day 0 and 14
Test organism	
Species:	*Lemna gibba*
Source:	In-house culture, originally obtained from Climate Stress Laboratory, USDA, Beltsville, Maryland
Test design	
Test vessels:	500 mL glass Erlenmeyer flasks containing 200 mL of test or control medium
Test medium:	M-Hoagland's media without sucrose or EDTA
Replication:	Three replicates per treatment
Starting density:	4 plants, each with 3 – 4 fronds (12 – 16 fronds per vessel)
Exposure regime:	Static
Aeration:	No
Duration:	14 days
Environmental conditions	
Test temperature:	23.6 to 24.5°C
pH:	test start: 5.0 to 5.1 test end: 5.5 to 5.9
Lighting:	Constant light, 500 footcandles

Study Design and Methods

Experimental dates: 26th October to 9th November 1995

A stock solution with a nominal concentration of 10 mg/L was prepared by dissolving 0.0202 g of the test item completely in sterile media and adjusting the final volume to 2000 mL. The stock solution was mixed for approximately 46 hours to aid dissolution. The test solutions were prepared by diluting appropriate aliquots of the stock solution in M-Hoagland's media.

An aliquot of test solution was dispensed into three replicate test vessel and the test started by distributing four *L. gibba* plants each with 3 or 4 fronds into each replicate vessel. Test vessels were randomly arranged in an incubator and maintained at a constant temperature and under continuous illumination. The total number of fronds in each replicate was determined after 1, 4, 6, 8, 11, 13 and 14 days of

exposure. The number of chlorotic fronds, the presence of flowers and any abnormalities was also noted at these time intervals.

The pH was measured at the start and at the end of the test. The temperature within the incubator was continuously measured using a representative flask of water incubated among the test vessels.

The test concentrations were verified by chemical analysis of CGA219417 at days 0 and 14, using high performance liquid chromatography.

All statistical analysis were performed using the number of *L. gibba* fronds and the initial measured concentrations. The 14 day EC_{50} and EC_{25} were estimated using weighted least squares non-linear regression analysis. Determination of the 14 day NOEC (No Observed Effect Concentration) was performed using a parametric one-way analysis of variance (ANOVA) and Dunnett's t-test was used to compare treatment and control data.

Results and Discussion

At the start of the test, the measured concentrations were in the range 88 to 94 % of the nominal values and at the end of the test were below 70 % of nominal (see table below). Initial measured concentrations of 0.542, 1.17, 2.21, 4.42 and 9.38 mg/L were used for the calculation and reporting of results.

Table 8.2.7-1: Analytical results

Nominal concentrations (mg/L)	Measured Concentration of CGA-219417 (mg/L)	
	Day 0	Day 14
Control	<0.100	<0.100
0.6	0.542	<0.100
1.3	1.17	<0.100
2.5	2.21	0.233
5.0	4.42	1.54
10	9.38	5.65

Water quality throughout the test was within acceptable limits. The incubator temperature ranged from 23.6 to 24.5 ºC during test period and the measured pH ranged from 5.0 at test initiation to 5.9 at Day 14.

The *L. gibba* population in the control vessels grew well, increasing from an average 14 fronds per vessel on Day 0 to 234 fronds per vessel after 14 days. The average frond numbers in each treatment group and the estimated NOEC and EC_x values are presented in the table below.

Table 8.2.7-2: Mean values at each concentration of CGA219417 for growth rate and yield for *Lemna gibba*

Mean measured concentrations (mg/L)	Growth rate		Yield	
	Mean growth rate (day^{-1}) 0 – 72 hrs	Percentage inhibition (%)	Mean number of fronds	Percentage inhibition (%)
Control	0.199	-	219.3	-
0.542	0.199	0.0	204.3	6.84
1.17	0.203	0.0	231.3	0.0
2.21	0.202	0.0	221.0	0.0
4.42	0.198	0.57	207.0	5.62
9.38	0.110	44.96	52.0	76.29
$E_{ry}C_{10}$	n.d		4.884 (2.627 – 6.069)	
$E_{ry}C_{20}$	n.d.		5.639 (3.501 – 6.728)	
$E_{ry}C_{50}$	n.d.		7.424 (5.906 – 8.414)	

Numbers in brackets represent 95% confidence intervals

Validity criteria

Not reported.

Conclusions

Based on initial measured concentrations and measurement of frond number, the 14 day E_yC_{50} of CGA219417 to *Lemna gibba* was 7.42 mg/L.

(Ward *et al.* 1995)

In accordance with **Commission Regulation (EU) No 283/2013**, the EC_{10} and EC_{20} values have been estimated and these are provided below.

Report:	Submitted for purposes of renewal due to change in data requirements guidance: K-CA 8.2.7/01Taylor, S., Pickering, F. (2015~~e6b~~) Cyprodinil (CGA219417) – Growth and reproduction toxicity test with CGA219417 and the duckweed, *Lemna gibba* G3. Statistical re-analysis. Final Report. Report Number: CEA.1418. Cambridge Environmental Assessments, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK. Amended (Syngenta File No: CGA219417_11589)

Summary

Report number 792-CG (Ward *et al*., 2001) did not provide EC_{10} and EC_{20} estimates relating to yield or average specific growth rate for *Lemna gibba*. Consequently the data generated in this study have been re-analysed in order to provide these values.

Reliable calculations of the 14 day E_yC_x values were generated. E_rC_x values could not be reliably determined due to a lack of dose response.

Statistical Analysis

The yield represents the frond number/biomass generated during the test or evaluation period minus the frond number/biomass at the start of the exposure or evaluation period. The average specific growth rate represents the slope of the growth curve.

All computations were carried out in ToxRat Professional version 2.10 (ToxRat Solutions GmbH, 2001-2010). Probit analysis with linear maximum likelihood regression was used to determine the concentration response function. Chi² was used as a goodness of fit measure.

Results

Average Specific Growth Rate

There were clear treatment related effects observed and the relationship between dose and response was not significant at 14 days (p(F) = 0.055). No E_rC_x values or confidence limits were reliably determined.

Yield

There were clear treatment related effects observed and the relationship between dose and response was significant at 14 days (p(F) = 0.008). E_yC_x values and the 95% and 99% confidence limits were reliably determined.

EC_{10} and EC_{20} Estimates

Parameter	14 days
EyC_{20} (mg/L) **95% CL**	5.639 (3.501-6.728)
EyC_{10} (mg/L) **95% CL**	4.884 (2.627-6.069)

CL: Confidence Limits

Conclusion

Reliable calculations of the 14 day E_yC_x values were generated. E_rC_x values could not be reliably determined due to a lack of dose response.

(Taylor, S., Pickering F. 2015~~c~~16b)

Relevant Literature on Aquatic Macrophytes

No relevant scientifically peer-reviewed open literature could be found on cyprodinil or its major metabolites. Details of the literature search undertaken can be found in M-CA Section 9 of the current submission.

CA 8.2.8 Further testing on aquatic organisms

A mesocosm study (Kennedy & Reed, 1995) previously submitted in the EU was summarised in point B.9.2.9 of the DAR therefore additional an additional summary is not included here.

For purposes of active substance renewal it is a requirement for micro-mesocosm studies to be re-evaluated and minimum detectable differences of effects observed between test concentrations should be

estimated. However, the study conducted by Kennedy & Reed (1995) was deficient in several areas. This study was reviewed by the EFSA PPR Panel and the opinion reported in ***EFSA Journal (2006) 329, 1-77***. Following the conclusions of this panel the mesocosm described below was conducted.

A study conducted since the previous review of cyprodinil is included below along with an independent re-evaluation of it. The endpoints are summarised in Table 8.2-10.

Report:	K-CA 8.2.8/01 Ashwell J., Benyon K., Powley W., Richardson M. (2007) Cyprodinil (CGA219417) 300 g/L EC formulation A14325E: Effects on aquatic organisms in an outdoor microcosm. Report Number T008777-05-REG. Syngenta, Jealott's Hill International Research Centre, UK. Experimental dates March 2006 – March 2007. (Syngenta File No. CGA219417/1683)

Guidelines

Campbell P.J., Arnold D.J.S., Brock T.C.M., Grandy N.J., Heger W., Heimbach F., Maund S.J., Streloke M. (1999) PP178: Guidance document on higher-tier aquatic risk assessment for pesticides (HARAP). Lacanau Océan, France: Setac-Europe Publication.

Giddings J.E., Brock T.C.M., Heger W., Heimbach F., Maund S.J., Norman S.M., Ratte H.T., Schäfers C., Streloke M. (2002) PP60: CLASSIC - Community - level aquatic system studies - interpretation criteria. Pensacola, Fl., USA: Society of Environmental Toxicology and Chemistry (Setac).

de Jong F.M.W., Brock T.C.M., Foekema E.M., Leeuwangh P. (2007): Guidance for summarizing and evaluating aquatic micro- and mesocosm studies - Draft version February 2007. A guidance document of the Dutch Platform for the Assessment of Higher Tier Studies, Rivm Report 601506009.

GLP: Yes

Executive Summary

This microcosm study was conducted to investigate the effects of cyprodinil (formulated as A14325E) on freshwater invertebrates and algae under field conditions, in accordance with the guidance documents on Higher Tier Aquatic Risk Assessment for Pesticides (HARAP, 1999), Community-Level Aquatic System Studies-Interpretation Criteria (CLASSIC, 2002) and the draft document "Guidance for summarizing and evaluating aquatic micro- and mesocosm studies", (de Jong *et al.,* 2007): A guidance document of the Dutch Platform for the Assessment of Higher Tier Studies, Rivm Report 601506009).

Materials

Test Material:	CGA219417 EC (300) A14325E
Lot/Batch #:	SMU5BP002
Purity:	303 g/L, corresponding to 29.9% w/w
Stability of test compound:	May 2007
Treatments:	
Test concentrations:	1.5, 5, 10, 20 and 50 µg a.s./L and a deionised water control
Vehicle and/or positive control:	Deionised water / None
Analysis of test concentrations:	Yes

Test design

Test systems:	The test systems were rectangular varied depth microcosms, each containing approximately 1,300 litres of water over 10 cm of sediment, with established communities of plants and invertebrates.
Exposure regime:	The replicated study design consisted of five treatments of cyprodinil (1.5, 5, 10, 20 and 50 µg a.s./L) and an untreated control. The treatment levels were chosen to incorporate known toxicity levels from laboratory studies and the maximum predicted environmental concentrations from proposed use patterns. Three, weekly, applications of each treatment were made during June 2006. Application was by direct addition to the microcosms followed by thorough mixing to rapidly distribute the formulation through the water column (taking the toxicological approach favoured by HARAP and CLASSIC).
Replication:	The treatments were unequally replicated: Control (4 replicates), 1.5 µg a.s./L (3 replicates), 5 µg a.s./L (4 replicates), 10 µg a.s./L (4 replicates), 20 µg a.s./L (3 replicates), 50 µg a.s./L (2 replicates). The disproportionate allocation of replicates to treatments was to enable increased precision for statistical analysis compared to the control for the critical treatment levels (i.e. around the expected no effect levels).

Study Design and Methods

Experimental dates: 3rd March 2006 to 1st March 2007

Sampling procedures

Physico-chemical properties of the water, phytoplankton, zooplankton and macroinvertebrate communities were studied for approximately one month prior to application and for approximately four months after application. The following sampling methods were employed:

Water column sampling: For zooplankton, phytoplankton, chlorophyll a, alkalinity and hardness measurements

"Hydrolab" water quality multi-probe: For in situ measurements of water physico-chemical properties

ESAS (Enhanced Surface Area Substrate) samplers: Artificial colonising substrates to sample benthic and epiphytic dwellers

Sweep-netting (NETS): To sample swimming and epiphytic organisms

Emergence traps (TRAPS): To sample emergent adult insects

Litterbags (LLB): To sample organisms processing leaf litter and to determine differences in the rate of decomposition of leaf material in the microcosms

Water residue analysis: Microcosm water was sampled for measurement of cyprodinil residues at the following intervals:

-1, +1, +6 and +24 hours and 3 days after the first application.

-1 hour (7 days after first application), +1, +6 and +24 hours and 3 days after the second application.

-1 hour (7 days after second application), +1, +6 and +24 hours, 3, 7, 14 and 21 days after the third application.

From 28 days after the third application, microcosms were not sampled once residues had declined to below the LOQ. In microcosms where residues remained above the LOQ, further samples were taken 28, 35, 42, 49, 56 and 63 days after the third application.

All four control microcosms were only sampled prior to the first application (-1 hour) and then at +1 hour after each of the three applications. On all other sampling occasions just one control microcosm (M02) was sampled.

Preparation of test solutions

Treatment concentrations, nominally 1.5, 5, 10, 20, and 50 µg a.s./L were selected to cover known toxicity levels determined from laboratory studies, maximum modelled, worst-case, predicted environmental concentrations (PEC), and the 50 µg a.s./L (highest) rate was included as it was considered likely to give clear effects on a variety of taxa.

A stock solution of cyprodinil was prepared by taking 2 g (within ± 1%) of formulation A14325E (300 g a.s./L) and making it up to 2000 mL with deionised water. Known volumes of this solution were then diluted with deionised water to give concentrations of cyprodinil appropriate for treating the microcosms at each of the treatment rates. For each application, the test solutions were prepared the day before application. Prior to addition to the microcosms, sub-samples of the application solutions were taken to verify concentrations.

Analytical method

Application solutions and microcosm water samples for residue analysis were analysed for cyprodinil by liquid chromatography with mass spectroscopic detection (LC/MS/MS).

Statistical analyses

Multivariate (Principal Response Curves method) was employed to identify and treatment-related changes in community structure. Analysis of variance was also used to look at population level differences for selected taxa; those not analysed were considered to be too variable in occurrence or not abundant enough to permit meaningful conclusions to be drawn.

Results and Discussion

Analytical data

Residues of cyprodinil in the microcosm water were measured following application of the test item. One hour after the first application, cyprodinil residues were measured at between 75 and 80% of the nominal applied. Residues declined to below 50% of nominal 3 days after application and at 7 days (1 hour prior to the second application) were between 22 and 27% of nominal. Following the second application, residues of between 119 and 154% of nominal were observed. These had declined to between 38 and 80% of nominal by 7 days after the second application (1 hour prior to the third application). Residues between 118 and 156% of the nominal rates were measured 1 hour after the third application. The water column DT_{50} for cyprodinil was approximately 2-3 days.

Table 8.2.8-1: Cyprodinil concentrations measured in microcosm water in a microcosm study with three applications of cyprodinil (CGA219417) 300 g/L EC formulation A14325E

Application number	Timepoint	Nominal treatment concentration (µg a.s./L)	Mean % of nominal
Application 1	- 1 hour	0 (Control)	ND
		1.5	ND

Application number	Timepoint	Nominal treatment concentration (µg a.s./L)	Mean % of nominal
		5	ND
		10	ND
		20	ND
		50	ND
	+ 1 hour	0 (Control)	ND
		1.5	76
		5	75
		10	79
		20	79
		50	80
	+6 hours	0 (Control)	ND
		1.5	58
		5	66
		10	67
		20	71
		50	71
	+24 hours	0 (Control)	ND
		1.5	54
		5	57
		10	57
		20	65
		50	64
	+3 days	0 (Control)	ND
		1.5	<LOQ
		5	35
		10	39
		20	43
		50	39
Application 2	-1 hour	0 (Control)	ND
		1.5	<LOQ
		5	22
		10	22
		20	24
		50	27
	+1 hour	0 (Control)	ND
		1.5	119
		5	139
		10	146
		20	154
		50	135
Application 2 (continued)	+6 hours	0 (Control)	ND
		1.5	94
		5	97

Application number	Timepoint	Nominal treatment concentration (µg a.s./L)	Mean % of nominal
		10	97
		20	107
		50	111
	+24 hours	0 (Control)	ND
		1.5	77
		5	88
		10	92
		20	63
		50	43
	+3 days	0 (Control)	ND
		1.5	55
		5	56
		10	62
		20	67
		50	72
Application 3	-1 hour	0 (Control)	ND
		1.5	80
		5	63
		10	38
		20	66
		50	47
	+1 hour	0 (Control)	ND
		1.5	118
		5	156
		10	142
		20	142
		50	120
	+6 hours	0 (Control)	ND
		1.5	112
		5	113
		10	121
		20	127
		50	115
	+24 hours	0 (Control)	ND
		1.5	83
		5	89
		10	93
		20	100
		50	104
	+3 days	0 (Control)	ND
		1.5	69
		5	77
Application 3 (continued)	+3 days (continued)	10	79

Application number	Timepoint	Nominal treatment concentration (µg a.s./L)	Mean % of nominal
		20	90
		50	86
	+7 days	0 (Control)	ND
		1.5	<LOQ
		5	48
		10	49
		20	57
		50	62
	+14 days	0 (Control)	ND
		1.5	<LOQ
		5	22
		10	25
		20	27
		50	34
	+21 days	0 (Control)	ND
		1.5	<LOQ
		5	15
		10	12
		20	15
		50	19
	+28 days	0 (Control)	ND
		5	<LOQ
		10	<LOQ
		20	8
		50	10
	+35 days	0 (Control)	ND
		20	4
		50	7
	+42 days	0 (Control)	ND
		20	<LOQ
		50	4
	+49 days	0 (Control)	ND
		50	3
	+56 days	0 (Control)	ND
		50	2
	+63 days	0 (Control)	ND
		50	<LOQ

ND = None detected (limit of detection: 0.3 µg a.s./L)
<LOQ = Less than limit of quantification (0.75 µg a.s./L)

The application solutions, prepared the day before each application were also analysed for cyprodinil. These were measured at between 59-75%, 73-83%, and 64-82% of nominal for each of the 3 applications, respectively. Owing to the high concentrations of the application solutions, considerable dilution of these was required prior to analysis, while the water residue samples taken from the microcosms were analysed by direct injection with no dilution step. These are therefore considered a reliable measure of the

concentrations achieved in the microcosms and appropriate dosing is considered to have been achieved. Effect concentrations are based on the nominal treatment rates.

Biological data

Three applications of cyprodinil at 1.5, 5 and 10 µg a.s./L (maximum measured concentrations of 1.8, 7.8 and 14.6 µg a.s./L, respectively) had no clear treatment-related effects on physico-chemical parameters, leaf litter decomposition rates, phytoplankton communities or zooplankton communities and populations. Transient effects on phytoplankton populations were not believed to be treatment effects. Similarly, for the macroinvertebrates, effects at 5 µg a.s./L on the isopod crustacean *Asellus aquaticus* were not supported by a dose response and not believed to be treatment-related.

Three applications of cyprodinil at 20 µg a.s./L (maximum measured concentration 30.8 µg a.s./L) had no clear treatment-related effects on physico-chemical parameters or on leaf litter decomposition rates. Only transient effects occurred on phytoplankton and zooplankton communities and populations. Long term effects were observed on macroinvertebrate communities, driven by reductions in *Asellus aquaticus*.

Three applications of cyprodinil at 50 µg a.s./L (maximum measured concentration 67.5 µg a.s./L) had no clear treatment-related effects on physico-chemical parameters and while a marked reduction in leaf litter decomposition was observed, this was not statistically significant. There were only slight effects on phytoplankton communities and populations. Long term and more marked effects occurred on zooplankton and macroinvertebrate communities and populations, principally on Copepoda (zooplankton) and *Asellus aquaticus* (macroinvertebrates).

The lack of recovery demonstrated in different sample types by *Asellus* at 20 and 50 µg a.s./L is likely a result of the fact that the entire population was exposed to the test item in the microcosms, due to the conservative toxicological exposure scenario. The dosing and mixing of the test item throughout the water column resulted in a lack of refugia and since *Asellus* has a completely aquatic life cycle, short term recovery by immigration was extremely unlikely.

The responses observed in this study are also summarised below according to the effect classes suggested in the draft document “Guidance for summarizing and evaluating aquatic micro- and mesocosm studies”, (de Jong et al (2007): A guidance document of the Dutch Platform for the Assessment of Higher Tier Studies, Rivm Report 601506009).

Table 8.2.8-2: Overall summary of effect classes observed for several categories of endpoints in the outdoor mesocosm study receiving three applications of cyprodinil (CGA219417) 300 g/L EC formulation A14325E

	Treatment levels (µg a.s./L nominal)				
	1.5	**5**	**10**	**20**	**50**
Population responses					
Macroinvertebrate;					
Asellus aquaticus (ESAS)	1	2	2	5B	5B
Crangonyx pseudogracilis (ESAS)	1	1	1	1	3B
A*sellus aquaticus* (NETS)	1	3A[a]	2	5B	5B
Total Planorbidae (NETS)	1	1	1	1	3A
Asellus aquaticus (LLB)	1	1	1	5B	5B
Crangonyx pseudogracilis (LLB)	1	1	1	1	3A
Total Leaf Litter bags (LLB)	1	1	1	3A	3A
Zooplankton;					
Daphnia sp.	1	1	1	2	2
Nauplia sp.	1	1	1	1	5A
Cylopidae (Copepodit)	1	1	1	1	3A[b]
Total Zooplankton	1	1	1	1	5A
Phytoplankton;					
Carteria sp. (rough wall)	2	2	2	2	2
Scenedesmus aculeolatus	1	1	1	2	2
Rhodomonus sp.	1	1	1	2	2
Community responses					
Macroinvertebrates;					
ESAS (PRC)	1	2	1	5A	5B
Sweep Nets (PRC)	1	3A[c]	2	5B	5B
Leaf Litterbag (PRC)	1	1	2	5B	5B
Zooplankton (PRC)	1	1	1	1	5A
Phytoplankton (PRC)	1	1	1	2	2
Leaf Litterbag (PRC)	1	1	2	5B	5B
Physico-chemical measurements	1	1	1	1	1

Effect classes presented are based upon reductions in response for the most sensitive endpoints.[a,c] Statistically significant reductions seen later in the study are not supported by a dose response and are not believed to be treatment effects[b] Effect class based upon a trend; no statistically significant effects observed, but a clear reduction seen at this concentration.

Conclusion

The following NOECs/NOEAECs were derived following the guidance contained in the draft document "Guidance for summarizing and evaluating aquatic micro- and mesocosm studies", (de Jong *et al.,* (2007): A guidance document of the Dutch Platform for the Assessment of Higher Tier Studies, Rivm Report 601506009).

Table 8.2.8-3: Derived NOECs and NOEAECs from the outdoor mesocosm study receiving three applications of cyprodinil (CGA219417) 300 g/L EC formulation A14325E

Group	NOEC (µg a.s./L)	NOEAEC (µg a.s./L)
Macroinvertebrate community	10	10
Macroinvertebrate populations	10	10
Zooplankton community	20	20
Zooplankton populations	20	20
Phytoplankton community	20	50
Phytoplankton populations	20	50
Physico-chemical measurements	50	50

Considering the conservative nature of the study design (i.e. treatment of the entire water body with limited potential for internal recovery) and the type of effects observed, it was concluded that three nominal applications of 10 µg a.s./L would not result in unacceptable ecological effects.

(Ashwell, *et al*., 2007)

An independent review of these mesocosm studies has been conducted as briefly described below.

Report:	Submitted for purpose of renewal as conducted for compliance with new data requirements: K-CA 8.2.8/02 Taylor S & Dark R (2015). Cyprodinil (A14325E) – Statistical (MDD) Re-analysis of Existing Data from a Microcosm Study with Cyprodinil. Cambridge Environmental Assessments, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK (Syngenta File No. A14325E_10079)

For zooplankton, MDD values were calculated for Cyclopidae (Copepodit), Cyclopidae sp., *Daphnia* sp., *Keratella quadrata*, *Nauplia* sp. and total zooplankton.

Of these data, three taxa and the total zooplankton were considered suitable for ETO-RAC derivation, namely *Daphnia* sp. *Keratella quadrata*, *Nauplia* sp. and total zooplankton. Overall these data encompassed one copepod, one cyplopoid, one rotifer and total zooplankton, thus covering the three main zooplankton groups.

MDD analysis of the available data for zooplankton demonstrated that typically small to large effects could be determined throughout the study for five parameters. As these evaluations included sensitive taxa (*Daphnia* sp.) and organisms from the three main zooplankton groups (cladocera, copepoda and rotifera), the data generated are considered robust and reliable for ETO-RAC derivation and a NOEC (class 1) of 14.6 µg a.s./L (based on measured concentrations) is recommended for zooplankton. If an NOEAEC (class 3A) is required for ERO-RAC it can be considered to be 67.5 µg a.s./L.

MDD analysis of the available data for macroinvertebrates demonstrated that typically small to large effects could be determined throughout the study for nine parameters (15 endpoints) and for four parameters (eight endpoints), following at least one application of the test item. As these evaluations included sensitive taxa (*Asellus aquaticus* and *Crangonyx pseudogracilis*) and organisms from one isopod, one amphipod, two insecta, one gastropod, total bugs, total pond snails, total ramshorn snails, total flatworms, and total organisms from four different sampling techniques, the data generated are considered robust and reliable for ETO-RAC derivation. The overall NOEC (class 2) of 14.6 µg a.s./L is recommended for macroinvertebrates and if a NOEAEC (class 5B) is required for ERO-RAC derivation it is also considered to be 14.6 µg a.s./L.

The overall NOEC (class 2) from this study has been robustly determined as 14.6 µg a.s./L (based on measured concentrations).

CA 8.3 Effects on Arthropods

CA 8.3.1 Effects on bees

Table 8.3.1-1: Summary of Endpoints for Bees

Organism	Test item	Test type	Endpoint		Reference (author, date, Syngenta File No.)
Honey bee	Cyprodinil	Acute contact	EU	LD_{50} >784 µg a.s./bee	***Boeri et al. (1995d) CGA219417/0532***
				LD_{50} >100 µg a.s./bee	***Thompson (1990) CGA219417/0001***
	Cyprodinil [a]	Acute Oral		Oral 72h LD_{50} >150 µg/bee (>112.5 µg a.s./bee)	***Schmitzer (1995) CGA219417/0771***
		Acute Contact		Contact 72h LD_{50} >100 µg/bee (75 µg a.s./bee)	
	A8637C	Acute Oral		Oral 72h LD50 >250 µg/bee (125 µg a.s./bee)	***Candolfi (1995) CGA219417/0375***
		Acute Contact		Contact 72h LD50 >250 µg/bee (125 µg a.s./bee)	
		Adult chronic	New Study	10 day LD_{50} = 112.2 µg consumed a.s./bee/day 10 day NOED = 47.3 µg consumed a.s./bee/day 10 day NOEC = 1.284 g a.s./kg food	***Ruhland (2014) A8637C_10321***
		Chronic larval	New Study	7 day NOED = 13.3 µg a.s./larva 8 day NOED = 33.3 µg a.s./larva 7 day NOEC = 0.084 g a.s./kg diet 8 day NOEC = 0.211 g a.s./kg diet	***Kleebaum (2014) A8637C_10330***
	A14325E	Adult chronic	New Study	10 day LD_{50} = 69.7 µg consumed a.s./bee/day 10 day NOED = 44.2 µg consumed a.s./bee/day 10 day NOEC = 1.284 g a.s./kg food	***Ruhland (2014) A14325E_10065***
		Chronic larval	New Study	8 day NOED = 17.3 µg a.s./larva 8 day LD_{10} = 24.2 µg a.s./larva 8 day NOEC = 0.110 g a.s./kg diet	***Kleebaum (2014) A14325E_10067***

CA 8.3.1.1 Acute toxicity to bees

CA 8.3.1.1.1 Acute oral toxicity

The studies previously submitted in the EU were summarised in point B.9.4.1 of the DAR . At the request of ANSES summaries have been included in order to aid dossier evaluation ~~therefore an additional study summary is not repeated here.~~ The endpoints are summarised in Table 8.3.1-1.

For acute toxicity studies the LD_{10} and LD_{20} values have not been calculated since they are not required by the recommended test guidelines set out in Commission Communication 2013/C 95/01 (EPPO standard PP 1/170 (4): Side-effects on honeybees OECD Test Guideline 213: Honeybees, Acute Oral

Toxicity Test), or by the risk assessments according to the current Terrestrial Guidance Document (2009) [11].

Report:	Summary submitted at the request of ANSES for ease of dossier evaluation: *KIIA 8.3.1.1. Thompson H. M. (1990). Acute contact and oral toxicity of CGA219417 to honeybees. Report Number C90/0276. Environmental Research Group, ADAS Central Science Laboratory, Surrey, UK. (Syngenta file No. CGA219417/0001)*

Guidelines

UK Control of Pesticides Regulations

GLP: Yes

Executive Summary

The oral and contact toxicity of CGA219417 to the honey bees was was evaluated in 48-hour toxicity tests. Worker bees in groups of 10 were either topically dosed with CGA219417 at doses between 25 and 101 µg/bee, or fed solutions of CGA219417 in acetone and sucrose ranging from 1.25 to 5.03 mg/mL (25.2 – 110.5 µg/bee equivalent dose).

The results indicated that the 48-hour LD_{50} for contact and oral toxicity of CGA219417 to honey bees was >101 µg/bee and >316 µg/bee, respectively.

Materials

Test Material	CGA219417
Lot/Batch #:	P912004
Purity:	99.5%
Description:	Colourless solid
Reanalysis/expiry date:	Not reported
Treatments	
Test doses:	Oral test: 25.3, 50.3 and 100.5 µg/bee (nominal) Contact test: 25, 50 and 100 µg/bee Both tests included a control group
Solvent:	Acetone
Positive control:	None
Analysis of test concentrations:	None

[11] SANCO/10329/2002 rev 2 final 17 October 2002

Test organism

Species:	*Apis mellifera*, worker bees
Source:	Maintained at test facility
Feeding:	Not reported

Test design

Test vessels:	Not reported
Replication:	Three replicates per treatment, each containing 10 bees
Duration:	48 hours

Environmental conditions

Test temperature:	Not reported
Humidity:	Not reported
Lighting:	Not reported

Study Design and Methods

Experimental dates: 14th to 22nd August 1990

Test solutions for the contact and oral toxicity test were prepared by dissolving quantities of CGA219417 in acetone. The appropriate test solutions were then either topically applied to test organisms or feed to the test organisms within a sucrose and acetone mixture. The average consumption during the oral test was measured after 24 hours by weighing the remaining liquid. No details of the environmental conditions of the test chambers was included in the study report.

No statistical analysis was performed on the results as none of the test dosages resulted in greater than 50 % mortality.

Results and Discussion

During the contact study, no mortality occurred in the control group. A total of 10 bees (33%) exposed to the highest dose of 100.5 µg/bee had died by 48 hours.

Table 8.3.1.1.1-1: Observed mortality during the contact toxicity study

Dosage (µg/bee)	Number Dead		
	4 hours After Application	24 hours After Application	48 hours After Application
Control	0	0	0
25.2	0	0	0
50.3	3	3	3
100.5	5	7	10

A total of 15 of the 30 control bees died during the oral toxicity test. At the highest test dosage 17 % mortality was observed. Based on actual test material consumption the highest dosage that resulted in no mortality was 316.3 µg/bee.

Table 8.3.1.1.1-2: Observed mortality during the oral toxicity study

Nominal Dosage (µg/bee)	Replicate	Average Consumption (µg/bee)	Number Dead		
			4 hours After Application	24 hours After Application	48 hours After Application
Control	1	-	0	0	0
	2	-	0	8	8
	3	-	0	7	7
25.2	1	50.4	1	1	2
	2	61.1	0	0	0
	3	36.6	0	7	7
50.3	1	115.3	0	0	0
	2	87.3	0	1	1
	3	133.2	1	2	2
100.5	1	157.9	0	1	3
	2	242.2	0	2	2
	3	316.3	0	0	0

Validity Criteria

Not reported.

Conclusions

The results indicated that the 48-hour LD_{50} for contact and oral toxicity of CGA219417 to honey bees was >101 µg/bee and >316 µg/bee, respectively.

(Thompson, 1990)

Report:	Summary submitted at the request of ANSES for ease of dossier evaluation: *KIIIA 10.4.1. Schmitzer S. (1995). Laboraotry testing for toxicity (acute contact and oral LD_{50}) of CGD 20280 F to honey bees (Apis mellifera L.) (Hymenoptera, Apidae). Report Number 541036. IBACON, 64380 Rossdorf, Germany. (Syngenta file No. CGA219417/0771)*

Guidelines

EPPO (1992). Guideline on Test Methods for Evaluation the Side-Effects of Plant Protection Products on Honeybees (No. 170). Bulletin OEPP/EPPO Bulletin, 22, 203-215

EPA (1985). Hazard Evaluation Division Standard Evaluation Procedure, Honey Bee—Acute Contact LD50 Test

MAFF (1986). Working Document 7/3: Data Requirements for Approval under the Control of Pesticides Regulations: Laboratory Testing for Toxicity to Honey bees

GLP: Yes

Executive Summary

The oral and contact toxicity of CGD20280F, a formulation contained 75% CGA219417, to the honey bee (*Apis mellifera* L.) was evaluated in 48-hour toxicity tests. For the contact toxicity test the organisms were exposed to the following dose rates of the test material, 100, 50, 25, 12.5 and 6.25 µg/bee. During the oral toxicity test the test organisms were exposed to 144.3, 65.1, 36.9, 18.7 and 9.1 µg/bee of the test material. Each study also included a solvent control, untreated control and positive control performed with dimethoate.

The results of this study did not reveal any acute toxic effects of CGD20280F to honey bees in the contact and oral toxicity test. No critical mortality was observed in both toxicity tests up to the highest dosage tested. The contact LD_{50} of CGD20280F to honey bees was in excess of 100 µg/bee and the oral LD_{50} was concluded to be in excess of 150 µg/bee.

Materials

Test Material	CGD20280F
Lot/Batch #:	P301087
Formulation code:	A8779A
Active ingredient content:	75% CGD219417
Description:	Grey brown, solid
Reanalysis/expiry date:	August 1995
Treatments	
Test concentrations:	Oral test: 150, 67.9, 38.2, 19.8 and 9.5 µg/bee Contact test: 100, 50, 25, 12.5, 6.25 µg/bee Both tests included a solvent control, untreated control and positive control
Solvent:	Acetone
Positive control:	Perfekthion (37 % dimethoate) at 0.2 µg a.s./bee
Analysis of test concentrations:	No
Test organism	
Species:	*Apis mellifera* L., four to six weeks old worker bees
Source:	Maintained at test facility
Feeding:	Commercial syrup for honey bees (Apiinvert) provided *ad libitum* during the tests
Test design	
Test vessels:	Stainless steel chambers (width 10.5 cm, height 8.5 cm, depth 5.5 cm). Front was closed with removable glass sheets; bottom was perforated with ventilation holes and the top contained two openings for food tube and entry for bees
Replication:	Three replicates per treatment, each containing 10 bees
Duration:	48 hours

Environmental conditions

Test temperature:	26 to 30°C
Humidity:	40 to 82%
Lighting:	Tests performed in dark

Study Design and Methods

Experimental dates: 2nd to 5th May 1995

For the contact toxicity test, five application solutions of the test substance in acetone were prepared at adequate concentrations to yield target nominal test dosages of 100, 50, 25, 12.5 and 6.25 µg/bee, when applied to the bees. The test organisms were anaesthetised with CO_2 and one 5.0 µL drop of the appropriate application solution was placed on the ventral thorax of each test organisms using a Burkhard Applicator. For the solvent control, 5 µL/bee of acetone was applied to the test organisms. For the untreated control the bees were anaesthetised but not treated with any solution. For the positive control 0.2 µg/bee dimethoate in 5 µL acetone was applied to the test organisms.

For the oral toxicity test, five dilutions of the test substance in acetone and ready to use syrup (1 part solvent to 19 parts syrup) were prepared at adequate concentrations to yield the target test dosages when *ca.* 30 mg of dilution was administered per bee. The test organisms were starved for 1 to 2 hours after which approximately 330 mg of the prepared dilutions in tips of 1 mL syringes were hung inside each test cage. The bees were provided with untreated food after complete uptake of the test solutions had occurred or after three hours. The exact amount of test material consumed was determined by reweighing the syringes. The control bees were feed syrup without the test material, while the solvent control bees were feed syrup containing acetone. For the positive control 0.2 µg/bee dimethoate was administered in acetone/syrup.

During both tests the bees were continuously observed for the first 30 minutes after application of the test substance. Subsequently the bees were observed 45 minutes, 60 minutes, 2 hours, 4 hours, 24 hours and 48 hours after application. Any mortality or abnormal behaviour was recorded at each timepoint.

No statistical analysis was performed on the results of these tests as no toxic effects were observed at the highest test dosage.

Results and Discussion

During the contact study, six of the 150 honey bees (4%) exposed to the test item died during the 48 hour experimental period. Intensive cleaning behaviour and apathy was observed in the treated bees up to 4 hours after application. After 4 hours no further behavioural abnormalities were observed. In the solvent control two bees (6.7%) died and no mortality was observed in the untreated control. In the positive control mortality of 86.7% was observed.

Table 8.3.1.1.1-3: Observed mortality during the contact toxicity study

Dosage (µg/bee)	% Mortality				
	30 minutes after application	1 hour after application	4 hours after application	24 hours after application	48 hours after application
Control	0	0	0	0	0
Solvent Control	0	3.3	6.7	6.7	6.7
6.25	0	6.7	10.0	10.0	10.0
12.5	3.3	3.3	3.3	3.3	3.3
25	0	3.3	3.3	3.3	3.3
50	0	0	0	0	0.0
100	0	0	3.3	3.3	3.3
Positive control	0	0	43.3	86.7	86.7

The actual dosages consumed by the bees during the oral study was determined to be 9.5, 19.8, 38.2, 67.9 and 150 µg/bee. A total of 28 of the 150 (18.7 5) bees exposed to the test item died during during the oral toxicity test. Apathy and and coordination problems were observed in the three highest treatment groups in the first hour after application. No mortality occurred in the untreated control group and a total of 2 (6.7%) bees died in the solvent control group. Complete mortality occurred in the positive control treatment.

Table 8.3.1.1.1-4: Observed mortality during the oral toxicity study

Actual Dosage (µg/bee)	% Mortality				
	30 minutes after application	1 hour after application	4 hours after application	24 hours after application	48 hours after application
Control	0	0	3.3	3.3	3.3
Solvent Control	0	6.7	6.7	6.7	6.7
9.1	0	0	0	0	0
18.7	0	6.7	13.3	13.3	16.7
36.9	3.3	3.3	10.0	20.0	20.0
65.1	0	0	30.0	30.0	30.0
144.3	0	10.0	23.3	26.7	26.7
Positive control	0	3.3	70.0	100.0	100.0

Validity Criteria

The study was considered valid as the following criterion was met:

- Mortality of ≤10 % occurred in the control groups

Conclusions

The 24 and 48-hour contact LD_{50} of CGD20280F to the honey bee (*Apis mellifera* L.) was concluded to be >100 µg/bee (equivalent to 75 µg a.s./bee). The 24 and 48-hour oral LD_{50} of CGD20280F to the honey bee (*Apis mellifera* L.) was concluded to be >150 µg/bee (equivalent to 112.5 µg a.s./bee).

(Schmitzer S. 1995)

CA 8.3.1.1.2 Acute contact toxicity

See CA 8.3.1.1.1 above.

Report:	Summary submitted at the request of ANSES for ease of dossier evaluation: *KIIA 8.3.1.1. Boeri R. L., Magazu J. P. & Ward T. J. (1995). Acute Toxicity of CGA-219417 to the Honey Bee, Apis mellifera. Report Number 664-CG. T. R. Wilbury Laboratories, Inc., Massachusetts 01945, USA. (Syngenta file No. CGA219417/0532).*

Guidelines

U.S. EPA-FIFRA, Guideline 141-1

GLP: Yes

Executive Summary

The contact toxicity of CGA219417 to the the honey bee (*Apis mellifera*) was evaluated in 48-hour toxicity test. The organisms were exposed to nominal dose rates of 50, 100, 200, 400 and 800 µg/bee alongside a untreated control and a solvent control. The concentration of test material within the application solutions was measured and resulted in actual measured dose rates of 54, 89, 196, 396 and 784 µg/bee.

Following the 48-hour exposure, 96% of the control and 92% of the solvent control bees were alive. Contact exposure of the honey bees to CGA219417 resulted in a 48-hour LD_{50} of >784 µg.

Materials

Test Material	CGA219417
Lot/Batch #:	P012012
Purity:	98.8%
Stability of the test compound:	Not verified
Description:	Off-white granules
Reanalysis/expiry date:	31 January 1996
Treatments	
Test concentrations:	Solvent control, untreated control and nominal dose rates 50, 100, 200, 400 and 800 µg/bee (actual dose rates: 54, 89, 196, 396 and 784 µg/bee)
Solvent:	Acetone
Positive control:	None
Analysis of test concentrations:	Yes, HPLC analysis of dosing solutions

Test organism

Species:	*Apis mellifera*
Source:	Apiarist, Mark Jordan, Essex, Massachusetts, USA
Acclimation:	Hive received one day prior to start of test. There were no mortalities during the 15 hours prior to the test
Feeding:	Continuously supplied with 50% sugar / 50% water solution

Test design

Test vessels:	Clear plastic 1 pint containers, approximately 9 cm diameter and 9 cm tall
Replication:	Two replicates per treatment, each containing 25 bees
Duration:	48 hours

Environmental conditions

Test temperature:	21.8 to 22.7°C
Humidity:	Not reported
Lighting:	Test performed in dark

Study Design and Methods

Experimental dates: 17th to 19th May 1995

Stock solutions of the test material were prepared at concentrations of 25, 50, 100, 200 and 400 mg/mL by dissolving appropriate amounts of the test material in 10 mL acetone. The actual concentrations of the stock solutions were determined using HPLC analysis with UV detection. The test organisms were anaesthetised with CO_2 and 2 µL of the appropriate application solution was placed on the anterior abdomen of each bee. For the solvent control, 2 µL/bee of acetone was applied to the test organisms, control bees were anaesthetised only. A total of 50 bees were exposed to each dose rate and the controls.

The number of surviving bees and the occurrence of sublethal effects were determined visually and recorded initially and after 1, 4, 24 and 48 hours. The incubator temperature was recorded daily.

The contact LD_{50} of CGA219417 to honey bees could not be determined as none of the dose rates tested resulted in greater than 50% mortality.

Results and Discussion

Samples collected from the stock solutions used to dose the bees had measured concentrations of 89 to 108% of the target nominal concentration. The actual measured concentrations within the stock solutions were 27.1, 44.5, 98/1. 198 and 392 mg/mL. Based on these measured values the actual dose rates were 54, 89, 196, 396 and 784 µg/bee.

Table 8.3.1.1.2-1: Analytical results

Nominal Stock Concentrations (mg/mL)	Measured Stock Concentration (mg/mL)	% Nominal
Solvent Control	<LOD	-
25	27.1	108
50	44.5	89
100	98.1	98
200	198.0	99
400	392.0	98

LOD: 50 mg/L

After 48 hours contact exposure, survival of 96% and 92% was observed in the control and solvent control respectively and no sublethal effects were observed in these treatment groups. Four of the 42 surviving bee exposed to 784 µg/bee exhibited a loss of equilibrium after 24 hours of exposure and two bees of the same treatment group exhibited loss of equilibrium after 48 hours. No other sublethal effects were noted during the study.

Table 8.3.1.1.2-2: Observed mortality during the contact toxicity study

Actual Dosage (µg/bee)	Number of Surviving Bees			
	1 hour After Application	4 hours After Application	24 hours After Application	48 hours After Application
Control	50	50	50	48
Solvent Control	50	50	48	46
54	50	50	48	48
89	50	50	50	49
196	50	50	47	46
396	50	50	49	47
784	50	50	45	42

Validity Criteria

Not reported.

Conclusions

Contact exposure of honey bees to CGA219417 resulted in a 48-hour LD_{50} of >784 µg/bee and a NOEC of 396 µg/bee.

(Boeri *et al.* 1995)

CA 8.3.1.2 Chronic toxicity to bees

Chronic toxicity data for bees is a new data requirement under **Commission Regulation (EU) No 283/2013**, applicable where there is a possibility that bees may be exposed.

Since the use of cyprodinil may lead to minor potential exposure to bees, a test has been carried out with the representative formulations in lieu of the technical active substance, and are summarised below.

Report:	Submitted for purposes of renewal due to change in data requirements guidance: K-CA 8.3.1.2/01 Ruhland S. (2014) Cyprodinil EC (A14325E) – Chronic toxicity to the honey bee *Apis mellifera* L. in a 10 day continuous laboratory feeding study. Report Number 141048147B. BioChem agrar. Labor für biologische und chemische Analytik GMbH, Kupeferstraβe 6, 04827 Gerichshain, Germany. (Syngenta File No. A8637C_10321)

Guidelines

Decourtye *et al*. (2005)

Suchail *et al*. (2001)

AFPP method CEB No. 230 (2012)

EFSA Guidance Document (2013)

Ring test protocol of the AG-Bienenschutz (2014)

GLP: Yes.

Executive Summary

The toxicity of A8637C to honey bees was determined in a 10-day continuous feeding test in the laboratory at dose levels of 400.0, 200.0, 100.0, 50.0 and 25.0 µg a.s./bee.

The 10 day LD_{50} was determined to be 112.2 µg consumed a.s./bee/day. The 10 day NOED was determined to be 47.3 µg consumed a.s./bee/day. The 10 day LC_{50} was determined to be 3.829 g a.s./kg food. The 10 day NOEC was determined to be 1.284 g a.s./kg food.

Materials

Test Material	A8637C Cyprodinil WG
Lot/Batch #:	SMO2C304
Actual content of active ingredients:	50.2% w/w
Description:	Brownish granules
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	End of December 2016
Density:	Not stated
Treatments	
Test rates:	400.0, 200.0, 100.0, 50.0 and 25.0 µg a.s./bee.
Control:	50% (w/v) sucrose solution.
Toxic standard:	Dimethoate EC 400
Administration:	Ingestion in aqueous sucrose solution
Test organisms	
Species:	*Apis mellifera* L. (subspecies *iberica* P.)

Age and sex:	Young female workers 1-4 days old
Source:	Beekeeper Bienenfarm Kern GmbH, Rehbacher Anger 10, 04249 Leipzig, Germany
Food:	50% w/v aqueous sucrose solution
Test design	
Test cage description:	Aluminium cages with the dimensions: 20 cm (width) x 15 cm (height) x 10 cm (depth); with holes in the lateral walls for sufficient air supply and ventilation and two glass plates (one in front and one in the back) for observations of the bees
Replication:	3
No. of bees/arena :	20
Duration of test:	10 days
Environmental test conditions	
Temperature:	33.5 – 34.5 °C
Humidity:	47.2 – 54.3%
Photoperiod:	Constant darkness

Study Design and Methods

Experimental dates: 1st to 10th June 2014

Honeybees (*Apis mellifera*) were exposed to A8637C via oral ingestion for a period of 10 days. A stock solution of the highest test concentration was directly prepared in acetone. All lower test solutions were freshly prepared daily, by appropriate dilution of stock solution with 50 % (w/v) aqueous sucrose solution.

The treated/untreated food was provided *ad libitum* in a plastic syringe, which had been weighed before application. The actual consumption was determined by reweighing the syringe containing the remaining test solution each day after removal from the test units. Any unconsumed food was rejected and replaced with fresh treated /untreated food. Observations for mortality, sublethal effects and behavioural abnormalities were recorded daily.

Due to their social feeding behaviour, the honeybees of a distinct group are assumed to share the application solution (trophallaxis) and thus receive similar doses of the applied respective item.

The mortality [%] per treatment was calculated from the number of dead bees and the total number of introduced bees per treatment group. Mortality in the test item treatment was corrected for corresponding control mortality, where necessary, according to the formula of Abbott (1925), modified by Schneider-Orelli (1947).

The statistical calculations were performed with a computer program (for example ToxRat Professional 2.10.06).

For statistical calculation of the mortality results the Fisher's Exact Binomial test (with Bonferroni Correction) was used. The accepted significance level was $p < 0.05$ (one-sided greater). For calculation of the LD_X/LC_X value of test and reference item a Probit/Weibull model was used (linear maximum likelihood regression).

Results and Discussion

Mortality data for the test material and control are summarised in the table below.

Table 8.3.1.2-1: Summary of chronic toxicity of A8637C to the honeybee (*Apis mellifera* L)

Treatment		Mortality after 10 days (%)	Corrected 10-day mortality (%)
Consumed (µg / bee / day)	Dose (g /kg food)		
Control		3.3	-
365.268	10.271	93.3*	93.1
126.800	5.136	68.3*	67.2
69.657	2.568	16.7*	13.8
47.282	1.284	15.0	12.1
24.426	0.642	8.3	5.2
10 d LC_{50} (95% confidence limits)		3.829 g a.s./kg food (2.183 – 6.714)	
10 d LD_{50} (95% confidence limits)		112.2 µg consumed a.s./bee/day (72.8 – 172.9)	
NOEC[a]		1.284 g a.s./kg food	
NOED[a]		47.3 µg consumed a.s. /bee /day	

Corrected mortality (according to SCHNEIDER-ORELLI 1947), negative values are treated as "0"
* Statistically significant difference in pairwise comparison between treatment and untreated control
Median lethal dose/conc. (and 95 % CL / lower-upper) was calculated by using Logit/Weibull analysis (linear max. likelihood regression)
[a] - Fisher`s Exact Binominal Test with Bonferroni Correction; α=0.05; one sided greater

Validity of the test

Control mortality in the test was 3.3 % (must be <15 % to be considered valid). Therefore the test was considered valid.

Conclusions

The chronic toxicity of A8637C was tested on honeybees under laboratory conditions.

The 10 day LD_{50} was determined to be 112.2 µg consumed a.s./bee/day. The 10 day NOED was determined to be 47.3 µg consumed a.s./bee/day. The 10 day LC_{50} was determined to be 3.829 g a.s./kg food. The 10 day NOEC was determined to be 1.284 g a.s./kg food.

(Ruhland S, 2014)

Report:	Submitted for purposes of renewal due to change in data requirements guidance: K-CA 8.3.1.2/02 Ruhland S. (2014a) Cyprodinil EC (A14325E) - Chronic toxicity to the honeybee *Apis mellifera* L. in a 10 day continuous laboratory feeding study. Report Number 14 10 48 147 B. BioChem agrar Labor für biologische und chemische, Analytik GmbH, Kupferstraße 6 04827 Gerichshain, Germany. (Syngenta file No. A14325E_10065).

Guidelines

Decourtye *et al*. (2005)

Suchail *et al*. (2001)

AFPP method CEB No. 230 (2012)

EFSA Guidance Document (2013)

Ring test protocol of the AG-Bienenschutz (2014)

GLP: Yes.

Executive Summary

The toxicity of A14325E to honey bees was determined in a 10 day continuous feeding test in the laboratory at dose levels of 399.9, 200.0, 100.0, 50.0 and 25.0 µg a.s./bee.

The 10 day LD_{50} was determined to be 69.7 µg consumed a.s./bee/day. The 10 day NOED was determined to be 44.2 µg consumed a.s./bee/day. The 10 day LC_{50} was determined to be 2.062 g a.s./kg food. The 10 day NOEC was determined to be 1.284 g a.s./kg food.

Materials

Test Material	A14325E Cyprodinil EC
Lot/Batch #:	SMO3A100
Actual content of active ingredients:	29.1 % w/w corresponding to 295 g/L
Description:	Light yellow liquid
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	End of January 2017
Density:	1014 kg/m³
Treatments	
Test rates:	399.9, 200.0, 100.0, 50.0 and 25.0 µg a.s./bee.
Control:	50 % (w/v) sucrose solution.
Toxic standard:	Dimethoate EC 400
Administration:	Ingestion in aqueous sucrose solution
Test organisms	
Species:	*Apis mellifera* L. (subspecies *iberica* P.)
Age and sex:	Young female workers 1-4 days old
Source:	Beekeeper Joaquin Cordero, Paseo de Moro No. 19, 41370 Cazalla (Seville), Spain
Food:	50% w/v aqueous sucrose solution
Test design	
Test cage description:	Aluminium cages with the dimensions: 20 cm (width) x 15 cm (height) x 10 cm (depth); with holes in the lateral walls for sufficient air supply and ventilation and two glass plates (one in front and one in the back) for observations of the bees
Replication:	3
No. of bees/arena :	20
Duration of test:	10 days
Environmental test conditions	
Temperature:	33.5 – 34.5 °C
Humidity:	47.2 – 54.3%
Photoperiod:	Constant darkness

Study Design and Methods

Experimental dates: 1st to 10th June 2014

Honeybees (*Apis mellifera*) were exposed to A14325E via oral ingestion for a period of 10 days. A stock solution of the highest test concentration was directly prepared in acetone. All lower test solutions were freshly prepared daily, by appropriate dilution of stock solution with 50% (w/v) aqueous sucrose solution.

The treated/untreated food was provided *ad libitum* in a plastic syringe, which had been weighed before application. The actual consumption was determined by reweighing the syringe containing the remaining test solution each day after removal from the test units. Any unconsumed food was rejected and replaced with fresh treated/untreated food. Observations for mortality, sublethal effects and behavioural abnormalities were recorded daily.

Due to their social feeding behaviour, the honeybees of a distinct group are assumed to share the application solution (trophallaxis) and thus receive similar doses of the applied respective item.

The mortality [%] per treatment was calculated from the number of dead bees and the total number of introduced bees per treatment group. Mortality in the test item treatment was corrected for corresponding control mortality, where necessary, according to the formula of Abbott (1925), modified by Schneider-Orelli (1947).

The statistical calculations were performed with a computer program (for example ToxRat Professional 2.10.06).

For statistical calculation of the mortality results the Fisher's Exact Binomial test (with Bonferroni Correction) was used. The accepted significance level was $p < 0.05$ (one-sided greater). For calculation of the LD_X/LC_X values of test and reference item a Probit/Weibull model was used (linear maximum likelihood regression).

Results and Discussion

Mortality data for the test material and control are summarised in the table below.

Table 8.3.1.2-2: Summary of chronic toxicity of A14325E to the honeybee (*Apis mellifera* L)

Treatment		Mortality after 10 days (%)	Corrected 10-day mortality (%)
Consumed (µg / bee / day)	**Dose (g /kg food)**		
Control		3.3	-
219.210	10.270	100.0*	99.9
189.734	5.135	98.3*	98.3
84.533	2.567	76.7*	75.9
44.182	1.284	11.7	8.6
28.039	0.642	0.0	0.0
10 d LC_{50} (95% confidence limits)		2.062 g a.s. /kg food (1.623 – 2.621)	
10 d LD_{50} (95% confidence limits)		69.7 µg consumed a.s./bee/day (63.6 – 76.5)	
NOEC[a]		1.284 g a.s./kg food	
NOED[a]		44.2 µg consumed a.s /bee /day	

Corrected mortality (according to SCHNEIDER-ORELLI 1947), negative values are treated as "0"
* Statistically significant difference in pairwise comparison between treatment and untreated control
Median lethal dose/conc. (and 95 % CL / lower-upper) was calculated by using Probit analysis (linear max. likelihood regression)
[a] Fisher`s Exact Binominal Test with Bonferroni Correction; α=0.05; one sided greater

Validity of the test

Control mortality in the test was 3.3 % (must be <15 % to be considered valid). Therefore the test was considered valid.

Conclusions

The chronic toxicity of A14325E was tested on honeybees under laboratory conditions.

The 10 day LD_{50} was determined to be 69.7 µg consumed a.s./bee/day. The 10 day NOED was determined to be 44.2 µg consumed a.s./bee/day. The 10 day LC_{50} was determined to be 2.062 g a.s./kg food. The 10 day NOEC was determined to be 1.284 g a.s./kg food.

(Ruhland S, 2014a)

CA 8.3.1.3 Effects on honeybee development and other honeybee life stages

Data on larval and brood development for bees is a new data requirement under **Commission Regulation (EU) No 283/2013**, applicable where there is a possibility that bees may be exposed.

New tests have been carried out with two representative formulations in lieu of the technical active substance, and are summarised below.

Report:	Submitted for purposes of renewal due to change in data requirements guidance: K-CA 8.3.1.3/01 Kleebaum K. (2014). Cyprodinil WG (A8637C) – Chronic toxicity to honeybee larvae *Apis mellifera* L. under laboratory conditions (*in vitro*). Report Number 141048145B. BioChem agrar. Labor führ biologische und chemische Analytik GMbH, Kupeferstraβe 6, 04827 Gerichshain, Germany. (Syngenta File No. A8637C_10330).

Guidelines

OECD DRAFT Guidance Document for testing chemicals: Honey bee (*Apis mellifera*) larval toxicity test, repeated exposure (February 2014)

OECD 237 Guidelines for testing chemicals: Honey bee (*Apis mellifera*) larval toxicity test, single exposure (2013)

GLP: Yes

Executive Summary

The purpose of this study was to determine the semi-chronic toxicity of A8637C (active ingredient: cyprodinil) to honeybee larvae *Apis mellifera* L. in an *in vitro* test after repeated oral application. The 7 and 8 day NOED was determined to be 13.3 and 33.3 µg cyprodinil/larva, respectively. The 7 and 8 day LD_{50} was determined to be 43.9 and 47.0 µg cyprodinil/larva, respectively.

Materials

Test Material	A8637C Cyprodinil WG (50)
Lot/Batch #:	SMO2C304
Actual content of active ingredients:	Cyprodinil: 50.2% w/w
Description:	Brownish granules
Stability of test compound:	Stable under the given conditions
Reanalysis/Expiry date:	31 December 2016
Density:	Not applicable

Treatments

Test rates:	Total µg A8637C/larva: 10.7, 26.6, 66.6, 166.5 and 416.3 Total µg cyprodinil/larva: 5.3, 13.3, 33.3, 83.3 and 208.2 g cyprodinil/kg diet: 0.034, 0.084, 0.211, 0.526 and 1.316
Control:	Untreated artificial diet
Toxic standard:	Dimethoate tech. (BAS 152 I), purity 99.8%
Administration:	Oral application using a sterile pipette

Test organisms

Species:	Worker honey bee larvae *Apis mellifera* L. subspecies *carnica* P. (Insecta, Hymenoptera, Apoidea)
Age:	First instar (L1) during grafting
Source:	Colonies purchased from Bienenfarm Kern GmbH, Rehbacher Anger 10, 04249 Leipzig, Germany
Food:	Aqueous sugar solution (comprising yeast, glucose, fructose and water) mixed with royal jelly at a 1:1 ratio w/w

Test design

Test cage description:	Crystal polystyrene grafting cells (e.g. CNE Nicoplast, internal diameter 9 mm) were placed in 48 well plates. The well plates were filled up to 1/3 with dental roll and the grafting cells were placed on wetted and disinfected dental rolls.
Replication:	3
No. of bees/arena :	12

Environmental test conditions

Temperature:	34.2 – 35.0 °C
Humidity:	92 - 97% (RH)
Photoperiod:	Constant darkness
Duration of test:	Pre-grafting (*in vivo*): days -3 to 0 Grafting: day 1 Pre-exposure (*in vitro*): days 1 to 3 Application: days 3 to 6 Post exposure (*in vitro*): days 7 and 8

Study Design and Methods

Experimental dates: 20th June to 28th August 2014

The test/reference item was mixed into sterile filtered aqueous sugar solution (stock A). Several dilutions were prepared by adding further sugar solution (stocks B and C) and royal jelly was added to each stock solution at a ratio of 1 : 1, based on (w/w), to reach the final test concentrations. Dosages were adjusted to reflect the target amounts of A8637C.

Honeybee larvae *Apis mellifera* L. were exposed to repeated oral application of 5.3, 13.3, 33.3, 83.3 and 208.2 µg cyprodinil/larva (equivalent to 0.034. 0.084, 0.211, 0.526 and 1.316 g cyprodinil/kg diet) in an *in vitro* test. One control group was included in the test. The larvae of the control treatment were fed with untreated artificial diet, which served as a vehicle for the test item and reference item.

On Day 1 the combs containing the larvae were transported from the hive to an acclimated laboratory room. Larvae were transferred from the combs to the crystal polystyrene grafting cells using a suitable grafting tool (e.g. grafting needle Swiss type). During grafting the C-shaped larvae were placed on the surface of the artificial diet within the grafting cells. Cells were placed in 48 well plates filled up to 1/3 with a piece of dental roll. Each replicate unit consisted of 12 larvae, and there were 3 replicates per treatment and control. Each larva was fed the treated diet daily between Day 3 and Day 6 using a sterile pipette.

The number of dead larvae was recorded daily on Day 4 to Day 8. Any large amounts of unconsumed food were recorded on Day 7 and Day 8. After the last assessment (Day 8) the culture plates with all organisms were placed in a freezer.

All observations were made in comparison to the control larvae. For each concentration, the corrected mortality was calculated according to Abbott (1925) modified by Schneider-Orelli (1947).

The LD_{50} values were calculated by Moving Average Computation. The statistical significance of the mortality values and the NOEC was calculated using Fisher's Exact Binomial Test with Bonferroni Correction ($P \leq 0.05$).

Results and Discussion

Mortality data and other observations for the test material and reference item are summarised in the table below.

Table 8.3.1.3-1: Summary of semi-chronic toxicity of A8637C to honeybee larvae

Item applied	Dosage[1] [µg cyprodinil/larva]	Concentration [g cyprodinil/kg diet]	Day 7			Day 8		
			Mortality mean %		OO[3]	Mortality mean %		OO[3]
			Absolute	Correct.[2]	Mean %	Absolute	Correct.[2]	Mean %
Control	-	-	2.8	-	2.8	11.1	-	2.8
Test item	5.3	0.034	5.6	2.9	0.0	5.6	0.0	0.0
	13.3	0.084	0.0	0.0	5.6	11.1	0.0	0.0
	33.3	0.211	22.2*	20.0	14.5	22.2	12.5	0.0
	83.3	0.526	100.0*	100.0	-	100.0*	100.0	-
	208.2	1.316	100.0*	100.0	-	100.0*	100.0	-
Reference item	6.2	0.039	86.1	85.7	0.0	88.9	87.5	0.0
Treatment	Endpoints		Day 7			Day 8		
Test item doses	NOED [µg cyprodinil/larva]		13.3			33.3		
	LD_{50} [µg cyprodinil/larva] (95 %-CL/lower-upper)		43.9 (36.9 – 52.1)			47.0 (40.7 – 54.2)		
Test item concentrations	NOEC [g cyprodinil/kg diet]		0.084			0.211		

Results were average based on 3 replicates, containing 12 larvae each
Calculations were performed with non-rounded values
* Statistically significant difference in pairwise comparison between treatment and untreated control (Fisher's Exact Binomial Test with Bonferroni Correction; one sided greater; $\alpha = 0.05$)
[1] All test item doses were based on a sum of applications on days 3 to 6
[2] Corrected mortality (according to Schneider-Orelli 1947), negative values are set to "0"
[3] OO: Other observations (large quantities of remaining food, smaller body size of larva)
- Not applicable

Validity of the test

All of the validity criteria were met:

- Control mortality should be ≤ 15% for larvae across all control replicates at day 7 and day 8 (actual values were 2.8 and 11.1)
- Reference item mortality should be ≥ 50% for larvae across all reference replicates at day 7 and day 8 (actual values were 86.1 and 88.9%)
- Concentration of the active substance in analysed sample of test item stock solution A should be ± 20 % of the nominal concentration of A (actual value ranged between 108 and 110 %)

Conclusions

The 7 and 8 day NOED was determined to be 13.3 and 33.3 µg cyprodinil/larva, respectively. The 7 and 8 day LD_{50} was determined to be 43.9 and 47.0 µg cyprodinil/larva, respectively.

(Kleebaum K, 2014)

Report:	Submitted for purposes of renewal due to change in data requirements guidance: K-CA 8.3.1.3/02 Kleebaum K. (2014a) Cyprodinil EC (A14325E) – Chronic toxicity to honeybee larvae *Apis mellifera* L. under laboratory conditions (*in vitro*). Report Number 141048146B. BioChem agrar. Labor führ biologische und chemische Analytik GMbH, Kupeferstraβe 6, 04827 Gerichshain, Germany. (Syngenta File No. A14325E_10067).

Guidelines

OECD DRAFT Guidance Document for testing chemicals: Honey bee (*Apis mellifera*) larval toxicity test, repeated exposure (February 2014)

OECD 237 Guidelines for testing chemicals: Honey bee (*Apis mellifera*) larval toxicity test, single exposure (2013)

GLP: Yes

Executive Summary

The purpose of this study was to determine the semi-chronic toxicity of A14325E (active substance: cyprodinil) to honeybee larvae *Apis mellifera* L. in an *in vitro* test after repeated oral application. The 8 day NOED was determined to be 17.3 µg cyprodinil/larva. The 8 day LD_{10} was determined to be 24.2 µg cyprodinil/larva. The 8 day LD_{50} was determined to be 45.7 µg cyprodinil/larva.

Materials

Test Material	A14325E Cyprodinil EC (300)
Lot/Batch #:	SMO3A100
Actual content of active ingredients:	Cyprodinil: 29.1% w/w corresponding to 295 g/L
Description:	Light yellow liquid
Stability of test compound:	Stable under the given conditions
Reanalysis/Expiry date:	31 January 2017
Density:	1014 kg/m^3

Treatments

Test rates:	Total µg A14325E/larva: 29.3, 58.6, 117.3, 234.5 and 469.0 Total µg cyprodinil/larva: 8.7, 17.3, 34.7, 69.4 and 138.8 g cyprodinil/kg diet: 0.055, 0.110, 0.219, 0.439 and 0.877
Control:	Untreated artificial diet
Toxic standard:	Dimethoate tech. (BAS 152 I), purity 99.8%
Administration:	Oral application using a sterile pipette

Test organisms	
Species:	Worker honey bee larvae *Apis mellifera* L. subspecies *carnica* P. (Insecta, Hymenoptera, Apoidea)
Age:	First instar (L1) during grafting
Source:	Colonies purchased from Bienenfarm Kern GmbH, Rehbacher Anger 10, 04249 Leipzig, Germany
Food:	Aqueous sugar solution (comprising yeast, glucose, fructose and water) mixed with royal jelly at a 1:1 ratio w/w
Test design	
Test cage description:	Crystal polystyrene grafting cells (e.g. CNE Nicoplast, internal diameter 9 mm) were placed in 48 well plates. The well plates were filled up to 1/3 with dental roll and the grafting cells were placed on wetted and disinfected dental rolls.
Replication:	3
No. of larvae/replicate :	12
Environmental test conditions	
Temperature:	34.0 – 34.8 °C
Humidity:	91 – 98% (RH)
Photoperiod:	Constant darkness
Duration of test:	Pre-grafting (*in vivo*): days -3 to 0 Grafting: day 1 Pre-exposure (*in vitro*): days 1 to 3 Application: days 3 to 6 Post exposure (*in vitro*): days 7 and 8

Study Design and Methods

Experimental dates: 18th July to 2nd September 2014

The test/reference item was mixed into sterile filtered aqueous sugar solution (stock A). Several dilutions were prepared by adding further sugar solution (stocks B and C) and royal jelly was added to each stock solution at a ratio of 1 : 1, based on (w/w), to reach the final test concentrations. Dosages were adjusted to reflect the target amounts of A14325E.

Honeybee larvae *Apis mellifera* L. were exposed to repeated oral application of 8.7, 17.3, 34.7, 69.4 and 138.8 µg cyprodinil/larva (equivalent to 0.055, 0.110, 0.219, 0.439 and 0.877 g cyprodinil/kg diet) in an *in vitro* test. One control group was included in the test. The larvae of the control treatment were fed with untreated artificial diet, which served as a vehicle for the test item and reference item.

On Day 1 the combs containing the larvae were transported from the hive to an acclimated laboratory room. Larvae were transferred from the combs to the crystal polystyrene grafting cells using a suitable grafting tool (e.g. grafting needle Swiss type). During grafting the C-shaped larvae were placed on the surface of the artificial diet within the grafting cells. Cells were placed in 48 well plates filled up to 1/3 with a piece of dental roll. Each replicate unit consisted of 12 larvae, and there were 3 replicates per treatment and control. Each larva was fed the treated diet daily between Day 3 and Day 6 using a sterile pipette.

The number of dead larvae was recorded on daily on Day 4 to Day 8. Any large amounts of unconsumed food were recorded on Day 7 and Day 8. After the last assessment (Day 8) the culture plates with all organisms were placed in a freezer.

All observations were made in comparison to the control larvae. For each concentration, the corrected mortality was calculated according to Abbott (1925) modified by Schneider-Orelli (1947).

The LD_x values were calculated by Probit using Linear maximum likelihood regression. The statistical significance of the mortality values and the NOEC was calculated suing Fisher's Exact Binomial Test with Bonferroni Correction ($P \leq 0.05$)

Results and Discussion

Mortality data and other observations for the test material and reference item are summarised in the table below.

Table 8.3.1.3-2: Summary of semi-chronic toxicity of A14325E to honeybee larvae

Item applied	Dosage[1] [µg cyprodinil/larva]	Concentration [g cyprodinil/kg diet]	Day 8		
			Mortality mean %		OO[3]
			Absolute	Correct.[2]	Mean %
Control	-	-	0.0	-	0.0
Test item	8.7	0.055	0.0	-	0.0
	17.3	0.110	5.6	-	3.0
	34.7	0.219	22.2*	-	0.0
	69.4	0.439	80.6*	-	0.0
	138.8	0.877	100.0*	-	-
Reference item	6.2	0.039	91.7	-	0.0
Treatment	**Endpoints**		**Day 8**		
Test item doses	NOED [µg cyprodinil/larva]		17.3		
	LD_{10} [µg cyprodinil/larva] (95 %-CL/lower-upper)		24.2 (19.5 – 30.2)		
	LD_{50} [µg cyprodinil/larva] (95 %-CL/lower-upper)		45.7 (39.6 – 52.6)		
Test item concentrations	NOEC [g cyprodinil/kg/diet]		**0.110**		

Results were average based on 3 replicates, containing 12 larvae each
Calculations were performed with non-rounded values
* Statistically significant difference in pairwise comparison between treatment and untreated control (Fisher's Exact Binomial Test with Bonferroni Correction; one sided greater; $\alpha = 0.05$)
[1] All test item doses were based on a sum of applications on days 3 to 6
[2] Corrected mortality (according to Schneider-Orelli 1947), negative values are set to "0"
[3] OO: Other observations (large quantities of remaining food, smaller body size of larva)
- Not applicable

Validity of the test

All of the validity criteria were met:

- Control mortality should be ≤ 15% for larvae across all control replicates at day 7 and 8 (actual values were 0.0 and 0.0%)
- Reference item mortality should be ≥ 50% for larvae across all reference replicates at day 7 (actual values were 83.3 and 91.7%)
- Concentration of the active substance in analysed sample of test item stock solution A should be ± 20 % of the nominal concentration of A (actual value ranged between 89 and 97%)

Conclusions

The 8 day NOED was determined to be 17.3 µg cyprodinil/larva. The 8 day LD_{10} was determined to be 24.2 µg cyprodinil/larva. The 8 day LD_{50} was determined to be 45.7 µg cyprodinil/larva.

(Kleebaum K, 2014a)

CA 8.3.1.4 Sub-lethal effects

Further tests investigating sub-lethal effects, such as behavioural and reproductive effects, on bees or colonies are not required since the risk assessments based on acute and chronic data indicate an acceptable risk to bees, and there are no indications of sublethal effects based on either the mode of action or existing tests.

Relevant Literature on Bees

No relevant scientifically peer-reviewed open literature could be found on cyprodinil or its major metabolites. Details of the literature search undertaken can be found in M-CA Section 9 of the current submission.

CA 8.3.2 Effects on non-target arthropods other than bees

Studies conducted on non-target arthropods with the representative formulations A8637C and A8779A were submitted for the previous EU review to represent the active substance cyprodinil. Endpoints from these studies are presented in Tables 8.3.2-1 and 8.3.2-2.

Since the previous EU review of cyprodinil studies have been conducted with the representative formulation A14325E. Endpoints from these studies are presented in Table 8.3.2-3.

Table 8.3.2-1: Summary of endpoints for non-target arthropods for A8637C

Species	Test type	Treatments (kg a.s./ha) and spray interval	Endpoints [a]	Reference (author, date, Syngenta File No.)
Aphidius rhopalosiphi	Tier 1, glass plate	0.045, 0.225, 0.45	No adverse effects on mortality at any rate. Parasitisation affected at 0.45 kg a.s./ha, therefore: LR_{50} >0.45 kg a.s./ha; ER_{50} >0.225 kg a.s./ha	***Grimm (1999) CGA219417/0925***
	Tier 1, glass plate	Rate-response 0.3, 0.45, 0.6, 0.75, 0.9	LR_{50} = 0.71 kg a.s/ha; parasitisation affected >50% at both rates tested (0.3 and 0.45 kg a.s./ha)	***Vinall (2001) CGA219417/1048***
	Semi-field test on wheat	4 x 0.225 (2-3 d int), 2 x 1.5 (7 d int)	No adverse effect at 4 x 0.225 kg a.s./ha; >>50% effect at 2 x 1.5 kg a.s./ha	***Kleiner (1997) CGA219417/0813***
	Semi-field test on apple	1 x 1.5, 2 x 1.5 (7 d int)	No adverse effects on mortality or parasitisation	***Aldershof (2000) CGA219417/0965***
	Extended laboratory aged-residue test	3 x 375 (7 d int). Bioassays initiated at 0 and 7 d after final application	5.1 and 5.0% corrected mortality for 0 and 7 DAT bioassays; 3.6% reduction and 8.8% increase in parasitisation, respectively.	***Stevens (2016) A8637C_10377***
Typhlodromus pyri	Tier 1, glass plate	0.045, 0.225, 0.45	No effects on mortality or fecundity >50% at any rate, therefore: (LR/ER_{50} >0.45 kg a.s./ha)	***Grimm (1999) CGA219417/0923***

Species	Test type	Treatments (kg a.s./ha) and spray interval	Endpoints [a]	Reference (author, date, Syngenta File No.)
	Tier 1, glass plate	0.045, 0.225, 0.90	Mortality not affected at any rate (LR_{50} >0.9 kg a.s./ha); >50% effect on fecundity at 0.225 and 0.9 kg a.s./ha; <50% effect on fecundity at 0.045 kg a.s./ha	***Taruza (2001) CGA219417/1057***
	Field test in apple	4 x 0.045, 4 x 0.225 (9-10 d int)	No statistically significant difference from control plot was found	***Aldershof (2000) CGA219417/0949***
	Field test in apple	4 x 0.075, 4 x 0.375 (5-8 d int)	No biologically significant difference from control plot was found	***Aldershof (2000) CGA219417/0974***
Coccinella septempunctata	Tier 1, glass plate	0.075, 0.375, 0.75	100% mortality within 4 days in all treatments	***Halsall (2000) CGA219417/0946***
	2-D extended laboratory test on bean leaves	Rate-response 0.15, 0.225, 0.30, 0.375, 0.45	LR_{50} = 0.444 kg a.s./ha; >50% effect on reproduction at 0.45 kg a.s./ha; no effect on fecundity at 0.375 kg a.s/ha and below	***Halsall (2001) CGA219417/1051***
	Semi-field test on bean plants	4 x 0.045, 4 x 0.225 (7-8 d int), 2 x 0.3, 2 x 1.5 (7 day int)	Mortality >50% at 2 x 1.5 and 2 x 0.3 kg/ha; mortality <50% at 4 x 0.045 and 4 x 0.225 kg/ha. No effect on fecundity at any rate	***Kleiner (1999) CGA219417/0899***
	Semi-field test on apple	4 x 0.045, 4 x 0.225 (7 day interval)	No effects >50%	***van. Stratum (2002) CGA219417/1066***
Chrysoperla carnea	Tier 1, glass plate	0.075, 0.375, 0.75	<50% effect on mortality at 0.75 kg a.s./ha; >50% effect on mortality at 0.375 kg a.s./ha No effect on reproduction at the two rates tested (0.075 and 0.375 kg a.s./ha), therefore: LR_{50} >0.075<0.75 kg a.s./ha); $ER_{50(reproduction)}$ >0.375 kg a.s./ha	***Halsall (2000) CGA219417/0969***
	Semi-field test on apple	1 x 0.225; 4 x 0.225 (7 d int)	Neither mortality of fecundity were adversely affected for any treatment scenario	***Bakker (2002) CGA219417/1058***
Orius laevigatus	Semi-field test on quince	2 x 1.5 (4 week int), 4 x 0.225 (7 d int)	>50% effect on mortality and fecundity at 2 x 1.5 kg a.s./ha (unreliable fecundity result); <50% effect on mortality and fecundity at 4 x 0.225 kg/ha.	***Kleiner (1997) CGA219417/0815***
	Semi-field test on apple	2 x 0.3, 2 x 1.5 (11 d int); 4 x 0.225 (7-11 d int)	No effects >50% in any treatment [b]	***Aldershof (1999) CGA219417/0938***
Poecilus cupreus	Tier 1, on sand	0.045, 0.225, 0.45	Neither mortality or feeding were adversely affected at any rate, therefore: LR_{50} >0.45 kg a.s./ha; $ER_{50(feeding)}$ 0.45 kg a.s./ha	***Grimm (1999) CGA219417/0916***

[a] Endpoints are assessed according to ESCORT 2 (***Candolfi et al. 2001***). For worst-case Tier 1 laboratory tests on inert substrates: LR_{50}/ER_{50} and 50% effect level of any sublethal effects evaluated (the latter are not strictly required by ESCORT 2). For higher tier tests: 50% effects level for lethal or sublethal effects.
[b] There was a 51% corrected mortality after a single application of 3.0 kg/ha, but only 29% mortality after 2 applications of 3.0 kg/ha, and 17% after 2 weeks ageing following application of 2 x 3.0 kg/ha.

Table 8.3.2-2: Summary of endpoints for non-target arthropods for A8779A

Organism	Test type	Test rates/study design	Endpoints	Reference (author, date, Syngenta File No.)

Organism	Test type	Test rates/study design	Endpoints	Reference (author, date, Syngenta File No.)
Aphidius rhopalosiphi	Tier 1, glass plates	0.03, 0.75, 1.5 kg a.s./ha	No adverse effects on mortality. Adverse effects on parasitisation at 0.75 and 1.5 kg a.s./ha, therefore: LR_{50} > 1.5 kg a.s./ha; $ER_{50(parasitisation)}$ = 0.030 kg a.s./ha	***Calis (1999) CGA219417/0913***
	Tier 1, glass plates	0.375, 0.75, 1.5, 3.75 kg a.s./ha	Adverse effects on mortality at top rate. No acceptable effects on parasitisation at either rate tested (0.75 and 1.5 kg a.s./ha), therefore: LR_{50} >1.5 kg a.s./ha; ER_{50} <0.75 kg a.s./ha	***Kelly (2001) CGA219417/1060***
	Tier II (barley seedlings)	0.030, 0.75, 1.5 kg a.s./ha	No adverse effects at any rate, therefore: LR_{50} >1.5 kg a.s./ha; $ER_{50(parasitisation)}$ >1.5 kg a.s./ha	***Schuld (2000) CGA219417/0947***
Typhlodromus pyri	Tier 1, glass plates	0.75, 1.5 kg a.s./ha	No adverse effects on mortality at either rate. Adverse effects on reproduction at both rates, therefore: LR_{50} > 1.5 kg a.s./ha; $ER_{50(reproduction)}$ <0.75 kg a.s./ha	***Candolfi (1998) CGA77102/0871***
	Tier 1, glass plates	0.75, 1.5 kg a.s./ha	No adverse effects on mortality at either rate. Adverse effects on reproduction at lowest rate, therefore: LR_{50} > 1.5 kg a.s./ha; $ER_{50(reproduction)}$ >0.75 kg a.s./ha	***Calis (1999) CGA219417/0914***
	Tier 1, glass plates	0.375, 0.75, 1.5, 2.25 kg a.s./ha	No adverse effects on mortality or reproduction at any rate, therefore: LR_{50} > 2.25 kg a.s./ha; $ER_{50(reproduction)}$ >2.25 kg a.s./ha	***Kelly (2001) CGA219417/1059***
	Field test	Vines were sprayed twice with 750 g a.s./ha and the numbers of mites on leaves were assessed 7 days after the first application, and 6 and 22 days following the second application.	A8779A caused no adverse effects on the predatory mites	***Oberwalder (1998) CGA219417/0887***
Coccinella septempunctata	Tier 1, glass plates	1.5 kg a.s./ha	100% mortality at 1.5 kg a.s./ha after 3 days, therefore LR_{50} <1.5 kg a.s./ha	***Kleiner (1995) CGA219417/0766***
	Semi-field (trays of broad beans)	0.75 kg a.s./ha	30.7% mortality at 750 g a.s./ha	***Nienstedt (1999) CGA219417/0932***
	Semi-field aged-residue (trays of broad beans)	Plants were sprayed twice with 0.0375 and 0.75 kg a.s./ha at 7 day intervals.	Three bioassays were set up at 0, 14 and 28 days after the 2nd application. No adverse effects on mortality of fecundity for either rate in any bioassay	***Grimm (2000) CGA219417/1004***
Aleochara bilineata	Tier II (sand)	0.75 and 1.5 kg a.s./ha	66.6% effect on number of F1 progeny at 1.5 kg a.s/ha; 16% effect on number of F1 progeny at 1.5 kg a.s/ha therefore: ER_{50} >0.75 <1.5 kg a.s./ha	***Kemmeter (1999) CGA219417/0935***
	Tier II (LUFA 2.1 soil)	1.275 kg a.s./ha	No reduction in number of F1 progeny relative to the control, therefore: ER_{50} >1.275 kg a.s./ha	***Grimm (2001) CGA219417/1022***
Poecilus cupreus	Tier II (sand)	0.75 and 1.5 kg a.s./ha	No mortality or effects on feeding at either rate, therefore: LR_{50} > 1.5 kg a.s./ha; $ER_{50(feeding)}$ = 1.5 kg a.s./ha	***Beech (1994) CGA219417/0240***

Organism	Test type	Test rates/study design	Endpoints	Reference (author, date, Syngenta File No.)
Pardosa sp.	Tier II (sand)	0.03 and 0.75 kg a.s./ha	No mortality or effects on feeding at either rate, therefore: LR_{50} > 0.75 kg a.s./ha; $ER_{50(feeding)}$ = 0.75 kg a.s./ha	***Kleiner (1999) CGA219417/0968***
Episyrphus balteatus	Tier 1, glass plates	0.75 and 1.5 kg a.s./ha	No adverse effects on mortality or reproduction at either rate, therefore: LR_{50} > 1.5 kg a.s./ha; $ER_{50(reproduction)}$ = 1.5 kg a.s./ha	***Kühner (1995) CGA219417/0830***

Note: Adverse effects refers to >50% mortality or >50% reduction in parasitisation, other measures of reproduction or feeding relative to the control

Table 8.3.2-3: Summary of endpoints for non-target arthropods for A14325E

Species	Test type[a]	Treatment rate (mL/ha)	Result	Reference (author, date, Syngenta File No.)
Typhlodromus pyri (predatory mite)	T1 Laboratory, glass plate	Dose response: 93.67, 375, 750, 1950, 3000	LR_{50} = 583 g a.s./ha Reproduction: no effect >50% up to 225 g a.s./ha (the highest rate tested for fecundity)	***Vaughan (2005) CGA219417/1298***
Aphidius rhopalosiphi (aphid parasitoid wasp)	T1 Laboratory, glass plate	Dose response: 23.33, 93.67, 375, 750, 1950, 3000	LR_{50} = 72.3 g a.s./ha Reproduction: no effect >50% at 7 g a.s./ha (the only rate tested)	***Vinall (2005) CGA219417/1297***
Typhlodromus pyri (predatory mite)	2-D extended laboratory, plant substrate	Dose response: 23.33, 93.67, 375, 750, 1950, 3000	LR_{50} >900 g a.s./ha. Reproduction: no effect >50% at up to 585 g a.s./ha; >50% effect at 900 g a.s./ha	***Vaughan (2005) CGA219417/1332***
Typhlodromus pyri	Extended laboratory aged-residue test	2 x 1500 (14 d int). Bioassays initiated at 0 and 14 d after final application	8.0 and 10% corrected mortality for 0 and 14 DAT bioassays; 16.1 and 12.2% reduction in reproduction, respectively.	***Fallowfield (2017) A14325E_10107***
Aphidius rhopalosiphi (aphid parasitoid wasp)	3-D extended laboratory, on whole plant	Dose response: 23.33, 93.67, 375, 750, 1950, 3000	LR_{50} >900 g a.s./ha. Reproduction: no effect >50% at up to 900 g a.s./ha	***Vinall 2005 CGA219417/1390***
Chrysoperla carnea (green lacewing)	2-D extended laboratory, plant substrate	Dose response: 23.33, 93.67, 375, 750, 1950, 3000	LR_{50} = 718 g a.s./ha. Reproduction: no unacceptable effect up to 225 g a.s./ha (the highest tested for reproduction)	***Spincer (2005) CGA219417/1375***
Chrysoperla carnea	Extended laboratory aged-residue test	2 x 1500 (14 d int). Bioassays initiated at 0 and 7 d after final application	14.7 and 5.9% corrected mortality for 0 and 7 DAT bioassays; no adverse effects on reproduction for either bioassay	***Vaughan (2016) A14325E_10106***
Coccinella septempunctata (ladybird beetle)	2-D extended laboratory, plant substrate	Dose response: 23.33, 93.67, 375, 750, 1950, 3000	LR_{50} > 900 g a.s./ha; Reproduction: no unacceptable effect up to 900 g a.s./ha	***Spincer 2005 CGA219417/1392***

CA 8.3.2.1 Effects on *Aphidius rhopalosiphi*

The studies previously submitted in the EU were evaluated in point B.9.5.1 of the DAR. At the request of ANSES summaries have been included in order to aid dossier evaluation ~~, therefore additional study summaries are not repeated here~~.

Report:	Summary submitted at the request of ANSES for ease of dossier evaluation: *KIIA 8.3.2.2. Grimm C. (1999). Acute Toxicity of CGA 219417 WG 50 (A 8637 C) to the Parasitic Wasp Aphidius rhopalosiphi (Hymenoptera: Aphidiidae). Report Number 983965. Novartis Crop Protection AG, CH-4002 Basel, Switzerland. (Syngenta file No. CGA219417/0925).*

Guidelines

Mead-Briggs M. (1992). A laboratory method for evaluating the side-effects of pesticides on the cereal aphid parasitoid *Aphidius rhopalosiphi*. Aspects of Applied Biology 31:179-189

Polgar, L. (1988). Guideline for testing the effects of pesticides on *Aphidius matricariae* Hal. Hym., Aphidiidae. IOBC/WPRS Bulletin XI/4: 29-34

GLP: Yes.

Executive Summary

The aim of the study was to determine the effects of dry residues of A8637C on the parasitic wasp, *Aphidius rhopalosiphi* (De Stefani-Perez) (Hymenoptera, Braconidae), under worst-case laboratory test conditions on glass.

A8637C was evaluated at three application rates, equivalent to 90, 450 and 900 g product/ha. No significant mortality was observed in any A8637C treatment group during the 48-hour exposure. The LR_{50} can therefore be assumed to be >900 g A8637C/ha.

The mean number of parasitized aphids per surviving female was significantly lower in the 900 g product/ha treatment, compared to the control.

Materials

Test Material	CGA219417 WG 50
Lot/Batch #:	609025
Formulation code:	A8637C
Active Ingredient Content:	51.3% w/w CGA219417
Stability of the test compound:	Not reported
Description:	Beige granules
Reanalysis/expiry date:	April 2000
Treatments	
Test treatments:	90, 450 and 900 g/ha of A8637C
Solvent:	None
Positive control:	Perfekthion (dimethoate), applied at 1 mL/ha
Application method:	Calibrated laboratory sprayer
Spray volume:	200 L spray solution/ha
Test organism	
Species:	*Aphidius rhopalosiphi* De Stefani-Perez. (Hymenoptera: Aphidiidae)

Source:	PK Nützlingszuchten, D-73642 Welzheim, Germany
Acclimation:	Prior to exposure the parasitoids were held under the following conditions; Temperature 20 ± 2°C to 25 ± 2°C, Relative humidity 75 ± 15%, Photoperiod 16 hours light : 8 hours dark
Feeding:	During exposure wasps were feed with a piece of cotton soaked in 33% honey and water solution. Water was supplied via a cotton wick connecting the test units to a water bath

Test design

Test vessels:	Exposure phase: Two treated glass plates (10 x 10 cm) fitted to a square frame of stainless steel (internal dimensions 9.4 x 9.4 cm and 2 cm high). The test cages were ventilated with a vacuum pump to prevent pesticide vapour build up Reproduction phase: Potted barley plants, approximately 10 plants per pot, each 2 to 10 cm tall. The pots were enclosed within a clear acrylic cylinder sealed at the top with 0.1 mm nylon mesh. The plants were infested with approximately 80 *Rhopalosiphum padi*
Substrate:	Glass plates
Replication:	Exposure phase: Four replicates per treatment each containing 10 wasps Reproduction phase: 15 replicates per treatment each containing a single female wasp
Duration:	Exposure phase: 48 hours Reproduction phase: 11 days

Environmental conditions

Test temperature:	19 to 22 °C
Humidity:	71 to 90 %
Lighting:	Exposure phase: 885 to 1215 lux Reproduction phase: 6810 to 10220 lux Photoperiod of 16 hours light : 8 hours dark

Study Design and Methods

Experimental dates: 22nd June to 20th July 1999

The study was performed in two phases: the exposure phase and the reproduction phase. During the exposure phase adult *A. rhopalosiphi* were exposure to residues of the test material for 48 hours and mortality assessed. Treatments were applied to glass plates which were then used to form the floor and ceiling of shallow arenas. Ten adult wasps (including a minimum of five females) were placed in each replicate arena (n = 4 per treatment rate). Assessments of treatment effects were made at 2, 24 and 48 h.

During the reproduction phase the effects of the test item on fecundity was assessed. Fifteen female wasps, impartially selected from each treatment group were confined individually over untreated aphid-infested barley plants for 24 h, before being removed. The plants were left for a further 11 days before the number of aphid mummies that developed was recorded.

Mortality data were analysed for significance with 2 x 2 contingency tables using one-way chi squared test. The results from the reproduction phase of the test were compared to the control values using the Dunnett test.

Results and Discussion

No significant mortality was observed in any A8637C treatment group compared to the control after 48 hours exposure. The 7.5% mortality observed in the 450 g/ha treatment group was due to two test organisms escaping from the test chamber. Complete mortality was observed in the toxic reference (Perfekthion) group after 48 hours exposure, therefore no wasps were available from this group for the reproduction phase of the test.

Table 8.3.2.1-1: Effects of fresh residues of A8637C on glass on mortality and fecundity of *Aphidius rhopalosiphi* under laboratory test conditions

Treatment	Rate (g product/ha)	% mortality at 48 h	Mean number mummies per surviving female	% of control reproduction
Control	0	0	26.1	-
A8637C	90	0	20.9	80
	450	7.5	24.3	93
	900	0	11.9[a]	46
Perfekthion	0.2	100[a]	-	-

[a] Statistically significant difference from control

A significant reduction in the number of mummies per surviving females was observed in the 900 g product/ha treatment group. No attempt was made to estimate the LR_{50} values for the effects of A8637C on *Aphidius rhopalosiphi*.

Validity criteria

Not reported.

Conclusion

The effects of A8637C on the parasitic wasp, *Aphidius rhopalosiphi*, were evaluated under worst-case laboratory test conditions on glass. No attempt was made to estimate the LR_{50} values for the effects of A8637C on *Aphidius rhopalosiphi*. A8637C caused no significant mortality at 900 g product/ha. The LR_{50} can therefore be assumed to be >900 g A8637C/ha.

The mean number of parasitized aphids per surviving female was significantly lower in the 900 g product/ha treatment, compared to the control.

(Grimm C. 1999)

Report:	Summary submitted at the request of ANSES for ease of dossier evaluation: *KIIA 8.3.2.2. Vinall S. (2001). CGA219417: A rate-response laboratory test to evaluate the effects of a 500 g/kg WG formulation (A-8637 C) on the parasitic wasp, Aphidius rhopalosiphi. Report Number 2003797. Mambo-Tox Ltd., Southampton, England. (Syngenta file No. CGA219417/1048).*

Guidelines

Mead-Briggs M. (2000). A laboratory method for evaluating the effects of plant protection products on the parasitic wasp, *Aphidius rhopalosiphi* (DeStephani-Perez) (Hymenoptera, Braconidae)

GLP: Yes

Executive Summary

The aim of the study was to determine the effects of dry residues of A8637C on the parasitic wasp, *Aphidius rhopalosiphi* (De Stefani-Perez) (Hymenoptera, Braconidae), under worst-case laboratory test conditions on glass.

A8637C was evaluated at five application rates, equivalent to 600, 900, 1200, 1500 and 1800 g product/ha.

The 48-hour LR_{50} was estimated to be 1422.5 g product/ha. In addition, a significant reduction in wasp fecundity was observed in the 600 and 900 g product/ha treatment groups.

Materials

Test Material	CGA219417 WG (50)
Lot/Batch #:	WM910165
Formulation code:	A8637C
Active Ingredient Content:	50.9% w/w CGA219417
Stability of the test compound:	Not reported
Description:	Light-brown extruded granules
Reanalysis/expiry date:	March 2003
Treatments	
Test treatments:	600, 900, 1200, 1500 and 1800 g/ha of A8637C
Solvent:	None
Positive control:	Perfekthion (415.7 g/L dimethoate), applied at 0.425 mL/ha
Application method:	Potter laboratory spray tower
Spray volume:	200 L spray solution/ha
Test organism	
Species:	*Aphidius rhopalosiphi* De Stefani-Perez. (Hymenoptera: Braconidae)
Source:	PK Nützlingszuchten, Welzheim, Germany
Acclimation:	Emerged wasps were held at 19 - 21 °C, relative humidity 58 - 92 %, Photoperiod 16 hours light : 8 hours dark at an intensity of 700 – 900 Lux. Wasps used in the study were less than 2 days old.
Feeding:	Cotton wool soaked with a 1:3 v/v honey and water solution
Test design	
Test vessels:	Exposure phase: Two treated glass plates fitted to a square frame (10 x 10 cm) made from metal casing. Three holes (10 mm diameter) drilled through each of the side walls provided ventilation and these were covered with discs of fine-gauge, stainless steel mesh. One hole was left uncovered as an access hole for introduction of the test organisms and was sealed with a cotton wool bung. The test cages were ventilated with an aquarium pump to prevent pesticide vapour build up Reproduction phase: Potted barley plants, enclosed within a clear acrylic

	cylinder (9 cm diameter, 20 cm high) sealed at the top with nylon netting. The plants were infested with host aphids *R. padi* and *M. dirhodum*
Substrate:	Glass plates
Replication:	Exposure phase: Four replicates per treatment each containing 10 organisms Reproduction phase: 15 replicates per treatment each containing a single organism
Duration:	Exposure phase: 48 hours Reproduction phase: 11 days
Environmental conditions	
Test temperature:	Exposure phase: 20 to 21°C Reproduction phase: 17 to 23°C
Humidity:	Exposure phase: 67 to 91% relative humidity Reproduction phase: Not reported
Lighting:	Exposure phase: 1300 to 1700 lux Reproduction phase: 5000 to 6500 lux Photoperiod of 16 hours light : 8 hours dark

Study Design and Methods

Experimental dates: 13th June to 16th July 2001

The study was performed in two phases: the exposure phase and the reproduction phase. During the exposure phase adult *A. rhopalosiphi* were exposure to residues of the test material for 48 hours and mortality assessed.

Treatments were applied to glass plates which were then used to form the floor and ceiling of shallow arenas. Ten adult wasps (including a minimum of five females) were placed in each replicate arena (n = 4 per treatment rate). Assessments of treatment effects were made after 48 hours exposure.

During the reproduction phase the effects of the test item on fecundity were assessed. Fifteen female wasps, impartially selected from each of 600 and 900 g product/ha treatment groups and the control were confined individually over untreated aphid-infested barley plants for 24 h, before being removed. The plants were left for a further 11 days before the number of aphid mummies that developed was recorded.

The percentage mortality at each treatment rate was calculated both before and after correction for mortality in the control treatment using Abbott's formula. The LR_{50} was estimated using the moving average method.

Results and Discussion

The effects of A8637C on mortality and fecundity of *A. rhopalosiphi* is summarised in the table below.

Table 8.3.2.1-2: Effects of fresh residues of A8637C on glass on mortality and fecundity of *Aphidius rhopalosiphi* under laboratory test conditions

Treatment	Rate (g product/ha)	% mortality at 48 h	Corrected % mortality	Mean mummies per memale	% reduction in fecundity (%)
Control	0	3	-	7.5	-
A8637C	600	23	21	0.5	93

Treatment	Rate (g product/ha)	% mortality at 48 h	Corrected % mortality	Mean mummies per memale	% reduction in fecundity (%)
	900	37	35	0.3	96
	1200	58	57	-	
	1500	40	38	-	
	1800	63	62	-	
Perfekthion	0.425 mL/ha	100	100	-	

A 93 % and 96 % decrease in the number of mummies produced compared to the control was observed in the 600 and 900 g product/ha treatment groups, respectively.

Validity criteria

The validity criteria were met in that:

- Mortality in the control treatment did not exceed 13% (actual was 3%).
- Mortality in the toxic reference was >50% (actual was 100%).
- Female wasps produced >5 mummies/wasp (actual was 7.5) and <2 females produced mummies (actual was 0).

Conclusion

The effects of CGA219417 WG (50) (A8637C) on the parasitic wasp, *Aphidius rhopalosiphi*, were evaluated under worst-case laboratory test conditions on glass. The 48-hour LR_{50} was estimated to be 1422.5 g product/ha and treatment rates of 600 and 900 g product/ha were found to significantly reduce wasp fecundity.

(Vinall, 2001)

A summary of a study conducted with A14325E which has not previously been submitted is presented below:

Report:	K-CA 8.3.2.1/01 Vinall S. (2005) Cyprodinil (CGA219417) 300 g L^{-1} EC formulation (A14325E): A laboratory bioassay of the effects of fresh residues on the parasitic wasp *Aphidius rhopalosiphi* (Hymenoptera, Braconidae). Mambo-Tox Ltd, Southampton, UK. Report Number SYN-05-4. (Syngenta file No. CGA219417/1297)

Guidelines

Mead-Briggs *et al*. (2000). A laboratory test for evaluating the effects of plant protection products on the parasitic wasp, *Aphidius rhopalosiphi* (De Stefani-Perez) (Hymenoptera, Braconidae).

GLP: Yes.

Executive Summary

The aim of the study was to determine the effects of fresh dry residues of A14325E on the parasitic wasp, *Aphidius rhopalosiphi* (De Stefani-Perez) (Hymenoptera, Braconidae), under worst-case laboratory test conditions on glass.

A14325E was evaluated at six application rates, equivalent to 3000, 1950, 750, 375, 93.67 and 23.33 mL product/ha. Also included in the definitive test were a water-treated control and a toxic reference treatment of BASF Perfekthion (nominally 400 g/L dimethoate), applied at a rate of 0.20 mL product/ha (0.08 g a.s./ha). All treatments were applied to glass plates at a volume rate of 200 L/ha. Assessments of treatment effects were made over 48 h. To assess any sub-lethal effects, reproduction assessments were then carried out for insects from the control and from all treatment rates of the test item that had resulted in < 50% corrected mortality. Fifteen female wasps were confined individually over untreated aphid-infested barley plants for 24 h, before being removed. The plants were left for a further 10 days before the number of aphid mummies that developed was recorded.

The median lethal rate (LR_{50}) was estimated to be 241.1 mL product/ha. A14325E had no effect >50% on the fecundity of the wasps at 23.33 mL product/ha, the only rate tested for fecundity.

Materials

Test Material:	A14325E
Description:	Clear amber-coloured fluid, nominally containing 300 g/L CGA219417 (cyprodinil)
Lot/Batch #:	SMU5BP002
Purity:	303 g/L
Stability of test compound:	The test item is assumed to be stable for the period of use in the study, pending concurrent batch analysis.
Test rates:	3000, 1950, 750, 375, 93.67 and 23.33 mL A14325E/ha
Vehicle and control:	Purified water
Toxic reference:	Perfekthion EC (400 g dimethoate/L) in deionised water (0.2 mL/ product ha)
Spray volume rate:	200 L spray solution/ha
Application method:	Potter Laboratory Spray Tower, calibrated for each treatment preparation.
Test organisms	
Species:	*Aphidius rhopalosiphi* De Stefani-Perez. (Hymenoptera: Braconidae)
Source:	Culture maintained at Test Facility on cereal aphids (*Metopolophium dirhodum* and *Rhopalosiphum padi*).
Food:	1:3 v/v solution of honey in water or 10% w/v solution of fructose in water.
Test substrate:	Glass plates
Environmental test conditions	
Temperature:	Mortality assessment phase: 20 to 21°C Fecundity assessment phase: 19 to 21°C
Humidity:	Mortality assessment phase: 65 to 84% relative humidity
Photoperiod:	Mortality assessment phase: 16 h daily photoperiod (1100-1650 lux) Fecundity assessment phase: 16 h daily photoperiod (4100-4500 lux)

Study Design and Methods

Experimental dates: 29th March to 11th April 2005.

Treatments were applied to glass plates which were then used to form the floor and ceiling of shallow arenas. Ten adult wasps (including a minimum of five females) were placed in each replicate arena (n = 4 per treatment rate). Assessments of treatment effects were made at 2, 24 and 48 h. To assess any sub-lethal effects, fecundity assessments were then carried out for insects from the control and from all treatment rates of the test item that had resulted in < 50% corrected mortality. Fifteen female wasps were confined individually over untreated aphid-infested barley plants for 24 h, before being removed. The plants were left for a further 10 days before the number of aphid mummies that developed was recorded.

Results and Discussion

Table 8.3.2.1-13: Effects of fresh residues of A14325E on glass on mortality and fecundity of *Aphidius rhopalosiphi* under laboratory test conditions

Treatment	Rate (mL product/ha)	% mortality at 48 h [a]	Corrected % mortality at 48 h	Mean number mummies per surviving female [a]	Effect on reproduction [b] (%)
Control	0	0	-	40.4	-
A14325E	3000	80	100	n.a.	-
	1950	83	55	n.a.	-
	750	65	0	n.a.	-
	375	65	0	n.a.	-
	93.67	55	0	n.a.	-
	23.33	0	0	30.8	24
Perfekthion	0.2	100	95	n.a.	-

[a] The results for the test item treatment and control were compared by t-test for unmatched pairs but they did not differ significantly (P > 0.05).
[b] A positive value indicates a decrease in reproduction, relative to the control.
n.a. not assessed in this treatment.

A Probit analysis on these data indicated that the 48-h LR_{50} was 241.1 mL A14325E/ha (95% confidence limits = 123.6 and 410.7mL product/ha).

Conclusion

The effects of A14325E on the parasitic wasp, *Aphidius rhopalosiphi*, were evaluated under worst-case laboratory test conditions on glass. The median lethal rate (LR_{50}) for the test item was estimated to be 241.1 mL product/ha. A14325E had no statistically significant or >50% effect on the fecundity of the wasps at 23.33 mL product/ha, the only rate tested for fecundity.

(Vinall S, 2005)

CA 8.3.2.2 Effects on *Typhlodromus pyri*

The studies previously submitted in the EU were evaluated in point B.9.5.1 of the DAR. At the request of ANSES summaries have been included in order to aid dossier evaluation ~~therefore additional study summaries are not repeated here.~~

Report:	Summary submitted at the request of ANSES for ease of dossier evaluation: *KIIA 8.3.2.2. Grimm C. (1999). Acute Toxicity of CGA 219417 WG 50 (A 8637 C) to the Predacious Mite Typhlodromus pyri Scheuten (Acari: Phytoseiidae). Report Number 983969. Novartis Crop Protection AG, CH-4002 Basel, Switzerland. (Syngenta file No. CGA219417/0923).*

Guidelines

Blümel *et al*. (1998). Laboratory residual contact test of plant protection products on the predatory mite *Typhlodromus pyri* (Scheuten). Ringtest protocol, revised version.

Louis, F. and Ufer, A. (1995). Methodical improvements of standard laboratory tests for determining the side-effects of agrochemicals on predatory mites (Acrai: Phytoseiidae). Anz. Schädlingskde, Pflanzenschutz, Umweltschutz 68: 153-154

Overmeer, W. P. J. (1988). Laboratory method for testing side-effects of pesticides on the predacious mites *Typhlodromus pyri* and *Amblyseius potentillae* (Acrai: Phytoseiidae). IN: IOBC Working Groups Pesticides and Beneficial Organisms. IOBC/WPRS Bulletin XI/4: 65-69

GLP: Yes

Executive Summary

The aim of the study was to determine the effects of dry residues of A8637C on the predacious mite, *Typhlodromus pyri*, under worst-case laboratory test conditions.

A8637C was evaluated at three application rates, equivalent to 90, 450 and 900 g product/ha.

No significant mortality was observed in any A8637C treatment group during the exposure. The mean number of eggs laid per female was significantly lower in the 900 g product/ha treatment, compared to the control.

Materials

Test Material	CGA219417 WG 50
Lot/Batch #:	609025
Formulation code:	A8637C
Active Ingredient Content:	51.3% CGA219417
Stability of the test compound:	Not reported
Description:	Beige granules
Reanalysis/expiry date:	April 2000
Treatments	
Test treatments:	90, 450 and 900 g/ha of A8637C
Solvent:	None
Positive control:	Perfekthion (dimethoate), applied at 9 mL/ha
Application method:	Calibrated laboratory sprayer
Spray volume:	200 L spray solution/ha

Test organism

Species:	*Typhlodromus pyri* (Acari: Phytoseiidae), protonymph stage
Source:	Culture maintained at Test Facility
Feeding:	Mixture of walnut (*Juglans regia*) and apple (*Malus vulgaris*) pollen

Test design

Substrate:	Glass plates formed from two microscope slide cover slips, each approximately 2.5 cm x 5 cm
Replication:	Five replicates, each containing 20 test organisms, for each treatment group
Duration:	14 days

Environmental conditions

Test temperature:	24.5 to 25.5°C
Humidity:	74 to 80%
Lighting:	Intensity 1116 to 1500 lux Photoperiod of 16 hours light : 8 hours dark

Study Design and Methods

Experimental dates: 15th to 29th June 1999

The test substrate comprised glass plates formed from two microscope slides, each approximately 2.5 cm x 5 cm. The microscope slides were treated with stock solutions of the test material using a calibrated laboratory sprayer. Following treatment, the glass plates were left to dry and then placed onto damp filter paper, with their treated surface uppermost. The bioassay was initiated approximately 1 h after treatments were applied, *i.e.* once residues on the treated glass plates had dried. The test arena measured 13 cm^2 and was limited by a barrier of "Tangle-Trap" glue to prevent mites escaping.

Twenty protonymphal *T. pyri* were placed within each replicate arena, with five replicates (100 mites in total) prepared per treatment. The mites were fed regularly with untreated pollen for food. Their survival was assessed over a 7-day period. The sex of the adult mites was determined and they were then left *in situ* so that their survival and reproduction could be assessed over a further 7 days. These further assessments were carried out for the control and for all treatments, with the exception of the toxic reference group. The mean number of eggs produced per female between 7 and 14 days after treatment (DAT) was calculated.

Results and Discussion

No significant mortality was observed in any A8637C treatment group compared to the control after 14 days exposure. No attempt was made to estimate the LR_{50} values for the effects of A8637C on *Typhlodromus pyri*. No treatment A8637C group resulted in ≥50 % mortality and therefore the LR_{50} can be considered to be >900 g A8637C/ha.

Table 8.3.2.2-1: Effects of fresh residues of A8637C on glass on mortality and fecundity of *Typhlodromus pyri*, under laboratory test conditions

Treatment	Rate (g/ha)	Mean % mortality 14 DAT	Corrected % mortality 14 DAT	Mean number of eggs per female
Control	-	10	0	1.46
A8637C	90	12	-3.2	1.43
	450	9	-3.2	1.34
	900	18	2.2	1.03[b]
Perfekthion	9 mL/ha	99[a]	98.9	-

[a] Statistically significant difference from control (2 x 2 contingency table; $p \leq 0.05$)
[b] Statistically significant difference from control (Dunnett test; $p \leq 0.05$)

A significant reduction in the number of eggs laid per surviving females was observed in the 900 g product/ha treatment group.

Validity Criteria

The study was considered valid as the following criteria were fulfilled;

- Mortality in the control was not above 20 % (actual: 0 %)
- Mortality in the toxic reference treatment was not below 50 % (actual: 98.9 %)

Conclusion

The effects of A8637C on the predacious mite, *Typhlodromus pyri*, were evaluated under worst-case laboratory test conditions on glass. No A8637C application rate resulted in ≥50 % mortality and therefore the LR_{50} can be considered to be >900 g product/ha. A8637C caused no significant mortality at 900 g product/ha and had no statistically significant effects on the fecundity of the mites at 450 g product/ha.

(Grimm, 1999)

Report:	Summary submitted at the request of ANSES for ease of dossier evaluation: *KIIA 8.3.2.2. Taruza S. (2001). CGA 219417: A rate-response laboratory test to evaluate the effects of a 500 g/kg WG formulation (A-8637 C) on the predatory mite, Typhlodromus pyri. Report Number 2003798. Mambo-Tox Ltd., Southampton, UK. (Syngenta file No. CGA219417/1057).*

Guidelines

Blümel *et al*. (2000). Laboratory residual contact test with the predatory mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) for regulatory testing of plant protection products.

GLP: Yes

Executive Summary

The aim of the study was to determine the effects of dry residues of A8637C on the predacious mite, *Typhlodromus pyri*, under worst-case laboratory test conditions.

A8637C was evaluated at three application rates, equivalent to 90, 450 and 1800 g product/ha.

No significant mortality was observed in any A8637C treatment group during the exposure. The mean number of eggs laid per female was significantly lower in the 450 and 1800 g product/ha treatment groups, compared to the control.

Materials

Test Material	CGA219417 WG 50
Lot/Batch #:	WM910165
Formulation code:	A8637C
Active Ingredient Content:	50.9% CGA219417
Stability of the test compound:	Not reported
Description:	Not reported
Reanalysis/expiry date:	March 2003
Treatments	
Test treatments:	90, 450 and 1800 g/ha of A8637C
Solvent:	None
Positive control:	Perfekthion (dimethoate), applied at 15 ml product/ha (6 g a.s./ha)
Application method:	Calibrated Potter Laboratory Spray Tower at spray pressure of 0.7 bar
Spray volume:	200 L spray solution/ha
Test organism	
Species:	*Typhlodromus pyri* (Acari: Phytoseiidae), protonymph stage
Source:	Culture maintained at test facility originating from PK Nützlingszuchten, Welzheim, Germany
Feeding:	1:1 v/v mixture of walnut (*Juglans regia* L.) and apple (*Malus* sp. var. Winter Banana)
Test design	
Substrate:	Glass plates formed from two microscope slides, each 22 mm x 40 mm in area and thickness No.1 (i.e. 0.13-0.16 mm).
Replication:	Five replicates, each containing 20 test organisms, for each treatment group
Duration:	14 days
Environmental conditions	
Test temperature:	23 to 25 °C
Humidity:	64 to 76%
Lighting:	Intensity 350 to 435 lux

Photoperiod of 16 hours light : 8 hours dark

Study Design and Methods

Experimental dates: 15th to 29th June 1999

The test substrate comprised glass plates formed from two microscope slide cover each 22 mm x 40 mm in area and thickness No.1 (i.e. 0.13-0.16 mm). The test material was applied to the surface of the glass slides using a laboratory sprayer and the bioassay was initiated approximately 1 h after treatments were applied, *i.e.* once residues on the treated glass plates had dried. A ring of 'Non-Drying Insect Glue' was drawn on each plate to make an arena in which to confine the mites.

Twenty protonymphal *T. pyri* were placed within each replicate arena, with five replicates (100 mites in total) prepared per treatment. The mites were fed regularly with untreated pollen for food. Their survival was assessed at approximately 24 hours and 7 days after treatment. The sex of the adult mites was determined and they were then left *in situ* so that their survival and reproduction could be assessed over a further 7 days. These further assessments were carried out for the control and for all treatments, with the exception of the toxic reference group. The mean number of eggs produced per female between 7 and 14 days after treatment (DAT) was calculated.

Results and Discussion

No significant mortality was observed in any A8637C treatment group compared to the control after 14 days exposure. No attempt was made to estimate the LR_{50} values for the effects of A8637C on *Typhlodromus pyri*. No treatment A8637C group resulted in ≥50% mortality and therefore the LR_{50} can be considered to be higher than the top test application rate.

Table 8.3.2.2-2: Effects of fresh residues of A8637C on glass on mortality and fecundity of *Typhlodromus pyri*, under laboratory test conditions

Treatment	Rate (g/ha)	Mean % mortality 7 DAT	Corrected % mortality 7 DAT	Mean number of eggs per female [a]	% egg production relative to control
Control	-	15	-	7.2	-
A8637C	90	15	0	6.3	-13
	450	6	0	3.5[a]	-51
	1800	14	0	1.9[a]	-74
Perfekthion	6 g a.s./ha	94[a]	93	-	-

[a] Statistically significant difference from control (ANOVA, $p < 0.001$)

A significant reduction in the number of eggs laid per surviving females was observed in the 450 and 1800 g product/ha treatment groups.

Validity Criteria

The study was considered valid as the following criteria were fulfilled;

- Mortality in the control was not above 20 % (actual: 0 %)
- Mortality in the toxic reference treatment was not below 50 % (actual: 98.9 %)
- Mean number of eggs produced between days 7 to 14 was >4 in the control group (actual: 7.2)

Conclusion

The effects of A8637C on the predacious mite, *Typhlodromus pyri*, were evaluated under worst-case laboratory test conditions on glass. No A8637C application rate resulted in ≥50 % mortality and therefore the LR_{50} can be considered to be >1800 g product/ha. A8637C caused no significant mortality at 1800 g product/ha and had no statistically significant effects on the fecundity of the mites at 90 g product/ha. At rates of 450 and 1800 g product/ha mite fecundity was significantly reduced by A8637C.

(Taruza, 2001)

A summary of a study conducted with A14325E which has not previously been submitted is presented below:

Report:	K-CA 8.3.2.2/01 Vaughan R. (2005) Cyprodinil (CGA219417) 300 g L^{-1} EC formulation (A14325E): A laboratory bioassay of the effects of fresh residues on the predatory mite, *Typhlodromus pyri* (Acari: Phytoseiidae). Mambo-Tox Ltd, Southampton, UK. Report Number SYN-05-5. (Syngenta file No. CGA219417/1298)

Guidelines

Blümel *et al*. (2000). Laboratory residual contact test with the predatory mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) for regulatory testing of plant protection products.

GLP: Yes.

Executive Summary

The aim of the study was to determine the effects of fresh dry residues of A14325E on the predatory mite, *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae), under worst-case laboratory test conditions on glass.

A14325E was evaluated in a bioassay at five rates, equivalent to 3000, 1950, 750, 375 and 93.67 mL product/ha. Also included in the definitive test were a control treatment of purified water and a toxic reference treatment of BASF Perfekthion (nominally 400 g/L dimethoate) applied at a rate of 15 mL product/ha (nominally 6 g a.s./ha). All treatments were applied to glass plates at a volume rate equivalent to 200 L spray solution/ha. Mortality was evaluated over 7 days exposure, and fecundity over a further 7 days.

Under worst-case laboratory test conditions, the 7-day LR_{50} (median lethal rate) for *Typhlodromus pyri* was equivalent to 1943 mL A14325E/ha. A14325E had no effects >50% on fecundity at rates up to 750 mL/ha, the highest tested for fecundity.

Materials

Test Material:	A14325E
Description:	Clear amber-coloured fluid, nominally containing 300 g/L CGA219417 (cyprodinil)
Lot/Batch #:	SMU5BP002
Purity:	303 g/L
Stability of test compound:	The test item is assumed to be stable for the period of use in the study, pending concurrent batch analysis.
Test rates:	3000, 1950, 750, 375 and 93.67 mL A14325E/ha
Vehicle and control:	Purified water
Toxic reference:	Perfekthion EC (400 g dimethoate/L) in deionised water (15 mL product/ha)
Spray volume rate:	200 L spray solution/L

Application method:	Potter Laboratory Spray Tower, calibrated for each treatment preparation.
Test organisms	
Species:	*Typhlodromus pyri* (Acari: Phytoseiidae).
Source:	Culture maintained at Test Facility.
Food:	1:1 v/v mixture of walnut (*Juglans regia* L.) and apple (*Malus* sp. var. Winter Banana)
Test substrate:	Glass plates formed from two microscope slide cover slips, each 22 mm x 40 mm in area and thickness No.1 (i.e. 0.13-0.16 mm).
Environmental test conditions	
Temperature:	24 to 26°C
Humidity:	49 to 81% relative humidity (fluctuations below 60% RH were for short periods, not more than 2 h).
Photoperiod:	16 h daily photoperiod (600-1500 lux)

Study Design and Methods

Experimental dates: 22nd March to 5th April 2005.

The test substrate comprised glass plates formed from two microscope slide cover slips, each 22 mm x 40 mm in area. Following treatment, the glass plates were left to dry and then placed onto damp tissue paper, with their treated surface uppermost. The bioassay was initiated approximately 1 h after treatments were applied, *i.e.* once residues on the treated glass plates had dried. An oblong-ring of 'Non-Drying Insect Glue' was drawn on each plate to make an arena in which to confine the mites. Twenty protonymphal *T. pyri* were placed at the centre of each replicate arena, with four replicates (80 mites in total) prepared per treatment. The mites were fed regularly with untreated pollen for food. Their survival was assessed over a 7-day period, by which time they were adult. The sex of the adult mites was determined and they were then left *in situ* so that their reproduction could be assessed over a further 7 days. These further assessments were carried out for the control and for treatment rates of the test item that had resulted in < 50% corrected mortality. The mean number of eggs produced per female between 7 and 14 days after treatment (DAT) was calculated.

Results and Discussion

Table 8.3.2.2-1: Effects of residues of A14325E on glass on mortality and fecundity of the mite, *Typhlodromus pyri*, under laboratory test conditions

Treatment	Rate (mL/ha)	Mean % mortality 7 DAT	Corrected % mortality 7 DAT	Mean number of eggs per female [a]	Effects on reproduction [b] (%)
Control	-	19	-	8.2	-
A14325E	3000	70	63	n.a.	-
	1950	61	52	n.a.	-
	750	38	23	4.5 *	45
	375	29	12	5.9	28
	93.67	21	3	9.2	-12
Perfekthion	15	99	98	-	-

[a] Treatments compared by one-way ANOVA and Dunnett's Test ($\alpha = 0.05$). Asterisks indicate test item treatments that differed significantly from the control (* $P < 0.05$).

[b] Change in numbers of eggs per female, relative to control (after Blümel *et al*., 2000). A positive value indicates a decrease and a negative value an increase.
n.a. not tested at this rate.

A Probit regression analysis indicated that the 7-day LR_{50} (median lethal rate) was 1943 mL product/ha (95% confidence limits = 1437 and 2924 mL product/ha).

Conclusion

The effects of A14325E on the predatory mite, *Typhlodromus pyri*, were evaluated under worst-case laboratory test conditions on glass. The 7-day LR_{50} (median lethal rate) was determined as being 1943 mL product/ha. A14325E had no statistically significant effect on the reproduction of mites at rates of up to and including 375 mL product/ha, and no effects >50% up to 750 mL/ha (the highest rate tested for fecundity).

(Vaughan R, 2005)

Relevant Literature on Non-Target Arthropods other than Bees

No relevant scientifically peer-reviewed open literature could be found on cyprodinil or its major metabolites. Details of the literature search undertaken can be found in M-CA Section 9 of the current submission.

CA 8.4 Effects on Non-Target Soil Meso- and Macrofauna

Table 8.4-1: Table of endpoints for worm studies with cyprodinil and metabolites

Organism	Test item	Test type	Endpoint		Endpoint used for the risk assessment	Reference (author, date, Syngenta File No.)
Earthworm	Cyprodinil	Acute	EU	LC_{50} = 192 mg/kg soil d.w	-	***Vial (1990) CGA 249417/0015***
	Cyprodinil (A8779A)			LC_{50} = 272 mg UNIX /kg soil d.w. (204 mg/kg a.s./kg soil d.w.)		***Grade (1993) CGA 219419/0218***
				LC_{50} = >1000 mg/kg soil d.w.		***Koenig (1995) CGA219417/0769***
	CGA249287			LC_{50} = >1000 mg/kg soil d.w		***Batscher (1999) CGA249287/0009***
	CGA275535			LC_{50} = >1000 mg/kg soil d.w		***Pfeifle (2001) CG275535/0012***
	Cyprodinil (A8779A)	Chronic and reproductive	EU	NOEC = 3.75 kg a.s./ha (≅ 5 mg a.s./kg)	20 mg a.s./kg [b]	***Nienstedt (2001) CGA219417/1029 EC_{10}/EC_{20} estimate Taylor & Joyce (2015) CGA8779A_10235***
			EC_{10}/EC_{20} [a]	Not possible to estimate due to lack of a significant concentration response		
	Cyprodinil (A8779A)		EU	NOEC = 15 kg a.s./ha (≅ 20 mg a.s./kg)		***Ehlers (2001) CGA219417/1028 EC_{10}/EC_{20} estimate Taylor & Pickering (2015) CGA8779A_10237***
			EC_{10}/EC_{20} [a]	Not possible to estimate due to lack of a significant concentration response		
	CGA249287		EU	NOEC = 1.13 mg/kg soil d.w.	NOEC = 1.13 mg/kg	***Pfeifle (2001) CGA249287/0020 Taylor & Pickering (2015) CGA249287_10008***
			EC_{10}/EC_{20} [a]	Not possible to estimate due to lack of a significant concentration response		
	CGA275535		New	NOEC (reproduction) = 556 mg/kg soil d.w.; EC_{10} = 385 mg/kg; EC_{20} = 638 mg/kg	EC_{10} = 385 mg/kg	***Lührs (2014) CGA275535_10002***
	CGA321915			NOEC/EC_{10}/EC_{20} (reproduction) = 1000 mg/kg soil d.w.	NOEC/EC_{10} = 1000 mg/kg	***Lührs (2015) CGA321915_10012***

[a] Values estimated in accordance with **Commission Regulation (EU) No 283/2013**

[b] For the tests conducted by Neinstedt and Ehlers the NOEC values represent the highest concentrations tested. Therefore the endpoint of 20 mg a.s./kg derived by Ehlers 2101 will be used for the risk assessment

Table 8.4-2: Table of endpoints for soil meso- and macro-fauna (other than earthworms) with cyprodinil and metabolites

Organism	Test item	Test type	Endpoint		Endpoint used for the risk assessment	Reference (author, date, Syngenta File No.)
Folsomia candida	Cyprodinil [a]	28 day chronic	New	NOEC = 29.4 mg A14325E/kg (8.67 mg a.s./kg); EC_{10} = 53.2 mg A14325E//kg (15.7 mg a.s./kg); EC_{20} = 67.7 mg A14325E (20 mg a.s./kg)[c]	NOEC = 29.4 mg a.s./kg ~~; EC_{10} = 15.7 mg a.s./kg~~	***Lührs (2014) A14325E_10061***
	Cyprodinil [b]			NOEC/EC_{10}/EC_{20} = 105 mg A8637C/kg (52.5 mg a.s./kg) [d, e]		***Lührs (2014) A8637C_10314***
			$EC_{10/20}$	Not possible to estimate due to lack of a significant concentration response		***EC_{10}/EC_{20} estimate Taylor & Pickering 2016) A8637C_10368***
	CGA249287			NOEC = 31 mg/kg soil; EC_{10} = 7.9 mg/kg; EC_{20} = 22.7 mg/kg	EC_{10} = 7.9 mg/kg	***Vinall (2012) CGA249287/10003***
	CGA275535			NOEC = 171.5 mg/kg soil [f]	NOEC = 171.5 mg/kg	***Lührs (2014) CGA275535_10004***
	CGA321915			NOEC/EC_{10}/EC_{20} = 1000 mg/kg soil d.w. [e]	NOEC/EC_{10} = 1000 mg/kg	***Lührs (2015) CGA321915_10010***
Hypoaspis aculeifer	Cyprodinil [a]	14 day chronic		NOEC/EC_{10}/EC_{20} = 1000 mg A14325E/kg soil (295 mg a.s./kg) [c, e]	NOEC/EC_{10} = 277.8 mg/kg	***Lührs (2014) A14325E_10062***
	Cyprodinil [b]			NOEC/EC_{10}/EC_{20} = 555.6 mg A8637C/kg (277.8 mg a.s./kg) [d, f]		***Lührs (2014) A8637C_10312***
	CGA249287			NOEC = 74 mg/kg soil; EC_{10} = 70.5 mg/kg; EC_{20} = 321.3 mg/kg	EC_{10} = 70.5 mg/kg	***Schultz (2014) CGA249287_10005***
	CGA275535			EC_{10} = 171.5; EC_{10} = 104.6 mg/kg; EC_{20} = 272.5 mg/kg	EC_{10} = 104.6 mg/kg	***Lührs (2014) CGA275535_10000***
	CGA321915			NOEC/EC_{10}/EC_{20} = 1000 mg/kg soil	NOEC/EC_{10} = 1000 mg/kg	***Lührs (2015) CGA321915_10011***

[a] Tested as A14325E
[b] Tested as A8637C
[c] Concentrations converted to active substance content based on nominal formulation composition of 295 g cyprodinil/L
[d] Concentrations converted to active substance content based on nominal formulation composition of 500 g cyprodinil/kg
[e] It was not possible to estimate EC_{10} or EC_{20} values as the NOEC was derived for the highest concentration tested
[f] It was not possible to estimate EC_{10} or EC_{20} values as a significant concentration response could not be derived

Comment from the RMS: *Effects on reproduction of A8637C on Folsomia candida (K-CA 8.4.2.1/0 1; Lührs, 2014a): The 22.4% reduction of reproduction at the NOEC (105 mg A8637C/kg) can be considered as biologically relevant. In this case, a NOEC of 58.3 mg A8637C/kg or calculating an EC10 should be more relevant for risk assessment. Please provide an EC10 calculation, as well as its confidence interval.*

Response from Syngenta: An attempt was made to re-analyse the data. It was not possible to determine EC_{10} or EC_{20} values. A summary of the statistical analysis is presented in Section 8.4.2.1.

Summaries of studies conducted following the original submission of cyprodinil are presented below. The endpoints are summarised in Table 8.4-2.

CA 8.4.1 Earthworms – sub-lethal effects

The studies previously submitted in the EU were summarised in point B.9.6.2 of the DAR At the request of ANSES summaries have been included in order to aid dossier evaluation ~~therefore additional study summaries are not repeated here.~~ The endpoints are summarised in Table 8.4-1 above.

Report:	Summary submitted at the request of ANSES for ease of dossier evaluation: *KIIA 8.4.2. Ehlers, H. A. (2001). Effects of CGA 219417 75 WG (A-8779 A) on Reproduction and Growth of Earthworms Eisenia fetida (Savigny 1826) in Artificial Soil. Report Number 2013503. IBACON GmbH, Rossdorf, Germany. (Syngenta file No. CGA219417/1028).*

Guidelines

BBA-Richtlinien für die Prüfung von Pflanzenschutzmitteln im Zulassungsverfahren, Teil VI, 2-2 (1994)

International Standard: ISO guideline 11268-2, Part 2 (1998)

GLP: Yes

Executive Summary

In a chronic toxicity test in which earthworms (*Eisenia fetida*) were exposed to CGA219417 75 WG (A8779A) the NOEC (based on reproduction) was determined to be 20.0 kg A8779A/ha. No significant effects were observed on adult survival, body biomass or reproduction at any of the A8779A rates tested.

Materials

Test Material	CGA219417 75 WG
Formulation code:	A8779A
Lot/Batch #:	WM 902997
Content of active ingredient:	75% CGA219417
Description:	Beige to light brown solid
Stability of test compound:	Stable under test conditions
Reanalysis/Expiry date:	November 2001
Treatments	
Test rates:	2, 10, 20 kg A8779A/ha
Control:	Deionised water
Toxic standard:	Derosal SC 360 g/L (31.1 % carbendazim)
Solvent:	None
Test organisms	
Species:	*Eisenia fetida andrei* (Savigny 1826)
Age and weight range at test start:	Adults (approximately 9 months old) with clitellum; Weight range was 345 to 545 mg
Source:	Culture maintained at test facility

Feeding:	10 g/kg finely ground cow manure was mixed into artificial soil at the start of the study. 5 g finely ground cow manure was scattered on the soil surface in each test vessel after application and weekly for the first 3 weeks of the exposure. Four weeks after application 5 g manure was mixed into the substrate in each test vessel.

Test design

Vessels:	Plastic containers (18.3 x 13.6 x 6 cm) covered with perforated lids. Filled with 646 g (wet weight) test soil (equivalent to 500 g dry weight)
Substrate:	Artificial soil comprising 10 % sphagnum peat, 20 % kaolin clay containing > 30 % kaolinite, 69.5 % fine quartz sand (> 50% of the particles between 0.05 mm and 0.2 mm), approximately 0.5 % calcium carbonate
Replication:	Four replicate vessels per treatment and control
No. of worms/replicate :	10
Duration of test:	8 weeks

Environmental test conditions

Temperature:	19 to 21°C
pH of soil:	Test start: 5.9 Test end: 5.7 to 5.9
Water content of soil:	Test start: 54.1 % of water holding capacity Test end: 57.0 to 60.6 % of water holding capacity
Photoperiod:	16 hours light, 8 hours dark (intensity 407 to 700 lux)

Study Design and Methods

Experimental dates: 22nd March to 30th May 2001

Three application solutions were prepared by adding 3.33, 16.67 or 33.33 g of A8779A to a graduated flask and filling with 1000 mL deionised water. The application solutions were then sprayed onto the soil surface using a laboratory sprayer using a 8006 EVS spray nozzle and an application rate of 600 L spay liquid/ha. For the reference treatment 5.36 g Derosal SC was suspended in 1000 mL deionised water and sprayed onto the soil. The applications occurred after the test organisms had been introduced into the test vessels and had burrowed into the soil. The control treatment was sprayed with deionised water only.

The worms were washed, dried and individually weighed prior to addition to the test vessels. After four weeks, the adult worms were removed from the test vessels. Mortality and body weight of the surviving worms was determined. The test soils were then incubated for an additional four weeks, following which the number of hatched juveniles per test container was determined. Once a week, during the exposure period, the water content was checked by weighting each test vessel. Water was added using a hand sprayer, where necessary to maintain the water content within 10 % of the initial values.

Data concerning adult worm bodyweight and reproduction in the A8779A treatments were compared to the control group using Dunnett-test ($\alpha = 0.05$, two-sided).

Results and Discussion

Mortality and reproduction are summarised in the table below.

Table 8.4.1-1: Effect of A8779A on mortality, growth and reproduction of *Eisenia fetida*

Endpoints	Treatment groups (kg A8779A/ha)				
	Control	2.0	10.0	20.0	Toxic Standard
Mean adult mortality at 28 days (%)	0	0	0	0	0
Mean % bodyweight change of adults from 0-28 days	29.3	34.5	32.1	34.2	4.0[a]
Mean number of juveniles after 8 weeks	261	216	268	264	48[a]
NOEC (biomass, reproduction)	20 kg A8779A/ha				n.a.

[a] Significantly different from control (Student t-test $\alpha = 0.05$)

No significant effects were observed on mortality, bodyweight change or reproduction at any of the A8779A application rates tested.

Validity criteria

The test is considered valid as:

- Adult mortality was 0 % in the control (≤ 10 % required)
- The mean number of juveniles in the four control replicates was 261 (> 30 required)
- The coefficient of variation in the control was 12.8 % (< 30 % required)
- Control worms weight increased by 29.3 % (≤ 20 % bodyweight loss required)

The reproduction and bodyweight of the worms in the toxic standard treatment were significant different from the control group, indicating the test organisms were sufficiently sensitive.

Conclusions

Exposure for 28 days to A8779A at rates up to 20.0 kg A8779A/ha did not affect the survival, body weight or repordution of the earthworm, *Eisenia fetida*.

(Ehlers, 2001)

Study data have been re-evaluated for the purposes of calculating EC_{10} and EC_{20} values. The outcome of the re-evaluation is provided below.

Report:	Submitted for purposes of renewal due to change in data requirements guidance: K-CA 8.4.1/01 Taylor S. & Joyce F. (2015) Cyprodinil (A8779A) – Effects of A8779A on reproduction and growth of earthworms *Eisenia fetida* (Savigny 1826) in artificial soil. Statistical Re-analysis. Report Number CEA.1410. Cambridge Environmental Assessments, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK. (Syngenta File No: A8779A_10236)

Summary

Report number 10291022 (Ehlers, 2001) did not provide estimates of the EC_{10} and EC_{20} for the effects of A8779A on body weight change, feeding activity or reproductive toxicity to the earthworm *Eisenia fetida.* Consequently the data generated in this study have been re-analysed in order to provide these values.

No EC_{10} or EC_{20} values for reproduction or biomass could be reliably calculated due to a lack of significant dose response relationships.

Statistical Analysis

The total amount of food provided and the number of juveniles at the end of the test were analysed. Probit analysis with linear maximum likelihood regression was used to determine the concentration response function. Chi² was used as a goodness of fit measure.

The sum of bodyweight of surviving adult earthworms were analysed as a proportion of the sum of initial body weight. Probit analysis with linear maximum likelihood regression was used to determine the concentration response function. Chi² was used as a goodness of fit measure and the proportion of variance explained by the dose/response function was determined.

All computations were carried out in the Statistical program: ToxRat Professional 2.10.05 (ToxRat Solutions GmbH, 2001-2010).

Results

There was no difference between treatments and controls and therefore no dose response in either the number of juveniles (p(F) = 0.371), feeding activity (p(F) = 0.156) or bodyweight changes (p(F) = 0.219). No EC_{10} or EC_{20} values could be calculated.

Conclusion

No EC_{10} or EC_{20} values for reproduction or biomass could be reliably calculated due to a lack of significant concentration response relationships.

(Taylor S. & Joyce F. 2015)

Report:	Summary submitted at the request of ANSES for ease of dossier evaluation: *KIIA 8.4.2. Nienstedt, K. M. (2001). A Chronic Toxicity and Reproduction Test Exposing Eisenia fetida to CGA 219417 75 WG (A-8779 A) in OECD Artificial Soil. Report Number 983972. Springborn Laboratories (Europe) AG, Horn, Switzerland. (Syngenta File No. CGA219417/1029).*

Guidelines

BBA-Richtlinien für die Prüfung von Pflanzenschutzmitteln im Zulassungsverfahren, Teil VI, 2-2 (1994)

International Standard: ISO guideline 11268-2, Part 2 (1998)

GLP: Yes

Executive Summary

In a chronic toxicity test in which earthworms (*Eisenia fetida*) were exposed to CGA219417 75 WG (A8779A) the NOEC (based on reproduction) was determined to be 3750 g a.s./ha. No significant effects were observed on adult survival, body biomass or reproduction at any of the A8779A concentrations tested.

Materials

Test Material	CGA219417 75 WG
Formulation code:	A8779A
Lot/Batch #:	WM 902997
Content of active ingredient:	75% CGA219417
Description:	Beige to light brown granules
Stability of test compound:	Stable under test conditions
Reanalysis/Expiry date:	November 2001
Treatments	
Test concentrations:	750 and 3750 g a.s./ha
Control:	Deionised water
Toxic standard:	Carbendazime S, separate study performed between 25 May to 24 July 2000
Solvent:	None
Test organisms	
Species:	*Eisenia fetida*
Age and weight range at test start:	Adults (8 to 9 months old) with clitellum; Weight range was 480 to 588 mg
Source:	Culture maintained at test facility
Feeding:	0.01 g dry cattle manure / g dry soil was added to the artificial soil. Fed weekly with 6.0 g dried cattle manure starting one day after application
Test design	
Vessels:	Plastic containers (17 x 12.5 x 10 cm) covered with perforated lids. Filled with 550 g dry weight test soil
Substrate:	Artificial soil comprising 10 % sphagnum peat, 20 % kaolin clay with > 30 % kaolinite, 70 % industrial sand (> 50% of the particles between 0.05 mm and 0.2 mm), approximately 0.3 % calcium carbonate. Moisture content adjusted to 60 % of the maximum water holding capacity before start of test
Replication:	Six control replicates and four replicates per A8779A treatment
No. of worms/replicate :	10
Duration of test:	8 weeks
Environmental test conditions	
Temperature:	18 to 20.5 °C
pH of soil:	Test start: 5.8 to 5.9 Test end: 7.0 to 7.1
Water content of soil:	Test start: 61.0% of water holding capacity Test end: 69.0% of water holding capacity
Photoperiod:	16 hours light, 8 hours dark (intensity 424 to 546 lux)

Study Design and Methods

Experimental dates: 17th February to 14th April 2000

A8779A was applied to soil at 750 and 3750 g a.s./ha, which is equivalent to 1 times and 5 times the application rate of 750 g a.s./ha. A stock solution of 6.25 g a.s./L was prepared by dissolving 4.1664 g of A8779A in deionised water to a total volume of 500 mL, this solution was used as the application solution for the 3750 g a.s./ha treatment rate. An aliquot of 100 mL of the stock solution was diluted with deionised water to a total volume of 500 mL to obtain the application solution for the 750 g a.s./ha treatment.

After the worms had borrowed into the soil the stock solutions were applied using an SL Conformal Spray System with an application rate of 600 L/ha. The control treatment was sprayed with deionised water only.

The worms were washed, dried and individually weighed prior to addition to the test vessels. After four weeks, the adult worms were removed from the test vessels, and mortality and body weight of the surviving worms was determined. The test soils were then incubated for an additional four weeks, following which the number of hatched juveniles per test container was determined. Once a week, during the exposure period, the water content was checked by weighting each test vessel and if necessary lost water was replaced.

The Fisher exact test was used to compare the treatment group mortality to the control group. Data concerning adult worm bodyweight and reproduction were analysed by ANOVA.

Results and Discussion

Mortality and reproduction are summarised in the table below.

Table 8.4.1-2: Effect of A8779A on mortality, growth and reproduction of *Eisenia fetida*

Endpoints	Treatment groups (g a.s./ha)		
	Control	750	3750
Mean adult mortality at 28 days (%)	0	0	2.5
Mean % bodyweight change of adults from 0-28 days	53.2	52.7	48.5
Mean number of juveniles per test vessel	46.0	41.2	37.4
NOEC (biomass, reproduction)	3750 g a.s./ha		

No significant effects were observed on mortality, bodyweight change or reproduction at any of the A8779A application rates tested.

Validity criteria

The test is considered valid as:

- Adult mortality was 0 % in the control (≤ 10 % required)
- The mean number of juveniles per vessel in the control was 46 (> 30 required)
- The coefficient of variation in the control was 21.7 % (< 30 % required)

- Control worms weight increased by 53.2 % (≤ 20 % bodyweight loss required)

During the separate toxic reference test the reproduction of earthworms was significantly affected at 250 g carbendazim/ha, indicating the test organisms were sufficiently sensitive.

Conclusions

Exposure for 28 days to A8779 at rates up to 3750 g a.s./ha (equivalent to 5000 g A8779A/ha) did not affect the survival, body weight or reproduction of the earthworm, *Eisenia fetida*.

(Nienstedt, 2001)

Study data have been re-evaluated for the purposes of calculating EC_{10} and EC_{20} values. The outcome of the re-evaluation is provided below.

Report:	Submitted for purposes of renewal due to change in data requirements guidance: K-CA 8.4.1/02 Taylor S. & Pickering F. (2015da) Cyprodinil (A8779A) – A chronic toxicity and reproduction test exposing *Eisenia fetida* to A8779A in OECD artificial soil. Statistical Re-analysis. Report Number CEA.1411. Cambridge Environmental Assessments, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK. (Syngenta File No: A8779A_10237)

Summary

Report number 1047.094.631 (Neinstedt, 2001) did not provide estimates of the EC_{10} and EC_{20} for the effects of A8779A on mortality, biomass or reproduction to the earthworm *Eisenia fetida.* Consequently the data generated in this study have been re-analysed in order to provide these values.

As there were only two test item concentrations a clear dose response could not be generated and therefore no EC_{10} or EC_{20} values for mortality, reproduction or biomass could be reliably calculated.

Statistical Analysis

As there were only two test item concentrations no meaningful statistical analyses could be completed as there was no clear dose response.

Results

As there were only two test item concentrations no meaningful statistical analyses could be completed as there was no clear dose response and as such no EC_{10} or EC_{20} values could be calculated.

Conclusion

As there were only two test item concentrations a clear dose response could not be generated and therefore no EC_{10} or EC_{20} values for mortality, reproduction or biomass could be reliably calculated.

(Taylor S. & Pickering F. 2015d)

Metabolites of cyprodinil

Report:	Summary submitted at the request of ANSES for ease of dossier evaluation: *KIIA 8.4.2. Pfeifle, V. (2001). Effects of CGA249287 (Metabolite of CGA219417) on the Reproduction of Earthworm, Eisenia fetida. Report Number 2003516. Solvias AG, CH-4002 Basel, Switzerland. (Syngenta File No. CGA249287/0020).*

Guidelines

BBA-Richtlinien für die Prüfung von Pflanzenschutzmitteln im Zulassungsverfahren, Teil VI, 2-2 (1994)

International Standard: ISO guideline 11268, Part 2 (1998)

OECD Guideline for Testing Chemicals: Proposal for a new guideline: Earthworm Reproduction Test (*Eisenia fetida*/*andrei*). Draft document (2000).

GLP: Yes

Executive Summary

In a chronic toxicity test in which earthworms (*Eisenia fetida*) were exposed to CGA249287 the NOEC (based on reproduction) was determined to be 1.13 mg test item/kg soil d.w. No significant effects were observed on adult survival, adult biomass or reproduction at any of the CGA249287 concentrations tested. Therefore, the LOEC was concluded to be >1.13 mg/kg dry soil.

Materials

Test Material	CGA249287
Lot/Batch #:	KI 4014/11
Purity:	100 ± 2 %
Description:	White powder
Stability of test compound:	Not reported
Reanalysis/Expiry date:	December 2000
Treatments	
Test concentrations:	0.113, 0.566 and 1.13 mg test item /kg soil d.w.
Control:	Untreated soil
Toxic standard:	Separate study with carbendazim and benomyl
Solvent:	None
Test organisms	
Species:	*Eisenia fetida*
Age and weight range at test start:	Adults (approximately 11 months old); Weight range was 321 to 386 mg
Source:	Culture maintained at test facility
Feeding:	5 g dry, finely ground cow manure was spread on surface of the soil in each test container one day after worm addition and weekly thereafter up to Day 28.

Test design

Vessels:	Plastic containers (17 x 12.5 cm, height approximately 6 cm) covered with perforated lids. Filed with approximately 792 g (wet weight) test soil
Substrate:	Artificial soil comprising 10 % sphagnum peat, 20 % kaolinite clay, 69 % industrial quartz sand (> 50% of the particles between 0.05 mm and 0.2 mm), approximately 1 % calcium carbonate and 5 g finely ground cow manure per 500 g dry soil
Replication:	Four replicate vessels per treatment and control
No. of worms/arena :	10
Duration of test:	8 weeks

Environmental test conditions

Temperature:	20 ± 2 °C
pH of soil:	Test start: 5.7 to 5.8 Test end: 5.7 to 5.9
Water content of soil:	Test start: 55.0 to 57.4 % of dry weight Test end: 56.6 to 57.6 % of dry weight
Photoperiod:	16 hours light, 8 hours dark (average intensity 508 lux)

Study Design and Methods

Experimental dates: 29th January to 28th March 2001

A stock solution was prepared by dissolving 14.98 mg of test item with deionised water in a volumetric flask. The solution was treated with ultrasound for 10 minutes and then made up to 1000 mL. To obtain the test item treatment concentrations, appropriate aliquots of the stock solution were added directly to 2000 g of artificial soil for each treatment concentration. The moisture content of the test soil was adjusted to 60 % of the maximum water holding capacity. The treated soil was divided between four replicate test vessels each containing 500 g dry soil (792 g wet weight) and 10 weighed worms were placed on the soil surface in each test vessel.

After four weeks, the adult worms were removed from the test vessels, and mortality and the body weight of the surviving worms were determined. The test soils were then incubated for an additional four weeks, following which the number of hatched juveniles per test container was determined.

Changes in adult worm biomass in the CGA249287 treatments were compared to the control group using the Dunnett-test (α = 0.05, one-sided, smaller). The mean reproductive output for the treatments with CGA249287 were compared with the control using the U-test after Bonferroni-Holm (α = 0.05, one-sided, smaller). No attempt was made to estimate EC_x values.

Results and Discussion

Mortality and reproduction are summarised in the table below.

Table 8.4.1-3: Effect of CGA249287 on mortality, growth and reproduction of *Eisenia fetida*

Endpoints	Treatment groups (mg CGA249287/kg soil dry weight)			
	Control	0.113	0.566	1.13
Mean adult mortality at 28 days (%)	0	0	0	5
Mean % biomass change of adults from 0-28 days	123	119	125	119
Mean number of juveniles after 8 weeks	96	81	83	89
NOEC (biomass, reproduction)	1.13 mg/kg dry soil			

Validity criteria

The test is considered valid as:

- Adult mortality was 0 % in the control (≤ 10 % required)
- The mean number of juveniles per control replicate was 96 (> 30 required)
- The coefficient of variation in the control was 29.2 % (≤ 30 % required)
- Weight loss of the adults in the control was +23 % (≤ 20 % required)

In the two separate reference test with carbendazim performed in 2000, the mean number of juveniles counted for the reference item was 94 and 8, respectively. These tests were performed at concentrations of 1 and 1.5 mg carbendazim/kg, respectively and both results were significantly different compared to the control.

Conclusions

In a chronic toxicity test in which earthworms (*Eisenia fetida*) were exposed to CGA249287 no effects on reproduction, mortality or biomass were observed. Therefore, the NOEC was determined to be 1.13 mg test item/kg dry soil (the highest concentration tested)

The EC_x for the emerged number of juveniles on Day 56 could not be determined due to a lack of effect on reproduction of the earthworm *Eisenia fetida*.

(Pfeifle, 2001)

For Pfeifle (2001), (Syngenta File No. CGA249287/0020), study data have been re-evaluated for the purposes of calculating EC_{10} and EC_{20} values. The outcome of the re-evaluation is provided below.

Report:	Submitted for purposes of renewal due to change in data requirements guidance: K-CA 8.4.1/03 Taylor S., Pickering F. (2015eb) CGA249287 – Effects of CGA249287 on the reproduction of the earthworm *Eisenia fetida*. Statistical Re-analysis. Report Number CEA.1427. Cambridge Environmental Assessments, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK. (Syngenta File No: CGA249287_10008)

Summary

Report number L01-000120 (Pfeifle, 2001) did not provide estimates of the EC_{10} and EC_{20} for change in reproductive capacity or body weight of survivors of the earthworm *Eisenia fetida* treated with CGA249287. Consequently the data generated in this study have been re-analysed in order to provide these values.

No EC_{10} or EC_{20} values for reproduction or body weight could be reliably calculated due to a lack of dose response.

Statistical Analysis

Mean bodyweight of surviving adult earthworms was calculated as a proportion of mean initial body weight. Probit analysis with linear maximum likelihood regression was used to determine the concentration response function. Chi² was used as a goodness of fit measure and the proportion of variance was explained by the dose/response function.

Reproduction was calculated as total number of offspring hatched per replicate. Probit analysis with linear maximum likelihood regression was used to determine the concentration response function. Chi² was used as a goodness of fit measure.

All computations were carried out in the Statistical program: ToxRat Professional 2.10.05 (ToxRat Solutions GmbH, 2001-2010).

Results

There was no significant dose response in either reproductive output (p(F) = 0.370) or earthworm body weight (p(F) = 0.187) and therefore EC_{10} and EC_{20} values could not be reliably determined.

Conclusion

No EC_{10} or EC_{20} values for reproduction or body weight could be reliably calculated due to a lack of significant dose response.

(Taylor S. & Pickering F. 2015ec)

Summaries of studies conducted since the previous EU submission of cyprodinil are presented below:

Report:	Submitted for purposes of renewal as conducted on relevant soil metabolite: K-CA 8.4.1/04 Lührs U. (2014) CGA275535 - Effects on Reproduction and Growth of Earthworms *Eisenia fetida* in Artificial Soil Report Number 92791022. Institut für Biologische Analytik und Consulting, IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany. (Syngenta file No. CGA275535_10002)

Guidelines

OECD Guidelines No. 222: Earthworm Reproduction Test (*Eisenia fetida*/*Eisenia andrei*) (2004)

GLP: Yes

Executive Summary

In a chronic toxicity test in which earthworms (*Eisenia fetida*) were exposed to CGA275535 the NOEC (based on reproduction) was determined to be 556 mg /kg soil d.w. The EC_{10}, and EC_{20} values for reproduction were estimated to be 385 and 638 mg /kg soil d.w., respectively.

Materials

Test Material	CGA275535
Lot/Batch #:	Kl 6230/3
Purity:	99% w/w
Description:	Beige solid
Stability of test compound:	Stable under standard conditions.
Reanalysis/Expiry date:	End of February 2018
Density:	Not stated
Treatments	
Test rates:	16, 29, 53, 95, 171, 309, 556 and 1000 mg CGA275535/kg soil
Control:	Untreated
Toxic standard:	Luxan Carbendazim 500 FC
Test organisms	
Species:	*Eisenia fetida* (subspecies *Eisenia fetida andrei*)
Age and weight range at test start:	Approx 8 months 300 to 600 mg
Source:	Cultured at test facility
Feeding:	5 g finely ground cattle manure, each week for first 4 weeks.
Test design	
Vessels:	Plastic trays (18.3 × 13.6 × 6.4 cm) with a lid pervious to air and light.
Substrate:	Artificial soil comprising 10% sphagnum peat, 20 % kaolinite clay, 69.5 % industrial quartz sand (> 50% of the particles between 0.05 mm and 0.2 mm) and 0.5% calcium carbonate.
Replication:	Control:8 Treatment: 4
No. of worms/arena :	10
Duration of test:	8 weeks
Environmental test conditions	
Temperature:	18 to 22 °C
pH of soil:	5.6 to 5.7
Water content of soil:	51.3 to 60.4%
Photoperiod:	16 hour photoperiod (400 to 800 lux)

Study Design and Methods

Experimental dates: 15th July to 10th September 2014

Approximately 24 hours prior to test start, the artificial soil was prepared and deionised water was added to the dry components to adjust the water content to approximately 40-60% of its maximum water holding capacity (WHC). The worms were acclimatised in a separate batch of the untreated artificial substrate for approximately 24 hours before test start. The test concentrations were prepared by dispersing an exactly weighed amount of the test material in deionised water to make a stock solution. This stock solution was diluted with deionised water for each concentration and was thoroughly mixed with the artificial soil using a laboratory mixer, achieving a final nominal water content of 40-60% of WHC. The

acclimatised test animals were washed, gently dried on a paper towel, weighed and randomly placed onto the test substrate (10 animals per test vessel). Worms which had not dug themselves in after 30 minutes were replaced.

After four weeks, the adult worms were removed from the test vessels, and mortality and the body weight of the surviving worms were determined. After all of the adult worms had been removed, the soil in each vessel was mixed with 5 g horse manure. Four weeks later, the number of surviving juveniles and any morphological alterations were recorded. Observations of behavioural and pathological symptoms were observed weekly.

The EC values and their 95% confidence limits were calculated by applying Probit-Analysis (Finney, 1971).

The body weight change and reproduction data were tested for normal distribution and homogeneity of variance ($\alpha = 0.05$) using the Shapiro-Wilk´s test and the Levene´s test, respectively.
As the data for body weight changes and reproduction were normally distributed and homogeneous in both cases, Williams t-test was used to compare treatment and control values (multiple comparison, two-sided for weight and one-sided smaller for reproduction, $\alpha = 0.05$).

The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05.

Results and Discussion

Mortality and reproduction are summarised in the table below.

Table 8.4.1-14: Effect of CGA275535 on mortality, growth and reproduction of *Eisenia fetida*

Endpoints	Treatment groups (mg/kg soil dry weight)								
	Control	**16**	**29**	**53**	**95**	**171**	**309**	**556**	**1000**
Mean adult mortality at 28 days (%)	0	0	0	0	0	0	0	0	0
Mean % biomass change of adults from 0-28 days	95	112	100	123	133	122	100	111	96
Mean number of juveniles after 8 weeks	312	309	355	347	333	292	273	291	199*
% difference in reproduction relative to the control	-	1.0	-13.9	-11.4	-6.8	6.3	12.3	6.6	36.0
NOEC (biomass)	1000								
NOEC (reproduction)	556								
EC_{10} (reproduction)	385								
EC_{20} (reproduction)	638								

* significantly reduced compared to the control (Williams t-test, $\alpha = 0.05$, two-sided for weight changes and one-sided smaller for reproduction)

Validity criteria

The validity criteria are as follows:

- Adult mortality was 0% in the control (< 10% required)
- The mean number of juveniles for the control was 312 (> 30 required)
- The coefficient of variation for reproduction was 11.9 % (< 30% required)

Conclusions

In a chronic toxicity test in which earthworms (*Eisenia fetida*) were exposed to CGA275535 the NOEC (based on reproduction) was determined to be 556 mg /kg soil d.w. The EC_{10}, and EC_{20} values for reproduction were estimated to be 385 and 638 mg /kg soil d.w., respectively.

(Lührs U, 2014)

Report:	Submitted for purposes of renewal as conducted on relevant soil metabolite: K-CA 8.4.1/05 Lührs U. (2015) CGA321915 – Effects on reproduction and growth of earthworms *Eisenia fetida* in Artificial Soil. Report Number 96341022. IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany. (Syngenta file No. CGA321915_10012)

Guidelines

OECD Guideline No. 222: Earthworm Reproduction Test (*Eisenia fetida*/*Eisenia andrei*) (2004)

GLP: Yes

Executive Summary

In a chronic toxicity test in which earthworms (*Eisenia fetida*) were exposed to CGA321915 the NOEC (based on reproduction) was determined to be 1000 mg CGA321915/kg soil d.w. The EC_{10}, EC_{20} and EC_{50} could not be determined, but are assumed to be >1000 mg CGA321915/kg.

Materials

Test Material	CGA321915
Lot/Batch #:	MES 356/1
Purity:	98 % w/w
Description:	White solid
Stability of test compound:	Stable under standard conditions.
Reanalysis/Expiry date:	End of September 2016
Treatments	
Test rates:	16.3, 29.4, 52.9, 95.3, 171.5, 308.6, 555.6, 1000 mg CGA321915/kg soil d.w.
Control:	Deionised water
Toxic standard:	Carbendazim was tested at concentrations of 5 and 10 mg product/kg soil dry weight (separate study - No.: 91441022, dated July – September 2014).
Test organisms	
Species:	*Eisenia fetida* (subspecies *Eisenia fetida andrei*)

Age and weight range at test start:	Approximately 7 to 8 months with well developed clitellum; 301 – 600 mg
Source:	Bred under standard conditions at IBACON
Feeding:	Finely ground cattle manure
Test design	
Vessels:	Plastic boxes (18.3 × 13.6 × 6 cm) with a lid pervious to air and light.
Substrate:	Artificial soil comprising 10% sphagnum peat, 20 % kaolinite clay, 69.5 % industrial quartz sand (> 50% of the particles between 0.05 mm and 0.2 mm) and 0.5% calcium carbonate. 659.9 g wet weight soil, corresponding to about 500 g dry weight, of artificial soil was added to each test vessel.
Replication:	Control: 8 Treatment: 4
No. of worms/arena :	10
Duration of test:	8 weeks
Environmental test conditions	
Temperature:	18 – 22 °C
pH of soil:	Test start: 5.8 – 6.0 Test end: 6.0 – 6.1
Water content of soil:	Test start: 53.8 – 56.7% WHC Test end: 54.3 – 59.7% WHC
Photoperiod:	16 h light: 8 h dark

Study Design and Methods

Experimental dates: 22nd January to 20th March 2015

The test concentrations were prepared by dispersing an exactly weighed amount of the test material in deionised water to make a stock solution. This stock solution was diluted with deionised water for each concentration and was thoroughly mixed with the artificial soil using a laboratory mixer, achieving a final nominal water content of 40-60 % of WHC. The acclimatised test animals were washed, gently dried on a paper towel, weighed and randomly placed onto the test substrate (10 animals per test vessel). 5 g finely ground cattle manure was added each week for the first four weeks of the experiment.

After four weeks, the adult worms were removed from the test vessels, and mortality and the body weight of the surviving worms were determined. After all of the adult worms had been removed the soil in each vessel was mixed with 5 g horse manure. Four weeks later, the number of surviving juveniles and any morphological alterations were recorded. Observations of behavioural and pathological symptoms were observed at day 28.

The endpoints were mortality, change of biomass (difference in fresh weight of surviving worms between test start and four weeks after treatment) and reproduction (the number of juveniles present). The arithmetic mean and the standard deviation per treatment and per control for reproduction, mortality and biomass were calculated. The statistical analysis was performed with the software ToxRat Professional 2.10.05 (ToxRat Solutions GmbH). The LC_x/EC_x values were calculated by Probit analysis. For identifying the NOEC values Fisher`s Exact Binomial Test with Bonferroni Correction and the Williams-t-test were used to compare the control with the independent test item groups.

Results and Discussion

Mortality and reproduction are summarised in the table below.

Table 8.4.1-25: Effect of CGA321915 on mortality, growth and reproduction of *Eisenia fetida*

Endpoints	Treatment groups (mg CGA321915/kg soil dry weight)								
	Control	**16.3**	**29.4**	**52.9**	**95.3**	**171.5**	**308.6**	**555.6**	**1000**
Mean adult mortality at 28 days (%)	0.0	0.0	0.0	2.5	0.0	0.0	0.0	0.0	0.0
Mean % biomass change of adults from 0-28 days	43.8	46.5	48.4	42.8	43.0	46.4	42.2	42.9	36.9
Mean number of juveniles after 8 weeks	296	280	320	300	333	266	313	340	342
% difference in reproduction relative to the control	-	5.6	-8.1	-1.3	-12.4	10.2	-5.5	-14.6	-15.4
NOEC (reproduction)	1000								

Negative values indicate an increase compared to the control

Validity criteria

The test is considered valid as:

- Adult mortality was 0% in the control (< 10% required)
- The number of juveniles per control replicate was 244 to 356 (> 30 required)
- The coefficient of variation for reproduction was 15.9% (< 30% required)

Conclusions

In a chronic toxicity test in which earthworms (*Eisenia fetida*) were exposed to CGA321915 the NOEC (based on reproduction) was determined to be 1000 mg CGA321915/kg soil d.w. The EC_{10}, EC_{20} and EC_{50} could not be determined, but are assumed to be >1000 mg CGA321915/kg.

(Lürhs U, 2015)

CA 8.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

CA 8.4.2.1 Species level testing

New studies have been carried out for cyprodinil on *Folsomia candida* and *Hypoaspis aculeifer* to fulfil current data requirements in **Commission Regulation (EU) No 283/2013**. Studies were carried out with the two formulations (A8637C and A14325E), with both being considered representative for effects of the active substance. The endpoints for these studies are presented in Table 8.4-2. Study summaries are therefore presented below.

Report:	K-CA 8.4.2.1/01 Lührs U. (2014a) Cyprodinil WG (A8637C) – Effects on the reproduction of the Collembola *Folsomia candida* in artificial soil with 5% peat. Report Number 92771016. Institut für Biologische Analytik und Consulting, IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany. (Syngenta File No. A8637C_10314)

Guidelines

OECD Guidelines No. 232. Collembolan Reproduction test in soil (2009)

ISO 11267: Soil quality – inhibition of reproduction of Collembola (*Folsomia candida*) by soil pollutants. (1999)

GLP: Yes.

Executive Summary

The toxicity of A8637C to the reproduction and the parental mortality of collembola species *Folsomia candida* were determined. The NOEC for mortality and reproduction were determined to be 105 mg A8637C/kg soil d.w. (52.5 mg cyprodinil/kg), the highest concentration tested.

Materials

Test Material	Cyprodinil WG A8637C
Lot/Batch #:	SMO2C304
Actual content of active ingredients:	Cyprodinil: 50.2% w/w
Description:	Brownish granules
Stability of test compound:	Stable under standard conditions.
Reanalysis/Expiry date:	End of December 2016
Treatments	
Test rates:	1.72, 3.09, 5.56, 10.0, 18.0, 32.4, 58.3, 105 mg A8637C/kg d.w.
Control:	Untreated (moistened with deionised water)
Toxic standard:	Boric acid (content 100.3%) at 30.5, 48.8, 78.1, 125 and 200 mg/kg (separate study - No.: 61405016).
Application method:	A stock solution was prepared and combined with artificial soil
Test organisms	
Species:	*Folsomia candida*
Age:	10 – 12 days
Source:	Culture maintained at Test Facility
Feeding:	2 mg granulated dry yeast at the start of the test and after 14 days
Test design	
Arenas:	100 mL glass containers; diameter 5 cm; tightly closed lids
Substrate:	Artificial soil comprising 5% sphagnum peat, 20% kaolinite clay, 74.75% industrial quartz sand (> 50% of the particles between 0.05 mm and 0.2 mm) and 0.25% calcium carbonate.
Replication:	8 in the control; 4 per test item treatment; 1 additional per treatment for pH and water content checks
No./arena :	10
Duration of test:	28 days
Environmental test conditions	

Temperature:	18 – 22 °C
pH of soil:	At test start: 6.2 – 6.3 At test end: 6.0 – 6.1
Water content of soil:	At test start: 20.2 – 21.7% (49.2 – 52.9 % of MWHC) At test end: 18.9 – 20.2% (46.1 – 49.3 % of MWHC)
Photoperiod:	16:8 LD; 400 – 800 Lux

Study Design and Methods

Experimental dates: 20th August to 18th September 2014

The test concentrations were prepared by dispersing an exactly weighed amount of the test item in deionised water to make a stock solution. This stock solution was diluted with deionised water for each test concentration and was thoroughly mixed with the artificial soil using a mixing machine, achieving a final nominal water content of 40-60% of MWHC. The control was treated with deionised water only.

Ten juvenile collembolans were transferred after the application to the substrate surface of each test vessel using an aspirator. There were four replicates (+ one replicate not loaded with collembolans for measurement purposes) used per test concentration and eight replicates in the control. The test organisms were fed twice during the experiment (at the start of the test and after 14 days) with approximately 2 mg of granulated dry yeast per test vessel. Four weeks after introducing the test organisms, the surviving parental collembolans and offspring (juveniles) were counted.

The 28-day mortality data for the individual test-item treatments were compared to those in the control using Fisher's Exact Test ($\alpha = 0.05$, one-sided greater). Reproductive data were tested for normal distribution and homogeneity of variance using Shapiro-Wilk's test and Levene's test ($\alpha = 0.05$). Further statistical evaluation was conducted using Williams t-test (multiple comparison, $\alpha = 0.05$, one-sided smaller). Determination of the NOEC was based on results of the statistical evaluations.

EC_x values were not determined as no reduction in reproduction of more than 50% was observed.

Results and Discussion

Mortality and fecundity are summarised in the table below.

Table 8.4.2.1-1: Effects of residues of A8637C on mortality and reproduction of *Folsomia candida*

Endpoint	Treatment group (mg A8637C/kg soil d.w.)								
	Control	**1.72**	**3.09**	**5.56**	**10.0**	**18.0**	**32.4**	**58.3**	**105.0**
% Mortality of parental collembolans after 4 weeks	13	20	23	15	28	20	23	13	18
Mean number of juveniles after 4 weeks	428	426	488	357	430	395	399	400	332
SD	77	119	147	112	38	109	85	140	58
% reduction compared to control	-	0.6	-14.1	16.6	-0.3	7.7	6.9	6.6	22.4
NOEC (reproduction)	105 mg/kg soil d.w. (52.5 mg cyprodinil/kg)								

Validity criteria

The validity criteria are as follows:

- Control treatment mortality was 13 % (must be < 20%)

- The mean number of juvenile recorded in the control treatment was 300 to 552 (must be > 100 per replicate)
- The coefficient of variation of reproduction in the control was 18 % (must not be > 30%)

Conclusions

The toxicity of A8637C to the reproduction and the parental mortality of collembola species *Folsomia candida* were determined. The NOEC for reproduction was determined to be 105 mg A8637C/kg soil d.w. (52.5 mg cyprodinil/kg), the highest concentration tested.

(Lührs U, 2014a)

Study data have been re-evaluated for the purposes of calculating EC_{10} and EC_{20} values. The outcome of the re-evaluation is provided below.

Report:	Submitted for purposes of renewal due to change in data requirements guidance: K-CA 8.4.2.1/02 Taylor S., & Pickering, F. (2016c) Cyprodinil WG (A86637C) – Effects on Reproduction of the Collembola (*Folsomia candida*) in Artificial Soil with 5% Peat - Statistical Re-analysis. Report Number CEA.1713. Cambridge Environmental Assessments, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK. (Syngenta File No: A8637C_10368)

Summary

The report from IBACON GmbH study number 92771016 (Lührs, 2014) for the reproductive toxicity test of A8637C on the collembola (*Folsomia candida*) did not provide estimates of the EC_{10} or EC_{20} for the response variables (adult mortality and reproduction) evaluated as part of the original study. Consequently the data generated in this study have been re-analysed in an attempt to provide these values.

No EC_{10} or EC_{20} values for mortality or reproduction could be reliably calculated due to a lack of significant dose response.

Statistical Analysis

No mortality was observed after 28 days of exposure for any test concentration. In addition, there were no statistically significant differences in the feeding activity or biomass change between each of the treatment concentrations and the control in the original report. As a result, these parameters were not statistically analysed and no ECx values could reliably be determined.

Probit analysis with linear maximum likelihood regression was used in an attempt to determine the concentration response function for reproduction. Chi^2 was used as a goodness of fit measure. The proportion of variance explained by the dose/ response function was determined and is presented as the coefficient of determination, r2 (0 <= r2 <= 1).

All computations were carried out in the Statistical program: ToxRat Professional 2.10.05 (ToxRat Solutions GmbH, 2001-2010).

Results

No statistically significant (p(F)= < 0.05) dose response was observed for either mortality or reproduction and as a result, no EC_{10} or EC_{20} values were reliably calculated.

Conclusion

No EC_{10} or EC_{20} values for mortality or reproduction could be reliably calculated due to a lack of significant dose response.

(Taylor S. & Pickering F. 2016c)

Report:	K-CA 8.4.2.1/03 ~~02~~ Lührs U. (2014b) Cyprodinil EC (A14325E) - Effects on Reproduction of the Collembola *Folsomia* candida in Artificial Soil with 5% Peat. Report Number 92781016. Institut für Biologische Analytik und Consulting IBACON GmbH Arheilger Weg 17, 64380 Rossdorf, Germany. (Syngenta file No. A14325E _10061)

Guidelines

OECD Guidelines No. 232. Collembolan Reproduction test in soil (2009)

ISO 11267: Soil quality – inhibition of reproduction of Collembola (*Folsomia candida*) by soil pollutants. (1999)

GLP: Yes.

Executive Summary

The toxicity of A14325E to the reproduction and the parental mortality of collembola species *Folsomia candida* were determined. The NOEC was determined to be 29.4 mg/kg soil dry weight (8.67 mg cyprodinil/kg). The EC_{10} and EC_{20} values were estimated to be 53.2 mg and 67.7 mg test item/kg soil, respectively (15.7 and 20 mg cyprodinil/kg).

Materials

Test Material	A14325E Cyprodinil EC	
Lot/Batch #:	SMO3A100	
Actual content of active ingredients:	Cyprodinil:	29.1% w/w, corresponding to 295 g/L
Description:	Liquid	
Stability of test compound:	Stable under standard conditions.	
Reanalysis/Expiry date:	End of January 2017	
Density:	1014 kg/m^3	

Treatments

Test rates:	2.80, 5.04, 9.08, 16.3, 29.4, 52.9, 95.3 and 171.5 mg test item/kg soil
Control:	Untreated
Toxic standard:	Boric acid
Application method:	Mixed into artificial soil

Test organisms

Species:	*Folsomia candida*
Age:	10 – 12 day old juveniles
Source:	Culture maintained at Test Facility

Feeding:	2 mg granulated dry yeast at the start of the test and after 14 days

Test design

Arenas:	Glass containers (volume: 100 mL; diameter: 5 cm), closed tightly to avoid water evaporation, filled with 30 g artificial soil fresh weight.
Substrate:	Artificial soil comprising 5% sphagnum peat, 20% kaolinite clay, 74.7% industrial quartz sand (> 50% of the particles between 0.05 mm and 0.2 mm) and 0.3% calcium carbonate.
Replication:	Control: 8 Treatment: 4
No./arena :	10
Duration of test:	28 days

Environmental test conditions

Temperature:	18 to 22°C
pH of soil:	Test start: 6.2 – 6.3 Test end: 6.1
Water content of soil:	Test start: 50.8% to 52.9% of WHC Test end: 46.4% to 49.7% of WHC
Photoperiod:	16 hour photoperiod (400 – 800 lux)

Study Design and Methods

Experimental dates: 1st to 30th September 2014

The test concentrations were prepared by dispersing an exactly weighed amount of the test item in deionised water to make a stock solution. This stock solution was diluted with deionised water for each test concentration and was thoroughly mixed with the artificial soil using a mixing machine, achieving a final nominal water content of 40-60% of WHC. The control was treated with deionised water only.

Ten juvenile collembolans were transferred after the application to the substrate surface of each test vessel using an exhauster. Four replicates (+ two replicates not loaded with collembolans for measurement purposes) were used per test concentration and eight were used in the control. The test organisms were fed twice during the experiment (at the start of the test and after 14 days) with approximately 2 mg of granulated dry yeast per test vessel. Four weeks after introducing the test organisms, the surviving parental collembolans and offspring (juveniles) were counted.

The results represent rounded values calculated from the exact raw data.

Mortality data were statistically analyzed using Fisher's Exact Test (multiple comparison, $\alpha = 0.05$, one-sided greater).

Reproduction data were tested for normal distribution and homogeneity of variance using Shapiro-Wilk's test and Levene's test ($\alpha = 0.05$). Since variances were not homogeneous the further statistical evaluation was performed using Bonferroni-Welch t-test (multiple comparison, $\alpha = 0.05$, one-sided smaller).

The determination of the NOEC and LOEC values was based on the results of the statistical evaluation. The EC values for reproduction were calculated by Probit Analysis (Finney 1971). The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH

Results and Discussion

Mortality and fecundity are summarised in the table below.

Table 8.4.2.1-2: Effects of residues of A14325E on mortality and reproduction of *Folsomia candida*

Endpoint	Treatment group (mg A14325E /kg soil d.w.)								
	Control	**2.80**	**5.04**	**9.08**	**16.3**	**29.4**	**52.9**	**95.3**	**171.5**
% Mortality of parental collembolans after 4 weeks	6	5	15	10	8	5	15	8	55*
Mean number of juveniles after 4 weeks	491	566	483	456	466	472	392*	337	61*
SD	73	76	31	58	43	32	32	123	30
% reduction compared to control	-	-15.3	1.5	7.1	5.0	3.9	20.1	31.3	87.6
NOEC (reproduction)	29.4 (8.67 mg cyprodinil/kg)								
EC_{10}	53.2 (15.7 mg cyprodinil/kg)								
EC_{20}	67.7 (20 mg/kg cyprodinil)								

* significantly reduced compared to the control (Bonferroni-Welch t-test, $\alpha = 0.05$, one-sided smaller)
negative values indicate an increased reproduction relative to the control

Validity criteria

The validity criteria are as follows:

- Control treatment mortality was 6 % (must be < 20%)
- The mean number of juvenile recorded in the control treatment was 491 (must be > 100 per replicate)
- The coefficient of variation of reproduction in the control was 14.9% (must not be > 30%)

Conclusions

In a 28-day collembola (*Folsomia candida)* study with A14325E, the NOEC for reproduction was determined to be 29.4 mg A14325E/kg soil dry weight (8.67 mg cyprodinil/kg). The EC_{10} and EC_{20} values were estimated to be 53.2 mg and 67.7 mg test item/kg soil, respectively (15.7 and 20 mg cyprodinil/kg.

(Lührs U, 2014b)

Report:	K-CA 8.4.2.1/04 ~~03~~ Lührs U. (2014c) Cyprodinil WG (A8637C) - Effects on Reproduction of the Predatory Mite *Hypoaspis aculeifer* in Artificial Soil with 5% Peat Report Number 92771089. Institut für Biologische Analytik und Consulting, IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany. (Syngenta file No. A8637C_10312)

Guidelines

OECD (2008). OECD Guideline for Testing of Chemicals, Section 2 – Effects on Biotic Systems, Method 226 (adopted 3 October 2008): Predatory mite (*Hypoaspis (Geolaelaps) aculeifer*) reproduction test in soil.

GLP: Yes.

Executive Summary

The potential effects of A8637C on the mortality and reproductive success of the soil mite species *Hypoaspis aculeifer* (CANESTRINI) were determined during a 14-day test. The NOEC was determined to be 555.6 mg /kg soil dry weight (277.8 mg cyprodinil/kg soil).

Materials

Test Material	A8637C Cyprodinil WG
Lot/Batch #:	SMO2C304
Actual content of active ingredients:	50.2% w/w
Description:	Brownish solid
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	End of December 2016
Density:	Not stated
Treatments	
Test rates:	16.3, 29.4, 52.9, 95.3, 171.5, 308.6, 555.6 and 1000 mg A8637C/kg soil dry weight
Control:	Untreated substrate, i.e. purified water only
Toxic standard:	BAS 152 11 I Perfekthion (nominally 400 g dimethoate/L, analysed 400.9 g dimethoate/L)
Test organisms	
Species	*Hypoaspis aculeifer* (CANESTRINI)
Source:	Cultured in test facility
Food:	Cheese mites, *Tyrophagus putrescentiae* at 2 spatulas on day 0, 1 and 5, 1 spatula on day 7 and 9, ½ a spatula on day 12.
Age at test start:	12 days after reaching adult stage
Test design	
Vessels:	Glass containers (volume: 100 mL; diameter: 5 cm), tight screw top closure to avoid water evaporation, filled with approx 20 g soil d.w.
Substrate:	Artificial soil comprising 5% sphagnum peat, 20 % kaolinite clay, 74.75% industrial quartz sand and 0.25% calcium carbonate.
Replication:	Control group: 8 Treated group: 4
No. of mites/arena :	10 females
Duration of test:	14 days
Environmental test conditions	
Temperature:	18 to 22 °C
pH:	Test start: 6.1 – 6.2 Test end: 6.0 – 6.1
Water content of soil:	50.4 to 53.2% of maximum WHC
Photoperiod:	16 h light : 8 h dark, 400 to 800 lux

Study Design and Methods

Experimental dates: 20th August to 4th September 2014

Adult females of the soil mite *Hypoaspis aculeifer* were exposed to concentrations of A8637C incorporated into the test soil. An exactly weighed amount of the test item was mixed with purified water to make a stock solution, and appropriate volumes of this stock solution were further diluted with purified water to obtain the test concentrations such that, when added to pre-moistened artificial soil, a final moisture content value of 50% WHC was achieved. Adult females were transferred to the test vessels which contained untreated (control), reference item or test item treated artificial soil. Ten adult females were introduced to each test vessel. As a source of food, cheese mites (*Tyrophagus putrescentiae*) were added to the soil surface throughout the test. The test was carried out under controlled light-dark cycle. Fourteen days after introducing the test organisms, the surviving mites and the juveniles of *Hypoaspis aculeifer* were extracted by heat/light extraction. From these data the mortality of the adult females and the reproductive output were calculated.

The mean number of dead adult female mites for each treatment, the mean number of juvenile mites for each treatment, the NOEC, the LOEC values, and the LC_{50} at day 14 were not able to be determined by statistical analysis as no mortality above 50% was observed.

Mortality data were statistically analysed using Fisher's Exact test (multiple comparison, $\alpha = 0.05$, one-sided greater). Reproduction data were tested for normal distribution and homogeneity of variance using Shapiro-Wilk's test and Levene's test ($\alpha = 0.05$). As data were normally distributed and homogeneous, the further statistical evaluation was performed using Williams t-test (multiple comparison, $\alpha = 0.05$, one-sided smaller).

The determination of the NOEC and LOEC values was based on the results of the statistical evaluation. The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05.

Results and Discussion

Mortality and fecundity are summarised in the table below.

Table 8.4.2.1-3: Effects of residues of A8637C on mortality and reproduction of *Hypoaspis aculeifer*

Endpoint	Treatment group (mg A8637C/kg soil d.w.)								
	Control	**16.3**	**29.4**	**52.9**	**95.3**	**171.5**	**308.6**	**555.6**	**1000**
	Mortality of adult mites after 14 days								
% mortality	5	3	3	3	10	0	0	5	8
	Number of juveniles after 14 days								
Mean no. progeny per replicate	257	247	237	236	259	250	227	236	218*
standard deviation	16	25	16	22	32	39	35	19	13
% reduction compared to control	-	3.9	7.8	8.3	-0.8	2.7	11.5	8.2	15.0

* significantly reduced compared to the control (Williams t-test, $\alpha = 0.05$, one-sided smaller)
The results represent rounded values calculated from the exact raw data

Validity Criteria

The validity criteria for the control group were met:

- Mean mortality of adult females: ≤ 20% (observed: 5%)
- Mean number of juveniles per replicate: ≥ 50 (calculated: 257)

- Coefficient of variation (mean number of juveniles per replicate): ≤ 30% (calculated: 6.2%)

Conclusions

In a test conducted with A8637C reproduction was not reduced compared to the control up to and including the concentration of 555.6 mg test item/kg soil but was reduced at 1000 mg test item/kg soil. Therefore, the overall No Observed Effect Concentration (NOEC) was determined to be 555.6 mg test item/kg soil (277.8 mg cyprodinil/kg soil).

(Lührs U, 2014c)

Report:	K-CA 8.4.2.1/05 ~~04~~ Lührs U. (2014d) Cyprodinil EC (A14325E) - Effects on Reproduction of the Predatory Mite *Hypoaspis aculeifer* in Artificial Soil with 5% Peat. Report Number 92781089. Institut für Biologische Analytik und Consulting IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany. (Syngenta file No. A14325E_10062)

Guidelines

OECD Guideline 226: Predatory mite (*Hypoaspis (Geolaelaps) aculeifer*) reproduction test in soil. (2008)

GLP: Yes.

Executive Summary

In a test conducted with A14325E no statistically significant effects on reproduction of *Hypoaspis aculeifer* up to and including the concentration of 1000 mg test item/kg soil were observed.

Therefore, the overall NOEC was 1000 mg/kg soil dry weight (295 mg cyprodinil/kg), the highest concentration tested.

Materials

Test Material	A14325E Cyprodinil EC	
Lot/Batch #:	SMO3A100	
Actual content of active ingredients:	Cyprodinil:	29.1% w/w, corresponding to 295 g/L
Description:	Light yellow Liquid	
Stability of test compound:	Stable under standard conditions	
Reanalysis/Expiry date:	End of January 2017	
Density:	1014 kg/m^3	
Treatments		
Test rates:	16.3, 29.4, 52.9, 95.3, 171.5, 308.6, 555.6 and 1000 mg test item/kg soil	
Control:	Untreated	
Toxic standard:	BAS 152 11 I Perfekthion (nominally 400 g dimethoate/L, analysed 400.9 g dimethoate/L)	
Test organisms		
Species	*Hypoaspis aculeifer* (CANESTRINI)	
Source:	Cultured in test facility	
Food:	Cheese mites, *Tyrophagus putrescentiae* at 2 spatulas on day 0, 2 and 4, 1 spatula on day 7 and 9, ½ spatulas on day 11.	

Age at test start:	7 days after reaching adulthood
Test design	
Vessels:	Glass containers (volume: 100 mL; diameter: 5 cm), tight screw top closure to avoid water evaporation, filled with approximately 20 g artificial soil dry weight.
Substrate:	Artificial soil comprising 5% sphagnum peat, 20 % kaolinite clay, 74.7% industrial quartz sand and 0.3% calcium carbonate.
Replication:	Control group: 8 Treated group: 4
No. of mites/arena :	10
Duration of test:	14 days
Environmental test conditions	
Temperature:	18 to 22°C
pH:	Test start: 6.2 Test end: 6.1 – 6.2
Water content of soil:	Test start : 50.4% to 53.1% of maximum WHC Test end: 49.4% to 51.9% of maximum WHC
Photoperiod:	16 h light : 8 h dark, 400 to 800 lux

Study Design and Methods

Experimental dates: 1st to 17th September 2014

Adult females of the soil mite *Hypoaspis aculeifer* were exposed to concentrations of A14325E incorporated into the test soil. An exactly weighed amount of the test item was mixed with purified water to make a stock solution, and appropriate volumes of this stock solution were further diluted with purified water to obtain the test concentrations such that, when added to pre-moistened artificial soil, a final moisture content value of 50% WHC was achieved. Adult females were transferred to the test vessels which contained untreated (control), reference item or test item treated artificial soil. Ten adult females were introduced to each test vessel. As a source of food, cheese mites (*Tyrophagus putrescentiae*) were added to the soil surface throughout the test. The test was carried out under controlled light-dark cycle. Fourteen days after introducing the test organisms, the surviving mites and the juveniles of *Hypoaspis aculeifer* were extracted by heat/light extraction. From these data the mortality of the adult females and the reproductive output were calculated.

The mean number of dead adult female mites for each treatment, the mean number of juvenile mites for each treatment, the NOEC, the LOEC values, and the LC_{50} at day 14 were not able to be determined by statistical analysis as no mortality above 50% was observed.

Mortality data were statistically analysed using Fisher's Exact test (multiple comparison, $\alpha = 0.05$, one-sided greater).

Reproduction data were tested for normal distribution and homogeneity of variance using Shapiro-Wilk's test and Levene's test ($\alpha = 0.05$). As data were normally distributed and homogeneous, the further statistical evaluation was performed using Williams t-test (multiple comparison, $\alpha = 0.05$, one-sided smaller).

The determination of the NOEC and LOEC values was based on the results of the statistical evaluation. The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

Results and Discussion

Mortality and fecundity are summarised in the table below.

Table 8.4.2.1-4: Effects of residues of A14325E on mortality and reproduction of *Hypoaspis aculeifer*

Endpoint	Treatment group (mg A14325E/kg soil d.w.)								
	Control	**16.3**	**29.4**	**52.9**	**95.3**	**171.5**	**308.6**	**555.6**	**1000**
	Mortality of adult mites after 14 days								
% mortality	8	13	5	5	8	5	0	8	0
	Number of juveniles after 14 days								
Mean no. progeny per replicate [c]	258	271	291	285	257	277	280	254	266
standard deviation	24	30	28	23	39	12	21	38	37
% reduction compared to control	-	-5.0	-12.7	-10.6	0.3	-7.5	-8.6	1.6	-3.0

The results represent rounded values calculated from the exact raw data
Negative values indicate an increase relative to the control.

Validity Criteria

The validity criteria for the control group were met:

- Mean mortality of adult females: ≤ 20% (observed: 8%)
- Mean number of juveniles per replicate: ≥ 50 (calculated: 258)
- Coefficient of variation (mean number of juveniles per replicate: ≤ 30%

Conclusions

In a test conducted with A14325E no statistically significant effects on reproduction of *Hypoaspis aculeifer* up to and including the concentration of 1000 mg test item/kg soil were observed.

Therefore, the overall NOEC was 1000 mg/kg soil dry weight (295 mg cyprodinil/kg), the highest concentration tested.

(Lührs U, 2014d)

Metabolites of cyprodinil

Folsomia candida

Report:	Submitted for purposes of renewal as conducted on relevant soil metabolite: K-CA 8.4.2.1/06 ~~05~~ Vinall S. (2012) CGA249287 – A laboratory test to determine the effects of fresh residues on the springtail *Folsomia candida* (Collembola, Isotomidae). Mambo-Tox Ltd., 2 Venture Road, Chilworth Science Park, Southampton SO16 7NP, UK. (Syngenta File No. CGA249287_10003)

Guidelines

OECD Guidelines No. 232. Collembolan Reproduction test in soil (2009)

GLP: Yes.

Executive Summary

The effects of CGA249287 on the reproduction and the parental mortality of collembola species *Folsomia candida* were determined. Based on the mean number of F1 progeny the NOEC, EC_{10} and EC_{20} values were determined to be 31, 7.9 and 22.7 mg /kg soil dry weight, respectively.

Materials

Test Material	CGA249287
Lot/Batch #:	RL-2929
Purity:	100 ± 2% w/w
Description:	White solid
Stability of test compound:	Stable under standard conditions.
Reanalysis/Expiry date:	June 2018
Treatments	
Test concentrations:	2.8, 5, 9, 17, 31, 56, 100, 180 and 324 mg CGA249287/kg soil dry weight
Control:	Untreated
Toxic standard:	Betosip 114 (Sipcam (114 g/L phenmedipham)
Application method:	Mixed with artificial soil
Test organisms	
Species:	*Folsomia candida*
Age:	11 days old
Source:	Culture maintained at Test Facility
Feeding:	2 mg granulated dry yeast at the start of the test and after 14 days
Test design	
Arenas:	Glass containers (volume: 120 mL; diameter: 4.5 cm), closed tightly to avoid water evaporation, filled with 30 g artificial soil fresh weight.
Substrate:	Artificial soil comprising 5% sphagnum peat, 20% kaolinite clay, 74. % industrial quartz sand (> 50% of the particles between 0.05 mm and 0.2 mm) and 0.2% calcium carbonate.
Replication:	Control: 8 Treatment: 4
No./arena :	10
Duration of test:	28 days
Environmental test conditions	
Temperature:	19.2°C to 22.5°C
pH of soil:	Test start: 6.17 to 6.49 Test end: 5.66 to 6.23
Photoperiod:	16 hour photoperiod (440 to 510 lux)

Study Design and Methods

Experimental dates: 17th July to 26th September 2012

The test concentrations were prepared by dispersing an exactly weighed amount of the test item in deionised water to make a stock solution. This stock solution was diluted with deionised water for each

test concentration and was thoroughly mixed with the artificial soil using a mixing machine, achieving a final nominal water content of 40-60 % of WHC. The control was treated with deionised water only.

Ten juvenile collembolans were transferred after the application to the substrate surface of each test vessel using an exhauster. Four replicates (+ two replicates not loaded with collembolans for measurement purposes) were used per test concentration and eight were used for the control. The test organisms were fed twice during the experiment (at the start of the test and after 14 days) with approximately 2 mg of granulated dry yeast per test vessel. Four weeks after introducing the test organisms, the surviving parental collembolans and offspring (juveniles) were counted.

All values presented throughout this report were calculated using the original raw data and were not based on rounded values.

Mortality data were statistically analyzed using Fisher's Exact Test (multiple comparison, $\alpha = 0.05$, one-sided greater).

Probit regression analysis was performed on the data for the numbers of progeny in the test item, in order to derive the 'effect concentrations' (EC_X) for key points on the response curve (e.g. EC_{50}, EC_{20} and EC_{10}).

Results and Discussion

Mortality and fecundity are summarised in the table below.

Table 8.4.2.1-5: Effects of residues of CGA249287 on mortality and reproduction of *Folsomia candida*

Endpoint	Control	Treatment group (mg CGA249287/kg soil d.w.)									
		2.8	5	9	17	31	56	100	180	324	Tox ref
% Mortality of parental collembolans after 4 weeks	14	15	15	15	18	13	18	20	40**	43**	62***
Mean number of juveniles after 4 weeks	624	579	636	568	552	503	401**	394**	297***	214***	85***
% reduction compared to control	-	7.3	-1.8	9.1	11.7	19.4	35.7	36.9	52.4	65.8	86.4
NOEC	31										
EC_{10}	7.9 (95% CI = 3.3 to 13.7)										
EC_{20}	22.7 (95% CI = 13.1 to 34.5)										

Mortality in individual test item treatments compared to the control using Fisher's Exact Test($\alpha = 0.05$). Treatment means that differed significantly from the control are indicated with asterisks (*** $P < 0.001$, ** $P < 0.01$).

Fecundity assessment data from individual treatments were compared to the data from the control by one-way ANOVA and Dunnett's t-test ($\alpha = 0.05$). Values marked with asterisks differed significantly from the control (*** $P < 0.001$, ** $P < 0.01$).

Validity criteria

The validity criteria were met since:

- Control treatment mortality was 14% (must be < 20%)

- The mean number of juvenile recorded in the control treatment was 624 (must be > 100 per replicate)
- The coefficient of variation of reproduction in the control was 15% (must not be > 30%)

Conclusions

The NOEC based on mean number of progeny for exposure of *Folsomia candida* to CGA249287 was estimated to be 31 mg CGA249287/kg soil. EC_{10} and EC_{20} values were estimated to be 7.9 and 22.7 mg CGA249287/kg, respectively.

(Vinall S, 2012)

Report:	Submitted for purposes of renewal as conducted on relevant soil metabolite: K-CA 8.4.2.1/07 ~~06~~ Lührs U. (2014e) CGA275535 - Effects on Reproduction of the Collembola *Folsomia candida* in Artificial Soil with 5%. Report Number 92791016. Institut für Biologische Analytik und Consulting IBACON GmbH Arheilger Weg 17 64380 Rossdorf, Germany. (Syngenta File No. CGA275535_10004)

Guidelines

OECD Guidelines No. 232. Collembolan Reproduction test in soil (2009)

ISO 11267: Soil quality – inhibition of reproduction of Collembola (*Folsomia candida*) by soil pollutants. (1999)

GLP: Yes.

Executive Summary

The effects of CGA275535 on the reproduction and the parental mortality of collembola species *Folsomia candida* were determined. The NOEC based on number of F1 progeny was determined to be 171.5 mg /kg soil dry weight.

Materials

Test Material	CGA275535
Lot/Batch #:	Kl 6230/3
Purity:	99% w/w
Description:	Beige solid
Stability of test compound:	Stable under standard conditions.
Reanalysis/Expiry date:	End of February 2018
Density:	Not stated
Treatments	
Test rates:	16.3, 29.4, 52.9, 95.3, 171.5, 308.6, 555.6 and 1000 mg CGA275535/kg soil dry weight
Control:	Untreated
Toxic standard:	Boric acid tested once a year
Application method:	Mixed with artificial soil
Test organisms	
Species:	*Folsomia candida*

Age:	10 – 12 days
Source:	Culture maintained at Test Facility
Feeding:	2 mg granulated dry yeast at the start of the test and after 14 days
Test design	
Arenas:	Glass containers (volume: 100 mL; diameter: 5 cm), closed tightly to avoid water evaporation, filled with 30 g artificial soil fresh weight.
Substrate:	Artificial soil comprising 5% sphagnum peat, 20% kaolinite clay, 74.8% industrial quartz sand (> 50% of the particles between 0.05 mm and 0.2 mm) and 0.2% calcium carbonate.
Replication:	Control: 8 Treatment: 4
No./arena :	10
Duration of test:	28 days
Environmental test conditions	
Temperature:	18°C to 22°C
pH of soil:	Test start: 6.1 to 6.3 Test end: 5.8 to 5.9
Water content of soil:	Test start: 51.2% to 53.2% of WHC Test end: 47.3% to 49.7% of WHC
Photoperiod:	16 hour photoperiod (400 to 800 lux)

Study Design and Methods

Experimental dates: 8th August to 8th September 2014

The test concentrations were prepared by dispersing an exactly weighed amount of the test item in deionised water to make a stock solution. This stock solution was diluted with deionised water for each test concentration and was thoroughly mixed with the artificial soil using a mixing machine, achieving a final nominal water content of 40-60% of WHC. The control was treated with deionised water only.

Ten juvenile collembolans were transferred after the application to the substrate surface of each test vessel using an exhauster. Four replicates (+ two replicates not loaded with collembolans for measurement purposes) were used per test concentration and eight were used for the control. The test organisms were fed twice during the experiment (at the start of the test and after 14 days) with approximately 2 mg of granulated dry yeast per test vessel. Four weeks after introducing the test organisms, the surviving parental collembolans and offspring (juveniles) were counted.

All values presented throughout this report were calculated using the original raw data and were not based on rounded values.

Mortality data were statistically analyzed using Fisher's Exact Test (multiple comparison, $\alpha = 0.05$, one-sided greater).

Reproduction data were tested for normal distribution and homogeneity of variance using Shapiro-Wilk's test and Levene's test ($\alpha = 0.05$). Further statistical evaluation was performed using Williams t-test (multiple comparison, $\alpha = 0.05$, one-sided smaller).

The determination of the NOEC and LOEC values was based on the results of the statistical evaluation. EC values and their 95% confidence limits at day 28 were not determined by statistical analysis as no reduction of reproduction above 50% was observed. The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

Results and Discussion

Mortality and fecundity are summarised in the table below.

Table 8.4.2.1-6: Effects of residues of CGA275535 on mortality and reproduction of *Folsomia candida*

Endpoint	Treatment group (mg CGA275535/kg soil d.w.)								
	Control	**16.3**	**29.4**	**52.9**	**95.3**	**171.5**	**308.6**	**555.6**	**1000**
% Mortality of parental collembolans after 4 weeks	11	20	18	8	13	5	20	8	10
Mean number of juveniles after 4 weeks	553	517	479	459	488	582	443*	470*	452*
SD	68	98	87	44	92	109	49	77	47
% reduction compared to control	-	6.6	13.4	17.1	11.9	-5.2	20.0	15.1	18.3
NOEC (reproduction)	171.5								

* significantly different compared to the control (Williams t-test, $\alpha = 0.05$, one-sided smaller)

Validity criteria

The validity criteria are as follows:

- Control treatment mortality was 11% (must be <20%)
- The mean number of juvenile recorded in the control treatment was 553 (must be >100 per replicate)
- The coefficient of variation of reproduction in the control was 12.3 % (must not be >30%)

Conclusions

The effects of CGA275535 on the reproduction and the parental mortality of collembola species *Folsomia candida* were determined. The NOEC based on number of F1 progeny was determined to be 171.5 mg /kg soil dry weight.

(Lührs U, 2014e)

Report:	Submitted for purposes of renewal as conducted on relevant soil metabolite: K-CA 8.4.2.1/08 ~~07~~ Lührs U. (2015a) CGA321915 – Effects on Reproduction of the Collembola *Folsomia candida* in Artificial Soil with 5 % Peat. Report Number 96341016. Institut für Biologische Analytik und Consulting, IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany. (Syngenta file No. CGA321915_10010).

Guidelines

OECD Guidelines No. 232. Collembolan Reproduction test in soil (2009)

ISO 11267: Soil quality – inhibition of reproduction of Collembola (*Folsomia candida*) by soil pollutants. (1999)

GLP: Yes

Executive Summary

The effects of CGA321915 on the reproduction and the parental mortality of collembola species *Folsomia candida* were determined. The NOEC based on number of F1 progeny was determined to be 1000 mg CGA321915/kg soil dry weight.

Materials

Test Material	CGA321915
Parent:	CGA219417 (Cyprodinil)
Lot/Batch #:	MES 356/1
Purity:	98% w/w ± 2 %
Description:	White solid
Stability of test compound:	Stable under test conditions
Reanalysis/Expiry date:	30 September 2016
Density:	Not applicable
Treatments	
Test rates:	7.813, 15.63, 31.25, 62.5, 125, 250, 500 and 1000 mg CGA321915/kg soil dry weight
Control:	Deionised water
Toxic standard:	Boric Acid (purity: 100.3 %) at 30.5, 48.8, 78.1, 125 and 200 mg boric acid/kg dry soil weight (separate study - No.: 61405016, date: June to August 2014)
Application method:	CGA321915 dispersed in deionised water was mixed into artificial soil prior to introduction of collembolans
Test organisms	
Species:	*Folsomia candida* (Willem)
Age:	Juveniles, 10 to 12 days old
Source:	Culture maintained at Test Facility (origin not reported)
Feeding:	Approximately 2 mg granulated dried yeast at the start of the test and after 14 days
Test design	
Arenas:	Glass containers (100 mL capacity, 5 cm diameter) with close-fitting lids
Substrate:	Artificial soil comprising 5% sphagnum peat, 20% kaolinite clay, 74.8% fine quartz sand (> 50% of the particles between 0.05 mm and 0.2 mm) and 0.2% calcium carbonate. 30 g ± 1.0 g fresh weight of artificial soil was added to each test vessel
Replication:	Treated groups 4, control group 8, plus an additional jar per treatment for measurement purposes
No./arena :	10
Duration of test:	28 days
Environmental test conditions	
Temperature:	18 to 22 °C
pH of soil:	Test start: 6.0 to 6.1 Test end: 5.9 to 6.1
Water content of soil:	Test start: 50.4 to 51.7% of WHC Test end: 47.3 to 50.9% of WHC
Photoperiod:	16 hours light and 8 hours dark at 400 to 800 lux

Study Design and Methods

Experimental dates: 16th February to 17th March 2015

The test concentrations were prepared by dispersing an exactly weighed amount of the test item in deionised water to make a solution of the highest test concentration. This solution was then serially diluted to prepare the lower test concentrations and the treatments thoroughly mixed with the artificial soil using a laboratory mixer, achieving a final nominal water content of 40 - 60 % of WHC. The control was treated with deionised water only.

Ten juvenile collembolans were transferred after the application to the substrate surface of each test vessel using an aspirator. Four replicates were used per test concentration and eight replicates were used for the control (+ one replicate per treatment not loaded with collembolans for measurement purposes). The test organisms were fed twice during the experiment (at the start of the test and after 14 days) with approximately 2 mg of granulated dried yeast per test vessel. Four weeks after introducing the test organisms, the surviving parental collembolans and offspring (juveniles) were counted.

All values presented throughout this report were calculated using the original raw data and were not based on rounded values.

The percentage mortality of the springtails was calculated for each treatment. The 28-day mortality data for the individual test-item treatments were compared to those for the control using Fisher's Exact Binomial Test (multiple comparison, with Bonferroni Correction, $\alpha = 0.05$, one-sided greater). The percentage reduction in reproductive performance in the test item treatment groups, compared to the control group, was calculated.

The data from the test-item treatments were compared to the control data by Williams t-test (multiple comparison, $\alpha = 0.05$, one-sided smaller). The results were used to determine the NOEC with respect to reproduction. The EC_x values could not be determined due to the outcome of the test.

Results and Discussion

Mortality and fecundity are summarised in the table below.

Table 8.4.2.1-7: Effects of residues of CGA321915 on mortality and reproduction of *Folsomia candida*

Endpoint	Treatment group (mg CGA321915/kg soil d.w.)								
	Control	**7.813**	**15.63**	**31.25**	**62.5**	**125**	**250**	**500**	**1000**
Mean mortality of parental collembolans after 4 weeks[a] (%)	6	10	5	13	5	5	15	5	10
Mean number of juveniles after 4 weeks[b]	469	488	480	505	544	516	461	447	408
SD	33	98	55	28	69	84	67	50	45
% reduction compared to control[c]	-	-4	-2	-8	-16	-10	2	5	13
NOEC (reproduction)	1000								

[a] Mean mortality amongst springtails originally introduced. Individual treatments compared to the control data using Fisher's Exact Binomial Test (multiple comparison, with Bonferroni Correction, $\alpha = 0.05$, one-sided greater). No statistically significant differences were determined.
[b] Fecundity data were compared to the control by Williams t-test (multiple comparison, $\alpha = 0.05$, one-sided smaller). No statistically significant differences were determined.
[c] A positive value indicates a decrease in reproduction compared to the control

Validity criteria

The validity criteria are as follows:

- Control treatment mortality (mean) was 6% (must be < 20%)
- The mean number of juveniles recorded in the control treatment was 469 (must be > 100 per replicate)
- The coefficient of variation of reproduction in the control was 7% (must not be > 30%)

Conclusions

The effects of CGA321915 on the reproduction and the parental mortality of collembola species *Folsomia candida* were determined. The NOEC based on number of F1 progeny was determined to be 1000 mg CGA321915/kg soil dry weight.

It was not possible to derive EC_{10} or EC_{20} values from the data

(Lührs U, 2015a)

Hypoaspis acuelifer

Report:	Submitted for purposes of renewal as conducted on relevant soil metabolite: K-CA 8.4.2.1/09 ~~08~~ Schulz L. (2014) CGA249287 - Effects on the Reproduction of the Predatory Mite *Hypoaspis aculeifer*. Report Number 14 10 48 194 S. BioChem agrar Labor für biologische und chemische Analytik GmbH Kupferstraße 6, 04827 Gerichshain, Germany. (Syngenta file No. CGA249287_10005)

Guidelines

OECD (2008). Effects on Biotic Systems, Method 226 Predatory mite (*Hypoaspis* (Geolaelaps) *aculeifer*) reproduction test in soil.

GLP: Yes.

Executive Summary

In a 14-day *Hypoaspis aculeifer* study with CGA249287 the NOEC for reproduction was determined to be 74 mg test item/kg soil dry weight. EC_{10} and EC_{20} values were estimated to be 70.5 and 321.3 mg CGA249287/kg, respectively.

Materials

Test Material	CGA249287
Lot/Batch #:	RL-2929
Actual content of active ingredients:	100% w/w
Description:	White solid
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	End of June 2018
Density:	Not stated
Treatments	
Test rates:	23, 41, 74, 133, 240, 432, 778, 1400 mg test item/kg soil d.w. (spacing factor: 1.8)
Control:	Untreated substrate, i.e. purified water only
Toxic standard:	Dimethoate (analysed purity: 99.8%, tolerance ± 1.0%)
Test organisms	
Species	*Hypoaspis aculeifer*
Source:	Originally obtained from Bayer CropScience AG and maintained at the Test Facility.
Food:	Before and during test every 2-3 days, Cheese mites, *Tyrophagus putrescentiae* (SCHRANK)
Age at test start:	Adults with an age difference of 3 days
Test design	
Vessels:	100 mL SCHOTT-bottles with screw cap (4 cm diameter, 11 cm high)
Substrate:	Artificial soil comprising 5% sphagnum peat, 20 % kaolinite clay, 74.8% industrial quartz sand and 0.2% calcium carbonate. 300g soil d.w. in control. 200g in treatment.
Replication:	Control group: 8 Treated group: 4

No. of mites/arena :	10 females
Duration of test:	14 days
Environmental test conditions	
Temperature:	19.7 - 21.4°C
pH:	5.5 – 6.0
Water content of soil:	46.39 - 51.14% of WHC
Photoperiod:	16 h light : 8 h dark (513 lux)

Study Design and Methods

Experimental dates: 8th to 27th August 2014

Adult females of the soil mite *Hypoaspis aculeifer* were exposed to concentrations of CGA249287 incorporated into the test soil. An exactly weighed amount of the test item was mixed with purified water to make a stock solution, and appropriate volumes of this stock solution were further diluted with purified water to obtain the test concentrations such that, when added to pre-moistened artificial soil, a final moisture content value of 50% WHC was achieved. Adult females were transferred to the test vessels which contained untreated (control), reference item or test item treated artificial soil. Ten adult females were introduced to each test vessel. As a source of food, cheese mites (*Tyrophagus putrescentiae*) were added to the soil surface of each test arena at the beginning of the test, and cheese mites *ad libitum* (every 2-3 days) throughout the test. The test was carried out under controlled light-dark cycle. Fourteen days after introducing the test organisms, the surviving mites and the juveniles of *Hypoaspis aculeifer* were extracted by heat/light extraction. From these data the mortality of the adult females and the reproductive output were calculated.

Mortality (number of dead adults) in % for the treatment groups was calculated. Missing mites were counted as dead. Observations in the treatment group were expressed relative to the control group. The corrected mortality in the treatment groups was calculated using Abbott's formula.

The reduction of reproductive output (Rr) for the treatment groups was calculated in comparison to the control.

$$Rr\ (\%) = (1 - Rt / Rc) * 100\ \%$$

Rt and Rc are the absolute values observed in the treatment and control groups.

The statistical analysis was performed with the software ToxRat Professional 2.10.05. Fisher's Exact Binomial Test with Bonferroni Correction and the Williams-t-test were used to compare the control with the independent test item groups. Probit analysis was used for EC_x calculation.

Results and Discussion

Mortality and fecundity are summarised in the table below.

Table 8.4.2.1-8: Effects of residues of CGA249287 on mortality and reproduction of *Hypoaspis aculeifer*

Endpoint	Treatment group (mg CGA249287 /kg soil d.w.)								
	Control	**23**	**41**	**74**	**133**	**240**	**432**	**778**	**1400**
	Mortality of adult mites after 14 days								
% mortality[a]	1.3	0	0	0	7.5	2.5	0	0	0
	Number of juveniles after 14 days								
Mean no. progeny per replicate [c]	251.9	242.8	248.3	228.5	195.8*	205.3*	190.0*	196.8*	162.8*
standard deviation	35.1	47.1	17.8	29.8	17.3	9.6	21.8	18.7	47.0
coefficient of variation %	13.9	19.4	7.2	13.1	8.9	4.7	11.5	9.5	28.9
% reduction compared to control[d]	-	3.6	1.4	9.3	22.3	18.5	24.6	21.9	35.4

[a] Mortality amongst mites originally introduced. Individual test-item treatments compared to the control using Fisher's Exact Test ($\alpha = 0.05$). Treatments that differed significantly from the control are indicated with an asterisk (*).
[b] Calculated using Abbott's formula for corrected mortality (Abbott, 1925): M (%) = (1-t/c)*100%
[c] The results for each treatment were individually compared to the control by one-way ANOVA and Dunnett's t-test ($\alpha = 0.05$). Values marked with an asterisk (*) differed significantly from the control.
[d] Percent reduction: $(1-R_t/R_c)*100\%$, where Rt = mean number of juvenile mites observed in the treated group(s) and Rc = mean number of juvenile mites observed in the control group. A positive value indicates a decrease and a negative value indicates an increase in reproduction, relative to the control.

Validity Criteria

The validity criteria for the control group were met:

- Mean mortality of adult females: ≤ 20% (observed: 1.3%)
- Mean number of juvenile per replicate: ≥ 50 (calculated: 251.9)
- Coefficient of variation (mean number of juveniles per replicate): ≤ 30% (calculated: 13.9%)

Conclusions

In a 14-day *Hypoaspis aculeifer* study with CGA249287 the NOEC for reproduction was determined to be 74 mg test item/kg soil dry weight. EC_{10} and EC_{20} values were estimated to be 70.5 and 321.3 mg CGA249287/kg, respectively.

(Schulz L, 2014)

Report:	Submitted for purposes of renewal as conducted on relevant soil metabolite: K-CA 8.4.2.1/10 ~~09~~ Lührs U. (2014f) CGA275535 - Effects on Reproduction of the Predatory Mite *Hypoaspis aculeifer* in Artificial Soil with 5% Peat. Report Number 92791089. Institut für Biologische Analytik und Consulting, IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany. (Syngenta file No. CGA275535_10000)

Guidelines

OECD 266

GLP: Yes.

Executive Summary

In a *Hypoaspis aculeifer* test conducted with CGA275535, the NOEC for reproduction was determined to be 171.5 mg test item/kg soil dry weight. EC_{10} and EC_{20} values were estimated to be 104.6 and 272.5 mg CGA249287/kg, respectively.

Materials

Test Material	CGA275535
Lot/Batch #:	Kl 6230/3
Actual content of active ingredients:	CGA275535 99% w/w
Description:	Solid
Stability of test compound:	Stable under standard conditions.
Reanalysis/Expiry date:	End of February 2018
Density:	Not stated
Treatments	
Test rates:	16.3, 29.4, 52.9, 95.3, 171.5, 308.6, 555.6 and 1000 mg test item/kg soil
Control:	Untreated
Toxic standard:	Dimethoate: 400.0 g/L (nominal), 400.9 g/L (analysed)
Application method:	Mixed with artificial soil.
Test organisms	
Species:	*Hypoaspis aculeifer*
Age:	Adults, approximately 11 days after reaching the adult stage.
Source:	Culture maintained at Test Facility.
Feeding:	Two spatulas of cheese mites (*Tyrophagus putrescentiae* cultured by IBACON) at experimental start, 1-2 spatulas on day 3, 5, 7 and 10, ½ spatula on day 12.
Test design	
Arenas:	Glass containers (volume: 100 mL; diameter: 5 cm), tight screw top closure to avoid water evaporation, filled with approximately 20 g ± 1.0 g artificial soil dry weight.
Replication:	Control: 8 Treatment: 4
No. of mites/arena :	10
Duration of test:	14 days
Environmental test conditions	
Temperature:	18°C to 22°C
Humidity:	Not stated
Photoperiod:	16 h photoperiod (400 to 800 lux).

Study Design and Methods

Experimental dates: 8th to 25th August 2014

A stock solution was prepared by dissolving 481.5 mg of CGA275535 in 30 mL acetone. A dilution series was prepared from the stock solution by adding 12 mL of acetone to 15 mL of the second stock solution or the respective dilution. The test item blended sand was added to artificial soil with reduced sand fraction to result in a final net weight of 260 g. This resulted in the following nominal concentrations: 16.3, 29.4, 52.9, 95.3, 171.5, 308.6, 555.6 and 1000 mg test item/kg soil.

Mortality data were statistically analysed using Fisher's Exact test (multiple comparison, $\alpha = 0.05$, one-sided greater).

Reproduction data were tested for normal distribution and homogeneity of variance using Shapiro-Wilk's test and Levene's test ($\alpha = 0.05$). As data were normally distributed and homogeneous, the further statistical evaluation was performed using Williams t-test (multiple comparison, $\alpha = 0.05$, one-sided smaller).

The determination of the NOEC and LOEC values was based on the results of the statistical evaluation. The EC values were calculated by Probit Analysis. The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

Results and Discussion

Mortality and reproduction are summarised in the table below.

Table 8.4.2.1-9: Effects of CGA275535 on mortality and reproduction of *Hypoaspis aculeifer*

Endpoint	Treatment group (mg CGA275535/kg soil d.w.)								
	Control	**16.3**	**29.4**	**52.9**	**95.3**	**171.5**	**308.6**	**555.6**	**1000**
	Mortality of adult mites after 14 days								
% mortality[a]	1	3	3	5	5	5	8	3	3
	Number of juveniles after 14 days								
Mean no. progeny per replicate	226	223	202	223	219	228	143	162	140
Reduction as % of the control	-	99	89	99	97	101	63*	72*	62*

The results represent rounded values calculated from the exact raw data
* Significantly reduced compared to the control (Williams t-test, $\alpha = 0.05$, one-sided smaller)

Validity criteria

The validity criteria were met since:

- Control mortality was 1% (should be < 20%)
- Control reproduction was 183 to 274 (should be >50 juveniles per test unit)
- Coefficient of Variation (CV) of the control reproduction was 12.4% (should be < 30%)

Conclusions

In a *Hypoaspis aculeifer* test conducted with CGA275535, the NOEC for reproduction was determined to be 171.5 mg test item/kg soil dry weight. EC_{10} and EC_{20} values were estimated to be 104.6 and 272.5 mg CGA249287/kg, respectively.

(Lührs U, 2014f)

Report:	Submitted for purposes of renewal as conducted on relevant soil metabolite: K-CA 8.4.2.1/11 ~~10~~ Lührs U. (2015b), CGA321915 - Effects on Reproduction of the Predatory Mite *Hypoaspis aculeifer* in Artificial Soil with 5 % Peat. Report Number 96341089. Institut für Biologische Analytik und Consulting, IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany. (Syngenta file No. CGA321915_10011)

Guidelines

OECD Guideline 226: Predatory mite (*Hypoaspis (Geolaelaps) aculeifer*) reproduction test in soil. (2008)

GLP: Yes

Executive Summary

In a *Hypoaspis aculeifer* test conducted with CGA321915, the NOEC for reproduction was determined to be 1000 mg test item/kg soil dry weight, the highest concentration tested.

Materials

Test Material	CGA321915
Parent:	CGA219417 (Cyprodinil)
Lot/Batch #:	MES 356/1
Purity:	98% w/w (estimated error: ± 2 %)
Description:	White solid
Stability of test compound:	Stable under test conditions
Reanalysis/Expiry date:	30 September 2016
Density:	Not applicable
Treatments	
Test rates:	62.5, 125, 250, 500 and 1000 mg CGA321915/kg soil dry weight
Control:	Deionised water
Toxic standard:	BAS 152 11 I Perfekthion (nominally 400 g dimethoate/L, analysed 400.9 g dimethoate/L) applied at a rate of 1.3, 2.0, 3.0, 4.5 and 6.8 mg dimethoate/kg soil (separate study, Project 93461089, performed June 2014)
Test organisms	
Species	*Hypoaspis aculeifer* (CANESTRINI)
Source:	Cultured in test facility (origin not reported)
Food:	Cheese mites, *Tyrophagus putrescentiae* at 2 spatulas on day 0, 2 and 4, 1 spatula on day 7 and 9, ½ a spatula on day 11
Age at test start:	Adults. approximately 7 days after reaching the adult stage
Test design	
Vessels:	Glass containers (volume: 100 mL; diameter: 5 cm), tight screw top closure to avoid water evaporation, filled with approximately 20 g ± 1.0 g soil d.w.
Substrate:	Artificial soil comprising 5% sphagnum peat, 20% kaolinite clay, 74.8% fine quartz sand and 0.2% calcium carbonate
Replication:	Control group: 8 Treated group: 4 1 additional replicate per treatment for measurement purposes
No. of mites/arena :	10

Duration of test:	14 days

Environmental test conditions

Temperature:	18 to 22 °C
pH:	Test start: 6.0 to 6.1 Test end: 5.9 to 6.0
Water content of soil:	Test start: 50.4 to 51.7% of maximum WHC Test end: 48.1 to 51.2% of maximum WHC
Photoperiod:	16 h light : 8 h dark, 400 to 800 lux

Study Design and Methods

Experimental dates: 16th February to 4th March 2015

Adult females of the soil mite *Hypoaspis aculeifer* were exposed to concentrations of CGA321915 incorporated into the test soil. An exactly weighed amount of the test item was mixed with deionised water to make a stock solution of the highest test concentration, and the lower test concentrations were obtained using a series of dilutions such that, when added to pre-moistened artificial soil, a final moisture content value of 40 to 60% WHC was achieved. Adult females were transferred to the test vessels which contained untreated (control) or test item treated artificial soil. Ten adult females were introduced to each test vessel. As a source of food, cheese mites (*Tyrophagus putrescentiae*) were added to the soil surface throughout the test. The test was carried out under controlled light-dark cycle. Fourteen days after introducing the test organisms, the surviving mites and the juveniles of *Hypoaspis aculeifer* were extracted by heat/light extraction. From these data the mortality of the adult females and the reproductive output were calculated.

The mean number of dead adult female mites for each treatment, the mean number of juvenile mites for each treatment, the NOEC, the LOEC values, and the LC_{50} at day 14 were not able to be determined by statistical analysis as no mortality above 50% was observed.

Mortality data were statistically analysed using Fisher's Exact Binomial test (multiple comparison, with Bonferroni Correction, $\alpha = 0.05$, one-sided greater). Reproduction data were tested for normal distribution and homogeneity of variance using Shapiro-Wilk's test and Levene's test ($\alpha = 0.05$). As data were normally distributed and homogeneous, the further statistical evaluation was performed using Williams t-test (multiple comparison, $\alpha = 0.05$, one-sided smaller).

The determination of the NOEC and LOEC values was based on the results of the statistical evaluation. The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05.

Results and Discussion

Mortality and fecundity are summarised in the table below.

Table 8.4.2.1-10: Effects of residues of CGA321915 on mortality and reproduction of *Hypoaspis aculeifer*

Endpoint	Treatment group (mg CGA321915/kg soil d.w.)					
	Control	**62.5**	**125**	**250**	**500**	**1000**
	Mortality of adult mites after 14 days					
Mean mortality (%)	6	3	5	3	3	0
	Number of juveniles after 14 days					
Mean no. progeny per replicate	212	222	197	217	212	202
standard deviation	12	25	23	22	8	33
% reduction compared to control[a]	-	-5	7	-3	0	5

The results represent rounded values calculated from the exact raw data
[a] Negative values indicate an increased reproduction relative to the control

Validity Criteria

The validity criteria for the control group were met:

- Mean mortality of adult females in control: ≤ 20% (observed: 6%)
- Mean number of juveniles per replicate: ≥ 50 (calculated: 212)
- Coefficient of variation (mean number of juveniles per replicate): ≤ 30% (calculated: 5.7%)

Conclusions

The effects of CGA321915 on the mortality and reproductive output of the soil mite species *Hypoaspis aculeifer* were determined during a 14-day test. The NOEC was determined to be 1000 mg /kg soil dry weight, the highest concentration tested.

It was not possible to derive EC_{10} or EC_{20} values from the data

(Lührs U, 2015b)

Relevant Literature on Non-Target Soil Meso- and Macro-Fauna

No relevant scientifically peer-reviewed open literature could be found on cyprodinil or its major metabolites. Details of the literature search undertaken can be found in M-CA Section 9 of the current submission.

CA 8.5 Effects on Nitrogen Transformation

Table 8.5-1: Table of endpoints for cyprodinil

Organism	Test item	Test type	Endpoint		Endpoint used in the risk assessment (NOEC - mg/kg)	Reference (author, date, Syngenta File No.)
Soil micro-organisms	Cyprodinil	C & N transformation	EU	NOEC = 26.7 mg/kg	26.7	***Wütrich (1993) CGA219417/0209***
			LIT	NOEC not derived but effects <25% relative to the control at relevant concentrations [a]	-	***Jastrzębska (2010)***
	CGA249287		EU	NOEC = 3.33 mg/kg	3.33	***Grade (2000) CGA249287/010***
	CGA275535			Nitrogen transformation: 1.15 Carbon transformation: 2.3	1.15	***Seyfried (2001) CGA275535/020***
	CGA321915		New study	5.10	5.10	***Hammesfahr (2015) CGA321915_10008***

LIT = Scientific peer-reviewed literature article
[a] UNIX® (750 g cyprodinil/kg) was used as the test substance

The studies previously submitted in the EU were summarised in point B.9.8.1 of the DAR~~. therefore additional study summaries are not repeated here~~. At the request of ANSES summaries have been included in order to aid dossier evaluation .The endpoints are summarised in Table 8.5-1 above.

Report:	Summary submitted at the request of ANSES for ease of dossier evaluation: *KIIA 8.5. Wüthrich V. (1993). The effects of CGA219417 on soil respiration and nitrification. Report Number 329038. RCC UMWELTCHEMIE AG, CH-4452 Itingen, Switzerland. (Syngenta File No. CGA219417/0209).*

Guidelines

Biologische Bundesanstalt Deutschland (BBA). Richtlinien fuer die amtliche Pruefung von Pflanzenschutzmitteln Teil VI 1-1 (2. Auflage). “Auswirkungen auf die Aktivitaet der Bodenmikroflora, März, 1990.”

GLP: Yes

Executive Summary

The effect of CGA219417 on soil microorganisms was evaluated in two different soils types, a sandy loam and loam. CGA219417 was applied to soil at two concentrations of 2.67 and 26.7 mg test item/kg dry soil. The test item caused negligible effects on short-term respiration at 26.7 mg/kg dry soil. The observed effects ranged from 0 to -10.8 % deviation from the control after 28 days of incubation.

In the nitrification experiment CGA219417 did not significantly affect the ammonification in both soils at 26.7 mg/kg dry soil. Slight inhibitory effects on nitrification were observed after 28 days incubation in

the sandy loam soil at 2.67 mg/kg and the loam soil at 2.67 and 26.7 mg/kg dry soil. However, these effects were found to be transient and were not observed after 56 days incubation.

Materials

Test Material	CGA219417
Lot/Batch #:	P.912006
Purity:	99.6 %
Description:	Solid
Stability of test compound:	48 hours in water
Reanalysis/Expiry date:	February 1994
Treatments	
Test concentrations:	2.67 and 26.7 mg test item/kg dry soil
Solvent / carrier:	Acetone
Control:	Acetone spiked soil
Toxic standard:	None
Test design	
Soil type:	Soil 1: Sandy loam Soil 2: Loam
Test units:	250 mL Erlenmeyer flasks containing 100 g dry weight soil
Replication:	Triplicate soils samples were prepared for each treatment and control
Sampling intervals :	Carbon test: 0 - 3 hours, 14, and 28 days after application. Nitrification test: 0 - 3 hours, 14, 28 and 56 days
Duration of test:	Respiration test: 28 days Nitrification test: 56 days
Environmental test conditions	
Temperature:	20 ± 2 °C
pH of soil:	Soil 1: 6.9 to 7.6 Soil 2: 7.4 to 7.8
Soil moisture content:	Soil 1: 13.4 g water/100 dry soil Soil 2: 17.8 g water/100 dry soil
Photoperiod:	Constant darkness

Study Design and Methods

Experimental dates: 19th June to 11th September 1992

Soil samples were treated with CGA219417 at two concentrations, 2.67 and 26.7mg test item/kg dry soil. Test concentrations related to a soil depth of 5 cm and a soil density of 1.5 g/cm^3 and were equivalent to the maximum field rate (based on 2000 g a.s./ha) and ten times the maximum field rate. The study design included an untreated control.

During the application the soil was thoroughly mixed. Soils for use in the nitrification experiment were amended with 0.5 g Lucerne per 100 g dry soil. Water was added to the soil to achieve a water content of

40 % of MWC for soil 1 and 39 % for soil 2. The water content of the soil in each test vessel was monitored on a weekly basis and lost moisture was compensated by the addition of purified water.

Three replicate test vessel containing 100 g dry weight soil were prepared for each timepoint and treatment rate and the control for the nitrogen transformation test and carbon transformation test.

For the nitrogen transformation test, 40 g soil dry weight aliquots were collected from the triplicate test vessels and extracted with 45 mL 2 N KCl by shaking at room temperature. The samples were centrifuged and the supernatant decanted through filter paper. The extraction was repeated and the combined extracts were adjusted to 100 mL with 2 M KCl. The concentrations of nitrite, nitrate and ammonium were determined in triplicate using a flow injection analyser and the % deviation from the control group was determined.

For the carbon transformation test the respiration rates at each sampling point were determined by amending triplicate aliquots of each spiked soil with a glucose-talc mixture to induce respiration. The samples were then packed into glass-columns and respiration was measured by analyzing evolved CO_2 concentration in the outlet air stream for approximately 24 hours. CO_2 was measured semi-continuously with an infrared gas analyser. The mean respiration rates of the treatments after 28 days were compared to the control using Dunnett's test.

Results and Discussion

Results from the nitrogen transformation test and the carbon transformation test are summarised in the tables below.

Table 8.5-1: Effects on carbon transformation in soil after treatment with CGA219417

Soil Type	Treatment (mg/kg dry soil)	Incubation Time (Days)	Mean Respiration Rate (mg CO_2/h per 100 g dry soil)	% Deviation from Control
Soil 1: Sandy loam	Control	0	0.465	-
		14	0.704	-
		28	0.800	-
	2.67	0	0.392	-15.7
		14	0.842	+19.6
		28	0.803	0
	26.7	0	0.349*	-24.9
		14	0.840	+19.4
		28	0.763	-4.6
Soil 2: Loam	Control	0	0.936	-
		14	1.526	-
		28	1.522	-
	2.67	0	0.834	-10.9
		14	1.499	-1.8
		28	1.493	-1.9
	26.7	0	0.803	-14.1
		14	1.325*	-13.1
		28	1.357	-10.8

* statistically significantly different to control (Dunnett test, $\alpha = 0.05$)

Table 8.5-2: Effects of CGA219417 on nitrogen transformation in soil 1

Treatment (mg/kg dry soil)	Incubation Time (Days)	NH_4-N		NO_2-N		NO_3-N		Total N	
		Mean (mg/100 g dry soil)	% Deviation from Control	Mean (mg/100 g dry soil)	% Deviation from Control	Mean (mg/100 g dry soil)	% Deviation from Control	Mean (mg/100 g dry soil)	% Deviation from Control
Control	0	0.12	-	<0.01	-	1.61	-	1.75	-
	14	<0.01	-	0.01	-	1.21	-	1.23	-
	28	<0.01	-	<0.01	-	1.62	-	1.64	-
	56	0.02	-	<0.01	-	2.64	-	2.67	-
2.67	0	0.21	73.4	<0.01	n.a.	1.61	0.0	1.84	+5.2
	14	<0.01	n.a.	0.01	n.a.	0.65	-45.9	0.68	-45.0
	28	<0.01	n.a.	<0.01	n.a.	1.29	-20.4	1.31	-20.1
	56	<0.01	-6.0	<0.01	n.a.	2.49	-5.7	2.52	-5.6
26.7	0	0.20	64.7	<0.01	n.a.	1.63	+1.5	1.85	+6.0
	14	<0.01	n.a.	0.01	5.0	0.80	-33.5	0.83	-32.8
	28	<0.01	n.a.	<0.01	n.a.	1.61	-0.1	1.64	-0.1
	56	0.01	-12.3	<0.01	n.a.	2.75	4.3	2.78	4.1

n.a. not applicable

Table 8.5-3: Effects of CGA219417 on nitrogen transformation in soil 2

Treatment (mg/kg dry soil)	Incubation Time (Days)	NH_4-N		NO_2-N		NO_3-N		Total N	
		Mean (mg/100 g dry soil)	% Deviation from Control	Mean (mg/100 g dry soil)	% Deviation from Control	Mean (mg/100 g dry soil)	% Deviation from Control	Mean (mg/100 g dry soil)	% Deviation from Control
Control	0	0.09	-	<0.01	-	1.23	-	1.33	-
	14	<0.01	-	0.01	-	1.91	-	1.93	-
	28	<0.01	-	<0.01	-	2.51	-	2.53	-
	56	0.02	-	<0.01	-	3.96	-	3.99	-
2.67	0	0.21	128.8	<0.01	n.a.	1.39	+13.2	1.62	21.1
	14	<0.01	n.a.	0.01	2.9	0.87	-54.5	0.89	-53.8
	28	<0.01	n.a.	<0.01	n.a.	1.52	-39.5	1.54	-39.1
	56	0.02	-0.1	<0.01	n.a.	3.40	-14.1	3.43	-14.0
26.7	0	0.20	119.2	<0.01	n.a.	3.13	154.7	3.35	150.8
	14	<0.01	n.a.	0.01	-0.7	1.23	-35.7	1.25	-35.2
	28	<0.01	n.a.	<0.01	n.a.	1.84	-26.5	1.87	-26.2
	56	0.02	17.2	<0.01	n.a.	4.02	1.5	4.05	1.5

Validity criteria

Not reported.

Conclusions

CGA219417 showed negligible effects in short-term respiration experiments up to a concentration of 26.7 mg/kg dry soil. In addition, neither ammonification nor nitrification were significantly affected by

the test material. The slight inhibitory effects on nitrification observed at Day 28 were found to be transient and not evident by Day 56.

(Wüthrich V. 1993)

Metabolites of cyprodinil

Report:	Summary submitted at the request of ANSES for ease of dossier evaluation: *KIIA 8.5. Grade R. (2000). The Effects of CGA249287 (Metabolite of CGA219417) on Soil Respiration and Nitrification. Report Number 983835. Novartis Crop Protection AG, CH-4002 Basel, Switzerland. (Syngenta File No. CGA249287/0010).*

Guidelines

OECD Guidelines for Testing of Chemicals (Revised Draft Document, January 1999). Proposal for a new Guideline 216, Soil Microorganisms, Nitrogen Transformation Test and Proposal

GLP: Yes

Executive Summary

CGA249287 was applied to the soil at concentrations of 0.67 and 3.33 mg test item/kg dry soil.

The test item caused no significant effects on short-term respiration rates and on nitrification rates at 0.67 mg/kg dry soil. CGA249287 caused negligible and tolerable effects on N-mineralisation at 0.67 mg/kg and 3.33 mg/kg dry soil, respectively. For soil respiration negligible effects were observed at both the 0.67 and 3.33 mg/kg dry soil concentrations.

Materials

Test Material	CGA249287
Lot/Batch #:	RL-2929
Purity:	100 ± 2 %
Reanalysis/Expiry date:	March 2005
Treatments	
Test concentrations:	0.67 and 3.33 mg test item/kg dry soil
Solvent / carrier:	Acetone
Control:	Soil spiked with acetone treated sand
Toxic standard:	Dinoseb, 1.5 - 2.5 kg a.s./ha
Test design	
Soil type:	Loamy sand
Test units:	100 mL Erlenmeyer flasks
Replication:	Three replicates per test concentration for each sampling interval
Sampling intervals :	0-3 hours, 7, 14 and 28 days

Duration of test:	28 days
Environmental test conditions	
Temperature:	20 ± 1 °C
pH of soil:	7.4 to 7.6
Soil moisture content:	Not reported
Photoperiod:	Constant darkness

Study Design and Methods

Experimental dates: 9th November to 10th December 1999

Two experiments were performed to investigate the effects of CGA249287 on nitrogen conversions process and short-term respiration. For each test soil samples were treated with CGA249287 at two concentrations, 0.67 and 3.33 mg test item/kg dry soil. Test concentrations related to a soil depth of 5 cm and a soil density of 1.5 g/cm^3 and were equivalent to the maximum field rate (based on 500 g a.s./ha of parent) and five times the maximum field rate. The study design included a control and a toxic reference treatment, containing dinoseb at 12.5 kg a.s./ha.

The test item was dissolved in acetone and aliquots of this stock solution were applied to 20 g sand for each treatment rate. After complete evaporation of the acetone the treated sand was mixed with 1.0 kg soil (dry weight). Each treated soil was then mixed for approximately ten minutes. For the control treatment, sand was treated with an appropriate amount of acetone and once the acetone had evaporated the sand was mixed into the soil. In addition, soils used in the nitrification study were amended with powdered Lucerne directly after application of the test substance. The prepared soils were distributed between triplicate incubation flasks for each test concentration and sampling interval and incubated in the dark at 20 ± 1 °C. During incubation the soil moisture was controlled and adjusted with deionised water.

For the nitrogen transformation, triplicate samples of 10 g soil dry weight were sampled at each timepoint. The samples were extracted for 30 minutes with 1.9 M KCl. Extracts and residues were separated by centrifugation at 3000 rpm for 10 minutes. Extracts were passed through a folded paper filter. This treatment was repeated twice. The concentrations of nitrite, nitrate and ammonium in the extracts were determined in triplicate using a flow injection analyser.

For the carbon transformation test, the respiration rates at each sampling point were determined by amending triplicate 15 g soil aliquots of each spiked soil with 15 mg glucose. The samples were transferred to a glass column and the substrate induced respiration was followed for approximately 450 hours by means of an infrared (IR) gas analyser. Measurements were performed by flushing the glass columns about 5 times per hour with CO_2 free air for 30 seconds at a flow rate of 300 – 600 mL/min.

The mean respiration rate and nitrite, nitrate and ammonium concentrations were statistically evaluated using a Dunnett's test. The results were also compared and classified using the scheme detailed in Malkomes (1990).

Results and Discussion

Results from the nitrogen transformation test and the carbon transformation test are summarised in the tables below.

Table 8.5-4: Effects on carbon transformation in soil after treatment with CGA249287

Treatment (mg/kg dry soil)	Incubation Time (Days)	Respiration Rate (mL CO_2/h per kg dry soil)	% Deviation from Control
Control	0-3 hours	1.040	-
	7	1.002	-
	14	0.971	-
	28	0.938	-
0.67	0-3 hours	1.098	5.6
	7	1.071	6.9
	14	0.958	-1.3
	28	0.964	2.8
3.33	0-3 hours	1.036	-0.4
	7	0.998	-0.4
	14	0.838	-13.7
	28	0.920	-1.9
Toxic reference	0-3 hours	1.120	7.7
	7	0.891	-11.1
	14	0.678*	-30.2
	28	0.644*	-31.2

* statistically significantly different to control (Dunnett test)

Table 8.5-5: Effects of CGA249287 on total-N concentrations

Treatment (mg/kg dry soil)	Incubation Time (Days)	Total-N Concentrations (mg/kg dry soil)	% Deviation from Control
Control	0-3 hours	94.0	-
	7	124.0	-
	14	142.3	-
	28	156.3	-
0.67	0-3 hours	106.0	12.8
	7	133.3	7.5
	14	136.0	-4.4
	28	146.3	-6.4
3.33	0-3 hours	98.7	5.0
	7	117.0	-5.6
	14	119.0	-16.4
	28	124.3	-20.5
Toxic reference	0-3 hours	98.7	5.0
	7	162.0	30.6
	14	181.7	27.7
	28	197.5	26.4

Validity criteria

Not reported

Conclusions

In the tested soil CGA249287 showed no significant effects on short-term respiration rates and on nitrification rates at concentrations of 0.67 and 3.33 mg/kg dry soil. Based on the scheme proposed by Malkomes (1990) it can be concluded that at 0.67 and 3.33 mg/kg CGA249287 showed negligible or tolerable effects on N-mineralisation. On soil respiration negligible effects were observed at 0.67 and 3.33 mg/kg dry soil.

(Grade R. 2000)

Report:	Summary submitted at the request of ANSES for ease of dossier evaluation: *KIIA 8.5. Seyfried B. (2001). The Effects of CGA275535 (Metabolite of CGA219417) on Soil Respiration and Nitrification. Report Number 789761. RCC Ltd., CH-4452 Itingen, Switzerland. (Syngenta File No. CGA275535/0020).*

Guidelines

OECD guidelines 216, Soil Microorganisms: Nitrogen Transformation Test (2000)

OECD guidelines 217, Soil Microorganisms: Carbon Transformation Test (2000)

GLP: Yes

Executive Summary

CGA275535 was applied to the soil at concentrations of 0.23, 1.15 and 2.3 mg test item/kg dry soil. The test item caused no adverse effects on respiration in soil, as measured by CO_2 evolution at concentrations up to 2.3 mg test item/kg dry soil. CGA275535 had a negligible to tolerable effects on the ammonification processes in soil after 28, 44 and 57 days exposure at 2.3 mg test item/kg dry soil.

Materials

Test Material	CGA275535
Lot/Batch #:	KI 6230/3
Purity:	99 ± 2 %
Stability of test compound:	Not reported
Reanalysis/Expiry date:	October 2003
Treatments	
Test concentrations:	0.23, 1.15 and 2.3 mg test item/kg dry soil
Solvent / carrier:	Acetone
Control:	Untreated

Toxic standard:	Dinoseb acetate at 25 mg/kg dry soil

Test design

Soil type:	Sandy loam (Speyer 2.3)
Test units:	Two 1 litre incubation flasks, containing 150 g dry weight soil (one each for the carbon and nitrification tests)
Replication:	Single replicate for each treatment and control
Sampling intervals :	Carbon test: 0-3 hours, 7, 14, and 28 days after application Nitrification test: 0-3 hours, 7, 14, 28, 44 and 57 days after application
Duration of test:	Carbon: 28 days Nitrification test: 57 days

Environmental test conditions

Temperature:	20 ± 2 °C
pH of soil:	7.4 (water)
Soil moisture content:	40% MWHC (14.8 g water/100 g soil d.w.)
Photoperiod:	Constant darkness

Study Design and Methods

Experimental dates: 13th November to 17th January 2001

Soil samples were treated with CGA275535 at three concentrations, 0.23, 1.15 and 2.3 mg test item/kg dry soil. Test concentrations related to a soil depth of 5 cm and a soil density of 1.5 g/cm^3 and were equivalent to the maximum field rate (based on 750 g a.s./ha twice a year, of parent), five times and ten times the maximum field rate. The study design included a untreated control and a toxic reference treatment, containing 25 mg/kg dry dinoseb acetate.

The test item was dissolved in acetone and aliquots of this stock solution were applied to quartz sand. After complete evaporation of the acetone under a stream of nitrogen, each application was thoroughly mixed by means of a Vortex mixer. Each soil sample (150 g dry weight) received 1.5 g of the appropriate spiked sand mixture. During application, the soil was thoroughly mixed by careful swiveling the flasks. Water was added to the soil to achieve a water content of approximately 40 % of MWHC. The water content of the soil in each test vessel was monitored on a weekly basis during the first month and every two weeks thereafter. Lost moisture was compensated for by the addition of purified water.

One replicate test vessel containing 150 g dry weight soil was prepared for each timepoint and treatment rate and the control for each of the nitrogen transformation test and carbon transformation test.

For the nitrogen transformation test, the sub-samples of 150 g soil dry weight were weighed into each test vessel and the soils were amended with 0.7 g Lucerne meal. At each sampling point triplicate aliquots of approximately 47 g wet soil were extracted for 15 minutes with 2 M KCl. The samples were centrifuged and the supernatant decanted through filter paper. The extraction was repeated and the combined extracts were adjusted to 50 mL with 2 M KCl. The concentrations of nitrite, nitrate and ammonium were determined in triplicate using a flow injection analyser.

For the carbon transformation test the respiration rates at each sampling point were determined by amending triplicate aliquots of each spiked soil with 1.12 g glucose/kg dry soil. Respiration was measured by analyzing evolved CO_2 for approximately 22 hours. CO_2 was measured semi-continuously with an infrared gas analyser.

The mean respiration rate and nitrite, nitrate and ammonium concentrations at the end of their respective incubation period were statistically evaluated using a Dunnett test (Day 0 to 28) and Student t-test (Days 44 and 57).

Results and Discussion

Results from the nitrogen transformation test and the carbon transformation test are summarised in the tables below.

Table 8.5-6: Effects on carbon transformation in soil after treatment with CGA275535

Treatment (mg/kg dry soil)	Incubation Time (Days)	Respiration Rate (mL CO_2/h per kg dry soil)		% Deviation from Control
		Mean	SD	
Control	0	6.50	0.27	-
	7	6.28	0.32	-
	14	5.65	0.23	-
	28	5.07	0.16	-
0.23	0	6.90*	0.11	6.1
	7	6.72	0.23	7.0
	14	6.03	0.09	6.7
	28	5.65*	0.17	11.5
1.15	0	6.59	0.10	1.4
	7	6.42	0.12	2.3
	14	6.01	0.41	6.4
	28	5.41*	0.10	6.7
2.3	0	6.94*	0.13	6.6
	7	6.74*	0.04	7.4
	14	6.03	0.07	6.7
	28	5.67*	0.04	11.9
Toxic reference	0	4.44*	0.09	-31.8
	7	3.77*	0.09	-39.9
	14	2.95*	0.25	-47.8
	28	1.94*	0.10	-61.6

* statistically significantly different to control (Dunnett test, $\alpha = 0.05$)

Table 8.5-7: Effects of CGA275535 on nitrite formation

Treatment (mg/kg dry soil)	Incubation Time (Days)	Nitrite concentration (mg NO_2^--N/kg dry soil)		% Deviation from Control
		Mean	SD	
Control	0	0.1	<0.1	-
	7	<0.1	<0.1	-
	14	<0.1	<0.1	-
	28	<0.1	<0.1	-
	44	<0.1	<0.1	-
	57	<0.1	<0.1	-
0.23	0	0.1	<0.1	<0.1

Treatment (mg/kg dry soil)	Incubation Time (Days)	Nitrite concentration (mg NO_2^--N/kg dry soil)		% Deviation from Control
		Mean	SD	
	7	<0.1	<0.1	n.a.
	14	<0.1	<0.1	n.a.
	28	<0.1	<0.1	n.a.
1.15	0	0.1	<0.1	<0.1
	7	<0.1	<0.1	n.a.
	14	<0.1	<0.1	n.a.
	28	<0.1	<0.1	n.a.
2.3	0	0.1	<0.1	<0.1
	7	<0.1	<0.1	n.a.
	14	<0.1	<0.1	n.a.
	28	<0.1	<0.1	n.a.
	44	<0.1	<0.1	n.a.
	57	<0.1	<0.1	n.a.
Toxic reference	0	0.1	<0.1	<0.1
	7	1.6	<0.1	n.a.
	14	7.3	0.1	n.a.
	28	0.2	<0.1	n.a.

n.a. not applicable

Table 8.5-8: Effects of CGA275535 on nitrate formation

Treatment (mg/kg dry soil)	Incubation Time (Days)	Nitrate concentration (mg NO_3^--N/kg dry soil)		% Deviation from Control
		Mean	SD	
Control	0	8.3	0.2	-
	7	5.4	0.2	-
	14	8.9	0.4	-
	28	13.8	0.3	-
	44	20.0	0.3	-
	57	34.4	0.6	-
0.23	0	7.9	0.3	-4.8
	7	7.2*	0.3	33.3
	14	9.5	0.1	6.7
	28	14.5	0.2	5.1
1.15	0	8.5	0.1	2.4
	7	6.1*	0.1	13.0
	14	8.5	0.1	-4.5
	28	13.6	1.6	-1.4
2.3	0	8.1	0.1	-2.4
	7	7.3*	0.1	35.2
	14	8.9	0.4	<0.1
	28	15.9*	0.2	15.2

Treatment (mg/kg dry soil)	Incubation Time (Days)	Nitrate concentration (mg NO_3^--N/kg dry soil)		% Deviation from Control
		Mean	SD	
	44	12.5*	0.5	-37.5
	57	29.5*	0.3	-14.2
Toxic reference	0	7.5*	0.1	-9.6
	7	3.0*	0.1	-44.4
	14	20.9*	0.5	134.8
	28	23.2*	0.8	68.1

* statistically significantly different to control (Dunnett test or Student t-test, $\alpha = 0.05$)

Table 8.5-9: Effects of CGA275535 on ammonium formation

Treatment (mg/kg dry soil)	Incubation Time (Days)	Ammonium concentration (mg NH_4^+-N/kg dry soil)		% Deviation from Control
		Mean	SD	
Control	0	0.7	<0.1	-
	7	0.3	<0.1	-
	14	<0.1	<0.1	-
	28	<0.1	<0.1	-
	44	<0.1	<0.1	-
	57	<0.1	<0.1	-
0.23	0	0.8	0.1	14.3
	7	0.2	<0.1	-33.3
	14	<0.1	<0.1	n.a.
	28	<0.1	0.1	n.a.
1.15	0	0.9*	<0.1	28.6
	7	0.2	<0.1	-33.3
	14	<0.1	<0.1	n.a.
	28	<0.1	<0.1	n.a.
2.3	0	0.9*	0.1	28.6
	7	0.3	<0.1	<0.1
	14	<0.1	<0.1	n.a.
	28	<0.1	0.1	n.a.
	44	<0.1	<0.1	n.a.
	57	<0.1	<0.1	n.a.
Toxic reference	0	1.0*	<0.1	42.9
	7	10.3*	<0.1	3333.3
	14	6.7*	0.56	n.a.
	28	0.1	0.56	n.a.

* statistically significantly different to control (Dunnett test or Student t-test, $\alpha = 0.05$)
n.a. not applicable

Validity criteria

The study was considered valid as the following protocol defined criteria was fulfilled:

- A significant affect was observed in the toxic reference treatment in both the nitrification and respiration studies

Conclusions

The test item CGA275535 had no detrimental effect on microbial short-term respiration in soil, as measured by CO_2 evolution. Concentrations up to 2.3 mg/kg dry soil had negligible effects on soil respiration after 28 days of incubation.

The effects of CGA275535 on the nitrification and ammonification processes in the soil were low and transient. At concentrations of up to 1.15 mg/kg dry soil negligible effects were observed after 28 days of incubation. At 2.3 mg/kg dry soil, negligible to tolerable effects were observed after 28, 44 and 57 days of incubation. It may be concluded that no adverse effects of the test item on organic matter turnover and hence on soil fertility, are to be expected up to 2.3 mg/kg dry soil.

(Seyfried B. 2001)

A summary of a study not previously submitted is presented below:

Report:	Submitted for purposes of renewal as conducted on relevant soil metabolite: K-CA 8.5/01 Hammesfahr U. (2015) CGA321915 - Effects on Activity of Soil Microflora (Carbon and Nitrogen Transformation) in the Laboratory, Report Number 96341080, Institut für Biologische Analytik und Consulting, iBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany. (Syngenta file No. CGA321915_10008).

Guidelines

OECD guidelines 216, Soil Microorganisms: Nitrogen Transformation Test (2000)

OECD guidelines 217, Soil Microorganisms: Carbon Transformation Test (2000)

GLP: Yes

Executive Summary

CGA321915 was applied to the soil at concentrations of 1.53 mg CGA321915/kg soil d.w. and 5.10 mg CGA321915/kg soil d.w.. The test item CGA321915 caused no adverse effects on soil nitrogen transformation (measured as NO_3-N-production) or on soil carbon transformation (measured as CO_2-production) at the end of the 28-day incubation period.

Materials

Test Material	CGA321915 CSAA400257
Parent:	CGA219417 (Cyprodinil)
Lot/Batch #:	MES 356/1
Purity:	98 % w/w ± 2 %

Description:	White solid
Stability of test compound:	Stable under test conditions
Reanalysis/Expiry date:	30 September 2016
Density:	Not applicable

Treatments

Test rates:	1.53 and 5.10 mg CGA321915/kg soil d.w.
Control:	Deionised water only
Toxic standard:	Sodium chloride (99.9 % purity) at a concentration of 16.0 g/kg (Separate study – IBACON study code: 30699080, date May 2014 to August 2014)

Test design

Soil type:	Silty sand soil collected from a site in the district of Darmstadt-Dieburg, Germany; 7.5 % clay, 29.3 % silt and 63.2 % sand
Test units:	Nitrogen transformation test: 250 to 500 g soil dry weight in approximately 0.5 L plastic boxes (dimensions 0.10 m width x 0.10 m depth x 0.065 m height) filled up to 6 cm, covered with perforated lids Carbon transformation test: 750 to 1000 g soil dry weight in approximately 1 L plastic boxes (dimensions 0.12 m width x 0.165 m depth x 0.065 m height) filled up to 6 cm, covered with perforated lids
Replication:	3 per treatment rate and control
Sampling intervals :	≤ 6 hours, and 7, 14 and 28 days after application
Duration of test:	28 days

Environmental test conditions

Temperature:	20 ± 2 °C
pH of soil:	7.0 – 7.2
Soil moisture content:	48 to 55 % of WHC
Photoperiod:	Constant darkness

Study Design and Methods

Experimental dates: 27th January to 27th February 2015

Soil samples were treated with CGA321915 at two doses, 1.53 and 5.10 mg CGA321915/kg dry soil. Test concentrations related to a soil depth of 5 cm and a soil density of 1.5 g/cm^3

The test item was mixed with deionised water and the test solution was subsequently mixed with the soil in the laboratory mixer. Water was added to the soil to achieve a water content of 49 - 52 % of WHC. The water content of the soil in one replicate of each treatment group was determined at each sampling date, and water losses were compensated by adding pure water.

Three replicate soil samples were prepared for each treatment rate and the control for the nitrogen transformation test and carbon transformation test.

Mean nitrogen content (mg NO_3^--N/kg soil d.w), standard deviation and coefficient of variation as well as the mean nitrate nitrogen content/day (mg NO_3/kg soil d.w./day) were calculated for each treatment group and sampling date.

For the evaluation of the results the relative deviations (%) of the test item treatment groups from the control were calculated for each sampling date. A Student t-test (pair wise comparison, two-sided, α = 0.05) was performed for statistical evaluation.

Mean O_2 consumption after 12 hours was calculated using regression analysis. Furthermore, standard deviation and coefficient of variation were calculated for each treatment group and sampling date.

For the evaluation of the results the relative deviations (%) of the test item treatment groups from the control were calculated for each sampling date. A Student t-test (pair wise comparison, two-sided, α = 0.05) was performed for statistical evaluation.

Results and Discussion

Results from the Nitrogen transformation test and the Carbon transformation test are summarised in the tables below.

Table 8.5-210: Effects on nitrogen transformation in soil after treatment with CGA321915

Time Interval (days)	Control			1.53 mg CGA321915/kg soil dry weight				5.10 mg CGA321915/kg soil dry weight			
	NO_3-N [mg/kg soil d.w.]	NO_3-N [mg/kg soil d.w./day]	% CV	NO_3-N [mg/kg soil d.w.]	NO_3-N [mg/kg soil d.w./day]	Deviation from control [%][1)]	% CV	NO_3-N [mg/kg soil d.w.]	NO_3-N [mg/kg soil d.w./day]	Deviation from control [%][1)]	% CV
0 - 7	14.336	-0.275	-32.36	14.244	-0.279	1.45	-14.70	15.000	-0.204	-25.82	-25.98
0 - 14	19.869	0.258	5.43	19.563	0.240	-6.98	12.08	20.421	0.285	10.47	6.67
0 - 28	35.772	0.697	2.30	35.135	0.676	-3.01	0.89	37.189	0.741	6.31	4.72

The calculations were performed with non-rounded values
CV [%] = Coefficient of Variation
1) based on NO_3-nitrogen production; - = inhibition; + = stimulation
No statistically significant differences between the control and the test item treatments were calculated

Table 8.5-311: Effects on carbon transformation in soil after treatment with CGA321915

Days after application	Control		1.53 mg CGA321915/kg soil dry weight			5.10 mg CGA321915/kg soil dry weight		
	CO_2 – production [mg CO_2/kg soil d.w./h]	CV [%]	CO_2 –production [mg CO_2/kg soil d.w./h]	CV [%]	Deviation from control [%][1)]	CO_2 –production [mg CO_2/kg soil d.w./h]	CV [%]	Deviation from control [%][1)]
0	12.184	2.05	12.598	3.24	3.40	12.201	1.94	0.14
7	13.055	1.03	12.902	2.14	-1.17	12.862	0.83	-1.48
14	14.671	0.66	15.231	3.17	3.82	15.080	0.90	2.79
28	8.864	3.77	9.057	1.93	2.18	8.835	4.04	-0.33

The calculations were performed with non-rounded values
CV [%] = Coefficient of Variation
1) based on CO_2 production; - = inhibition; + = stimulation
No statistically significant differences between the control and the test item treatments were calculated

Validity criteria

The validity criteria are listed below:

- The coefficient of variation in the control replicates of the nitrogen and carbon transformation tests after 28 days were 2.30 % and 3.77 %, respectively (must be ≤ 15 %)
- The toxic standard caused an effect of -83.45 % at a concentration of 16 g/kg soil d.w. in the Nitrogen transformation test at Day 28, demonstrating the sensitivity of the test system (must be ≥ 25 %)

- The toxic standard caused an effect of -67.21 % at a concentration of 16 g/kg soil d.w. in the Carbon transformation test at Day 28, demonstrating the sensitivity of the test system (must be ≥ 25 %)

Conclusions

CGA321915 was applied to the soil at concentrations of 1.53 mg CGA321915/kg soil d.w. and 5.10 mg CGA321915/kg soil d.w. The test item CGA321915 caused no adverse effects on soil nitrogen transformation (measured as NO_3-N-production) and on soil carbon transformation (measured as CO_2-production) at the end of the 28-day incubation period.

(Hammesfahr U, 2015)

Relevant Literature on Nitrogen Transformation

Report:	K-CA 8.5/02 Jastrzebska E. (2010). The effect of fungicide Unix 75 WG and insecticides: Nomolt 150 SC and Dursban 480 EC on the number of soil microorganisms and the physicochemical properties of soil. *Nauka Przyroda Technologie*, 4(6) (Syngenta File No. A8779A_10240)

Guidelines

The study does not use standard test guideline. Methodologies applied were those as stated by Antheumisse (1963), Fenglerowa (1965), Lityński et al. (1976), Mulder and Antheumisse (1963), and Winogradski (1953).

GLP: No.

Executive Summary

A soil pot study was conducted with varying loadings of cyprodinil in parallel with a control tested. The tests studied the impact of the fungicide on ammonifying bacteria, *Azotobacter* spp. and *Arthrobacter* spp.. The tested concentrations were 0, 0.05, 0.5, 5.0, 50, 500 mg/kg of soil dry mass (dm).

There was significant correlation of increased Unix 75 WG application and a decrease in microbial colonies (with the exception of ammonifying bacteria at 50 days, which increased). Although correlations were presented, NOECs were not derived. Observation of the data reveals that there is no reduction of 25% in number of bacteria relative to the control for soil concentrations which are relevant for the use of cyprodinil. This will be discussed in further detail in the M-CP Section 10 documents.

Materials

Test Material 1	Unix 75 WG
Lot/Batch #:	Syngenta Agro
Purity:	N/A
Description:	Not stated
Treatments:	0, 0.05, 0.5, 5.0, 50, 500 mg/kg of soil dry mass (dm)

Treatments

Control:	No added fungicide macro-element medium only ($(g \cdot kg^{-1}$ of soil in terms of pure ingredient): N – 0.12 ($CO(NH_2)_2$], P – 0.05 (KH_2PO_4), K – 0.12 (KH_2PO_4 + KCl), Mg – 0.025 ($MgSO_4 \cdot 7H_2O$) and micro-elements (mg/kg of soil in terms of pure ingredient): Zn – 5.0 ($ZnSO_4 \cdot 7H_2O$), Cu – 5.0 ($CuSO_4 \cdot 5H_2O$), Mn – 5.0 ($MnCl_2 \cdot 5H_2O$), Mo – 5.0 ($Na_2MoO_4 \cdot 2H_2O$), B – 0.33 (H_3BO_3).

Toxic standard: None tested

Test design

Soil type: Granulometric loamy sand

Test units: 2.0 L plastic container (internal dimensions 16.7cm x 16.7cm x 9cm deep) with the lid perforated with a single 3 mm diameter hole to ensure aerobic conditions.

Replication: 3

Duration of test: 50 days (sampled at day 10 and 50)

Environmental test conditions

Temperature: Not stated

pH of soil: 6.62-6.90 (KCl)

Soil moisture content: 60 % WHC

Photoperiod: None stated

Study Design and Methods

A pot experiment was carried out in triplicate to test the effect of cyprodinil on microbial communities. Per experimental plot there were 10 pots, each plot had varying levels of cyprodinil applied as follows; 0, 0.05, 0.5, 5.0, 50, 500 mg·kg^{-1} of soil dm.

Soil was kept at a constant moisture content of 60% during the test by applying deionised water to replenish losses. The experiment lasted 50 days and samples were taken at day 10 and 50. At each sampling point, ammonifying bacteria (Am) and nitrogen-fixing bacteria (Im) – based on the Winogradski (1953) method, *Azotobacter* spp. (Az) using the Fenglerowa (1965) medium and *Arthrobacter* spp. (Arth) based on the Mulder and Antheumisse (1963) method, were determined.

Results and Discussion

At analysis point I the number of ammonifying bacteria negatively correlated with the dose of fungicide Unix 75 WG. At analysis point II, increasing doses of the product significantly stimulated proliferation of this bacterial group.

Table 8.5-4: The mean effect of Unix 75 WG on the number of nitrogen-fixing bacteria (IM), ammonyfying bacteria (Am), *Azotobacter* sp. (Az) and *Arthrobacter* spp. (Arth)

Dose of plant protection product (mg/kg of soil d.m.)	Im x 10^9	Am x 10^9	Az x 10^4	Arth x 10^6
$0_{control}$	12.26	11.46	6.06	13.17
Unix 75 WG				
0.05	15.86 (-29.4)	11.65 (-1.7)	5.76 (5.0)	11.51 (12.6)
0.5	18.59 (-51.6)	10.21 (10.9)	5.77 (4.8)	8.60 (34.7)
5	14.04 (-14.5)	13.17 (-14.9)	5.98 (1.3)	8.27 (37.2)
50	11.62 (5.2)	16.01 (-39.7)	4.11 (32.2)	4.21 (68.0)
500	9.32 (24.0)	14.24 (-24.3)	3.71 (38.8)	2.86 (78.3)
r (correlation coefficient)	-0.28	0.20	-0.35*	-0.75*

* significant correlation coefficient values for p = 0.01.

Numbers in brackets represent % difference from the control. Negative numbers represent an increase

Table 8.5-5: The effect at day 10 (I) and day 50 (II) of Unix 75 WG on the number of nitrogen-fixing bacteria (IM), ammonyfying bacteria (Am), *Azotobacter* sp. (Az) and *Arthrobacter* spp. (Arth)

Plant protection product dose ($mg \cdot kg^{-1}$ of soil d.m.)	Im x 10^9		Am x 10^9		Az x 10^4		Arth x 10^6	
	Analysis point							
	I	II	I	II	I	II	I	II
$0_{control}$	7.23	17.28	5.80	17.12	3.12	9.00	18.34	8.00
Unix 75 WG								
0.05	8.06 (-11.5)	23.65 (-36.9)	8.47 (-46.0)	14.82 (13.4)	3.14 (-0.6)	8.37 (7.0)	12.65 (31.0)	10.36 (-29.5)
0.5	10.20 (-41.1)	26.98 (-56.1)	6.21 (-7.1)	14.21 (17.0)	4.03 (-29.2)	7.50 (16.7)	8.58 (53.2)	8.61 (-7.6)
5	9.69 (-34.0)	18.38 (-6.4)	5.70 (1.7)	20.64 (-20.6)	4.48 (-43.6)	7.48 (16.9)	9.66 (47.3)	6.88 (14.0)
50	9.09 (-25.7)	14.14 (18.2)	4.04 (30.3)	27.97 (-63.4)	2.36 (24.4)	5.86 (34.9)	4.83 (73.7)	3.58 (55.3)
500	7.27 (-0.6)	11.37 (34.2)	3.71 (36.0)	24.76 (-44.6)	1.78 (42.9)	5.63 (37.4)	2.56 (86.0)	3.16 (60.5)
r (correlation coefficient)	0.07	-0.68*	-0.81*	0.79*	-0.48*	-0.85*	-0.86*	-0.74*

* significant correlation coefficient values for $p = 0.01$. I – 10 days, II – 50 days
Numbers in brackets represent % difference from the control. Negative numbers represent an increase

Conclusions

A soil pot study was conducted with varying loadings of cyprodinil in parallel with a control tested. The tests studied the impact of the fungicide on ammonifying bacteria, *Azotobacter* spp. and *Arthrobacter* spp.. The tested concentrations were 0, 0.05, 0.5, 5.0, 50, 500 mg/kg of soil dry mass (dm).

There was significant correlation of increased Unix 75 WG application and a decrease in microbial colonies (with the exception of ammonifying bacteria at 50 days, which increased). Although correlations were presented, NOECs were not derived. Observation of the data reveals that there is no reduction of 25% in number of bacteria relative to the control for soil concentrations which are relevant for the use of cyprodinil. This will be discussed in further detail in the M-CP Section 10 documents.

Study Reliability Evaluation	Yes	No	Not reported	Not applicable	Comments
Standardised test procedure followed		X			Methods are well reported and references above have been used.
Appropriate test procedure followed	X				Methodology is reasonably well documented and scientifically acceptable with basic requirements met. However, some limitations exist.
Data quality assured (GLP or equivalent)		X			
Controls appropriate	X				Controls contained a suitable amount of organic carbon as do the test treatments.
Control response acceptable, or accounted for statistically				X	
Temperature, pH & dissolved oxygen reported		X			Temperature not reported.
Alkalinity and hardness reported (metals)	X				Medium and substrate details are available.

Study Reliability Evaluation	Yes	No	Not reported	Not applicable	Comments
Statistics appropriate	**X**				
Effect levels above analytical limit of detection/quantification		**X**			
Material tested within limits of solubility, or effects above the limits of solubility sufficiently explained				**X**	
Analytical verification of test concentrations/doses				**X**	
Measurement of precipitate or undissolved material				**X**	
Appropriate dilution water used (e.g. not chlorinated tap, rain water etc)	**X**				Macro-element medium.

Study assessment	Score	Rationale
Reliability/Repeatability	Klimisch 2	Reliable with restrictions Non-GLP. Methodology is reasonably well documented and scientifically acceptable with basic requirements met.
Limitations		Recording of data mainly restricted to figures and tables, no raw data presented.
Relevance	Microbial inhibition results are relevant for ecotoxicity RA.	Study some adverse affect but at what level this becomes significant is not clear. Overall C_{org} is not impacted. As the endpoint is not obvious the relevance is in question.
Significance	Microbial inhibition results are suitable for use in risk assessment	

CA 8.6 Effects on Terrestrial Non-Target Higher Plants

Table 8.6-1: Table of endpoints for cyprodinil

Test substance	Species	Test type	Endpoints		Reference (author, date, Syngenta File No.)
Cyprodinil	Monocotyledons: Corn (*Zea mays*), oat (*Avena sativa*), onion (*Allium cepa*), ryegrass (*Lolium perenne*) Dicotyledons: Cabbage (*Brassica oleracea*), carrot (*Daucus carota*), cucumber (*Cucumis sativis*), lettuce (*Latuca sativa*), soybean (*Glycine max*), tomato (*Lycopersicon esculentum*)	Screen	Not previously submitted	No significant effects on any species at 560 g a.s./ha	***Schwab (1997) CGA219417/0863)***
A8637A ([cyprodinil 50 WG])	Monocotyledons: Maize (*Zea mays*), wild oat (*Avena fatua*), Yellow nutsedge (*Cyperus esculentus*) Dicotyledons: Sugar beet (*Beta vulgaris*), oilseed rape (Brassica napus), soyabean (*Glycine max*)	Screen	EU	No significant effects on any species at 0.45 kg/ha (225 g a.s./ha)	***Walder (2000) CGA219417/0989***
A8779A ([cyprodinil 75 WG])	Monocotyledons: Maize (*Zea mays*), wild oat (*Avena fatua*), Yellow nutsedge (*Cyperus esculentus*) Dicotyledons: Sugar beet (*Beta vulgaris*), oilseed rape (Brassica napus), soyabean (*Glycine max*)	Screen	EU	No significant effects on any species at 1 kg/ha (750 g a.s./ha)	***Walder (1999) CGA219417/0939***

CA 8.6.1 Summary of screening data

The studies previously submitted in the EU were summarised in point B.9.9.2 of the DAR therefore additional study summaries are not repeated here. The endpoints are summarised in Table 8.6-1 above.

A summary of a study not previously submitted is presented below:

Report:	K-CA 8.6.1/01 Schwab D. (1997) Evaluating the effects of CGA-219417 on the emergence and vegetative vigor of non-target terrestrial plants, Report Number 43506 . ABC Laboratories Inc., Analytical Chemistry and Field Services, 7200 E. ABC Lane, Columbia, Missouri. (Syngenta File No. CGA219417/0863).

Guidelines

EPA Guideline 122-1 Tier 1

GLP: Yes

Executive Summary

In a screening study in which the effects of cyprodinil on seedling emergence and vegetative vigour on 10 non-target plants species were evaluated, no adverse effects were observed at a treatment rate of 560 g a.s./ha.

Materials

Test Material	CGA-219417 75TF
Lot/Batch #:	P.507005
Actual content of active ingredients:	76.1% active substance
Description:	Not reported
Stability of test compound:	Not reported
Reanalysis/Expiry date:	Not reported

Treatments

Test rates:	0.5 lb a.s./A
Control:	Water control followed by formulation blank.
Application method:	Single nozzle overhead track sprayer with 4002E tip and 50-mesh sieve with spray width of 20 inches, 40 psi pressure and spray volume 20 GPA.

Test organisms

Species:	Cabbage (*Brassica oleracea*) Carrot (*Daucus carota*) Cucumber (*Cucumis sativis*) Lettuce (*Latuca sativa*) Soybean (*Glycine max*) Tomato (*Lycopersicon esculentum*) Corn (*Zea mays*) Oat (*Avena sativa*) Ryegrass (*Lolium perenne*) Onion (*Allium cepa*)

Test design

Vessels:	12-Nu pots (4 inch square x 5 inch deep) placed in individual saucers to contain all water applied.
Test soil:	Loam soil
Replication:	Four replicates for each treatment level
Duration of test:	3 weeks

Environmental test conditions

Temperature:	11 – 34 °C
Humidity:	12 - 100 %
pH of soil:	5.8
Light conditions:	16 hours daylight / 8 hours of darkness

Study Design and Methods

Experimental dates: 10 October 1996 to 07 November 1996

Ten species of higher plant were treated with a single concentration of test item, with a water control, followed by a formulation blank being treated before the application of the test item.

Approximately three weeks after the application of the test item, the number of emerged seedlings was used for statistical analysis to obtain the percent emergence data. Toxicological observations were made by comparing the growth and appearance of species in the CGA-219417 75TF treatment to the control.

The data collected from the vegetative vigour tests were the length of shoots, replicate dried shoot weight at the end of the test and toxicological effects rated.

Results

Phytotoxicity assessment

Table 8.6.1-1: Phytotoxicity rating

Species	Seedling emergence		Vegetative vigour	
	Control	560 g a.s./ha	Control	560 g a.s./ha
Cabbage (*Brassica oleracea*)	8	13	0	0
Carrot (*Daucus carota*)	0	0	0	0
Corn (*Zea mays*)	8	3	0	0
Cucumber (*Cucumis sativis*)	8	5	0	0
Lettuce (*Latuca sativa*)	3	0	0	0
Oat (*Avena sativa*)	0	0	0	5
Onion (*Allium cepa*)	0	0	0	0
Ryegrass (*Lolium perenne*)	3	0	0	0
Soybean (*Glycine max*)	3	5	0	0
Tomato (*Lycopersicon esculentum*)	0	0	0	0

Key to ratings: 10-39 slight effect; 40-69 moderate effect; 70-100 severe effect

Only sight phytotoxic effects from CGA219417 were noted. Table 8.6.1-1 shows the overall phytotoxicity rating from the final observation. For seedling emergence, slight effects were noted for cabbage, corn, cucumber and ryegrass. For vegetative vigour slight effects were observed solely for oat.

Table 8.6.1-2: Percent emergence

Species	Control	Vehicle	560 g a.s./ha	% effect
Cabbage	75	70	73	0
Carrot	83	78	68	-15
Corn	98	88	90	-3
Cucumber	98	95	90	-6
Lettuce	73	78	73	-3
Oat	100	98	93	-6
Onion	78	68	83	14
Ryegrass	83	78	88	10
Soybean	100	95	95	-3
Tomato	88	85	85	-1

Table 8.6.1-3: Shoot length (mm) for the pre-emergence assessment

Species	Control	Vehicle	560 g a.s./ha	% effect
Cabbage	65	63	63	-2
Carrot	117	116	105	-10
Corn	353	357	360	1
Cucumber	49	51	45	-10
Lettuce	57	61	59	0
Oat	339	349	357	4
Onion	131	143	145	6
Ryegrass	167	160	170	4
Soybean	106	104	110	5
Tomato	40	38	38	-3

Table 8.6.1-4: Shoot weight (g) for the pre-emergence assessment

Species	Control	Vehicle	560 g a.s./ha	% effect
Cabbage	0.135	0.142	0.145	5
Carrot	0.043	0.038	0.035	-15
Corn	0.233	0.248	0.252	5
Cucumber	0.344	0.351	0.339	-1
Lettuce	0.064	0.067	0.066	0
Oat	0.123	0.137	0.147	13
Onion	0.016	0.018	0.016	-6
Ryegrass	0.026	0.031	0.030	3
Soybean	0.416	0.401	0.401	-2
Tomato	0.095	0.087	0.100	10

Table 8.6.1-5: Shoot length (mm) for the vegetative vigour assessment

Species	Control	Vehicle	560 g a.s./ha	% effect
Cabbage	97	101	99	-2
Carrot	190	194	186	-3
Corn	888	913	892	-1
Cucumber	397	392	362	-8
Lettuce	107	98	111	8
Oat	421	427	413	-3
Onion	116	116	112	-3
Ryegrass	241	229	254	8
Soybean	176	176	177	1
Tomato	201	199	205	3

Table 8.6.1-6: Shoot weight (g) for the vegetative vigour assessment

Species	Control	Vehicle	560 g a.s./ha	% effect
Cabbage	1.487	1.447	1.541	5
Carrot	0.278	0.291	0.256	-10
Corn	3.551	2.623	3.078	0
Cucumber	3.344	3.398	3.389	1
Lettuce	1.039	1.023	1.084	5
Oat	0.696	0.706	0.704	0
Onion	0.030	0.029	0.029	-3
Ryegrass	0.544	0.525	0.534	0
Soybean	1.475	1.405	1.429	-1
Tomato	2.547	2.498	2.562	2

The results indicate that effects on emergence and growth were less than a 50% compared to the controls for all parameters tested

Validity criteria

All validity criteria were met as per guidelines. Immergence in controls was sufficient but survival was not reported.

Conclusions

In a screening study in which the effects of cyprodinil on seedling emergence and vegetative vigour on 10 non-target plants species were evaluated, no adverse effects were observed at a treatment rate of 560 g a.s./ha.

(Schwab D, 1997)

CA 8.6.2 Testing on non-target plants

As cyprodinil is not a herbicide and does not exhibit herbicidal activity Tier II tests are not required.

Relevant Literature on Non-Target Plants

No relevant scientifically peer-reviewed open literature could be found on cyprodinil or its major metabolites. Details of the literature search undertaken can be found in M-CA Section 9 of the current submission.

CA 8.7 Effects on Other Terrestrial Organisms (Flora and Fauna)

~~No other data is available on the effects of the active substance on other terrestrial organisms.~~

New biological activity testing was conducted with the metabolites CGA321915 and CGA249287 against two target pest species. The data are summarized fully in **MCA Section 3** and discussed in the **A8637C and A14325E Document N4** (Relevance Of Metabolites In Groundwater) documents. A brief summary is provided below.

Report:	Submitted for purposes of renewal as required in support of the risk assessment: K-CA 8.7/01, Schmid T. (2017) CGA321915/CGA249287 - Activity compared with Cyprodinil against *Alternaria solani* and *Blumeria graminis*. Syngenta Crop Protection AG, Stein, Switzerland. (Syngenta File No CGA219417_11766)

Executive Summary

This study was conducted to determine the biological activity of CGA321915 and CGA249287 when compared to that of cyprodinil in side by side tests on *Alternaria solani* on tomato and *Blumeria graminis* on wheat.

Spray solutions were prepared in an instant formulation (IF-050) which was applied as a foliar application to tomato plants at test rates of 200, 60, 20, 6, 2, 0.6 ppm. Control plants were treated with blank IF-050.

For the *Alternaria solani* evaluation, two days after application tomato plants were inoculated by spraying a spore suspension on to the test plants. For the *Blumeria graminis* test, the wheat plants were inoculated one day after application.

No activity was observed for CGA321915 and CGA249287 at rates of up to 200 mg/L for either pathogen. For cyprodinil, however, for the highest rate of 200 mg/L >90% efficacy was recorded against *Blumeria graminis* on wheat over 80% efficacy was observed against *Alternaria solani* on tomato.

(Schmid T, 2017)

Relevant Literature

No other relevant scientifically peer-reviewed open literature could be found on cyprodinil or its major metabolites. Details of the literature search undertaken can be found in M-CA Section 9 of the current submission.

CA 8.8 Effects on Biological Methods for Sewage Treatment

Table 8.8-1: Table of endpoints

Test item	Test species	Test type	Endpoints (EC_{50} – mg a.s./L)		Reference (author, date, Syngenta File No.)
Cyprodinil	Aquatic micro-organisms	Activated sludge respiration inhibition	EU	>100	***Grade (1992) CGA219417/0085***
CGA263208			New	48	***Bader (1991) CA1059/0011***
CA1139A				64.2	***Baumann (1992) CA1139/0012***

The study by Grade (1992) has been previously submitted in the EU and was summarised in point B.9.10.1 of the DAR. At the request of ANSES summaries have been included in order to aid dossier evaluation ~~therefore an additional study summary is not repeated here.~~ The endpoints are summarised in Table 8.6-1 above.

Report:	Summary submitted at the request of ANSES for ease of dossier evaluation: *KIIA 8.7. Grade R. (1992). Report on the test for inhibitory concentration on aerobic bacteria of CGA 219417. Report Number 928024. CIBA-GEIGY Ltd., CH-4002 Basel, Switzerland. (Syngenta File No. CGA219417/0085).*

Guidelines

87/302/EEC page 118-122. Activated sludge respiration inhibition test

GLP: Yes

Executive Summary

The effects of CGA219417 on the respiration of activated sludge microorganisms was tested. Aerobic activated sludge was incubated for 3 hours at the following nominal CGA219417 concentrations; 1.1, 3.3, 10.45, 32.1 and 105.0 mg/L. The EC_{50} for CGA219417 to activated sludge microorganisms was estimated to be >100 mg/L.

Materials

Test Material	CGA219417
Lot/Batch #:	P.012012
Purity:	99.2%
Stability of the test compound:	Not reported
Description:	White solid
Reanalysis/expiry date:	March 1995
Treatments	
Test treatments:	Blank control, and CGA219417 concentrations of 1.1, 3.3, 10.45, 32.1 and 105.0 mg/L
Solvent:	None
Positive control:	3, 5-dichlorophenol at 3.2, 10 and 32 mg/L
Analysis of test concentrations:	Not performed
Test organism	
Organisms:	Activated sludge microorganisms. Sludge concentration was 1.7 g/L (dry weight) in test vessels
Source:	Sewage treatment plant at CH-4153 Reinach on 4 February 1992
Test design	
Test vessels:	250 mL BOD flasks with gas inlet
Replication:	One replicate per test item and positive control concentration. Two replicates for blank control
Duration:	3 hours
Exposure regime:	Static
Environmental conditions	

Test temperature:	20 ± 2°C
pH:	Not reported
Aeration:	Yes
Dissolved oxygen:	Recorded during oxygen consumption determinations
Lighting:	Not reported

Study Design and Methods

Experimental dates: 6th February 1992

Activated sludge microorganisms were exposed to 1.1, 3.3, 10.45, 32.1 and 105.0 mg/L CGA219417 for 3 hours in BOD flasks. The sludge concentration within the test vessels was 1.7 g/L. The study design also included a blank control group and three concentrations (3.3, 10 and 32 mg/L) of a toxic reference substance. The test vessels were prepared by direct addition of the test substance to the test vessels without the preparation of a concentrated stock solution. Two replicate test vessels were prepared for the blank control group and a single vessel was prepared for each of the test item and toxic reference test concentrations.

After 3 hours exposure the oxygen consumption rate in each test vessel was determined and plotted and the inhibition was calculated based on the difference between treatment and control oxygen consumption.

Results and Discussion

Oxygen consumption of each test system together with the percent inhibition relative to the control is presented in the table below.

Table 8.8-1: Influence of CGA219417 on oxygen consumption of activated sludge in a 3-hour respiration inhibition test

Treatment	Concentration (mg/L)	O_2 consumption (mg/L/h)	Inhibition (%)
Blank 1	0	72.95	-2
Blank 2	0	70.11	2
Ref. 1	32	16.56	77
Ref. 2	10	51.76	28
Ref. 3	3.2	66.67	7
CGA219417	105	70.97	1
	32.1	73.64	-3
	10.5	70.74	1
	3.3	69.71	3
	1.1	65.63	8

No significant inhibition of respiration was observed at any CGA219417 concentration. The toxic reference treatments resulted in increasing inhibition with increasing concentration and the EC_{50} of 3, 5-dichlorophenol was estimated to be 15.4 mg/L.

Validity Criteria

Not reported

Conclusion

The EC_{50} for effects of CGA219417 to activated sludge microorganism was determined to be >100 mg/L.

(Grade R. 1992)

Metabolites of cyprodinil

Summarised of studies conducted on salts of phenyl guanidine not previously submitted are presented below.

Report:	Submitted for purposes of renewal as conducted on relevant aquatic metabolite: K-CA 8.8/01 Bader U. (1991) Report on the test for inhibitory concentration on aerobic bacteria of CGA 263208, Report Number 918089, Ciba-Geigy Ltd., Product Safety, Ecotoxicology, CH-4002 Basel, Switzerland. (Syngenta File No. CA1059/0011)

Guidelines:

OECD guideline No.: 209 (1984)

GLP:

Yes

Executive Summary:

The effect of CGA263208 on activated sludge respiration was tested. The EC_{50} for activated sludge microorganisms was determined to be 48 mg CGA263208/L (EC_{20} = 5 mg CGA263208/L and EC_{80} >100 mg CGA263208/L).

Materials and Methods:

Test Material	CGA263208
Description:	Grey solid
Lot/Batch #:	P.010010
Purity:	72.7%
Solubility	Slightly soluble at 20 °C
Stability of test compound:	31st December 1992
Test concentrations:	Nominal: 1.0, 3.2, 10.0, 32.1, 100.2 mg a.s./L, and a control (dechlorinated tap water).
Vehicle and/or positive control:	No vehicle/3,5-dichlorophenol; 3.2, 10 and 32 mg/L (nominal)
Analysis of test concentrations:	No
Test animals	
Sample/ specimen:	Activated sludge from sewage treatment plant (GH-4153, Reinach, 13/05/91)
Acclimatisation period:	27 days
Water:	Dechlorinated tap water
Nutrient solution	16 g peptone/L

	11 g meat extract/L 3.0 g urea/L 0.7 g NaCl/L 0.4 g $CaCl_2 \cdot 2H_2O$/L 0.2 g Mg $SO_4 \cdot 7 H_2O$/L 2.8 g K_2HPO_4/L
Test design	
Vessel:	250 mL BOD flasks with gas inlet
Duration:	3 h
Sludge concentration:	1.7 g/L
Environmental conditions	
Test temperature:	20 ± 2 °C
pH:	N.D.

Study Design and methods:

Experimental dates: 14th May 1991

Test procedure

The preparation of activated sludge from the aqueous layer was carried out using the settling method. Incorporation of the test material was not stated, from the data provided it would appear the test material is added to the sludge using an aqueous phase. The test vessels were stirred throughout keeping the substance homogenous throughout the test.

Measurements

Oxygen consumption per hour in mg/L was measured using an ORION-Electrode and recorded using a microprocessor.

Inhibition was determined on the basis of the measured oxygen consumption of a blank in comparison to a control (not test substance/ reference material added.) Nominal concentrations were used to determine inhibitory concentrations.

Results and Discussion:

Oxygen consumption of each test system together with the percent inhibition relative to the control is presented in the table below.

Table 8.8-2: Effects of CGA263208 on activated sludge microflora

Sample	Concentration (mg/L)	O_2 consumption (mg/L/h)	Inhibition (%)
Blank 1	0	53.9 (31.7)	5
Blank 2	0	59.3 (34.9)	-5
Ref. 1	3.2	52.4	7
Ref. 2	10	34.8	39
Ref. 3	32	9.7	83
CGA263208	1	53.7	5
	3	49.5	13
	10	43.5	23
	32	35.1	38
	100	18.1	68

Numbers in brackets represent the O_2 consumption in the blank controls per g of sludge

Validity criteria

Two out of the 3 validity criteria were met in that:

- Oxygen consumption in the blank controls should be ≥20 mg O_2/g of activated sludge/hour (mean consumption was 33.3 mg/g/h).
- The EC_{50} for the reference material should be within the range of 2-25 mg/L (actual estimated EC_{50} was 12.5 mg/L).
- The coefficient of variation for O_2 consumption between the controls should be < 30%. No reference was made to this parameter in the report.

Conclusions

The EC_{50} for effects of CGA263208 to activated sludge microorganism was determined to be 48 mg CGA263208/L

(Bader U, 1991)

Report:	Submitted for purposes of renewal as conducted on relevant aquatic metabolite: K-CA 8.8/02 Baumann W. (1992) Report on the test for inhibitory concentration on aerobic bacteria of CA 1139 A, Report Number 928176, Ciba-Geigy Ltd., Product Safety, Ecotoxicology, CH-4002 Basel, Switzerland. (Syngenta File No. CA1139/0012)

Guidelines:

OECD guideline No.: 209 (1984)

GLP:

Yes

Executive Summary:

The effect of CA1139A on activated sludge respiration was tested. The EC_{50} for activated sludge microorganisms was determined to be 64.2 mg CA1139A/L (EC_{20} = 7.3 mg CA1139A/L and EC_{80} >100 mg CA1139A/L).

Materials and Methods:

Test Material	CA1139A
Description:	Beige powder
Lot/Batch #:	P.201025
Purity:	76.2 %
Solubility	Soluble
Stability of test compound:	02/93
Test concentrations:	Nominal – 1.0, 3.2, 10.0, 32.1, 100.3 mg CA1139A/L, and a control (dechlorinated tap water).
Vehicle and/or positive control:	No vehicle/3,5-dichlorophenol: 3.2, 10 and 32 mg/L (nominal)
Analysis of test concentrations:	No
Test animals	
Sample/ specimen:	Activated sludge from sewage treatment plant (GH-4153, Reinach, 13/05/91)
Water:	Dechlorinated tap water
Nutrient solution	16 g peptone/L 11 g meat extract/L 3.0 g urea/L 0.7 g NaCl/L 0.4 g $CaCl_2 \cdot 2H_2O$/L 0.2 g Mg $SO_4 \cdot 7\ H_2O$/L 2.8 g K_2HPO_4/
Test design	
Vessel:	250 mL BOD flasks with gas inlet
Duration:	3 h
Sludge concentration:	1.69 g/L
Environmental conditions	
Test temperature:	20 ± 2 °C
pH:	8.1-8.4

Study Design and methods:

Experimental dates: 28th July 1992

Test procedure

The preparation of activated sludge from the aqueous layer was carried out using the settling method. Incorporation of the test material was not stated, from the data provided it would appear the test material is added to the sludge using an aqueous phase. The test vessels were stirred throughout keeping the substance homogenous throughout the test.

Measurements

Oxygen consumption per hour in mg/L was measured using an ORION-Electrode and recorded using a microprocessor.

Inhibition was determined on the basis of the measured oxygen consumption of a blank in comparison to

a control (not test substance/ reference material added. Nominal concentrations were used to determine inhibitory concentrations.

Results and Discussion:

Oxygen consumption of each test system together with the percent inhibition relative to the control is presented in the table below.

Table 8.8-3: Effects of CA1139A on activated sludge microflora

Sample	Concentration (mg/L)	O_2 consumption (mg/L/h)	Inhibition (%)
Blank 1	0	51.1 (30.2)	0
Blank 2	0	50.9 (30.1)	0
Ref. 1	3.2	14.2	6
Ref. 2	10	37.0	27
Ref. 3	32	47.8	72
CA1139A	1	50.6	1
	3.2	48.2	6
	10	44.1	14
	32	30.8	40
	100	18.9	63

Numbers in brackets represent the O_2 consumption in the blank controls per g of sludge

Validity criteria

Two out of the 3 validity criteria were met in that:

- Oxygen consumption in the blank controls should be ≥20 mg O_2/g of activated sludge/hour (mean consumption was 30.15 mg/g/h).
- The EC_{50} for the reference material should be within the range of 2-25 mg/L (actual estimated EC_{50} was 16.9 mg/L).
- The coefficient of variation for O_2 consumption between the controls should be < 30%. No reference was made to this parameter in the report.

Conclusions:

The EC_{50} for effects on activated sludge microorganism was determined to be 64.2 mg CA1139A/L

(Baumann W, 1992)

Relevant Literature on Sewage Treatment

No relevant scientifically peer-reviewed open literature could be found on cyprodinil or its major metabolites. Details of the literature search undertaken can be found in the M-CA Section 9 of the current submission.

CA 8.9 Monitoring Data

No monitoring data is available on the effects of the active substance in the EU.

Relevant Literature on Monitoring Data

No relevant scientifically peer-reviewed open literature could be found on cyprodinil or its major metabolites. Details of the literature search undertaken can be found in M-CA Section 9 of the current submission.